# Crystallographic structures of bacteriophage receptor-binding proteins and endolysins

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Most bacteriophages recognize their host cells via specialized spike or fibre proteins. The overall goal of our group is to determine their structures in complex with their natural receptors, which may lead to different biotechnological applications, such as elimination of specific bacteria. We also study the high-resolution structures of endolysin proteins, involved in bacterial wall degradation. This research aims to understand their mechanism of action and specificity, which, in turn, may lead to recombinant lytic enzymes with improved properties.

Bacteriophage epsilon15 infects *Salmonella enterica* subspecies enterica, serovar Anatum A1. This bacterium has a lipo-polysaccharide O-antigen consisting of units of D-O-acetyl-galactose-alpha1-6-D-mannose-beta1-4-L-rhamnose. Lysogenic epsilon15 blocks acetylation of the O-antigen and favours production of beta-linked O-antigen. The receptor-binding fibre protein is gp20. Gp20 consists of an N-terminal triple coiled-coiled virus binding domain (not resolved in our structures), a central beta-helix domain with O-antigen hydrolysis activity, a lectin domain and a C-terminal esterase domain. The O-antigen hydrolysis and esterase activities were proven by mutational analysis and NMR spectroscopy. The O-antigen hydrolysis proceeds through an inversion mechanism. The importance of the esterase activity for infection is unknown.

The RBP of bacteriophage S24-1 has been identified as orf16, a 642 amino acid protein. We have solved the structure and shown it is very similar to the RBP of Staphylococcus phages phi11 and P68. Teichoic acid may be a receptor for the protein, and we were able to co-crystallize the protein with a teichoic acid analogue.

*Pseudomonas* phage JG004 endolysin Pae87 is a monomodular lysozyme. We solved its structure without and with a peptidoglycan fragment NAG-MurNAc-LAla-DGlu, suggesting that a region of the muramidase domain functions as a *de facto* cell wall binding domain [1].

#### [1] Roberto Vázquez, Mateo Seoane-Blanco, Virginia Rivero-Buceta, Susana Ruiz, Mark J. van Raaij & Pedro García (2022). *Acta Cryst. D***78**, 435-454.