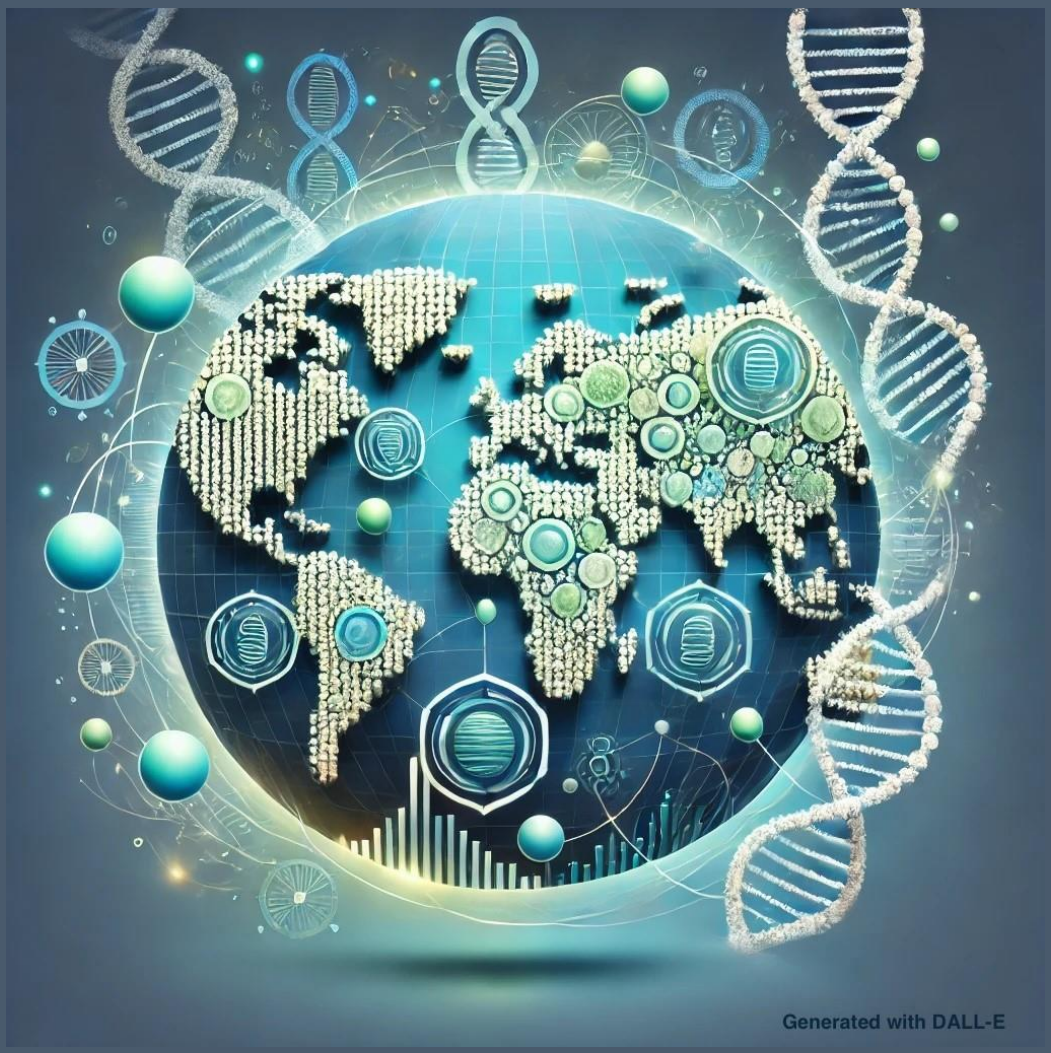
ISPB VIRTUAL SEMINAR SERIES 2025



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Welcome

Dear Plasmid Enthusiast,

On behalf of the Plasmid Biology Society, we say welcome you to the first ISPB Virtual Seminar Series! We are thrilled to have you join us online, from wherever you are, all throughout 2025. This seminar series brings together an exciting lineup of speakers covering a wide range of topics in plasmid biology**,** including molecular plasmid biology, ecology, evolution, plasmid-host interactions, and bacterial immunity**.**

These monthly meetings provide a fantastic opportunity to share our research, engage in scientific discussions, and connect with colleagues—until we meet again in person in Berlin in 2026**.** We look forward to inspiring talks and lively discussions!

