PTMBact 2024

5th International Congress on Post-Translational Modifications in Bacteria

May 29-30, 2024, Rouen, France

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"The ambition of the XL-Chem University Research School (EUR) is to become a benchmark training and research center in molecular chemistry at European level. The EUR XL-Chem is part of Normandie Université's "Chemistry, Biology, Health and Well-being" cluster. It aims to become a leading chemistry school in France and one of the major players in Europe for training and research in Organic Synthesis, Polymer Chemistry, Spectrochemistry and Cosmetics."

"The GDR 2038 entitled "Modifications Post-Traductionnelles Bactériennes" (BPTM) has been created for 5 years to study the roles played by post-translational modifications (PTMs) in bacterial physiology and the host/environment/bacteria relationship. It is now clear that the modified proteins and enzymes involved in these modification mechanisms are potential therapeutic targets for the development of new antibacterial strategies."

"The National Center for Scientific Research is one of the most important research institutions in the world. To meet the major challenges of the present and future, its scientists explore life, matter, the Universe and the functioning of human societies. Internationally recognized for the excellence of its scientific work, the CNRS is a reference both in the world of research and development and for the general public."

"The Rouen-Normandie Metropolis (Métropole Rouen Normandie) now comprises 71 municipalities, 498,822 inhabitants and a territory of 664 km2. Its responsibilities include (1) economic, social and cultural development and planning; (2) metropolitan spatial planning; (3) local housing policy; (4) urban policy; (5) management of services of public interest; and (6) environmental protection and enhancement."

"HeRacLeS brings together three platforms which have been nationally recognized for many years by the successive labels RIO then IBiSA: PRIMACEN (2001), PISSARO (2004) and SCAC (2008). These IBiSA platforms are also unique in Normandy not only because of their history and their label but also because of the very complete spectrum of technologies and methodologies mastered in their respective themes."

"The Norman Institute of Molecular, Medicinal and Macromolecular Chemistry (INC3M) is a Research Federation, certified by the Chemistry Department of the CNRS in January 2008 (FR 3038). The main objectives of the Federation are 1) the synergy of scientific skills, 2) the development of new research projects, 3) the thematic organization of projects around three well-defined axes and 4) the pooling of specific equipment."

"The Regional Research Federation “Health Safety, Well-being and Sustainable Food” is based on a strong and complementary structuring of research in microbiology and toxicology in the Normandy region, and is based on a history of collaborations in terms of research and training between Norman scientists in biology and analytical chemistry. It brings together seven research teams from Normandy universities and six technological structures."

"With over 32,000 students and 37 research units, the University of Rouen Normandy (URN) plays a leading role in teaching and research in Normandy. It is involved in regional teaching and research clusters, and promote interregional research activities by developing nationally and internationally renowned scientific partnerships. The URN therefore partake in numerous international projects in partnership with over 40 countries."

"The Faculty of Science and Technology offers a rich range of diplomas, both general and professional and research-oriented (doctoral schools) on 3 sites: Mont-Saint-Aignan, Le Madrillet and Évreux. This training offer relies heavily on the activity of our 14 recognized research laboratories (some are the first in France in their field)."

"The biscuit factory was created in 1850 and our century-old know-how has been recognized by the EPV (Living Heritage Company) label since 2017. We produce our specialties with local ingredients such as Isigny AOP butter, in order to facilitate our exchanges with our suppliers, develop the local economy and minimize our carbon footprint."

"The French Proteomics Society (FPS) is a scientific and technical association which aims to encourage, promote and carry out any action for the development of methods for the separation, characterization and quantification of peptides and proteins. It organizes alone, or in partnership with other learned societies, Congresses and Thematic Days. She also collaborates with the Youth Club to organize scientific meetings. It awards scholarships allowing our members, particularly the youngest, to participate in national or international conferences."

"The FHU RESPIRE aims to strengthen hospital-university-research unit relations within the North-West inter-region, in order to stimulate medical research on the theme of respiratory health and improve the quality of care through more rapid dissemination of innovations. The aim of the RESPIRE FHU is to improve respiratory health through an integrated approach that takes into account host characteristics from childhood onwards (immune response to aggression, microbiome, respiratory comorbidities), the environment, pathogens and their interactions at the pulmonary level."

"Pathogens is an international, peer-reviewed, open access journal on pathogens and pathogen-host interactions published monthly online by MDPI. The journal provides Open Access, high Visibility, rapid publication and recognition of reviewers."

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Program

Wednesday 29th May 2024

08:00 - 08:30	Registration of participants
08:30 - 08:45	Welcome and opening remarks
08:45 - 10:50	**Session 1 - Methods for PTM characterization (Chairwoman Julie Hardouin)**
08:45 - 09:30	Post-Translational Modifications in metaproteomics: a new level of complexity, but a new possible horizon - Jean Armangaud (France)
09:30 - 09:50	Quorum sensing in *Streptococcus thermophilus* : analytical condition from pheromones to RiPPs-modified peptides - Quentin Caillot (France)
09:50 - 10:10	FISH – flow cytometry reveals microbiome-wide changes in post-translational modification and altered microbial abundance among children with inflammatory bowel disease - Billy Bourke (Ireland)
10:10 - 10:30	Characterization of bacterial RiPPs involved in adaptation to copper: insights from top-down, bottom-up and native mass spectrometry approaches - Séverine Zirah (France)
10:30 - 10:50	On the merit of data reuse to infer protein co-regulation and PTM cross-talks - Nicolas Nalpas (France)
10:50 - 11:15	Coffee break
11:15 - 13:00	**Session 2 : What about glycans and derivatives ? (Chairman Nicolas Nalpas)**
11:15 - 12:00	Unusual glycan modifications on bacterial proteins - why are they there? - Christine Szymanski (USA)
12:00 - 12:20	Exploring the role of glucose-1,6-bisphosphate as a metabolic regulator - Sofia Doello (Germany)
12:20 - 12:40	Modular multi-step chemical derivatization of sialic acid for flagellin glycosylation in *Caulobacter* and a heterologous host for bio-conjugation - Silvia Ardissone (Switzerland)
12:40 - 14:00	Lunch
14:00 - 14:10	**Sponsors' talks**
14:10 - 15:55	**Session 3 - PTMs involved in stress response and adaptation (Chairman Christophe Grangeasse)**
14:10 - 14:55	Protecting Nascent Polypeptides from Premature Aging - Jean-François Collet (Belgium)
14:55 - 15:15	Connection between protein-tyrosine kinase inhibition and coping with oxidative stress in *Bacillus subtilis* - Ivan Mijakovic (Sweden)
15:15 - 15:35	The control of protein arginine phosphorylation facilitates protein homeostasis by an AAA+ chaperone protease system in *Bacillus subtilis* - Kürşad Turgay (Germany)
15:35 - 15:55	The CpxAR two-component system confers a fitness advantage for flea gut colonization by the plague bacillus - Robin Brandon (France)
15:55 - 17:30	**Coffee break and Poster**
18:15 - 19:45	Excursion
20:00	Dinner

Thursday 30th May 2024

09:00 - 10:45	**Session 4 - PTMs involved in bacterial resistance or virulence - part 1 (Chairman Nicolas Nalpas)**
09:00 - 09:45	Lipoprotein modification in bacteria, a novel target for antibiotics - Nienke Buddelmeijer (France)
09:45 - 10:05	The regulatory nitrogen-related phosphotransferase system PTSNtr participates in the cell envelope stress response in *Escherichia coli* - Boris Görke (Austria)
10:05 - 10:25	EnvZ-OmpR: a two-component system on all fronts of the *Yersinia pestis* infection cycle - Sébastien Bontemps-Gallo (France)
10:25 - 10:45	Molecular characterization of the putative Ser/Thr kinase HipA in *Klebsiella pneumoniae* - Payal Nashier (Germany)
10:45 - 11:15	Coffee break
11:15 - 13:00	**Session 5 - Roles of acylation actors (Chairwoman Julie Hardouin)**
11:15 - 12:00	Addressing the possibility of a histone-like code in bacteria - Valerie Carabetta (USA)
12:00 - 12:20	Post-translational modification of the bacterial ribosome - Christopher Rao (USA)
12:20 - 12:40	The Role of EF-P Post-translational Modifications in Bacterial Translation Stress Response - Alina Sieber (Germany)
12:40 - 13:00	Bioinformatics advances to uncover the viral and microbial acetylome - Hannelore Longin (Belgium)
13:00 - 14:15	Lunch
14:15 - 16:00	**Session 6 - PTMs involved in bacterial resistance or virulence - part 2 (Chairman Ivan Mijakovic)**
14:15 - 15:00	Control of bacterial peptidoglycan biosynthesis by an essential protein phosphorylation - Sven Halbedel (Germany)
15:00 - 15:20	hipL and hipIN are homologous toxin-antitoxin-like kinase systems regulated by auto-phosphorylation and internal translation initiation - Ditlev E. Brodersen (Denmark)
15:20 - 15:40	Exploring the role of lysine acetylation during phage infection - Nand Broeckaert (Belgium)
15:40 - 16:00	Role of bioenergetic pumps, F- and V-type ATP-synthase/ATPase in *Streptococcus pyogenes* pathogenesis - Vijay Pancholi (USA)
16:00 - 16:15	**Oral & Poster prize awards**
16:15 - 16:30	**Concluding remarks**

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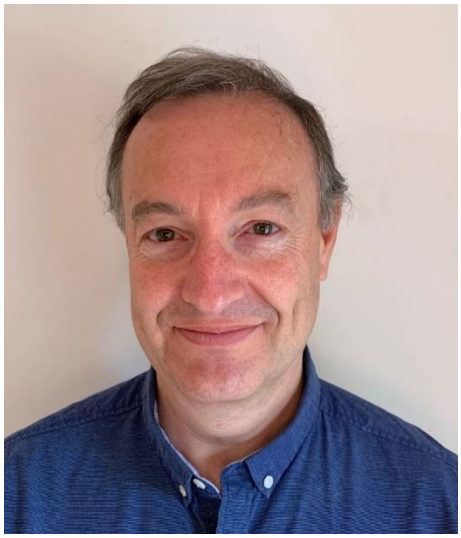
Keynotes resume

Jean ARMENGAUD

CEA-Li2D (ProGénoMix platform), Avignon, France

"Post-Translational Modifications in metaproteomics: a new level of complexity, but a new possible horizon"



Jean Armengaud (https://orcid.org/0000-0003-1589-445X) is specialized in mass spectrometry for biology and more specifically in proteogenomics and metaproteomics, having obtained his PhD in Biochemistry in 1994 from the University of Grenoble. He heads the ProGénoMIX platform located near Avignon in France, node of the ProFi Proteomics French Infrastructure. He is co-author of over 300 scientific publications. His aim is to contribute to a better understanding of the functioning of complex biological systems and to exploit this knowledge for medical and environmental purposes.

