Saturday, March 22, Session VII

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IN SITU VISUALIZATION OF THE *BORDETELLA* FILAMENTOUS HEMAGGLUTININ BY CRYO-ELECTRON TOMOGRAPHY

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Bordetella pertussis, the causative agent of whooping cough, relies on the surface adhesin filamentous hemagglutinin (FhaB) for adherence and colonization of respiratory tract cells. FhaB is initially translated as a 360-kDa precursor and later released into the external environment as a 'mature' 220-kDa protein; however, its structural properties remain poorly understood. Here, we describe two approaches for the preparation and characterization of bacterial minicells derived from *B. pertussis* and *B. bronchiseptica*. These minicells were generated by deleting the *minD* gene and introducing the A126V substitution in the *mreB* gene in the bacterial chromosome, as well as by overexpressing *FtsZQ* genes under the control of the *pBAD* promoter. Minicells were collected from cultures of the mutant *Bordetella* strains using differential and gradient centrifugation. Purified minicells were used for in situ visualization of the FhaB molecule via cryo-electron tomography. Tomograms revealed that the FhaB molecule forms a ~35-nm long filament protruding from the minicells surface. These findings highlight the potential of minicells as a powerful tool for structural investigations of *Bordetella* virulence factors *in situ*.

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TWO SOLUTIONS FOR EFFICIENT LIGHT-HARVESTING IN PHOTOTROPHIC *GEMMATIMONA*DOTA

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The anoxygenic phototrophic bacterium *Gemmatimonas phototrophica* prefers growth at lower-light intensities. It has evolved a unique type of photosynthetic complex that consists of a type-2 reaction centre surrounded by two rings of light harvesting antenna with each giving rise to a distinct near infrared absorbance band. A closely related species *Gemmatimonas groenlandica* grows better at higher-light intensities and contains the same photosynthesis gene cluster, yet its photosynthetic complex has a notably different near infrared absorption spectrum with only one large absorption band. In order to understand the origin of this difference, the structure of the photosynthetic complex from this species was determined by the cryogenic electron microscopy. The analysis revealed that it also contains two rings but that the outer antenna ring absorption is red-shifted. The shift was caused by rotation of a tryptophan residue side chain to form a H-bond with bacteriochlorophyll and increased the strength of the intra-dimer exciton coupling. In addition, the outer antenna ring lacks monomeric bacteriochlorophylls. This loss reduced the optical antenna cross-section in *Gemmatimonas groenlandica*, but the H-bond increased the probability of exciton exchange among the complexes (connectivity). Therefore, these evolutionary changes have changed the higher-light optimised complex present in *Gemmatimonas groenlandica* into a complex that has allowed *Gemmatimonas phototrophica* to grow well under lower-light intensities.

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2. Zeng, Y., Nupur, Wu, N., Madsen, A. M., Chen, X., Gardiner, A. T., and Koblížek, M. (2021) Gemmatimonas groenlandica sp. nov. Is an Aerobic Anoxygenic Phototroph in the Phylum Gemmatimonadetes, Frontiers in Microbiology 11 (2021) 606612.

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CELL ENTRY AND GENOME DELIVERY OF ENTEROVIRUSES

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Enteroviruses enter cells by receptor-mediated endocytosis. However, it is not fully understood how enteroviruses release their genomes and how enterovirus particles or RNA genomes cross the endosome membrane into the cytoplasm. We used cryo-electron microscopy to visualize enterovirus particles in the process of genome release. The exit of the RNA results in a loss of one, two, or three adjacent capsid-protein pentamers from a particle. The opening in the capsid, which is more than 120 Å in diameter, enables the release of the genome without the need to unwind its putative double-stranded RNA segments. We used cryo-electron tomography of infected cells to show that endosomes containing enteroviruses deform, rupture, and release virus particles into the cytoplasm. Blocking endosome acidification with bafilomycin A1 reduced the number of particles that released their genomes but did not prevent them from reaching the cytoplasm. Inhibiting post-endocytic membrane remodeling with wiskostatin promoted abortive enterovirus genome release in endosomes. Our results show that cellular membrane remodeling disrupts enterovirus-containing endosomes and thus releases the virus particles into the cytoplasm to initiate infection. The cells also contained empty capsids lacking pentamers of capsid proteins. Since the studied enteroviruses employ different receptors for cell entry but are delivered into the cytoplasm by cell-mediated endosome disruption, it is likely that most, if not all enteroviruses, and probably numerous other viruses from the family *Picornaviridae*, can utilize capsid opening and endosome rupture to infect cells.