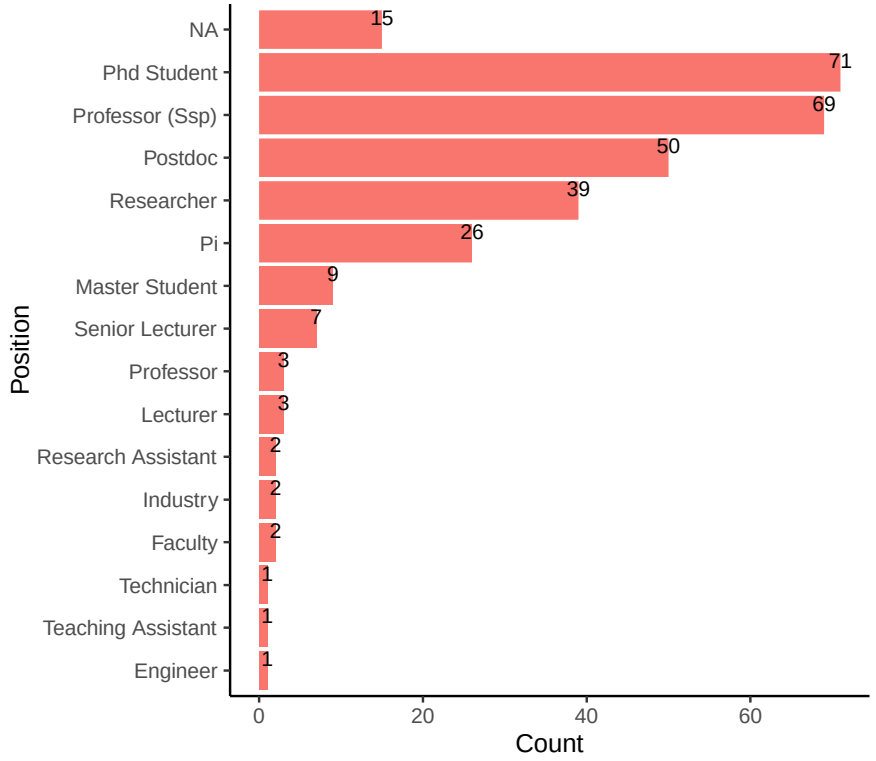
The Organizing Committee,

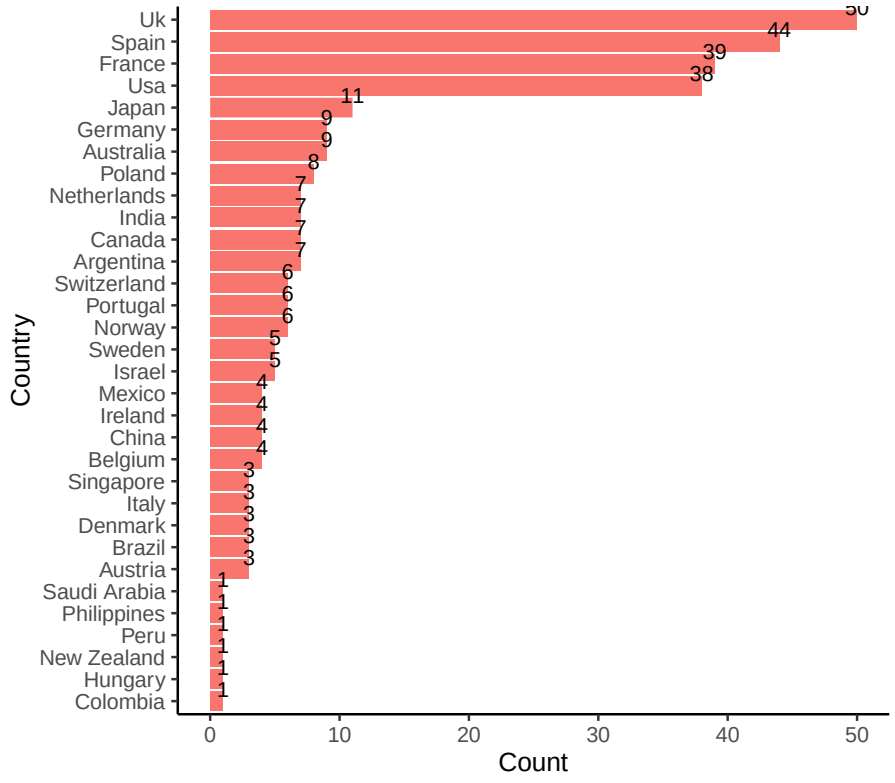
Manuel Ares Arroyo, Fabienne Benz, Pedro Dorado Morales

📢 Follow **@PlasmidSociety** on X for updates and announcements!

The Crowd

More than 300 plasmid enthusiasts from over 30 countries have signed up to hear 18 speakers share their research. Our participants represent all academic levels, from early-career researchers to established experts in the field.





Program

We will meet once a month on Tuesdays for two presentations and topic discussions. A reminder email with speaker details and login information will be sent out ahead of each session.

2025 Month	Day	GMT-7	GMT+1	GMT+8	Speaker 1	Speaker 2
March	Tue 11th	09:00 – 10:00	17:00 – 18:00	00:00 – 01:00 (1)	Laura Toribio Celestino	Laura Álvaro Llorente
April	Tue, 15th	01:00 – 02:00	09:00 – 10:00	16:00 – 17:00	Maho Tokuda	Célia Souque
May	Tue, 13th	17:00 – 18:00 (2)	01:00 – 02:00	08:00 – 09:00	Zhengqing Zhou	Alicia Calvo-Villamañan
June	Tue, 10th	09:00 – 10:00	17:00 – 18:00	00:00 – 01:00 (3)	Perrine Revoil	Yannick Baffert
July	Tue, 15th	01:00 – 02:00	09:00 – 10:00	16:00 – 17:00	Iris Veyrier	Célia Domingues
August 2025 Summer Break						
September	Tue, 16th	09:00 – 10:00	17:00 – 18:00	00:00 – 01:00 (4)	Ada Muñoz	Hon-Wing Liu
October	Tue, 14th	01:00 – 02:00	09:00 – 10:00	16:00 – 17:00	Nivedita Mitra	João Rebelo
November	Tue, 11th	17:00 – 18:00 (5)	01:00 – 02:00	08:00 – 09:00	Martin Zwanzig	Filipa Trigo da Roza
December	Tue, 16th	09:00 – 10:00	17:00 – 18:00	00:00 – 01:00 (6)	Jack Bravo	Michelle Angelique Boelcke

(1) Wed, 12th // (2) Mon, 12th // (3) Wed, 11th // (4) Wed, 17th // (5) Mon, 10th // (6) Wed, 17th

Due to the limited number of available slots, we were unfortunately unable to accommodate all submissions. In the selection process, we prioritized non-permanent/junior researchers, as well as candidates who were unable to present at the 2024 ISPB meeting. Additionally, we made a strong effort to maximize the diversity of presenting laboratories and to ensure broad geographical representation across the seminar series.