Christine SZYMANSKI

Department of Microbiology and Complex Carbohydrate Research Center, University of Georgia, Athens, USA

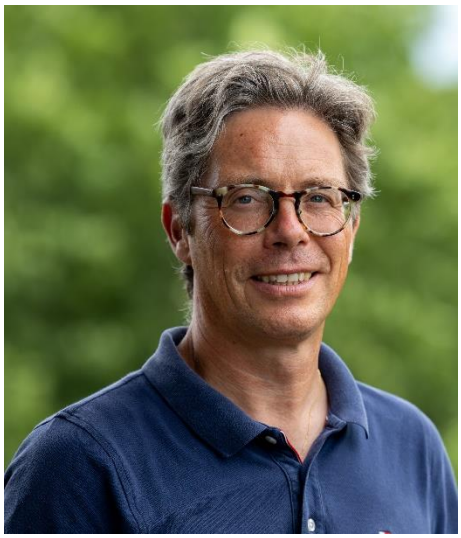
"Unusual glycan modifications on bacterial proteins - why are they there?"

Dr. Christine Szymanski first began working with *Campylobacter jejuni* during her PhD in the Department of Medical Microbiology and Immunology at the University of Alberta in Edmonton, Canada. She then joined the Enteric Disease Vaccine Program at the Naval Medical Research Center in Silver Spring, Maryland for her postdoctoral studies where they first described bacterial N-linked protein glycosylation. Dr. Szymanski then began her own independent research career first at the National Research Council in Ottawa, Canada and then back at the University of Alberta. It was in Edmonton where she formed the company, VaxAlta Inc. with a focus on carbohydrate-based vaccines for livestock. Christine joined the University of Georgia in 2016 where she continues to: 1) characterize bacterial glycoconjugate pathways, 2) exploit bacteriophage recognition proteins that bind these structures, and 3) understand the protective benefits of host milk oligosaccharides to develop novel therapeutics and vaccines for the prevention of diarrheal diseases and post-infectious neuropathies such as Guillain-Barré Syndrome. She is a fellow of the American Academy of Microbiology and Senior Member of the National Academy of Inventors.

Jean-François COLLET

Duve Institute, UCLouvain, Bruxelles, Belgium

"Protecting Nascent Polypeptides from Premature Aging"



Dr. Jean-Francois Collet is a Professor of Biochemistry at the de Duve Institute (UCLouvain, Brussels). The goal of Collet's lab is to better understand the mechanisms by which bacteria adapt to ever-changing environmental conditions and respond to stress. The lab focuses on questions important to both fundamental science and biomedical fields, contributing to the global effort to find new antibacterial approaches by identifying novel protein machineries that play crucial roles in bacterial physiology. Dr. Collet's lab has identified several systems that protect bacterial proteins from oxidation and uncovered molecular mechanisms allowing bacteria to detect stress in their cell envelope and to mount a protective response. As co-director of the de Duve Institute, Dr. Collet has received national and international awards and is a member of the Royal Belgian Academy, the Royal Belgian Academy of Medicine, and the European Academy of Microbiology.

Nienke BUDDELMEIJER

Institut Pasteur, Université Paris Cité, CNRS UMR6047, INSERM U1306Paris, France

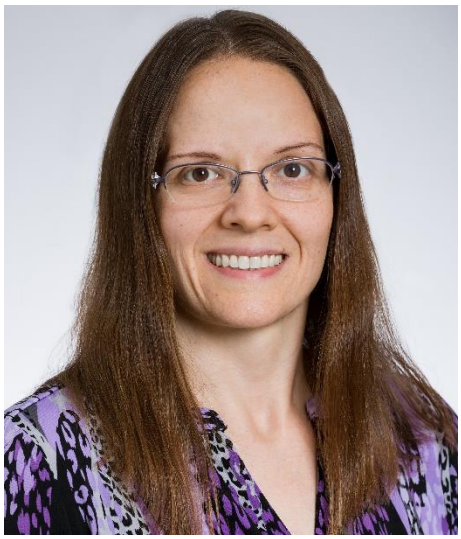
"Lipoprotein modification enzymes in bacteria – potential targets for novel antibiotics"

Dr. Nienke Buddelmeijer is an Assistant Professor in the Institut Pasteur of Paris. She earned her PhD in biology at the University of Amsterdam working on cell division. She did her postdoctoral research at the University of Amsterdam, Harvard Medical School and Institut Pasteur. Her group Cell Shape and Pathogenicity studies the bacterial lipoprotein modification pathway using a combination of bacterial genetics, biochemistry, structural biology, and inhibitor screening. This essential process involves integral membrane enzymes, two of which transfer a fatty-acid moiety from membrane phospholipids onto lipoproteins. The goal of her team is to obtain detailed insight on the role lipoproteins in the physiology of bacteria in relation to essential processes in the cell envelope and to understand how the degree of lipidation affects signaling of the innate immune system in pathogenic species. Furthermore, they develop assays to determine enzyme kinetics and substrate specificity, and design screening strategies to identify and characterize specific inhibitors of this essential pathway with the intention to find novel antibacterial agents. Their questions are how lipoproteins play a role in cell wall (peptidoglycan) biogenesis and how they contribute to cell envelope integrity in poorly studied organisms. As group leader, Dr. Buddelmeijer directly supervise students and young scientists and is involved in teaching and mentoring. She is co-coordinator of the Institut Pasteur Concerted Technological Actions on Drug Discovery and Screening and participates in national scientific evaluation committees and member of expert research groups.

Valerie CARABETTA

Cooper Medical School of Rowan University, Camden, USA

"Addressing the possibility of a histone-like code in bacteria"



Dr. Valerie Carabetta is an Assistant Professor in the Department of Biomedical Sciences at Cooper Medical School of Rowan University (CMSRU). She earned her PhD in molecular biology at Princeton University, characterizing stationary phase survival proteins in *Escherichia coli*. She did her postdoctoral research at the Public Health Research Institute at New Jersey Medical School, Rutgers University. The focus of her work was the study of the regulation of genetic competence, an important mechanism of the spread of antibiotic resistance genes among bacterial species. The overarching theme of her current laboratory is combatting antimicrobial resistance in bacteria. With the alarming trend of increasing antibiotic resistance and constant emergence of new "super bugs," the need to develop new antimicrobial strategies is critical. There are three active areas of ongoing research in the Carabetta lab, which approach this problem from different angles: 1) In Gram-positive bacteria, the role of lysine acetylation of the histone-like proteins on essential processes is being explored, with the goal of identifying novel drug targets, 2) Combinations of newly approved antibiotics, bacteriophage, and standard-of-care drugs are being evaluated to find effective new treatment options for highly drug-resistant infections, and 3) Antimicrobial peptide-loaded hydrogels are being developed to combat biofilm-related medical device infections.

Sven HALBEDEL

FG11 Division of Enteropathogenic bacteria and Legionella, Robert Koch Institute, Wernigerode, Germany

"Control of bacterial peptidoglycan biosynthesis by an essential protein phosphorylation"

Dr. Sven Halbedel is a bacterial geneticist currently working as a group leader at the Robert Koch Institute (the national public health institute in Germany) in Wernigerode. He earned his PhD at the University of Göttingen (DE), investigating regulatory protein phosphorylation in *Mycoplasma pneumoniae*, followed by postdoctoral work on *Bacillus subtilis* cell division proteins at Newcastle University (UK) and was recently appointed as an extraordinary professor at the University of Magdeburg (DE). His current research addresses the mechanisms synchronizing growth and cell division in Gram-positive bacteria, particularly in *Listeria monocytogenes*. A main focus lies on the role of PASTA kinases and their substrates in control of the proteolytic stability of MurA enzymes, which are the first committed step enzymes in bacterial peptidoglycan biosynthesis. Further research deals with bacterial systems for the sensing and detoxification of novel naturally occurring antimicrobial compounds and the genomic epidemiology of listeriosis within the frame of the national consultant laboratory for Listeria.

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Talks

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Methods for PTM characterization

1.1. Post-Translational Modifications in metaproteomics: a new level of complexity, but a new possible horizon

Jean Armangaud *† [1]

[1] CEA-Li2D – (ProGénoMix platform), Avignon, France – France

The exploration of microbial communities presents a formidable challenge owing to their vast diversity and intricate complexity. Metaproteomics, through the identification and quantification of proteins, offers a powerful avenue for delving into how microbiota function. Beyond elucidating fundamental functional attributes, which are inaccessible through alternative methodologies, metaproteomics empowers to establish the taxonomic constituents of the microbiota and track their fluctuations. Importantly, the interpretation of acquired data demands meticulous attention, with the choice of database and the mitigation of false positives emerging as pivotal considerations. The search of post-translational modifications within such a complex system escalates the challenge exponentially, given the sheer magnitude of the search space. These challenges inherent to metaproteomics, spanning mass spectrometry and data interpretation, will be addressed in the light of the performance of the latest generation of cutting-edge tandem mass spectrometers, exemplified by the Astral tandem mass spectrometer.

Keywords: metaproteomics, mass spectrometry, sequence database

*Speaker
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1.2. Quorum sensing in S. thermophilus: analytical condition from pheromones to RiPPs-modified peptides

Quentin Caillot *† [1], Alain Guillot , Lydie Oliveira Correia , Rozenn Gardan

[1] Quentin Caillot – Micalis Institute, INRA, AgroParisTech, Université Paris-Saclay, UMR 1319 – France

S. thermophilus, used in the dairy industry for fermentation, secretes 2 types of peptides as part of cell-cell communication called quorum sensing. SHP peptides, which are pheromones and control the activity of transcriptional regulators of the Rgg family, and RaS-RiPPs peptides, which are synthesised from operons whose transcription is controlled by these regulators. At least two these RaS-RiPPs are antimicrobial peptides. *S. thermophilus* strains have accumulated between 0 and 7 SHP/Rgg and their cognate RaS-RiPP operons in their genomes.
LC-MS/MS methods have already been developed for the specific detection of these peptides in culture supernatants. Of note, SHPs are hydrophobic whereas RaS-RiPPs are hydrophilic. The aim of this work was to develop an LC-MS/MS methodology for the exhaustive detection of SHPs and RaS-RiPPs peptides produced by *S. thermophilus* in a single analysis.

We optimized the separation, detection and fragmentation of these peptides. This enabled us to identify all the SHPs synthesised in four different wild-type strains of *S. thermophilus* including 3 new SHPs and 4 RaS-RiPPs including a new one never detected before in streptococci. Finally, we coupled the optimization of SHPs and RaS-RiPPs detection parameters to identify both types of peptide in a single and short run.

This methodology provides a comprehensive view of the peptides that are actually secreted and thus the systems that are functional. Developments dedicated to the quantification of the peptides are in progress. For SHPs, as their amino acid sequences can be very similar and their function interchangeable, these results will help to understand potential interferences at the cell level validated at the end with the detection of RaS-RiPPs.
In the future, this powerful tool will enable numerous applications for all bacteria including several pathogenics streptococcal possessing these systems, with the aim of better understanding and controlling quorum sensing.

