Project X: Structural basis of OMV immunogenicity in *Vibrio cholerae* infections

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Background and preliminary data:
Cholera, caused by the bacterium *Vibrio cholerae*, is a severe and potentially fatal diarrheal disease that is endemic in more than 47 countries across the globe. Every year, ~4 million cholera cases occur, causing ~100,000 deaths worldwide. Cholera outbreaks occur following infrastructure breakdowns, as exemplified by recent outbreaks in Yemen, Haiti and in many countries in Africa. Hence, *V. cholerae* remains a major public health concern, and better prevention and treatment measures are needed to alleviate the impact of cholera.
Biofilm formation, the development of extracellular matrix-enclosed, surface-associated microbial communities, is crucial in the infection cycle of many bacterial pathogens. *V. cholerae* forms biofilms in aquatic environments and human hosts, enhancing resistance to antibiotics and immune clearance. Hence, the biofilm growth mode is vital for both, environmental survival and transmission of the pathogen. The biofilm matrix consists of *Vibrio* polysaccharide (VPS) and specific matrix proteins. Recent studies identified bacterial outer membrane vesicles (OMVs) in the biofilm matrix [1]. OMVs are composed of lipopolysaccharides, phospholipids, outer membrane proteins, and periplasmic components. They are shed by bacteria and are integral to bacteria-bacteria and bacteria-host interactions by delivering proteins, toxins, and nucleic acids. OMVs are also immunogenic and provide *V. cholerae* protection against bacteriophages.
Until recently, the field has had a limited understanding of the protein content of the *V. cholerae* biofilm matrix and OMVs. To address this knowledge gap, the Yildiz group, a collaboration partner at the University of California, Santa Cruz, determined biofilm matrix and OMV proteomes via quantitative mass spectrometry [1]. Importantly, the proteome analyses revealed that about a tenth of all *V. cholerae* biofilm matrix proteins are outer membrane proteins (OMPs), elucidating the extent to which OMVs contribute to the matrix. In addition, the matrix and OMV proteomes overlapped by 26%, with highly pure OMVs carrying the major *Vibrio* matrix proteins. Strains that produce more OMVs also show an increase in colony corrugation, suggesting that OMV production and biofilm architecture are linked pathways. However, the specific roles that OMPs and OMVs play in biofilm matrix biogenesis and the host's immune response remain poorly understood.

Hypothesis: Understanding the role and structure of OMVs and their cargo has the potential to reveal new strategies for controlling *V. cholerae* infections and bacterial survival in the environment. Molecular insights into the mechanism of OMVs' immunogenicity may also inform on novel detection and vaccination strategies. We hypothesize that by determining the overall structure of OMVs decorated with host-derived antibodies we will gain a molecular understanding of the immunological interface of *V. cholerae* OMVs and the host, providing a detailed framework for novel, targeted intervention strategies.

Aims and Work Programme:
1. Structural characterization of *V. cholerae* matrix proteins in their native association with OMVs.
2. Isolation of OMV-elicited antibodies for the structural characterization of the OMV-host immunity interface.

In **Aim 1,** we will elucidate the structure of OMV-associated *V. cholerae* matrix proteins using electron cryotomography (cryoET). To do so, we will isolate OMVs from established, engineered *V. cholerae* strains, which produce the three major matrix proteins RbmA, RbmC, and Bap1 with distinct epitopes that can be recognized by commercially available, high-affinity antibodies (*e.g.*, GFP, SNAP or Myc tags). Signpost origami tags (SPOTs) developed in the

Grünewald group will be employed to detect and enumerate the tagged matrix proteins in tomograms of highly pure OMV preparations [2]. Resolution enhancement will be facilitated by sub-volume averaging, using SPOTs as markers for particle picking. Available crystal structures and computational models of target proteins (and their complexes) will be used to interpret tomograms. A particular interest will be on the structure of the matrix proteins on native membranes of OMVs compared to structures determined for isolated proteins using X-ray crystallography [3]. In addition, we anticipate to gain a molecular understanding of the anchoring mechanisms of matrix proteins on OMVs, providing insight into the biofilm/OMV interface, an attractive target for molecular interventions.

Immune responses in mice challenged with isolated OMVs mirror those of convalescent patients after a *V. cholerae* infection [4], indicating that the mouse model will provide relevant insight to human infections. Previous studies, using OMVs isolated from *V. cholerae* liquid culture, identified the O antigen component of lipopolysaccharides as the main epitope for immunogenic protection in mouse models. In **Aim 2,** we will compare the immune response elicited by OMVs isolated from planktonic vs. biofilm-grown cultures to uncover biofilm-specific host responses. We will contract immunization of mice with highly pure, native OMVs that were isolated from *V. cholerae* grown in liquid cultures or in biofilms. Antisera and spleen will be collected [4]. The sera will be used in immunoprecipitation experiments followed by mass spectrometry (MS)-based identification of OMV and OMV-associated immunogenic proteins. In parallel, isolated spleen B cells will be used to generate a library of monoclonal antibodies. Purified antigens identified in the proteomics screens completed by the Yildiz group (*i.e.*, RbmA, RbmC, Bap1, OmpU, OmpA, and OmpT) will be used to detect and isolate target-specific monoclonal cells lines. Other targets will be tested based on the MS data after immunoprecipitation. Purified, monoclonal antibodies with established targets will be coupled to SPOTs for detection and enumeration of target proteins on OMVs from planktonic or biofilm *V. cholerae* cultures. We anticipate that in the mid-term this approach will enable structural characterization of OMV proteins decorated with native antibodies from a host-pathogen model. In the long-run, the efficacy of such characterized monoclonal antibodies to prevent infections will be assessed in infant mouse models [5].

The project is supported by the combined expertise of the Project Leaders (Prof. Grünewald: cryoET, SPOTs, structure of biological vesicles; Prof. Sondermann: bacterial biofilms, membrane biology, structural biology) and their collaborators (Prof. Yildiz: *V. cholerae* microbiology, biofilms; Prof. Krey: infection and immunity). Together, the proposed work will enhance our understanding of *V. cholerae* infections involving three central pathways – biofilm formation, OMV shedding, and immunogenicity – and their interconnections. These pathways are intimately linked to the survival and infectivity of this important pathogen and are considered potent entry points for antiinfection strategies against *V. cholerae*. Outbreaks by *V. cholerae* and related bacteria remain a significant public health threat throughout the world, including in flooded areas and oceans experiencing the effects of global warming. Identifying ways to disperse bacterial biofilms by targeting the extracellular matrix has the potential to reduce the pathogens' environmental persistence and thus transmission and future outbreaks.

The proposed project will also contribute to expanding the international network of the fellow by strengthening a transatlantic collaboration, including training and exchange visits involving the groups in Hamburg and at the University of California, Santa Cruz.

Project-related publications: (max. 5)

[1] Potapova et al., doi: 10.1128/mbio.03304-23
[2] Silvester et al., doi: 10.1016/j.cell.2021.01.033
[3] Giglio et al., doi: 10.1128/JB.00374-13
[4] Schild et al., doi:10.1128/IAI.00532-08
[5] Gallego-Hernandez et al., doi: 10.1073/pnas.1916571117