Presentations

Each session features two speakers, with 20-minute presentations followed by 10 minutes of discussion and Q&A.

We encourage speakers to present ongoing research rather than published work. All presented data must be treated confidentially and cannot be shared without the presenter's consent.

While we strongly encourage live participation, we do have the challenge of time zones. Therefore, we are considering recording presentations (password-protected and available for 24 hours) for speakers who explicitly agree to this option.

Further details for speakers will be provided via email.

Abstracts

Title: Unravelling the molecular mechanism of collateral sensitivity induced by plasmid-mediated beta-lactam resistance

Authors: Laura Álvaro-Llorente[1], Cristina Herencias[1,2], Laura Jaraba[1], Álvaro San Martín[1,3], Ignacio de Quinto[1], Jerónimo Rodríguez-Beltrán[1,2]

Affiliation:
[1]Servicio de Microbiología, Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Hospital Universitario Ramón y Cajal, Madrid, Spain
[2]Centro de Investigación Biomédica en Red de Enfermedades Infecciosas CIBERINFEC, Instituto de Salud Carlos III, Madrid, Spain
[3]Microbial Pathogenesis Laboratory. Navarrabiomed-Complejo Hospitalario de Navarra (CHN)-Universidad Pública de Navarra (UPNA), IDISNA, Pamplona, Navarra, Spain

Position: PhD Student

Major antibiotic groups are losing effectiveness due to the uncontrolled spread of antimicrobial resistance (AMR) genes. In Enterobacterales, the most common resistance mechanism are β-lactam resistance genes -encoding β-lactamases- due to their frequent association with mobile genetic elements, such as plasmids. The dissemination of AMR genes encoded in plasmids and the critical shortage of new antibiotics underscore the urgent need of innovative therapeutic strategies that counter mobile AMR. One promising approach that capitalizes on the resistance acquisition event is to exploit collateral sensitivity (CS), which occurs when the acquisition of resistance to one antibiotic increases the susceptibility to a second antibiotic. In this context, we recently demonstrated that β-lactamase expression from plasmid-origin induces CS to the antibiotics azithromycin (AZI) and colistin (COL), a phenomenon that is pervasive in Enterobacterales.
Here, we aim to understand the molecular mechanism behind these CS patterns through transcriptomic analyses. Our findings suggest that β-lactamase accumulation in the periplasm destabilizes the bacterial envelope, thus increasing the permeability of AZI and enhancing the action of COL. Furthermore, we have simulated AZI treatments and found that bacterial strains carrying the plasmid die at significantly lower concentrations of AZI compared to plasmid-free strains. These findings highlight that the physiological side effects of β-lactamases can be leveraged therapeutically, paving the way for the rational design of specific therapies to block plasmid-mediated AMR or at least counteract their effects.

Title: Competition between conjugative plasmids by fertility inhibition protein

Authors: Yannick Baffert[1], Loïc Codemo[1], Yasmine Makhloufi[1], Sarah Djermoun[1], Marie-Eve Val[2], Eric Cascales[4], Christian Lesterlin[1] and Sarah Bigot[1]

Affiliation:
[1]Laboratory of Molecular Microbiology and Structural Biochemistry (MMSB), CNRS-University, Lyon, France,
[2]Pasteur Institute, Paris, France,
[4]Laboratoire d'Ingénierie des Systèmes Macromoléculaires (LISM), CNRS-Université Aix-Marseille, France

Position: PhD student

Bacterial conjugation is the primary mechanism for genetic material exchange, involving the transfer of DNA, primarily conjugative plasmids, via direct contact between a donor and a recipient cell. Conjugative plasmids face competition from co- resident plasmids and have evolved diverse strategies to ensure their successful dissemination. Among these, fertility inhibition (FI) systems play a critical role by preventing the transfer of unrelated co-resident conjugative plasmids. Despite the identification of numerous FI systems, their molecular mechanisms and functional roles remain poorly characterized.

One such FI factor is the Tir protein, first identified in 1985 on the R100 plasmid of the IncFII incompatibility group. Tir is known to inhibit the transfer of the IncP plasmid RP4, but its mechanism of action has remained elusive. In this study, we uncover the molecular mechanism by which Tir exerts its inhibitory effect. We demonstrate that Tir is an inner membrane protein that selectively inhibits the transfer of co-resident IncP and IncW plasmids by targeting a key component of the Type IV secretion system. Furthermore, the presence of Tir homologues across diverse conjugative plasmids from various incompatibility groups highlights its widespread use as a competitive strategy to outcompete co-resident plasmids.

Title: Developing a fluorescent monitoring tool for conjugative transfer of Gram-positive bacteria biofilms

Authors: Michelle Bölcke[1], Kirill Kuhlmann[2], Claudia Michaelis[1], Walter Keller[2], Tea Pavkov-Keller[2], Elisabeth Grohmann[1]

Affiliation:
[1]Faculty of Life Sciences and Technology, Department of Microbiology, Berliner Hochschule für Technik (BHT), Berlin, Germany
[2]Institute of Molecular Biosciences, University of Graz, Graz, Austria