Keywords: Streptococcus thermophilus, quorum sensing, LC/MS/MS, RaS RiPPs

*Speaker
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1.3. FISH – flow cytometry reveals microbiome-wide changes in post-translational modification and altered microbial abundance among children with inflammatory bowel disease

Mevlut Ulas* [1,2], Seamus Hussey [2], Annemarie Broderick [2], Emer Fitzpatrick [2], Cara Dunne [2], Sarah Cooper [2], Ania Dominik [2], Billy Bourke †‡ [1,2]

[1] University College Dublin [Dublin] – Ireland
[2] Children's Health Ireland at Crumlin – Ireland

Background: In previous studies we have shown altered p-Tyr in bacteria exposed to hydrogen peroxide following infection. The faecal microbiota are exposed to a surge in inflammatory ROS during active inflammatory bowel disease (IBD). However, metaproteomic analysis of microbiome post-translation modifications (PTM) is challenging, and little is known of the effects of inflammation on bacterial PTM landscape in IBD. Here we adapted and optimised fluorescence in situ hybridisation-flow cytometry (FISH-FC) to study microbiome wide tyrosine phosphorylation (p-Tyr) in children with and without IBD.
Results: Microbial p-Tyr signal was significantly higher in children with, compared to those without, IBD. *Faecalibacterium prausnitzii, Bacteroidota, Gammaproteobacteria* and *Bifidobacteria* tended to be more abundant in IBD than in non-IBD control children but there were only minor differences in p-Tyr among these bacterial communities in those with and without IBD. p-Tyr was significantly lower in non-IBD children older than 8yrs compared with those less than 8yrs and the effect was seen in all 4 bacterial subgroups studied. The opposite trend was seen in patients with IBD.
Conclusions: FISH-FC can be used to study the microbiome-wide PTM landscape. P-Tyr overall is higher in children with IBD but the affects of inflammation on p-Tyr vary according to the bacterial community and the overall microbiome p-Tyr signal changes with age in healthy children.

Keywords: Microbiome, post, translational modification, tyrosine phosphorylation, FISH, flow cytometry, Inflammatory Bowel Disease

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1.4. Characterization of bacterial RiPPs involved in adaptation to copper: insights from top-down, bottom-up and native mass spectrometry approaches

Séverine Zirah *† [1], Céline Guillaume [1], Laura Leprevost [2], Sophie Jünger [1], Lydie Oliveira [3], Céline Henry [3], Guy Lippens [4], Rudy Antoine [2], Svetlana Dubiley [4], Yanyan Li [1], Françoise Jacob-Dubuisson‡ [2]

[1] Molecules of Communication and Adaptation of Microorganisms (MCAM) – Museum National d'Histoire Naturelle, Centre National de la Recherche Scientifique – France
[2] Center for infection and Immunity of Lille (CIIL) – Institut Pasteur de Lille, Institut National de la Santé et de la Recherche Médicale, Université de Lille, Centre Hospitalier Régional Universitaire [CHU Lille], Centre National de la Recherche Scientifique – France
[3] MICrobiologie de l'ALImentation au Service de la Santé – AgroParisTech, Université Paris-Saclay, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement – France
[4] Toulouse Biotechnology Institute – Institut National des Sciences Appliquées - Toulouse, Centre National de la Recherche Scientifique, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement : UMR0792 – France

Copper homeostasis is crucial for bacteria to ensure acquisition of copper while preventing its adverse effects at high concentration. We identified a new class of ribosomally produced and post-translationally modified peptides produced by *Caulobacter crescentus*, that we called "bufferins", capable of enhancing bacterial growth under copper stress. *C. crescentus* produces two bufferins, Buf1 and Buf2, which harbor 4 and 6 cysteine residues, respectively. These peptides were characterized by top-down and bottom-up peptidomic approaches, which revealed that their maturation involves cleavage of the signal peptide together with a modification yielding a -10 Da and -18 Da shift for Buf1 and Buf2, respectively. In both peptides, the central cysteines carry a 4 Da shift modification while the peripheral ones are involved in a disulfide bridge. The complexation of bufferin 1 to copper was shown by native mass spectrometry. Finally, NMR analysis showed that the cysteines are modified into thiooxazole heterocycles, and in vivo analysis of the biosynthesis showed that thiooxazole formation is catalysed by multinuclear non-heme iron-dependent oxidative (MNIO) enzymes. Bufferins constitute a new family of RiPPs providing an additional line of bacterial defense against copper. This presentation will focus on their characterization by mass spectrometry.

Keywords: RiPP, copper, mass spectrometry

*Speaker
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1.5. On the merit of data reuse to infer protein co-regulation and PTM cross-talks

Nicolas Nalpas * [1], Lisa Brémard [1], Evan Croize [1], Emmanuelle Dé [1], Julie Hardouin† [1]

[1] University of rouen – Normandie Univ, University of Rouen, Laboratory of Polymers Biopolymers Surfaces (PBS),UMR 6270 – France

Acinetobacter baumannii is an opportunistic bacterial pathogen causing numerous deaths each year due to its ability to develop antibiotic resistance. As of 2020, only 43 antibiotics were in development with the vast majority targeting known mechanisms.It is urgent today to find new strategies to fight against this bacterium. In this context, the integration of already published data is an important source of information that can reveal the molecules interrelationships and derive clinical knowledge. Here, we re-analysed dozens of proteomic datasets across different strains of *A. baumannii* and different culture or stress conditions to predict novel co-regulated proteins and PTM cross-talks.

Over a thousand LC-MS2 files were reanalyzed to derive protein quantification across different experimental conditions. Using machine learning, we computed a co-regulation score for each protein pair and validated our prediction with a list of known protein interactions. Hundreds of novel protein co-regulation links were predicted, interestingly virulence-associated proteins were involved in several. For example, the general secretion pathway protein G (GspG) was implicated with 28 novel co-regulators, of which 16 proteins had functions related to the transport of molecules. Several proteomic datasets also investigated the O-phosphorylation, K-succinylation, K-acetylation and K-trimethylation of proteins. For each modified protein, we used the multi-sequence alignment of all its orthologs to compute the co-conservation score of the modified residues. Among 4,368 residues that were modified in *A. baumannii*, 19 residue pairs were co-evolving. These may represent acetyl-succinyl or phosphorylation-phosphorylation cross-talks in the L11 ribosomal protein (rplK) and acetylglutamate kinase (argB), respectively.

Currently our work focuses on the bioinformatic validation of the co-evolving residues, notably by verifying the modification status in other bacterial species. This resource of protein co-regulation and putative PTM cross-talks is the first of its kind for *A. baumannii* and contributes to our understanding of the cell signalling in this organism.

Keywords: PTM cross, talks, Acetylation, Succinylation, Phosphorylation, Trimethylation, Protein co, regulation, Bioinformatics

*Speaker

†Corresponding author: julie.hardouin@univ-rouen.fr

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What about glycans and derivatives ?

2.1. Unusual glycan modifications on bacterial proteins - why are they there?

Christine Szymanski *† [1,2], Nicholas De Mojana Di Cologna [3], Silke Andresen [4,5], Stephanie Archer-Hartmann [6], Nil Cortiella Vals [7], Parastoo Azadi [6,7], José Lemos [3], Camilo Perez [7], Jacqueline Abranches [3]

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[2] Department of Microbiology, University of Georgia, Athens, GA, USA – United States
[3] Department of Oral Biology, University of Florida, College of Dentistry, Gainesville, FL, USA – United States
[4] Complex Carbohydrate Research Center, University of Georgia, Athens, GA, USA – United States
[5] Department of Microbiology, University of Georgia, Athens, GA, USA – United States
[6] Complex Carbohydrate Research Center, University of Georgia, Athens, GA, USA – United States
[7] Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA, USA – United States

Dental caries affects 2.5 billion people worldwide. *Streptococcus mutans* is an important pathogen in dental caries due to its biofilm lifestyle, and acidogenic and aciduric nature. In addition, expression of the *S. mutans* collagen binding protein Cnm, present in 20% of the strains, is intimately associated with severe caries, systemic infections and auto-immune disorders. Our mass-spectrometry studies coupled with lectin detection have demonstrated that the threonine-rich repeat (TRR) domain of *S. mutans* Cnm, with > 70 threonine residues, undergoes extensive O-glycosylation with GalNAc and GlcNAc residues. We also found that *cnm* is co-transcribed with a downstream gene encoding a putative GT-A type glycosyltransferase, named *pgfS* (protein glycosyltransferase of streptococci). We identified three genes immediately downstream of *pgfS,* namely *pgfM1, pgfE* and *pgfM2,* that also contribute to Cnm modification. Importantly, the *pgf* genes are part of the *S. mutans* core genome and our findings suggest that we have uncovered an O-glycosylation pathway dedicated to modifying multiple carbohydrate binding adhesins. Surprisingly, we also detected phosphate modifications in the TRR domain of Cnm in *pgfS* and *pgfM2* mutants, and in low levels in wildtype Cnm. Phenotypic characterization of the *pgf* mutants in *S. mutans* OMZ175 revealed that protein glycosylation alters bacterial biofilm formation, surface charge, membrane stability, genetic competence, antibiotic sensitivity, human saliva survival, and rat oral colonization. This talk will summarize our current knowledge on Pgf-mediated O-glycosylation/phosphorylation and how these pathways impact *S. mutans* pathogenesis.

Keywords: Dental caries, Streptococcus mutans, O, glycosylation, Phosphorylation

*Speaker
†Corresponding author: cszymans@uga.edu

2.2. Exploring the role of glucose-1,6-bisphosphate as a metabolic regulator

Sofia Doello *† [1], Niels Neumann [1], Philipp Spaet [1], Boris Macek [1], Karl Forchhammer [1]

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Glycogen is a wide-spread sugar reserve important for the survival of a broad range of organisms and its metabolism is conserved from bacteria to humans. Glycogen synthesis and degradation require the interconversion between glucose-1-phosphate and glucose-6-phosphate, which is catalyzed by the bi-directional enzyme phosphoglucomutase (PGM1). This reaction represents a crucial point connecting glycogen synthesis and degradation with the central carbon metabolism (Doello and Forchammer, 2023). The catalytic activity of PGM1 relies on the phosphorylation of a serine residue located in the active site (Neumann et al., 2022). Phosphorylation of this residue and activation of the enzyme require the metabolic intermediate glucose-1,6-bisphosphate. Thus, this molecule plays an important regulatory role in the metabolism of carbon. We discovered that in cyanobacteria glucose-1,6-bisphosphate is produced by an enzyme of a different related family, PGM2 (Neumann et al., 2022). Interestingly, while cyanobacteria can survive without PGM1 under conditions in which glycogen is not required (photoautotrophic growth), a PGM2 knock out mutant could not be obtained under these conditions, suggesting that glucose-1,6-bisphosphate regulates other essential proteins (Ortega-Martinez et al., 2023). Here we used a phosphoproteomics approach to explore the role of glucose-1,6-bisphosphate as a metabolic regulator.

