Title - Structure guided characterization of bacterial antiviral defense systems

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Bacteria have evolved community-based pan-immune systems to defend against viral infection. There are several antiviral defense systems such as restriction endonucleases, CRISPR-Cas and CBASS (cyclic oligonucleotide based antiphage signaling systems) surveillance complexes, and some newly discovered and less-characterized systems like Gabija, Shedu, Zorya, Thoeris, Septu, Hachiman and Lamassu, as well as many more yet to be identified. The bacterial defense related genes are found to be spatially co-localized in bacterial genomes and they are shared by a population of bacteria by horizontal gene transfer, which reduces the energy burden from a single bacterium (Bernheim & Sorek, 2020; Makarova et al., 2011). Some of these systems involve different components, function in a synchronized fashion upon viral infection, whereas others form large complexes (Duncan-Lowey et al., 2023). In either case, the defense systems have the potential for viral infection recognition, and in some cases this step is enough to activate the effector molecule, which kills the infected bacteria and aborts further propagation of the virus. Whereas in some systems there are mediators, also referred to as cyclcic oligonucleotide secondary messengers, which are generated by bacterial enzymes like cyclases and relay the signal to the effector molecules upon viral infection (Cohen et al., 2019; Whiteley et al., 2019). The mechanism by which these enzymes are activated to generate the second messenger in the virus-infected bacterial cells remains unclear.

Moreover, the activated effector molecules follow different approaches to abortive infection – the most common effectors are nucleases, which degrade the viral genetic material selectively or without any self-discrimination; these kinds of effectors are relatively well characterized. However, there are other effectors which incorporate a transmembrane domain, that function either by membrane depolarization, metabolite depletion by pore formation or through disintegration of the bacterial cell membrane (Duncan-Lowey et al., 2021; VanderWal et al., 2023). To date, we have very limited understanding about the mechanism of the membrane-associated effectors.

Project – Elucidation of structure guided mechanism of a transmembrane effector protein Cam1 Type III CRISPR-Cas immune systems in bacteria provide adaptive immunity against viruses through usgage of CARF domain effector proteins. Though most of the CARF domain effectors which function as nucleases are relatively well characterized, the membrane associated CARF domain effectors remain poorly understood (Shah et al., 2019; Shmakov et al., 2018). Here, our efforts are focused on understanding the abortive infection mechanism of a transmembrane domain containing CRAF-effector protein, Cam1. We would like to perform cryo-EM studies to solve the structure of the full-length protein in detergent micelle in presence and absence of the second messenger to understand the mechanism of action of Cam1 protein. It has become very challenging for us to get good resolution in the membrane spanning domain of the protein as it is surrounded by the detergent and hence we need more particles to solve this problem. I belief if we can collect a large dataset using NCCAT cryo-EM facilities that will be very helpful for this study.

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