Position: PhD student

The emergence of multi-resistant bacteria poses an ever-increasing threat to humans. Horizontal exchange of antibiotic resistance and virulence genes mainly takes place via bacterial conjugation and transduction. However, while the mechanism of conjugation in Gram-negative bacteria has been intensively studied, findings on conjugation in Gram-positive (G+) bacteria are not that advanced. In my PhD studies, the plasmid pIP501 will serve as a model for conjugative transfer in G+ bacteria. pIP501 belongs to the Inc18 incompatibility group and has a very broad host range for conjugative transfer, including *Staphylococcus*, *Streptococcus*, *Enterococcus*, *Lactococcus* and *Streptomyces lividans*. The transfer region of the plasmid comprises 15 genes showing limited homologies to the type 4 secretion system (T4SS) from *Agrobacterium tumefaciens*. The role of 9 of these 15 gene products in the T4SS pIP501 has been determined to some molecular details. (T. Berger *et al.*, 2022; T. M. I. Berger *et al.*, 2022; Michaelis *et al.*, 2024; Michaelis & Grohmann, 2023; Miguel-Arribas *et al.*, 2022)
For this purpose, a mCherry labelled pIP501 based conjugative plasmid will be generated to monitor conjugative plasmid transfer in biofilms generated in biofilm flow cells. Plasmid transfer rates will be determined depending on the microbial community compositions and age of the biofilms. In addition, two putative key proteins TraE and TraJ of the pIP501 T4SS will be investigated on genetic and biochemical level.

Berger, T. M. I., Michaelis, C., Probst, I., Sagmeister, T., Petrowitsch, L., Puchner, S., Pavkov- Keller, T., Gesslbauer, B., Grohmann, E., & Keller, W. (2022). Small Things Matter: The 11.6-kDa TraB Protein is Crucial for Antibiotic Resistance Transfer Among *Enterococci*. Frontiers in Molecular Biosciences, 9, 867136. https://doi.org/10.3389/fmolb.2022.867136

Berger, T., Reisenbichler, A., Michaelis, C., Grohmann, E., & Keller, W. (2022). Structural and functional characterization of TraA, the Relaxase A Gram-positive Type IV secretion system (T4SS). Acta Crystallographica Section A Foundations and Advances, 78(a2), e28–e28. https://doi.org/10.1107/S2053273322096437

Michaelis, C., Berger, T. M. I., Kuhlmann, K., Ghulam, R., Petrowitsch, L., Besora Vecino, M., Gesslbauer, B., Pavkov-Keller, T., Keller, W., & Grohmann, E. (2024). Effect of TraN key residues involved in DNA binding on pIP501 transfer rates in *Enterococcus faecalis*. Frontiers in Molecular Biosciences, 11, 1268647. https://doi.org/10.3389/fmolb.2024.1268647

Michaelis, C., & Grohmann, E. (2023). Horizontal Gene Transfer of Antibiotic Resistance Genes in Biofilms. Antibiotics, 12(2), 328. https://doi.org/10.3390/antibiotics12020328

Miguel-Arribas, A., Wu, L. J., Michaelis, C., Yoshida, K., Grohmann, E., & Meijer, W. J. J. (2022). Conjugation Operons in Gram-Positive Bacteria with and without Antitermination Systems. Microorganisms, 10(3), 587. https://doi.org/10.3390/microorganisms10030587

Title: Plasmid targeting and destruction by DdmDE

Authors: Jack Bravo

Affiliation: Institute of Science and Technology Austria

Position: Assistant Professor

Plasmids are inherently selfish parasitic elements, and often do not share evolutionary interest with their hosts. While bacteria have evolved numerous anti-plasmid systems, the mechanisms that enable these systems to specifically target and eliminate plasmids without causing autoimmunity are poorly understood. Here we reveal the activation pathway of the DNA defence module DdmDE system, which rapidly eliminates small, multicopy plasmids from the *Vibrio cholerae* seventh pandemic strain (7PET). Through a combination of cryo-electron microscopy, biochemistry and in vivo plasmid clearance assays, we demonstrate that DdmE is a catalytically inactive, DNA-guided, DNA-targeting pAgo with a distinctive insertion domain. We observe that the helicase-nuclease DdmD transitions from an autoinhibited, dimeric complex to a monomeric state upon loading of single-stranded DNA targets. Furthermore, the complete structure of the DdmDE–guide–target handover complex provides a comprehensive view into how DNA recognition triggers processive plasmid destruction. Our work establishes a mechanistic foundation for how pAgos utilize ancillary factors to achieve plasmid clearance, and provides insights into anti-plasmid immunity in bacteria.

Title: Dissecting pOXA-48 fitness effects in clinical enterobacteria using plasmid-wide CRISPRi screens

Authors: Alicia Calvo-Villamañán, Jorge Sastre-Domínguez, Álvaro Barrera-Martín, Coloma Costas, Álvaro San Millán

Affiliation: Centro Nacional de Biotecnología, CSIC

Position: Postdoctoral researcher

Conjugative plasmids are the main vehicle for the spread of antimicrobial resistance (AMR) genes in clinical bacteria. AMR plasmids allow bacteria to survive antibiotic treatments, but they also produce physiological alterations in their hosts that commonly translate into fitness costs. Despite the key role of plasmid-associated fitness effects in AMR evolution, their origin and molecular bases remain poorly understood. In this study, we introduce plasmid-wide CRISPR interference (CRISPRi) screens as a tool to dissect plasmid-associated fitness effects. We designed and performed CRISPRi screens targeting the globally distributed carbapenem resistance plasmid pOXA-48 in 13 different multidrug resistant clinical enterobacteria. Our results revealed that pOXA-48 gene-level effects are conserved across clinical strains, and exposed the key role of the carbapenemase-encoding gene, blaOXA-48, as the main responsible for pOXA-48 fitness costs. Moreover, our results highlighted the relevance of postsegregational killing systems in pOXA-48 vertical transmission, and uncovered new genes implicated in pOXA-48 stability. This study sheds new light on the biology and evolution of carbapenem resistant enterobacteria and endorses CRISPRi screens as a powerful method for studying plasmid-mediated AMR.