Keywords: phosphorylation, glycogen, phosphoglucomutase, carbon metabolism

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2.3. Modular multi-step chemical derivatization of sialic acid for flagellin glycosylation in Caulobacter and a heterologous host for bio-conjugation

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Chemical diversification of sialic acids on surface structures including flagella, capsules or lipopolysaccharides is widespread across bacterial lineages. Despite the plethora of naturally occurring sialic acid derivatives, the genetic basis for derivatization remains unresolved. We discovered a tricistronic module, FlmEFX, that modifies the pseudaminic acid (Pse) moiety present on the six glycosylated flagellins of *Caulobacter crescentus,* a synchronizable α-proteobacterium assembling a polar flagellum. We found that FlmEFX controls the multi-step chemical derivatization of Pse and that balanced FlmEFX expression is important to prevent a block in Pse-dependent flagellin glycosylation, assembly and secretion. While the putative methyltransferase FlmE promotes a 28 Da modification of the basic Pse unit, together the FlmEFX unit enhances heterologous bio-conjugation of flagellins with the terminal Pse-derivative in recombinant *Escherichia coli* producing Pse and the flagellin glycosyltransferase FlmG. FlmEFX co-occur mostly with FlmG-dependent flagellin glycosylation systems, yet orthologs are also encoded in O-antigen gene clusters of pathogens.

Keywords: glycosylation, flagellin, pseudaminic acid, bioconjugation, sialic acid derivative

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PTMBact 2024

PTMs involved in stress response and adaptation

3.1. Protecting Nascent Polypeptides from Premature Aging

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Bacteria deal with many different types of stress. In my presentation, I will talk about a new mechanism that allows bacteria to handle oxidative stress. Specifically, I will explain how a helper protein called CnoX works with the GroEL chaperonin to fold proteins damaged by oxidation.

Keywords: stress response, oxidative stress, CnoX protein

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3.2. Connection between protein-tyrosine kinase inhibition and coping with oxidative stress in Bacillus subtilis

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We report that DefA, a minor peptide deformylase from *Bacillus subtilis*, contributes to resistance against oxidative stress. During oxidative stress, DefA catalytic function gets attenuated and DefA interacts with a bacterial protein-tyrosine kinase (BY-kinase) PtkA. High levels of PtkA activity are known to destabilize *B. subtilis* pellicle formation, which leads to higher sensitivity to oxidative stress. DefA inhibits PtkA autophosphorylation and phosphorylation of its substrate Ugd, which is involved in exopolysaccharide formation. Inactivation of *defA* does not affect the major oxidative stress regulons in *B. subtilis,* indicating that PtkA inhibition is the main pathway for DefA involvement in oxidative stress response. Our structural analysis identified DefA residues Asn95, Tyr150 and Glu152 as essential for interaction with PtkA. Inhibition of PtkA depends also on the presence of a C-terminal α-helix of DefA, which resembles PtkA-interacting motifs from known PtkA activators, TkmA, SalA and MinD. Loss of either the key interacting residues or the inhibitory helix of DefA abolishes inhibition of PtkA *in vitro*, and impairs post-oxidative stress recovery *in vivo*, confirming their involvement in the proposed mechanism.
Co-authors:

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Keywords: BY, kinase, oxidative stress, protein phosphorylation, protein, protein interaction, peptide deformylase

*Speaker

3.3. The control of protein arginine phosphorylation facilitates protein homeostasis by an AAA+ chaperone protease system in Bacillus subtilis

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AAA+ proteins, such as ClpC from *B. subtilis*, are chaperones with unfoldase activity that can form with ClpP an AAA+ protease complexes, participating together with adaptor proteins in the control of stress response and protein homeostasis. Here, we demonstrate that the protein arginine kinase and adaptor protein McsB, with its activator McsA, can recognize and phosphorylate misfolded and aggregated proteins and is responsible for targeting them for disaggregation and refolding by the AAA+ unfoldase ClpC. Interestingly, the presence of substoichometric YwlE phosphatase was necessary for the substrate protein refolding, most likely for the de-phosphorylation of the arginine-phosphorylated substrate protein extruded by ClpC. We observed that the successfully refolded, but not the still unfolded substrate proteins, could escape the transfer to ClpP degradation. This unique chaperone-protease system functions in the *B. subtilis* protein quality control system by disaggregating and refolding aggregated proteins, but can also remove more severely damaged protein aggregates by degradation.

Keywords: Stress response, protein homeostasis, chaperones, AAA+ proteins, adaptor proteins, protein arginine phosphorylation, McsB kinase, YwlE phosphatase, ClpCP

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3.4. The CpxAR two-component system confers a fitness advantage for flea gut colonization by the plague bacillus

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Yersinia pestis, the agent of flea-borne plague, must adapt to distinctly different environments in its flea vector and mammalian hosts. Successful transmission by the hematophagous insect requires the bacteria to adapt to the flea's lumen gut. Two-component systems are key players in bacterial adaptation. A previous transcriptomic study showed that the Cpx pathway is up-regulated in fleas compared to *in vitro* culture. The CpxAR two-component system is well described in other pathogens to be part of the envelope stress response and is critical for maintaining the integrity of the cell. Despite the CpxAR involvement in *Y. pestis*' resistance to the bactericidal activity of human neutrophils, no role in bubonic or pulmonary plague has been found. However, the role of the CpxAR system in the flea has not yet been investigated. Here, a phenotypic screening revealed a survival defect of the *cpx* mutant to oxidative stress and copper. Measured copper concentration in the blood meal isolated from the gut of fed fleas increased 4-fold during the digestive process. By direct analysis of phosphorylation of CpxR by a Phos-Tag retardation gel approach, we demonstrated that biologically relevant concentrations of copper triggered the system. Lastly, a competitive challenge highlighted the role of the CpxAR system in bacterial fitness during flea infection. Hence, we conclude that, during the flea's gut colonization, CpxAR is required to counter copper production by the flea.

Keywords: Yersinia pestis, TCS, phosphorylation, cpxAR, fitness

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PTMs involved in bacterial resistance or virulence - part 1

4.1. Lipoprotein modification in bacteria – a novel target for antibiotics

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Bacterial lipoproteins play an important role in bacterial physiology and virulence. They are part of protein complexes involved in cell envelope biogenesis and they function as signaling molecules of the innate immune system. The post-translational modification of lipoproteins is catalyzed by several integral membrane enzymes, some of which are essential for viability in diderm bacteria. We have developed a high-throughput screen for small compound inhibitors of apolipoprotein N-acyltransferase (Lnt) and we recently showed that phosphatidylglycerol:prolipoprotein diacylglyceryl transferase (Lgt), the first and highly conserved enzyme in the pathway, is essential in E. coli in the absence of major lipoprotein Lpp that covalently cross-links the outer membrane to the peptidoglycan. This suggests that other outer membrane lipoproteins have an important role in viability. Our results strengthen the hypothesis that lipoprotein modification is a good target for novel antibiotics. We are now asking to what extent the enzymes are conserved in bacteria to learn more about the molecular mechanism of the reaction and their substrate specificity, and to predict whether broad- or narrow spectrum inhibitors could be developed in the future.

Keywords: lipoproteins, virulence, outer membrane

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4.2. The regulatory nitrogen-related phosphotransferase system PTSNtr participates in the cell envelope stress response in Escherichia coli

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The "nitrogen-related" PTS(Ntr) constitutes an abridged phosphotransferase system transferring phosphoryl-groups originating from PEP via PtsP and NPr to the EIIA paralog PtsN. PtsN has no role for sugar transport but regulates various target proteins in a phosphorylation state-dependent manner. For instance, in *E. coli* the expression or activity of several potassium transport systems including the high affinity potassium transporter KdpFABC is controlled by non-phosphorylated PtsN, whereas phosphorylated PtsN binds and inhibits GlmS – the first enzyme in the pathway synthesizing UDP-GlcNAc, an essential precursor required for making the Gram-negative cell envelope. Recent results propose an even more extensive interaction network for PtsN including many metabolic enzymes. As far as known, the phosphorylation state of PTSNtr is controlled at two levels: A high glutamine/a-ketoglutarate ratio may inhibit PtsP autophosphorylation while phosphatase SixA is capable of removing phosphoryl-groups from the system by dephosphorylating NPr. Nonetheless, PtsN prevails predominantly in the phosphorylated form under standard laboratory conditions and conditions that would lead to dephosphorylation of PtsN in wild-type cells are basically unknown. Here, we report that the PTS(Ntr) is intimately interwoven with the envelope stress response governed by sigma E. Active sigma E increases expression of the *rpoN* operon including *ptsN* and *npr* by mediating read-through transcription from an upstream located promoter. Notably, sigma E also triggers partial dephosphorylation of PtsN involving SixA, which is itself a member of the sigma E regulon. Using a chemical screen, we identified osmotic stress and LPS damage elicited by antimicrobial peptides to cause dephosphorylation of PtsN in a SixA-dependent manner. We speculate that PTS(Ntr) activates potassium uptake to maintain turgor pressure and intracellular osmolarity. Concomitantly, it may redirect metabolism to achieve homeostasis of glutamate, which is required as counter-ion of potassium as well as for LPS modifications and synthesis of compatible osmolytes.

Keywords: nitrogen, related PTS, phosphorylation, envelope stress, potassium, sigma E, antimicrobial peptides

*Speaker

4.3. EnvZ-OmpR: a two-component system on all fronts of the Yersinia pestis infection cycle

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The plague agent *Yersinia pestis* mainly spreads among mammalian hosts and their associated fleas. Production of a successful mammal-flea-mammal life cycle implies that *Y. pestis* senses and responds to distinct cues in both host and vector. Two-component systems play a key role in sensing and adapting to fluctuating environmental conditions. An individual screening of mutants lacking each TCS allowed us to identify EnvZ-OmpR as required for production of bubonic and septicemic plague. Interestingly, we also demonstrated that the system ensures efficient transmission of *Y. pestis* to the mammalian host by increasing the likelihood of colonization of the flea's proventriculus. Notably, EnvZ-OmpR is involved in the consolidation of a soft bactericidal mass that entrapped *Y. pestis* within the proventriculus shortly after the infectious blood meal. Taken together, our results suggest that the EnvZ-OmpR system is used as a Swiss army knife by the plague bacillus to adapt to each stage of its infectious cycle. These results offer a unique - biologically relevant - perspective on how bacteria adapt their two-component system to invade a new host.

Keywords: Plague, Virulence, two component system, EnvZ OmpR, Yersinia pestis

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4.4. Molecular characterization of the putative Ser/Thr kinase HipA in Klebsiella pneumoniae

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Bacterial persisters are a subpopulation of antibiotic-tolerant bacteria produced by isogenic populations of antibiotic-sensitive bacteria. During antibiotic treatment, they can switch to slow growth with low metabolic activity (dormant-like state), resuming a normal growing state upon withdrawal of treatment. Persistent pathogens are a significant contributor to relapse in many chronic infections and frequently lead to antibiotic overuse and the development of antibiotic resistance. One of the best-studied drivers of persistence is the eukaryotic-type Ser/Thr kinase, HipA from *E. coli* and a recent study has identified homologs of the *hipA* in many bacterial species.

We aimed to characterize the putative *hipA* gene in the human pathogen *Klebsiella pneumoniae*, which shares 70.2% sequence identity with *hipA* in *E. coli*. *K. pneumoniae* belongs to the ESKAPE group of pathogens showing increasing resistance to antibiotics and the formation of multi-drug tolerant persister cells; however, mechanisms underlying persister formation in *Klebsiella* are poorly understood. To characterize *hipA* in *K. pneumoniae* (*hipAkp*), we performed a shotgun phosphoproteomics study and analyzed the impact of overproduced *hipAkp* in *E. coli* and *K. pneumoniae*.

