

Lactococcus lactis Complex Characterization

Lactococcus lactis is a Gram-positive bacterium widely used by the dairy industry. Several industrial strains are sensitive to various distinct bacteriophages constituting a major problem in causing milk fermentation failures. One strategy to minimize bacteriophage infections is to competitively block phage adsorption by adding a protein specifically binding to the phage Receptor Binding Proteins (RBPs, BppL trimer). These latter proteins, inserted in a large assembly termed baseplate, mediate phages anchoring to their receptors on the host cell surface.

We have selected three Designed Ankyrin Repeat Proteins (DARPin)s binding to the baseplate of the TP901-1 lactococcal phage. These DARPins were found to inhibit the infection of *L. lactis* by the TP901-1 phage. They thus constitute a potential tool to fight against phage and more generally against viral infections.

As a part of a structure/function study, we characterized both mass and stoichiometry of each DARPin in complex with either the RBP alone or a multi-protein complex, constituting part of the TP901-1 baseplate and formed by the RBP and the BppU protein. To reach this aim, we used a combination of UV_{280nm} absorbance, MALS, QELS and refractometry, coupled on line with an analytical SEC column.

We observed that all three selected DARPins could bind to the RBP-BppU complex or the RBP alone. Furthermore, addition of DARPins to the RBP-BppU complex resulted in a 5% increase of the R_h of the eluted species. The stoichiometry of binding was one DARPin per RBP independent of whether the RBP was alone or complexed to BppU (as illustrated in Figure 1 and 2). These data were later validated by the X-ray structures of a selected DARPin in complex with the RBP.

The method described here allows studying protein-protein complexes by monitoring complex formation and then accurately characterizing the size and stoichiometry of the resulting assembly.

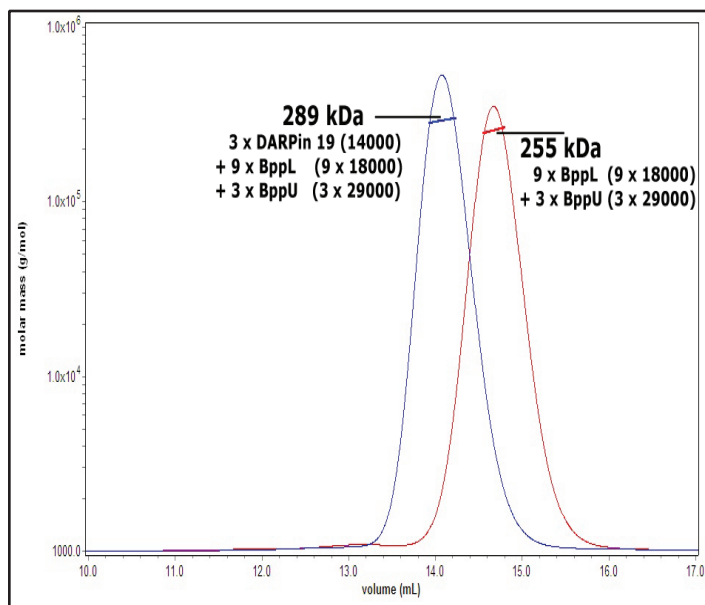


Figure 1. SEC/MALS/RI analysis of the DARPin19-BppL-BppU protein complex using a 24 mL Superose 6 10/30 column. The resulting complex is formed by 3 DARPin19 associated with 9 BppL (3 RBPs) and 3 BppU copies.

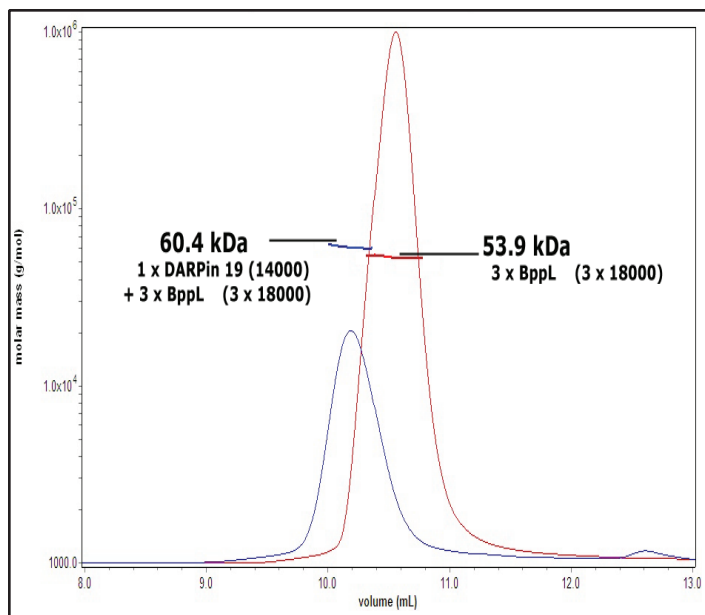
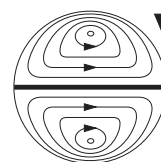


Figure 2. SEC/MALS/RI analysis of the DARPin19-BppL protein complex using a 15 mL Sdex KW-804 column. The resulting complex is formed by 1 DARPin19 associated with 3 BppL (1 RBP).

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