Title: The rare plasmid biosphere: a vast genetic reservoir in bacteria

Authors: Célia P. F. Domingues[1,2], João S. Rebelo[1], Teresa Nogueira[1,2], Francisco Dionisio[1]

Affiliation:
1 – CE3c — Centre for Ecology, Evolution and Environmental Changes & CHANGE, Global Change and Sustainability Institute, Faculdade de Ciências, Universidade de Lisboa, 1749- 016, Lisboa, Portugal
2 – INIAV — National Institute for Agrarian and Veterinary Research, 2780-157 Oeiras, Portugal

Position: PhD student

Bacterial communities often exhibit highly uneven abundance distributions, with dominant species coexisting with numerous rare taxa harboring a vast genetic diversity. While bacterial abundance patterns have been extensively studied, the distribution of plasmids remains poorly understood. Here, we investigated whether plasmids mirror the uneven abundance distributions of their hosts, using a dataset of 142,492 plasmids derived from bacterial isolates and metagenomes. There are 26,997 Plasmid Taxonomic Units (PTUs) in this plasmid dataset, revealing an intriguing pattern: a few PTUs are highly prevalent (with many plasmids), while the majority form a "rare plasmid biosphere," consisting of thousands of PTUs represented by a single plasmid. These findings have significant implications: the rare plasmid biosphere represents an exceptionally rich reservoir of genetic material, including antibiotic-resistance genes. Strategies to mitigate the spread of antibiotic resistance must target this largely unexplored genetic reservoir. Furthermore, potentially valuable genes for human applications, such as pollutant degradation, may also reside within this rare plasmid biosphere.

Title: Mechanism of size-selective plasmid elimination by bacterial SMC Wadjet systems

Authors: Hon-Wing Liu, Florian Roisné-Hamelin, Michael Taschner, Yan Li, Stephan Gruber

Affiliation: Department of Fundamental Microbiology (DMF), Faculty of Biology and Medicine (FBM), University of Lausanne (UNIL), 1015 Lausanne, Switzerland

Position: Postdoc

Wadjet systems exhibit the ability to specifically restrict plasmid transmission in diverse bacteria. They comprise a SMC (structural maintenance of chromosomes) motor component (JetABC) as sensor, and a TOPRIM-domain-containing executor (JetD) subunit. SMC complexes are known for folding DNA into chromosomes by actively pumping and extruding large DNA loops (DNA loop extrusion), thus it appears paradoxical that the Wadjet member performs a role in DNA elimination.
In vivo, Wadjet systems restrict plasmids provided they are of a smaller size (<~100kb) and of a circular shape. Purified Wadjet correspondingly cleaves circular DNA at a random position, resulting in variable DNA ends. In contrast, conjugating a large roadblock onto circular DNA prompts specific (non-random) cleavage at the anchor position, suggesting a requirement for the two motors of the complex to converge after extruding the plasmid before cleavage. Through cryo-EM, we visualize a cleavage-competent state of Wadjet with the two presumably converged and stalled JetABC motors entrapping a U-shaped DNA segment, which is further deformed upon docking of a JetD nuclease dimer. Our findings support a model in which near-complete plasmid DNA extrusion by JetABC results in mechanical bending of unextruded DNA, activating JetD and triggering DNA cleavage.
How plasmids larger than 100 kb, and chromosomes are exempt from this process is unclear. We propose and are currently evaluating two loop-extrusion based models for DNA size sensing – the “collision” and “timer” models. This work may provide broader insights into SMC behaviour on physiological substrates, as well as potential anti-Wadjet mechanisms.

Title: Mutational analysis of the F plasmid partitioning protein ParA reveals novel residues required for oligomerisation and plasmid maintenance.

Authors: Nivedita Mitra[1,2], Dipika Mishra[1,2 #], Manasi Mudaliyar[3,4], Irene Aniyan Puthethu[1,2], Pananghat Gayathri[5], Debnath Ghosal[3,4], Ramanujam Srinivasan[1,2 ‡]

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[1] School of Biological Sciences, National Institute of Science Education and Research, Bhubaneswar – 752050, India
[2] Homi Bhabha National Institutes (HBNI), Training School Complex, Anushakti Nagar, Mumbai, India 400094
[3] Department of Biochemistry and Pharmacology, Bio21 Institute, The University of Melbourne, Parkville, VIC, Australia
[4] ARC Centre for Cryo-electron Microscopy of Membrane Proteins, Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Parkville, VIC, Australia
[5] Biology, Indian Institute of Science Education and Research, Pune – 411008, India
Position- PhD Research Scholar in National Institute of Science Education and Research, India

Mobile genetic elements such as plasmids play a crucial role in the emergence of antimicrobial resistance. Hence, plasmid maintenance proteins like ParA of the Walker A type cytoskeletal ATPases/ ParA superfamily are potential targets for novel antibiotics. Plasmid partitioning by ParA relies upon ATP-dependent dimerisation and formation of chemophoretic gradients of ParA-ATP on bacterial nucleoids. Though polymerisation of ParA has been reported in many instances, the need for polymerisation in plasmid maintenance remains unclear. In this study, we provide novel insights into the polymerisation of ParA and the effect of polymerisation on plasmid maintenance. We first characterise two mutations, Q351H and W362E, in ParA from F plasmid ($ParA_F$) that form cytoplasmic filaments independent of the ParBS partitioning complex. Both mutants fail to partition plasmids, do not bind non-specific DNA and act as super-repressors to suppress transcription from the ParA promoter. Further, we show that the polymerisation of $ParA_F$ requires the conformational switch to the ParA-ATP* state. We identify two mutations, R320A in the C-terminal helix-14 and E375A helix-16 of $ParA_F$, that abolish filament assembly and affect plasmid partitioning. Our results thus suggest a role for higher-order structures or polymerisation of ParA in plasmid maintenance.