We showed that, in both *E. coli* and *K. pneumoniae,* overproduced HipAkp is highly toxic for the cells which can be rescued by overproduced antitoxin HipBkp. Phosphoproteomics results showed that HipAkp auto-phosphorylates at Serine150 and multiple putative substrates, including the GltX (glutamate tRNA synthetase) at Serine239. In *E. coli*, this phosphorylation leads to the accumulation of uncharged glutamate tRNA and activates the stringent response in cells, ultimately leading to antibiotic tolerance and persistence. Importantly, HipAkp over-expression showed increased protection against certain antibiotic classes compared to WT *K. pneumoniae,* as previously seen upon *hipAec* overproduction in *E. coli*. Therefore, we postulate that *hipAkp* plays a role in regulating antibiotic tolerance and persistence in *K. pneumoniae*.

Keywords: Phosphoproteome, Klebsiella pneumoniae, Antibiotic tolerance, Persistence, Mass Spectrometry

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Roles of acylation actors

5.1. Addressing the possibility of a histone-like code in bacteria

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Ne-lysine acetylation was originally thought to be rare in bacteria but is now appreciated to affect hundreds of bacterial proteins with diverse cellular functions. Acetylation was initially discovered as a post-translational modification (PTM) on the unstructured, highly basic N-terminal tails of eukaryotic histones. Histone acetylation constitutes part of the "histone code," and regulates chromosome compaction and various DNA processes, such as gene expression, replication, repair, and recombination. In bacteria, the nucleoid is compacted and organized by the action of nucleoid-associated proteins (NAPs). The Bacillus subtilis protein HBsu is a member of the most widely conserved NAP family and is considered a functional equivalent of eukaryotic histones. We found that HBsu contains seven novel acetylation sites, and this raised the exciting possibility that these modifications represent a "histone-like" code in bacteria. Previously, we discovered that one function of HBsu acetylation is to maintain normal chromosome compaction. To further decipher this code, we are exploring the physiological significance of protein acetylation. We have determined that acetylation regulates the process of sporulation, in part by regulating gene expression. Moreover, we have shown that deacetylation at a few specific sites is important for this regulation. In addition, we have explored the role of HBsu acetylation on antibiotic challenge and survival. So far, our findings have implicated that acetylation at specific sites of HBsu influences different bacterial properties, supporting the existence of a bacterial histone-like code.

Keywords: histone, like, acetylation, HBsu protein

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5.2. Post-translational modification of the bacterial ribosome

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Lysine acetylation and methylation are important regulators of enzyme activity and transcription in eukaryotes. However, little is known about these post-translational modifications in bacteria. In this work, we investigated how lysine acetylation and methylation affects translation in *Escherichia coli.* In multiple species of bacteria, ribosomal proteins are highly acetylated and methylated at conserved lysine residues, suggesting that this modification may regulate translation. In the case of lysine acetylation, we found that the addition of either of the acetyl donors acetyl phosphate and acetyl-coenzyme A inhibits translation but not transcription using an *E. coli* cell-free system. Further investigations using *in vivo* assays revealed that acetylation does not appear to alter the rate of translation elongation but, rather, increases the proportions of dissociated 30S and 50S ribosomes, based on polysome profiles of mutants or growth conditions known to promote lysine acetylation. Furthermore, ribosomal proteins are more acetylated in the disassociated 30S and 50S ribosomal subunits than in the fully assembled 70S complex. The effect of acetylation is also growth rate dependent, with disassociation of the subunits being most pronounced during late-exponential and early-stationary-phase growth-the same growth phase where protein acetylation is greatest. Among the proteins with the most conserved methylation sites is ribosomal protein L11 (bL11). bL11 methylation has been a mystery for five decades, as the deletion of its methyltransferase PrmA causes no cell growth defects. Our data revealed that bL11 methylation is important for stringent response signaling. Moreover, we show that the ΔprmA mutant has an abnormal polysome profile, suggesting a role in ribosomal homeostasis during stationary phase growth. Collectively, our data demonstrate that lysine acetylation inhibits translation, most likely by interfering with subunit association, and that bL11 methylation is important for stringent response signaling and ribosomal homeostasis.

Keywords: acetylation, methylation, translation

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5.3. The Role of EF-P Post-translational Modifications in Bacterial Translation Stress Response

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Deciphering genetic information at the ribosome is a fundamental trait shared among all living organisms. However, specific sequence motifs can induce translational halts, with consecutive prolines being a common culprit (Doerfel, Wohlgemuth *et al.* 2013, Krafczyk, Qi *et al.* 2021). The universal translation elongation factor P (EF-P), known as initiation factor 5A in eukaryotes and archea, plays a crucial role in resolving these stalls, thereby allowing continuation of protein biosynthesis (Ude, Lassak *et al.* 2013). EF-P's functionality is often augmented by post-translational modifications (PTMs), as highlighted by Lassak *et al.* (Lassak, Sieber *et al.* 2022). Nature has independently evolved unique modification strategies, such as arginine-α-rhamnosylation (Lassak, Keilhauer *et al.* 2015), lysine-5-aminopentanolylation (Rajkovic, Hummels *et al.* 2016), and lysine-β-lysylation (Yanagisawa, Sumida *et al.* 2010). Here, we introduce a newly identified EF-P paralog in *Escherichia coli*, named EfpL, which undergoes extensive acylation (Weinert, Iesmantavicius *et al.* 2013, Kuhn, Zemaitaitis *et al.* 2014). Notably, unlike other EF-P PTMs, EfpL acylation impedes rescue activity. In contrast to the constitutively functional EF-P, EfpL enhances protein biosynthesis depending on the metabolic state of the cell. With our functional analysis of EfpL, we can introduce a new chapter, how PTMs play a pivotal role in facilitating efficient translation in bacteria.

Keywords: Acylation, PTM of Lysine, Translational Stress, Elongation factor, P

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5.4. Bioinformatics advances to uncover the viral and microbial acetylome

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Today, microbial acetylome studies frequently report hundreds of acetylation sites, shifting the bottleneck in acetylomics from site identification to functional elucidation. Indeed, the number of identified acetylation sites routinely eclipses the number of sites that can be investigated experimentally. Consequently, the enzyme-target-function relationship often remains unexplored. However, advances in bioinformatics, including AlphaFold2, the groundbreaking protein structure predictor, have the potential to significantly support and accelerate acetylome research (1). Here, we highlight our use of bioinformatics advances to (i) identify acetyltransferases, (ii) investigate acetylation conservation and (iii) simulate the biomolecular impact of acetylations.
Using a combination of sequence- and AlphaFold2-based techniques, the genomes of 190 *Pseudomonas*-infecting bacteriophage species were mined to identify acetyltransferases. Eight distinct acetyltransferase clades were delineated, all displaying the main characteristics of Gcn5-related N-acetyltransferases (GNATs). However, they presented clear taxonomic preferences and remarkable structural variability, most strikingly in the GNAT motif B helix, that binds acetyl-CoA. Moreover, AlphaFold2 and molecular dynamics were integrated to elucidate the impact of detected acetylations on *P. aeruginosa* proteins MetE and MetK, revealing that acetylation likely impacts the MetE ligand binding and MetK multimerization. To assess whether these acetylations had been reported previously, we developed FLAMS: a post-translational modification (PTM) search engine, matching modifications to dbPTM and CPLM (2). Based on FLAMS, acetylation of MetE's K738 and MetK's K285 appear to be widely conserved.

While these results focus on specific examples, our techniques are broadly applicable. Indeed, the GNAT detection pipeline could easily be repurposed to detect any PTM enzyme in any species. In addition, FLAMS also operates on large PTM datasets. To conclude, these results show how bioinformatics advances can boost acetylation (and other PTM) research, on the enzyme-, target- and function-front.

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Keywords: acetylation, bioinformatics, AlphaFold, methodological advances

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PTMs involved in bacterial resistance or virulence - part 2

6.1. Control of bacterial peptidoglycan biosynthesis by an essential protein phosphorylation

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Biosynthesis of the peptidoglycan (PG) mesh consumes substantial amounts of resources and energy and therefore needs to be harmonized with nutrient supply and growth conditions. In Gram-positive bacteria, PG biosynthesis is controlled by PASTA domain containing protein serine/threonine kinases, such as PrkA of the foodborne pathogen Listeria monocytogenes. Recently, it was shown that PrkA-dependent control of PG biosynthesis is exerted through phosphorylation of the small cytosolic protein ReoM. Phosphorylation of ReoM influenced the proteolytic stability of L. monocytogenes MurA, the first committed step enzyme in PG biosynthesis, in a ClpCP-dependent manner, and was linked to growth, PG biosynthesis and β-lactam resistance (1,2). However, many details on the mechanisms explaining how ReoM controls proteolytic degradation of MurA, how the _˜20 other known PrkA targets contribute to PG biosynthesis regulation and how ReoM phosphorylation itself is controlled stayed unclear. We have isolated murA mutants that escape ClpCP-dependent proteolytic degradation in suppressor screens. The murA escape mutations fully suppressed otherwise essential prkA-dependent phenotypes as well as the toxicity of a phosphoablative reoM mutation, suggesting that MurA stability control through regulation of ReoM phosphorylation is the key purpose of PASTA kinase signaling in L. monocytogenes. ReoM interacted directly with MurA and this interaction was sensitive to phosphorylation. Phosphorylation of ReoM was dependent on growth phase and genetic constellations were discovered, under which ReoM was not phosphorylated. Our results support a model according to which PrkA-dependent phosphorylation of ReoM activates PG production in response to signals related to cell cycle progression and/or metabolic workload in PG biosynthesis. They also have important implications for the high intrinsic β-lactam resistance levels of L. monocytogenes and related Gram-positive bacteria.

Keywords: peptidoglycan, PASTA kinase, phosphorylation

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6.2. hipL and hipIN are homologous toxin-antitoxin-like kinase systems regulated by auto-phosphorylation and internal translation initiation

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HipA-like kinases are widespread in bacteria and have been implicated in antibiotic persistence.1,2 Some HipA-like kinases are expressed from bicistronic toxin-antitoxin (TA) loci in which the active kinase (the toxin) has a cell growth-inhibitory effect that is negated by the product of the co-transcribed gene (the antitoxin). Phylogenetic analysis has revealed an intriguing plethora of different operon and protein structures among HipA-like kinases across the bacterial kingdom, including monocistronic and tripartite systems.1-4 Many of these kinases have serine residues close the active site and are regulated by auto-phosphorylation using complex mechanisms that include kinase inactivation (*hipBA*) and inhibition by the antitoxin (*hipBST*). Here we show that autophosphorylation is needed for the activity in the monocistronic *hipL* system, while not affecting toxicity of the HipI kinase encoded in bicistronic *hipIN* systems. Moreover, we show that the monocistronic system is regulated by internal translation initiation generating a unique, two-domain antitoxin. Together, our results highlight the complex regulation governing the activity of bacterial kinases.

