

Mega-Dalton protein complexes characterization.

Recent technological advances in macromolecular crystallography led to focus on the study of more and more sophisticated biological systems, such as protein-protein complexes. Nevertheless, they represent one of the most challenging areas in modern structural biology.

Baseplates are large multi-protein assemblies found at the tip of bacteriophage tails, which constitute key components during host infection. In Gram-positive bacteria, infection is initiated by binding of the phage Receptor Binding Protein (RBP, BppL trimer) to a saccharidic receptor on the host surface. Several RBPs are present in each baseplate. A detailed analysis of these complexes would result in a better understanding of the mechanisms underlying phage-host interaction.

We report here a method to characterize protein-protein interactions and stoichiometry in MegaDalton macromolecular assemblies as illustrated by the study of the TP901-1 lactococcal phage baseplate. Our set-up involves the combined measurements from MALS, QELS, refractometry and UV_{280nm} absorbance coupled on line with an analytical SEC.

First, we confirmed that BppL is homotrimeric in solution, as observed in the crystal structure. Then, operon co-expression of BppU and BppL proteins resulted in a complex containing 9 BppL and 3 BppU copies (Figure 1).

Then, co-expressing BppL, BppU and Dit proteins in the same way yielded a 1.7 MDa complex constituted by 54 BppL, 18 BppU and 6 Dit copies (Figure 2) and characterized by R_h and R_g values of 12.5 nm and 11.4 nm, respectively.

These data provided a picture of how these proteins assemble to form a baseplate, and, that 54 host binding sites are displayed by this phage suggesting that avidity contribute to host anchoring.

The reported method illustrates the characterization of giant macromolecular complexes providing their size and stoichiometry in solution. When studying such high molecular-weight protein complexes ($R_g > 8$ nm), the R_g becomes accessible, in contrast to normal-size proteins. We describe here the biggest macromolecular entity, to our knowledge, characterized to date using this approach.

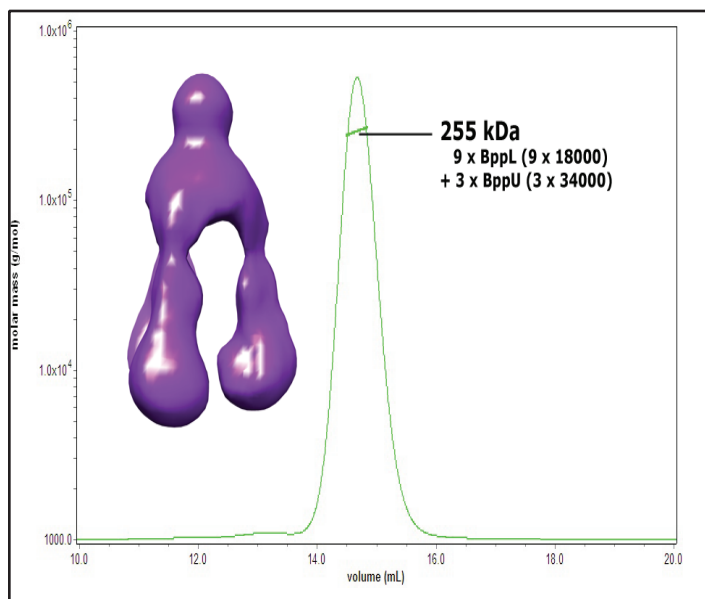


Figure 1. SEC/MALS/RI analysis of the BppU-BppL protein complex using a Superose 6 10/30 column. The resulting complex is formed by nine BppL (three RBPs) and three BppU copies. A picture of this complex obtained by negative staining electron microscopy is shown.

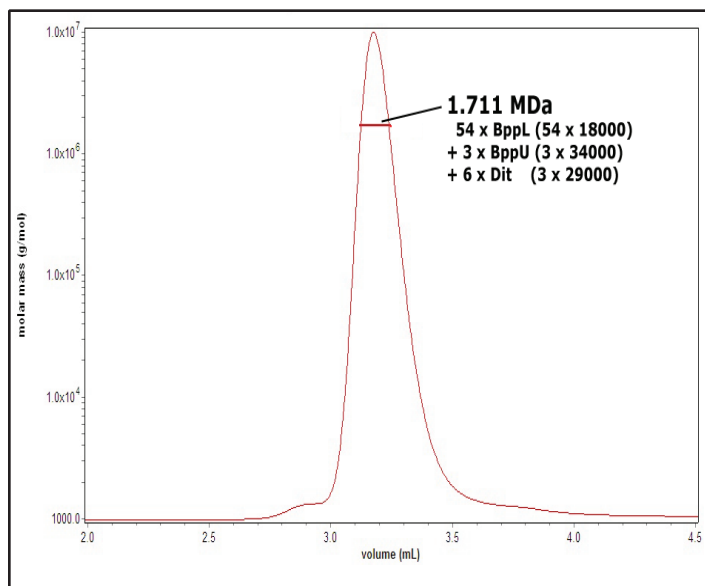
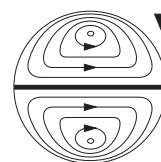


Figure 2. SEC/MALS/RI analysis of the BppU-BppL-Dit protein complex using a 15 mL Shodex KW804 column. The resulting complex is formed by 54 BppL, 18 BppU and 6 Dit copies.

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