Poster

New structural insights into bacterial colonization by *Vibrio cholerae*

H. V. Sørensen[1], M. Montserrat-Canals[1], Kaare Bjerregaard-Andersen[1], U. Krengel[1]

[1]*Department of Chemistry, University of Oslo*

ute.krengel@kjemi.uio.no

We set out to investigate the structure and function of GbpA, an important colonization factor of *Vibrio cholerae.* GbpA aids colonization by attaching to chitin in environmental reservoirs as well as mucins in the human host (Fig. 1). Curiously, this colonization factor is secreted. One of the main challenges of this work is the complex crystalline nature of chitin. We sought to overcome this challenge by employing small-angle neutron scattering (SANS) and electron microscopy, and were rewarded with glimpses into the colonization mechanism, and how GbpA aggregates chitin fibers in the process [1].

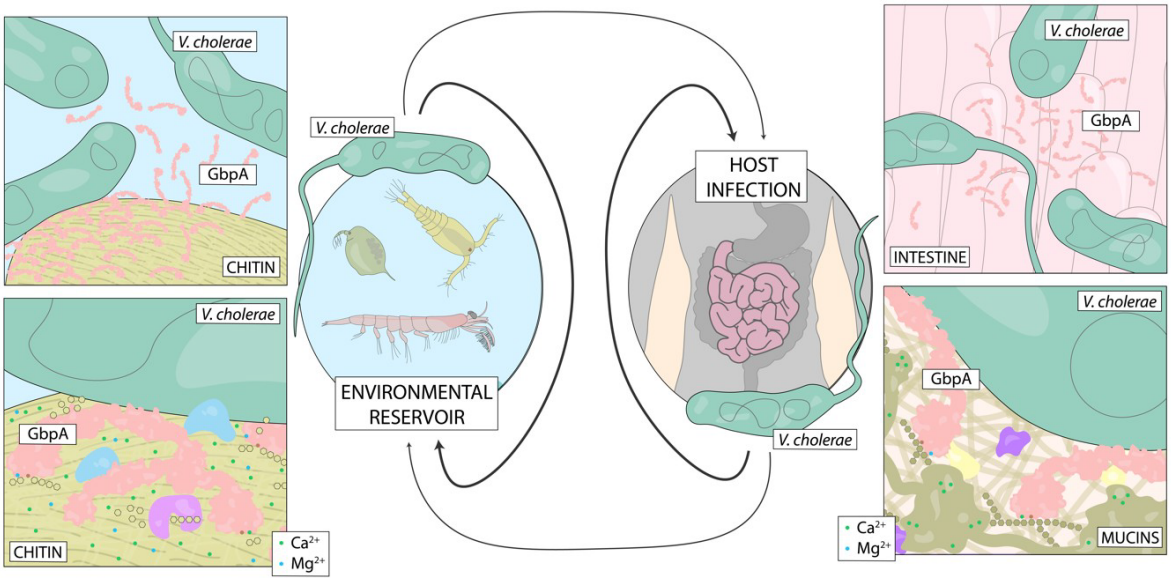

Figure 1. *V. cholerae* uses GbpA to colonize the small intestine of the host as well as aquatic reservoirs (from [2])

When structurally characterizing the lytic polysaccharide monooxygenase (LPMO) domain of GbpA by high-resolution X-ray crystallography, we unexpectedly found calcium or potassium ions next to the active site [2]. Following up on this observation, we analysed if mono- and divalent ions affect GbpA stability and catalysis. While monovalent ions (Na^+, K^+) had unspecific positive effects, Ca^{2+} strongly and specifically enhanced GbpA stability, whereas Mg^{2+} had a small, but specific destabilizing effect. The newly discovered metal-binding site only exists in a select number of AA10 LPMOs. Other AA10 LPMOs often possess a Lys at this site, which occupies the same position as the metal ions in GbpA. Indications are that GbpA (and related colonization factors) use cations like calcium, which is abundant in chitin and known to serve important functions in mucins, to modulate binding and catalytic activity also *in vivo*. This may have important consequences for *V. cholerae*´s life cycle in different niches, including environmental survival and infectivity.

[1] Sørensen, H. V., Montserrat-Canals, M., Prévost, S., Vaaje-Kolstad, G., Bjerregaard-Andersen, K., Lund, R. & Krengel, U. (2023). *Tangled up in fibers: How a lytic polysaccharide monooxygenase binds its chitin substrate. bioRxiv 2023.09.21.558757*

[2] Montserrat-Canals, M., Bjerregaard-Andersen, K., Sørensen, H. V., Cordara, G., Vaaje-Kolstad, G. & Krengel, U. (2023). *Calcium binding site in AA10 LPMO from Vibrio cholerae suggests modulating effects during environment survival and infection. bioRxiv 2023.12.22.573012*

We thank Gustav Vaaje-Kolstad and his group at NMBU, Norway as well as the staff at ILL, NIST and ESRF for a very stimulating collaboration, and acknowledge funding from the Norwegian Research Council.