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Keywords: toxin, antitoxin, kinase, HipA, persistence

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6.3. Exploring the role of lysine acetylation during phage infection

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Bacteriophages are the natural predators of bacteria and represent one of the most abundant entities in the biosphere. For a successful infection, these bacterial viruses take over and repurpose the host cellular machinery to produce new phage particles. In recent years, it has become apparent that posttranslational modifications – including lysine acetylation - can play a crucial role in host take-over, as well as in host defence(1).
To gain general insight on how phages utilize acetylation as a regulatory tool, we performed a comparative TMT-based acetylomics study. More precisely, we infected *P. aeruginosa* with five distinct phages individually. The infection process was then halted at the middle stage, after which the acetylation changes were measured (in triplicate). In total, 7,373 sites in 1,961 different proteins were detected. Around 29.6% (1,648/5,564) of the *P. aeruginosa* proteome was acetylated, a number markedly higher than previously reported for this species (8.9%)(2). Interestingly, multiple *P. aeruginosa* proteins – including several involved in the carbon metabolism (TCA, amino acid biosynthesis, ...) – contained sites which were significantly altered upon infection by several of our phages, suggesting a common viral stress response. Most site acetylation levels of *P. aeruginosa* proteins were only altered after exposure to specific phages, suggesting phage-induced acetylation events. These feature proteins involved in various processes, such as LPS biosynthesis, RNA degradation and more. Interestingly, 271 acetylated phage proteins were also detected. On average, 40.6 % of each phage proteome was acetylated.

Overall, this dataset represents the first large-scale acetylomics – and even PTM - study during phage infection. It has helped us shed more light on phage biology and how bacteria deal with specific stress conditions. In addition, this data has provided us with a first glimpse at the prevalence of PTMs in phage proteins.

1Longin,H.*et al.*Curr_Opin_Microbiol 77,102425.(2024)
2Gaviard,C.*et al.*J.Proteome_Res 17,7,2449-2459.(2018)

Keywords: phage biology, acetylomics, phage, host interactions

*Speaker

6.4. Role of bioenergetic pumps, F- and V-type ATP-synthase/ATPase in Streptococcus pyogenes pathogenesis

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F-type (F1F0) and V-type (vacuolar-V1V0) bioenergetic nanomotor pumps are conserved membrane-bound rotary enzyme complexes encoded by an operon of eight genes. Their clock and counter lock rotary movements create proton-motive force, allowing them to be ATP-synthase or ATPase. The structure and mechanistic functions of these nanomotors been established in eukaryotes and a few prokaryotes; however, their role in pathophysiology in group A *Streptococcus* (GAS, or *S. pyogenes*) pathogenesis is presently unknown. ATP plays a central role in bacterial metabolism and kinase- and phosphatase-mediated post-translational modifications (PTM) targeting Ser/Thr/Tyr phosphorylation (STYP). Based on several published transcriptome analyses of *S. pyogenes*, we have found that the expression of genes encoding F1F0 and V1V0 in specific GAS mutants lacking two-component systems, stand-alone regulators, eukaryote-type Ser/Thr/Tyr kinases and phosphatases, and similarly differential temporal regulation during mouse and primate GAS colonization and infection models. These initial findings have prompted us to test the hypothesis that F1F0 and V1V0 ATPases(synthases) play a crucial role in the modulation of GAS pathophysiology and hence in GAS pathogenesis. To that end, two GAS-derived mutants, each lacking the entire F1F0 or V1V0-coding operon genes, have been characterized to understand the contribution of these two enzyme complexes to the modulation of GAS gene transcriptome, metabolism, and protein STYP-PTMs, underpinning the molecular mechanism of GAS pathogenesis. The mutants lacking complexes of two gene clusters showed aberrant cell division and sensitivity to pH, impacting their growth. RNA-seq analyses have revealed altered expression of several genes responsible for two-component regulatory systems and those directly or indirectly involved in cell division, metabolism, and biofilm processes. Western immunoblot-based and global phosphoproteomic analyses showed altered Ser/Thr/Tyr phosphorylation patterns in these mutants.These results and reduced virulence of these GAS mutants in animal infection model studies, suggested that F-/V-ATPases may serve as important therapeutic targets.

Keywords: Bioenergetic pump, F and V, ATPases, Streptococcus pyogenes, virulence, proteomic, PTM, Ser/Thr/Tyr kinase, phosphatase

*Speaker

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Posters

P01. Enzyme mining of novel Prolyl 4-Hydroxylases for the efficient microbial synthesis of collagen

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Collagen is a polymeric protein with unique functional properties, such as high water-binding capacity and gel-forming abilities, making it a popular ingredient in the food, pharmaceutical, and biomedical industries. Traditionally, collagen has been obtained from animal sources, but concerns over safety, batch-to-batch variability, high cost, and ethical issues have led to increased interest in developing alternative sources.
Microbial cell factories offer a promising alternative for sustainable and standardized collagen production. *Bacillus subtilis*, known for its high expression levels of recombinant proteins and ease of genetic manipulation, has emerged as a potential host for collagen production. However, collagen requires complex post-translational modifications, mainly proline hydroxylation, which are necessary but challenging to replicate in microbial hosts. Previous studies have demonstrated that cloning of animal prolyl 4-hydroxylases (P4Hs) on microbial hosts either leads the enzymes to stay insoluble or act on recombinant collagen inefficiently. Since P4Hs are widely spread across the kingdoms of life, efforts have been made to express P4Hs from other organisms, bearing positive but insufficient results. Major setbacks have been the difficulty to distinguish between the two types of P4Hs which act either on proteins or on free L-proline and to maintain sufficient levels of enzyme activity.

In this study, we employ a systematic approach, utilizing enzyme mining tools such as the EFI-Sequence Similarity Network (SSN), to map the P4H diversity across the kingdoms of life. This approach aims to provide more insights about the mechanisms governing substrate selection and identify a P4H with sufficient activity on recombinant collagen.

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Keywords: P4H, enzymes, hydroxylation, hydroxyproline, SSN, bioinformatics, recombinant, col-

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lagen, heterologous, hydroxylase, protein

P02. Structural insights into the regulation of a bacterial tyrosine kinase by its cognate phosphatase

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Tyrosine phosphorylation, a post-translational modification ubiquitous in eukaryotes, also plays a pivotal role in prokaryotic physiology by affecting diverse processes including the rapid adaptation to environmental changes. Majority of tyrosine phosphorylation in bacterial cells is driven by members of family of atypical tyrosine kinases, the bacterial tyrosine kinases (BY-kinases) that encode catalytic domains (CDs) closely related to P-loop nucleoside triphosphatases and are structurally and mechanistically distinct from eukaryotic tyrosine kinases. Members of the BY-kinase family regulate numerous cellular processes most notably the synthesis, polymerization, and export of polysaccharides that form key components of biofilms or virulence-determining capsules. In *Escherichia coli*, the activity of the archetypal BY-kinase Wzc relies on the intricate interplay between auto-phosphorylation and dephosphorylation mediated by its counteracting low molecular-weight protein tyrosine phosphatase (LMW-PTP), Wzb. Wzc encodes a cytoplasmic catalytic domain (CD) that terminates in a tyrosine-rich C-terminal tail that serves as a substrate for intermolecular auto-phosphorylation and subsequent dephosphorylation by Wzb. Activity is related to the phosphorylation level of this C-terminal tail, rather than that of any specific tyrosine residue/s therein. Despite the fundamental importance of the interaction between Wzc and Wzb, its precise structural determinants, as well as the influence on the underlying regulatory mechanisms that drive Wzc activity, have remained poorly defined. Utilizing a variety of solution NMR techniques, we have unraveled the structural details of the transient complex formed between the catalytic core of Wzc with Wzb. Our study involves a docking protocol driven by a large number of experimental restraints, yielding the first model depicting the interaction between a BY-kinase CD and its regulatory LMW-PTP in atomic detail. In combination with our computational and experimental analyses on the catalytic core of Wzc, this structure allows the development of a mechanistic model of BY-kinase activation and activity.

Keywords: Bacterial, Tyrosine Kinase, Low, Molecular Weight Protein Tyrosine Phosphatase, Solution NMR

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P03. In Salmonella enterica, uncharacterized protein YjaB upregulates proline metabolism through post-translational acylation of glutamate dehydrogenase

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In *Salmonella* Typhimurium, L-proline is converted to glutamate by the proline and L-glutamate-gamma-semialdehyde dehydrogenase activities of the PutA protein and the glutamate dehydrogenase activity of GdhA, resulting in alpha-keto-glutarate (a-KG), an intermediate of the TCA Cycle. We have observed that overexpression of the uncharacterized gene *yjaB+* (encoding a putative Gcn5-family *N*-acetyltransferase, or GNAT) enhances growth of *S.* Typhimurium with proline as a sole source of carbon and energy. Others have observed downregulation of YjaB under pathogenic conditions, suggesting that YjaB may be a regulator with a positive effect in a free-living context but a negative effect during pathogenic growth. In *Escherichia coli* (a close relative of *S.* Typhimurium) GdhA is a substrate for the Lon protease and is known to associate with YjaB. In this work we show that a *lon* mutant strain phenocopies the behavior of the strain overexpressing *yjaB+* in minimal proline medium, that gdhA+ overexpression is beneficial to growth in proline, and that GdhA becomes acetylated in the presence of acetyl-CoA. Our working hypothesis is that YjaB acylates GdhA, protecting the protein from degradation and accelerating proline catabolism. Consistent with this idea, a mutation known to protect GdhA from protease activity occurs at a lysine residue. Notably, overexpression of the *cobB+* gene (encodes the NAD+-dependent protein deacylase CobB sirtuin) also enhances the growth effect of *yjaB+* with proline, suggesting that transient reversal of acylation may be necessary for maximum growth. Current work is testing our model that YjaB prevents GdhA proteolysis by lysine acylation. We are also investigating whether YjaB modulates the activity of Lon by lysine acetylation, resulting in faster proline catabolism.

Keywords: acetylation, GNAT, proline, glutamate, sirtuin, Lon

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P04. Protein glycosylation in Neisseria meningitidis effects susceptibility to the bactericidal activity of human sera

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Neisseria species exhibit a broad-spectrum *O*-linked glycosylation system targeting extracytoplasmic proteins. *Neisseria meningitidis*, a commensal bacterium of humans with varying carriage prevalence, can occasionally cause severe meningitis and/or septicemia. Antimicrobial resistant *Neisseria gonorrhoeae* represents an emerging global health problem and there is an urgent need for the development of a gonorrhea vaccine. Multiple studies have reported decreased rates of gonorrhea after vaccination with meningococcal serogroup B outer membrane vesicles (OMV) – based vaccines. Here, we examined the potential impact of bacterial protein glycosylation on susceptibility to complement-mediated killing of bacteria (serum bactericidal activity) elicited by human antibodies induced by OMV-based vaccines in humans.
Meningococcal OMV vaccines contain various outer membrane proteins and a MenBvac vaccine (based on the strain 44/76) was previously used to control serogroup B epidemics in Norway and Normandy, France. Glycan-specific, monoclonal antibodies identified multiple glycoproteins both in the vaccine strain and MenBvac. Immunoblotting against a panel of strains expressing different (or no glycans) demonstrated that the majority of individuals had IgG antibodies against neisserial glycan antigens. However, the MenBvac vaccine (despite containing several glycoproteins), did not induce production of additional antibodies against glycans. We therefore suggest that these antibodies have been have engendered by carriage of meningococci or commensal *Neisseria*.
In a complement – based, bactericidal assay using these human sera, we observed significantly higher bactericidal titres against a glycosylation null mutant versus the wildtype strain. In a similar fashion, the effect of meningococcal OMV vaccination against *N. gonorrhoeae* was investigated. However, meningococcal OMV vaccination did not induce bactericidal activity against *N. gonorrhoeae* regardless of glycosylation status. Altogether, we demonstrate that protein glycosylation can contribute to the ability of *N. meningitidis* to resist serum bactericidal activity. Further studies are necessary to understand the underlying molecular mechanisms behind these findings.

