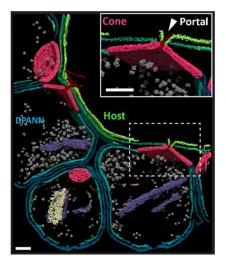
## Characterization of a large protein complex in Nanobdellota archaeon YN1

Milad Reyhani<sup>1</sup>, Matthew D Johnson<sup>1,2</sup>, Hiroyuki D. Sakai<sup>3,4</sup>, Shubha Udupa<sup>1,2</sup>, Debnath Ghosal<sup>1,2</sup>

<sup>1</sup>Department of Biochemistry and Pharmacology, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Melbourne, VIC, Australia. <sup>2</sup>ARC Centre for Cryo-electron Microscopy of Membrane Proteins, Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Parkville, VIC, Australia. <sup>3</sup>Department of Science and Engineering for Sustainable Innovation, Faculty of Science and Engineering, Soka University, Hachioji, Tokyo 192-8577, Japan. <sup>4</sup>Japan Collection of Microorganisms, RIKEN BioResource Research Center, Tsukuba, Ibaraki 305-0074, Japan, E-mail: mreyhani@student.unimelb.edu.au

DPANN archaea are a mysterious superphylum that is difficult to isolate and culture in the laboratory due to their specific culture conditions. DPANNs are characterized by their small size and limited metabolic capabilities<sup>1</sup>.Recently, our laboratory characterized the structural basis of the symbiotic relationship between a newly discovered DPANN Nanobdellota archaeon, YN1, and its host. Cryo-electron tomography (CryoET) revealed the presence of a large cone-shaped attachment organelle that facilitates the interaction between the nanoarchaeon and its host. This organelle, characterized by its large size and complexity, appears crucial for the stable association between YN1 and its host, suggesting a specialized mechanism for symbiosis in this system. However, to comprehensively understand the mechanism of symbiosis, it is imperative to examine the structure of these attachment organelles at both molecular and structural levels. Our current efforts are focused on purifying these attachment organelles to conduct detailed analyses through proteomic and biochemical assays, complemented by structural studies using CryoET and subtomogram averaging techniques. Our findings will shed light on the evolutionary dynamics and ecological significance of symbiotic relationships in archaea and provide valuable insights into how microbial interactions shape diverse ecosystems<sup>2</sup>.



**Figure**: Segmentation analysis of host-DPANN interaction. DPANN envelope = light/dark blue, Host envelope membrane = light/dark green, cones = pink. Attachment organelle = hot pink. Scale bar 100 nm.References: [1] Rinke, C. et al. Insights into the phylogeny and coding potential of microbial dark matter. Nature 499, 431–437 (2013).[2] Johnson, M et.al. A large attachment organelle mediates interaction between a novel Nanobdellota archaeon YN1 and its host, bioRxiv 2024.05.04.592509; doi: https://doi.org/10.1101/2024.05.04.592509.