Title: Conjugative plasmids as biological weapons of donor bacterial cells

Authors: João S. Rebelo[1], Célia P. F. Domingues[1,2], Francisca Monteiro[1], Teresa Nogueira[1,2], Francisco Dionisio[1]

Affiliation:
1 – CE3c — Centre for Ecology, Evolution and Environmental Changes & CHANGE, Global Change and Sustainability Institute, Faculdade de Ciências, Universidade de Lisboa, 1749- 016, Lisboa, Portugal
2 – INIAV — National Institute for Agrarian and Veterinary Research, 2780-157 Oeiras, Portugal

Position: PhD student

Plasmids' acquisition often lowers cells' growth rate, even when recipient cells belong to the same species as the donor cell, so plasmid ubiquity has been debated. Chromosomes occasionally mutate, rendering plasmids cost-free, but these compensatory mutations typically take hundreds of generations to appear after plasmid arrival. By then, it could be too late to successfully compete with fast-growing plasmid-free cells. Therefore, we must understand why plasmids would rely on the appearance of these mutations to ensure maintenance among bacterial communities.

Plasmids may also integrate host-beneficial genes (e.g., antibiotic resistance genes), but this event may also take too long. This presentation discusses the hypothesis that bacteria use conjugative plasmids as 'weapons' to compete with plasmid-free cells, particularly in structured environments. Cells already adapted to plasmids increase their inclusive fitness through plasmid transfer to impose a cost on nearby plasmid- free cells and increase the replication opportunities of nearby relatives. Experimental results, a population genetics model, and computer simulations corroborate this hypothesis, explaining the maintenance of conjugative plasmids (still) not coding for beneficial genes.

Title: Mechanism of bacterial DNA segregation: LLPS behavior, beyond trend and reality

Authors: Perrine Revoil, Jérôme Rech, Jean-Yves Bouet

Affiliation: Laboratoire de Microbiologie et génétique moléculaires (LMGM) – Centre de Biologie Intégrative (CBI) Toulouse

Position: PhD student

In bacteria, low-copy-number replicons carry partition systems to ensure faithful segregation. Among these, ParABS systems, comprising a Walker-type ATPase (ParA) and a DNA-binding protein (ParB), and *parS* centromere sites, are the most prevalent on plasmids and the only one present on chromosomes. ParB, a CTP-dependent molecular switch, self-assembles into partition complexes that exhibit liquid-liquid phase separation (LLPS).

To elucidate the assembly dynamics of partition complexes, we investigated the fusion behavior of ParB condensates *in vivo*. Such fusion behavior is hindered by ParA ATPases, which separate and localize ParB condensates. To observe ParB condensates fusion, we disrupted ParA activity by removing the nucleoid matrix over which ParA is mediating their anchoring, through an inducible, chromosome specific DNA degradation system. We showed that, in absence of nucleoid, ParB condensates still form on *parS* centromere sites, and rapidly fuse. In addition, we found that fusion strictly depends on ParB proteins and clusters all ParB proteins in a single condensate.

Moreover, we have shown that hexanediol, a disruptor of weak hydrophobic interactions, rapidly disassembles ParB condensates within seconds. It thus indicates that weak ParB-ParB interactions are crucial to ParB condensates assembly.

Lastly, we further characterized the fusion/fission dynamics of ParB condensates. We found that fusions occur within seconds, independently of ParA. These rapid dynamics confirm that partition complexes assembly is mediated by LLPS. Our work sheds light on the intricate interplay between LLPS-driven assembly of partition complexes and the counteractivity of ParA ATPases to efficiently ensure DNA partition.

Title: Causes and consequences of an uropathogenic F plasmid evolutionary success

Authors: Celia Souque[1,2], Tatiana Ruiz-Bedoya[1], Kepler Mears[1], Michael Baym[1]

Affiliation:
Department of Biomedical Informatics and Laboratory of Systems Pharmacology, Harvard Medical School, Boston, USA
Norwegian Institute of Public Health, Oslo, Norway

Plasmids are crucial drivers of antibiotic resistance in *E. coli*. However, despite their importance, we still poorly understand the drivers of their prevalence and success in clinical bacterial populations. To bridge this gap, we investigated the plasmid landscape of more than 2000 *E. coli* samples, representing all *E. coli* isolates collected over a year in one US healthcare network. We first identified one highly successful plasmid cluster, similar to the F-plasmid pUTI89, present in more than 30% of all samples. We then aimed to understand the diversity, origins, and potential mechanisms driving pUTI89 evolutionary success. This plasmid cluster was surprisingly conserved, with a shared backbone of 100kb found across more than 600 isolates, as well as ancient, as we identified a plasmid of nearly identical gene content isolated in 1931. We found the plasmid population structure to be divided in two clades, with differing transmission strategies: the first clade was strongly associated with acquisition of antibiotic resistance and limited to a few host sequence types. However the second clade (the most prevalent) was distributed across a wide variety of host backgrounds, pointing towards extensive horizontal gene transfer. Additionally, its success did not seem associated with acquisition of additional virulence or resistance genes, but instead variation in core plasmid functions. We then investigated in the lab the potential for this plasmid to impact the transmission of other F plasmids into its host cell, and found it to be surprisingly permissive to F plasmid co-infection. In conclusion, this plasmid cluster is a surprising counter-example to most well studied *E. coli* plasmids: pUTI89 is a highly transmissible plasmid that can be successful without carrying antibiotic resistance gene, and seems highly stable over decades, despite extensive horizontal gene transfer and opportunities for acquisition or loss of new genes by recombination. These results hint at the existence of extensive selective pressures acting on plasmid structures.