Keywords: Neisseria, bactericidal activity, O linked protein glycosylation

*Speaker

P05. Phosphoproteome of clinical strains of Acinetobacter baumannii

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Acinetobacter baumannii is a multi-drug-resistant (MDR) bacterium that has been attracting attention in recent years for its involvement in numerous hospital-acquired infections (1). It has been classified by the World Health Organization as a "Critical Priority" for which there is an urgent need to find new therapeutic solutions (2). Post-translational modifications (PTMs) play an important role in bacterial resistance, and their characterization by proteomic analysis is necessary (3). Many studies have been carried out on laboratory strains, but very few on clinical isolates.
In this study, we focused on phosphorylation on serine, threonine and tyrosine. We analysed the phosphoproteomes of different clinical isolates. The phosphopeptides were enriched using titanium dioxyde and analysed by LC-MS/MS on an Orbitrap Eclipse Tribrid. Overall, we identified 246 non-redundant phosphorylated peptide isoforms. Our results highlight different phosphorylation profiles between the different isolates. The majority of phosphopeptides (81%) are unique per clinical strains.

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Keywords: Acinetobacter baumannii, Antibiotic resistance, Phosphoproteome

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P06. The switch from cell wall-bound polysaccharides to secreted exopolysaccharides in Lacticaseibacillus rhamnosus results from altered tyrosine kinase phosphorylation

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Bacterial exopolysaccharides (EPS) are carbohydrate polymers secreted in the surrounding medium, which can be distinguished from cell wall polysaccharides (CWPS) covalently attached to the cell wall peptidoglycan. EPS produced by lactic acid bacteria (LAB) possess many valuable properties in the food or medical fields. They contribute to the texture of fermented milk products. Also, they are suggested to influence bacteria-host interactions, notably by affecting bacterial adhesion to the host cells and mucosa, or bacterial-mediated immunomodulation. We have developed a method to isolate spontaneous non-GMO LAB mutants that overproduce EPS. This method was successfully applied to *Lacticaseibacillus rhamnosus*, a LAB used in food fermentation or marketed as a probiotic. The obtained mutants were studied in detail to decipher the mechanisms involved in EPS overproduction. Whole genome sequence analysis of several EPS-overproducing mutants revealed the presence of missense mutations in gene *epsD* encoding a bacterial tyrosine kinase (BY-kinase) and located in a gene cluster encoding polysaccharide biosynthesis. The mutations were located in the vicinity of the ATP-binding site of EpsD. Moreover, phosphoproteomic analysis revealed the absence of autophosphorylation of the mutant EpsD kinase. The chemical structure of the overproduced EPS was established by NMR. Remarkably, in the wild-type parental *L. rhamnosus* strain, we found a polysaccharide with an

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identical structure covalently bound to the cell wall and covering the bacterial surface as shown by HR-MAS NMR. When the BY-kinase was deleted, a different phenotype was observed with a low amount of the same polysaccharide produced and attached to the cell wall. In conclusion, our results indicate that the switch from cell-wall bound polysaccharides to EPS released into the environment results from the impairment of the EpsD BY-kinase autophosphorylation.

Keywords: Exopolysaccharides, lactobacilli, cell wall, tyrosine kinase

P07. Proteome dynamics of bacterial pathogens during infection - New Drug Targets

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Antimicrobial resistance presents a constant global danger, with antibiotic escape mechanisms evolving at an unprecedented pace. *Staphylococcus aureus* ranks as the second most critical gram-positive bacteria on the WHO's priority list, predominantly spreading through nosocomial transmission. These strains carry drug-resistant genes, complicating treatment efforts. The conventional approach of targeting proteins and nucleic acids to inhibit microbial survival or resistance has proven insufficient, with bacteria easily rebounding. Current research on drug targets necessitates revisiting bacterial susceptibility and persistence. Understanding the mechanisms behind *S. aureus*'s escape from the phagolysosome is crucial, yet ambiguous due to undiscovered escape mechanisms. The phagolysosome, a degradative organelle rapidly acidifies internally, creates hypoxic environment, facilitates ROS & RNS species formation and undergoes rapid fusion and fission of different endosomes to degrade the encapsulated prey. The sensory proteins and the transmembrane lipids on *S. aureus* recognize the degradative environment and consequentially adapt to it via proteins that counteract the harsh effect by externally pumping them or internally altering the conditions. Phosphorylation, particularly through Two-Component systems (TCS), plays a vital role in *S. aureus*'s survival. However, the specific phospho-events underlying its survival remain cryptic. Apart from *WalRK* (a TCS vital for bacterial growth), none of the TCS's are not yet known to interfere with the cell viability in in-vitro conditions. In this project's initial phase, we aim to deduce the phosphorylation events occurring in *S. aureus* when exposed to phagolysosome-like conditions in vitro. By comparing their viability under normal and stress-inducing environments, such as acidic, osmotic, and oxidative stress, we seek to identify differentially expressed phosphoproteins that could serve as targets for disrupting bacterial survival. Subsequent evaluation in mutants and cell cultures will deepen our understanding. While phosphorylation research has gained importance for mechanistic insights, visualizing bacterial-specific drug interventions in these mechanisms requires urgent attention.

Keywords: Antimicrobial Resistance (AMR), S.aureus, PTMs, Phosphoproteomic, Phosphoproteins, Proteins, Drug Targets, TCS

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P08. Efficient and repeatable peptide desalting with AttractSPE®Disks Tips C18 in manual and fully automated proteomics workflows

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Bottom-up approaches are commonly used for proteomics analysis in LC-MS/MS. However, salts present in digestion buffers can generate ion-suppression that limits peptide detection and greatly impacts proteins identification.Several AttractSPE®Disks Tips C18 were evaluated for peptide desalting. These SPE Tips, packed with a SPE membrane combining high capacity and small dead volume, are particularly adapted for centrifugation or positive pressure assays.First, 200μL AttractSPE®Disks Tips C18 with different binding capacities were tested for the manual desalting of 100ng of HeLa digest by centrifugation. The nanoLC-MS/MS analyses of peptides were compared to those obtained after direct injection of a non-purified loading solution. This comparison showed that 97% of proteins could be identified after desalting on the C18 tips, with high recovery of all peptides on the whole polarity range. Low variability was obtained for intra- and inter-tips assays, highlighting the Tips reliability.Then, 10μL AttractSPE®Disks Tips C18 were compared to home-made StageTips (with Empore C18 SPE disks) for the automated desalting of 2μg and 100ng of protein digest on the DigestPro MSi robot (CEM) prior to analysis on a nanoElute – timsTOF Pro LC-MS/MS system. This comparison showed a 10% increase in the number of identified proteins and higher peptide intensities with the AttractSPE®Disks Tips C18. AttractSPE®Disks Tips C18 performed better in the hydrophilic range, thus demonstrating than this C18 sorbent offers a wider spectrum of interactions compared to home-made StageTips. In addition, the P3S team has optimized the program on the DigestPro MSi robot to reduce the time from 40min down to 10min per sample.Thus, this study has shown that the AttractSPE®Disks Tips C18 can be used both for manual or automated desalting workflows, and are perfectly adapted to single cell-like analysis as well as high peptide amounts, thus covering a wide range of applications.

Keywords: Peptide Desalting, SPE Tips, Protein identification, bottom, up proteomics

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P09. Phosphorylation of the juxtamembrane domain of StkP is required for cell division of Streptococcus pneumoniae

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The membrane eukaryotic-like Ser/Thr protein-kinase (eSTPK) StkP plays a key role in the regulation of peptidoglycan assembly and cell division in the pathogenic bacterium *Streptococcus pneumoniae*. StkP consists of an extracellular domain, a single transmembrane helix and a cytoplasmic domain. Within this cytoplasmic domain, the kinase catalytic domain is linked to the transmembrane helix through a juxtamembrane domain (JMD). While the structure and function of the extracellular domain of StkP have been well studied (Zucchini *et al.*, Nat. Microbiol., 2018), the structural organization of its cytoplasmic domain and the mechanism triggering its activity remain elusive. In this study, we first show that StkP autophosphorylates on multiple threonines located in the JMD. To investigate the role of these phosphorylation sites, we then perform an integrative structure-function analysis combining liquid-state NMR, cell imaging and proteomic. We show that the phosphorylation of the JMD influences cell morphogenesis and affects the dynamic of StkP at the division septum. Conversely, it does not affect the ability of StkP to phosphorylate its endogenous targets. We also demonstrate that the JMD is intrinsically disordered and that its conformation is not influenced by its phosphorylation. Strikingly, we demonstrate that the phosphorylated form of the JMD preferentially interacts with several proteins required for cell division and peptidoglycan synthesis. Altogether, our data show that phosphorylation of the StkP juxtamembrane domain modulates the establishment of the division machinery at the division septum. This work also provides the first molecular and structural insights towards the understanding of the role of the JMD domain that could serve as a model for other bacterial eSTPKs.

Keywords: Protein kinase, Streptococcus pneumoniae, juxtamembrane domain

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P10. Role of pupylation for iron homeostasis and oxidative stress responses in Corynebacterium glutamicum

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Pupylation, a post-translational modification resembling ubiquitination in eukaryotes, plays a crucial role in protein turnover and adaptation processes in actinobacteria. Recent studies in *Corynebacterium glutamicum*, which possesses the pupylation machinery but lacks a proteasome for the degradation of pupylated proteins, have revealed a role of pupylation for adaptation to iron starvation by targeting ferritin and Dps. These iron storage proteins serve as a defense mechanism against oxidative stress by sequestering iron ions, thus reducing the production of reactive oxygen species (ROS) via the Fenton reaction. Pupylation-triggered iron release from ferritin not only affects iron utilization and storage but also modulates oxidative stress responses. When a ferritin deletion mutant was exposed to hydrogen peroxide stress, expression of putative iron uptake systems was downregulated, whereas expression of genes involved in coping with oxidative stress was increased. These responses suggest the existence of a compensatory mechanism to adapt iron uptake to the absence of ferritin and respond to an increased susceptibility to oxidative damage. Further studies will explore the nature of this mechanism in order to understand the intricate interplay between pupylation, iron metabolism, and oxidative stress responses in *C. glutamicum* and other actinobacteria.