Title: Comprehensive classification of *Pseudomonas* plasmids carrying antimicrobial resistance genes

Authors: Maho Tokuda [(1)], Xie Hui [(2)], Nanako Isogai [(1)], Chiho Suzuki-Minakuchi [(2)], Masato Suzuki [(3)], Hideaki Nojiri [(2)], Yosuke Nishimura [(4)], Masaki Shintani [(1)]

Affiliation: [(1)] Shizuoka University, [(2)] The University of Tokyo, [(3)] National Institute of Infectious Diseases, [(4)] Japan Agency for Marine-Earth Science and Technology (JAMSTEC)

Position: Post-doc

The dissemination of antimicrobial resistance genes (ARGs) via the conjugative transfer of plasmids poses a significant public health threat. Carbapenemases, enzymes capable of hydrolysing a broad range of β-lactams, are of particular concern. *Pseudomonas* species are widespread not only the clinicals but also the natural environments, emphasizing their relevance in ARG dissemination. This study aimed to classify plasmids carrying carbapenem resistance gene(s) within *Pseudomonas* species using the public plasmid database, PLSDB[1)].

Among 846 *Pseudomonas* plasmids in PLSDB, 192 plasmids were found to harbour carbapenem resistance gene(s). However, when using the PlasmidFinder classification system[2)], only 14 of these plasmids could be classified, highlighting the limited applicability of existing classification schemes for *Pseudomonas* resistance plasmids.

To address this limitation, we developed a custom replication initiation protein (RIP) gene library, incorporating sequences from IncP-1 to P-14, PromA, pSN1216-29-like, pQBR103-like and pSTY-like plasmids[3)], and the Mob typer database[4)]. In addition, unclassified plasmids were subjected to all-against-all blastn analyses. This approach enabled the classification of 191/192 plasmids with carbapenem resistance genes in *Pseudomonas*. Notably, IncP-2 (58/192) and IncP-10 (26/192) emerged as the predominant plasmid groups carrying carbapenem resistance genes in *Pseudomonas*. These founding provide valuable insights into the classification and distribution of resistance plasmids contributing to a better understanding of ARG dissemination mechanisms.

[1)]Schmartz GP et al., 2022 (D1):D273-D278
[2)]Carattoli A et al., Antimicrob Agents Chemother 2014 58(7):3895-903
[3)]Nishimura et al., BioRxiv doi: https://doi.org/10.1101/2024.09.03.610885
[4)] Robertson J et al., 2018 Microb Genom. 4(8):e000206

Title: A plasmid-chromosome crosstalk in multidrug resistant enterobacteria

Authors: Laura Toribio-Celestino, Alicia Calvo-Villamañán, Cristina Herencias, Aida Alonso-Del Valle, Jorge Sastre-Dominguez, Susana Quesada, Didier Mazel, Eduardo PC Rocha, Ariadna Fernández-Calvet, Alvaro San Millan

Affiliation: Centro Nacional de Biotecnología (CNB-CSIC), Madrid, Spain

Position: PhD Student

Conjugative plasmids promote the dissemination and evolution of antimicrobial resistance in bacterial pathogens. However, plasmid acquisition can produce physiological alterations in the bacterial host, leading to potential fitness costs that determine the clinical success of bacteria-plasmid associations. In this study, we use a transcriptomic approach to characterize the interactions between a globally disseminated carbapenem resistance plasmid, pOXA-48, and a diverse collection of multidrug resistant (MDR) enterobacteria. Although pOXA-48 produces mostly strain-specific transcriptional alterations, it also leads to the common overexpression of a small chromosomal operon present in *Klebsiella* spp. and *Citrobacter freundii* strains. This operon includes two genes coding for a pirin and an isochorismatase family proteins (*pfp* and *ifp*), and shows evidence of horizontal mobilization across Proteobacteria species. Combining genetic engineering, transcriptomics, and CRISPRi gene silencing, we show that a pOXA-48-encoded LysR regulator is responsible for the plasmid-chromosome crosstalk. Crucially, the operon overexpression produces a fitness benefit in a pOXA-48-carrying MDR *K. pneumoniae* strain, suggesting that this crosstalk promotes the dissemination of carbapenem resistance in clinical settings.

Title: A biotechnological tool to detect integron cassettes

Authors: Filipa Trigo da Roza[1,2], André Carvalho[1,2], Paula Blanco[1,2], Amalia Prieto[1,2], Ester Vergara[1,2], Rocío Lopéz-Igual[3], Modesto Redrejo[4], Melanie Blokesch[5] & José Antonio Escudero[1,2]

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[3]
[4] Departamento de Bioquímica, UAM, Madrid, Spain
[5] UPBLO, GHI-SV, EPFL, Lausanne, Switzerland

Position: PhD student

Integrons are genetic platforms that capture, stockpile, and rearrange integron cassettes (IC), which encode genes with diverse functions, including antimicrobial resistance, phage resistance, or metabolic functions. However—and despite their dissemination to the clinical setting trough plasmids—detecting ICs is still challenging, and many functions they encoded are still unknown.