Keywords: Corynebacterium glutamicum, Pupylation, Oxidative stress, Hydrogen peroxide, Iron storage proteins, Transcriptional regulation

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P11. Design of fluorescent biosensors of Ser/Thr kinases to study the bacterial cell cycle

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The bacterium *Streptococcus pneumoniae* (pneumococcus) is a human pathogen that encodes a single Ser/Thr kinase called StkP. This kinase is acknowledged as a central regulator of pneumococcal cell division and cell wall synthesis. While StkP localizes at the septum and necessarily phosphorylates its substrates there, it is still not understood when and in which order StkP phosphorylates its substrates in the course of the cell cycle. We are thus currently lacking all the knowledge regarding the temporal dynamics of phosphorylation. To tackle this, it is necessary to design fluorescent biosensors of Ser/Thr phosphorylation and to implement single cell studies. While those sensors are abundant for use in eukaryotic cells, they are considerably lacking for use in bacteria to study this type of phosphorylation *in vivo*. Here, we created fluorescent hybrid proteins able to report StkP activity in single pneumococcal cells. These sensors allow us to gain crucial insights about the dynamics of StkP substrates phosphorylation over the course of the cell cycle and pave the way toward a better understanding of pneumococcal cell division. Considering that StkP-like kinases have also been associated to antibiotic escape in various bacterial species, especially to beta-lactam resistance, these sensors represent also a promising approach to study the contribution of phosphorylation in responding to antibiotic stress.

Keywords: Phosphorylation, single cell, biosensor, Streptococcus pneumoniae

*Speaker

P12. Lysine trimethylation in planktonic and pellicle modes of growth in Acinetobacter baumannii

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Over the past 30 years, *Acinetobacter baumannii* has been described as an important nosocomial pathogen due to frequent ventilator-associated infections. Many biological processes of *A. baumannii* remain elusive, such as the formation of an air-liquid biofilm (pellicle). Several studies demonstrated the importance of post-translational modifications (PTM) in *A. baumannii* physiology. Here, we investigated K-trimethylation in *A. baumannii* ATCC 17978 in planktonic and pellicle modes using proteomic analysis. To identify the most high-confidence K-trimethylated peptides, we compared different sample preparation methods (i.e. Strong cation exchange, antibody-capture) and processing software (i.e. different database search engines). We identified, for the first time, 84 K-trimethylated proteins, many of which are involved in DNA and protein synthesis (HupB, RplK), transporters (Ata, AdeB) or lipid metabolism processes (FadB, FadD). In comparison with previous studies, several identical lysine residues were observed acetylated or trimethylated, indicating the presence of proteoforms and potential PTM cross-talks. This is the first large-scale proteomic study of trimethylation in *A. baumannii* and will be an important resource for the scientific community (availability in Pride repository under accession PXD035239).

Keywords: Lysine trimethylation, Acinetobacter baumannii, biofilm, proteomics, bacteria, post, translational modifications

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P13. On the role of the HTH-domain of the HipA-like kinase YjjJ

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Persister cells are phenotypic variants of normal bacterial cells that are able to survive prolonged antibiotic treatments in a state of dormancy and present an urgent clinical threat. The first discovered gene conferring persistence was the eukaryotic type Ser/Thr kinase *hipA* which is part of the *hipBA* toxin-antitoxin (TA) system in *Escherichia. coli*. If not neutralized by its antitoxin HipB, HipA phosphorylates different substrate pools in *E. coli*, promoting growth stop and inducing persistence. A recent phylogenetic analysis revealed that homologues of *hipA* are widespread among various bacterial species, including clinically relevant strains (1). In a recent study we investigated the role of the monocistronic *hipA* homologue YjjJ (HipH) and showed its effect on cell division and were able to identify several novel phosphorylation targets (2). While YjjJ has no clearly assigned antitoxin, it carries a putative DNA binding helix-turn-helix (HTH) domain at its N-terminus which is usually found in HipB-like antitoxins to regulate expression of its own TA module.
In this work we show that the HTH-domain of YjjJ, in contrast to HipB, is not involved in regulating its own expression but is essential for the toxicity of YjjJ. Thereby, we show via shotgun proteomics that the HTH-domain of YjjJ regulates a variety of different genes, including potential virulence factors. We therefore postulate, that this domain of YjjJ like proteins provides additional regulation on the transcriptional level next to kinase activity.

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Keywords: Persistence, Toxin, antitoxin system, Serine/threonine protein kinase

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P14. The phosphoproteome signature of Listeria monocytogenes dormancy

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When exposed to stressful conditions, some bacteria can transition to a viable but non-culturable (VBNC) state. VBNC bacteria have lost their ability to grow on standard culture media, despite remaining viable and with a relatively active metabolism. By hibernating into a VBNC state, bacteria become more protected against environmental stresses, thus maximizing their chances of survival. The VBNC state is particularly problematic in the case of pathogens, as standard methods used for bacterial identification and enumeration are reliant on growth, and thus unsuitable for VBNC cells. Moreover, as the VBNC state is potentially reversible (resurrection), pathogenic bacteria may recover their virulence potential. Although hundreds of bacterial species have been reported to transition into the VBNC state, the underlying molecular mechanisms remain elusive. We have recently addressed this question by characterizing the VBNC state in the environmentally ubiquitous Gram-positive pathogen *Listeria monocytogenes*. By combining fluorescence microscopy and cryo-electron tomography with genetic and biochemical approaches, we discovered that starvation in mineral water drives *L. monocytogenes* into a VBNC state via a unique mechanism of cell wall shedding that generates osmotically stable cell wall-deficient coccoid forms. Here, we aim to investigate the regulatory role of protein post-translational modifications, in particular phosphorylation on tyrosine, serine and threonine residues, in VBNC state transition. To do so, phosphoproteome will be recovered via TiO2 beads enrichment and then analyzed by LC-MS/MS. Identification and statistical analysis will be performed by using i2MassChroQ and R scripts, respectively. By profiling the phosphoproteome dynamics of *L. monocytogenes* transitioning into a VBNC state, we anticipate that our results will provide novel insights on bacterial dormancy and could lead to the identification of molecular targets to be used in the fight against drug-tolerant dormant bacteria.

Keywords: VBNC, Listeria, bacterial persistence, phosphoproteome

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P15. Non-enzymatic acetylation in Acinetobacter baumannii

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Acinetobacter baumannii is an opportunistic pathogen classified by the WHO as one of the three pathogens causing the most threat to public health. With the emergence of carbapenem-resistant and multidrug resistant strains, the search for new antibacterial and antibiofilm agents against *A. baumannii* is crucial (1,2). The persistence of this pathogen is due to its ability to form biofilms and adapt quickly to extreme conditions, by using several molecular mechanisms including post-translational modifications (PTMs).
Lysine acetylation is a widespread PTM in bacteria, that can be added through two distinct mechanisms: non-enzymatic, through direct addition of acetyl groups mainly from the donor acetyl-phosphate (AcP) produced by the enzyme phosphate-acetyltransferase Pta ; and enzymatic, through the action of lysine acetyltransferases (KATs). The importance of enzymatic acetylation on *A. baumannii* physiology was already demonstrated through excess acetylation after deletion of KDACs (data not shown). Non-enzymatic acetylation was shown to impact central metabolism and translation in some bacteria (3), but to date no study in *A. baumannii* was carried out.

The aim of this study is to characterize proteins acetylated by the addition of AcP. The knock-out mutant Δ*pta* was constructed in *A. baumannii* strain ATCC 17978. We then compared the acetylome of the wild-type and mutant strains using mass spectrometry. Results showed that proteins undergoing chemical acetylation belong to different functional categories: translation, lipid and nucleotide metabolism, PTMs, cell wall biogenesis and signal transduction mechanisms. We will discuss on proteins acetylated by the addition of AcP.

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Keywords: Acinetobacter baumannii, Antibiotic resistance, Acetylome, Enzymatic acetylation, Non, enzymatic acetylation

P16. Global analysis of global protein phosphorylation during diurnal cycles in a cyanobacterium

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The cyanobacterial metabolism is widely governed by the circadian rhythm, causing a cyclic switch between photoautotrophic metabolism during light and heterotrophic metabolism during dark. In contrast to metabolite and transcript abundance levels, which oscillate between light and dark phases, protein levels remain widely stable during the circadian rhythm. This includes many enzymes specific for the photoautotrophic and the heterotrophic phase, which are co-occurring in the cytoplasmic fraction. However, the activity of antagonistic enzymes, which are characteristic for these fundamentally different metabolic states, must be tightly regulated to ensure cellular functionality.
Here, we aimed to investigate if post-translational phosphorylation, which is a frequently observed modification of metabolic proteins in the cyanobacterium *Synechocystis* sp. PCC 6803, is a feature of the circadian regulation. Therefore, global protein and phosphorylation abundances were compared between six consecutive time points in the diurnal cycle, covering close and distant time points, to resolve fast and slow regulatory events. More than 200 phosphorylation events (p-events) located on 128 phosphoproteins could be identified, including multiple p-events on 13 recently re-annotated and newly discovered proteins. Quantitative information of 80 p-events covering all time points revealed mostly stable phosphorylation levels during the light/dark cycle, with exception of redox-related proteins.

These results indicate that protein phosphorylation is predominantly utilized in cyanobacteria to adapt to stress situations such as during nutrient starvation (Ref.1,2) rather than to regular daily changes.

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Keywords: Protein phosphorylation, diurnal cycle, regulation, cyanobacteria, Synechocystis sp.

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PCC6803

P17. Identification of the mycoloylome in C. glutamicum

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Corynebacteriales is an order of bacteria that comprises several human pathogens, including *Mycobacterium tuberculosis* and *Corynebacterium diphtheriae*, as well as economically valuable species like *Corynebacterium glutamicum*. All these species share an atypical cell envelope composed of arabinogalactan-modified peptidoglycan and an outer membrane mainly composed of mycolic acid-derived glycolipids. In 2010, Huc et al. discovered the presence of protein O-mycoloylation in *C. glutamicum*, a post-translational modification catalyzed by the enzyme mycoloyltransferase C (MytC). MytC has been shown to transfer a mycolate chain from trehalose monomycolate (TMM) to the serine of certain small proteins, namely PorA/H, PorB/C, and ProtX. In this study, we undertook a proteomic global approach to identify the mycoloylome of *C. glutamicum*. We performed specific metabolic labelling of mycoloylated proteins using a unique synthetic alkyne-tagged TMM (alk-TMM) analogue. Labelled proteins were purified after biotin addition, and about 30 potential candidates were identified through mass spectrometry. In a parallel approach, we compared the cell envelope proteome of the wt strain to that of a MytC knockout mutant (ΔC). Found by both approaches: A list of 15 new potential mycoloylated proteins was proposed with high confidence. Interestingly, all these candidates are exported in the cell envelope and contain an unstructured region (Alphafold prediction) with a serine-rich sequence. Six out of twelve proteins were successfully produced in both WT and ΔC strains and displayed a different SDS PAGE migration pattern in the two strains. Four proteins were purified, and their mass was determined by LC-MS analysis, confirming the mycoloylation. Interestingly, mycoloyltransferases A and B were also found to be mycoloylated, opening new questions regarding the function of these enzymes in the assembly of the mycomembrane. Future work will focus on determining the mycoloylation sites on these proteins through mass spectrometry of digested proteins and single-point mutations to define a consensus sequence.

Keywords: Mycobacterium, Mycoloylation, Membrane proteins

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