To address these challenges, we developed a biotechnological tool to identify ICs independently of their sequence, phenotype, or genetic background. We have redesigned a class 1 integron to serve as a capture platform, incorporating *ccdB* or *sacB* as counter-selectable markers with an embedded integration site, *attI*. These markers enable the selection of recombinants in specific conditions, ensuring viability only if disrupted by incoming ICs, with a low escape mutant rate of 10^{-6}, providing a broad range for detecting cassettes.

We first demonstrated that both counter-selectable markers report IC capture in a classical recombination assay. We then developed three distinct applications for the tool: 1) a chromosomal version generating a conjugation-sentinel *E. coli* that allowed us to detect ICs from conjugative plasmids in clinical isolates, 2) a plasmidic version for harvesting ICs from large chromosomal integrons, producing libraries of up to 10^5 colonies per assay, and 3) a chromosomal version implemented in naturally competent, genetically modified *V. cholerae*, enabling the efficient IC capture directly from exogenous DNA, including samples with plasmidic and chromosomal integrons.

This tool uncovers integron content from diverse samples with high specificity. Its sequence-independent design represents a groundbreaking advance for understanding integron cassette ecology.

Title: Regulation of TopoIV activity in *Escherichia coli*

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During bacterial cell cycle, the replication of the chromosome and plasmids is followed by the segregation of each copy in daughter cells. As replication and segregation must be finely coordinated with cell division many proteins are recruited. Notably the MatP protein, and its DNA binding site *matS,* which participates in the positioning of the *ter* regions of sister chromatids at midcell[1]; and the recombinases XerC and XerD which separate dimers by site-specific recombination at the *dif* site[2]. Nevertheless, the entire mechanism that drive this coordination is not fully understood. It is known that a lot of proteins involved can interact with the Topoisomerase IV (TopoIV), which has a key role in the regulation through its catenane and pre-catenane resolution activity[3]. Catenanes are interlinked DNA molecules, and theses links must be removed to allow their segregation. To better understand the decatenation activity *in vivo*, I use the model bacterium *Escherichia coli* with temperature sensitive mutants of TopoIV. The activity is investigated by analyzing catenated plasmids accumulation at non-permissive temperature, as well as their resolution by recovering TopoIV.
Experiments are carried out with a small (2.7kb) and high copy number plasmid, pUC18; its derivative where a *dif* site has been added, TopoIV activity is strong at this site [4]. To study the effect of subcellular localization on the activity of TopoIV, a *matS* site has been added on theses plasmids. My results suggest that subcellular position at midcell by *matS,* and *dif* sites on the plasmid can improve decatenation, but also that the combination of *matS* and *dif* have a synergistic effect on TopoIV activity.

1. Mercier, R. *et al.* The MatP/matS Site-Specific System Organizes the Terminus Region of the E. coli Chromosome into a Macrodomain. *Cell* **135**, 475–485 (2008).
2. Blakely, G. *et al.* Two Related Recombinases Are Required for Site-Specific Recombination at dif and cer in E, coli K12, Cell **75**, 351-361 (1993).
3. Kato, J. *et al.* New Topoisomerase Essential for Chromosome Segregation in E. coli, Cell **63**, 393-404 (1990).
4.El Sayyed, H. *et al.* Mapping Topoisomerase IV Binding and Activity Sites on the E. coli Genome. *PLoS Genet* **12**, e1006025 (2016).

Title: Population ghost effect prolongs plasmid carriage following antibiotic pulse

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As major carriers and spreaders of antibiotic resistance, the abundance of plasmids within microbial communities can dictate the efficacy of antibiotic treatments. While quantitative metrics have been used to predict the long-term survival (steady state) of plasmids within single bacterial strains and communities, the rate at which a plasmid's abundance will converge to its steady state remains elusive. In this work, we quantified the dynamics of unstable plasmids that cannot survive in bacterial populations. We observed a slowed-down decay of plasmid abundance when the population was transiently treated with antibiotic selection. The half-life of decay increased from days to months after one-day of antibiotic selection. Through mathematical modelling, we attributed this effect to the decoupling of time scales between plasmid segregation loss and growth competition, which corresponds to the ghost effect in nonlinear dynamics. We further showed that sublethal doses of antibiotics could sufficiently induce a ghost effect. This phenomenon was experimentally demonstrated in synthetic microbial communities and resulted in the persistence of plasmids for up to a month. Together, our results suggest that transient, sublethal antibiotic exposure could result in unexpected persistence of burdensome plasmids.

Title: Simulated plasmid community assembly in biofilm vs. planktonic environments

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Spatial structure is a feature arising from local interaction of organisms. It can manifest in the observation of spatially correlated traits such as species abundance, organismal traits or genetic similarity, governed by local competition for space and the inheritance of traits to locally evolved offspring. With increasing local similarity, the importance of intraspecific competition increases compared to interspecific competition, which can have consequences for population dynamics and community composition. In this study, an individual-based model of the plasmidome is used to assess how spatial structure affects the simulated assembly of plasmid communities. For this purpose, two scenarios are compared: one in which the bacterial cells remain in their spatial position in order to imitate biofilms, and another in which the position of the bacterial cells is continuously changed at random to imitate conditions in planktonic environments. Other settings, such as cell densities, were not changed. The modeling results on the assembly of plasmid communities under both conditions show that plasmid diversity is higher in spatially structured environments. Also the sum of the total costs that all the plasmids impose on the bacterial population. In planktonic environments, the maintained conjugative plasmid types had on average a higher cost and transfer proability, and, when less than four different plasmid incompatibility groups were considered, their proportion on all plasmid types was lower. The results indicate that spatially structured conditions similar to those given in biofilms or bacterial flocs foster the maintenance of genetic diversity, while this can be comparatively reduced in planktonic environments.