Oligomerization and Stability of an AAA-ATPase from Acidinus Two-Tailed Virus (ATV)

ATV is a virus infecting archaea of the hyperthermophilic genus *Acidianus*, which has the unique property of undergoing a major morphological development outside and *independently* of the host cell. Virions develop long tails at each pointed end, at temperatures close to that of the host natural habitat, 85 °C.

ORF618 (69.3 kDa) has AAA-ATPase activity (ATPase Associated with various cellular Activities). Apart from the AAA-ATPase domain, ATV-ORF618 carries a C-terminal coiled-coil domain. In order to study the oligomeric state of the protein, samples were analysed by the combination of UV spectrophotometry, multi-angle light scattering (MALS), and refractometry, all coupled on-line to analytical size exclusion chromatography (SEC). UV, MALS, and refractometry measurements were made with a Photo Diode Array (Waters 2996), a miniDAWN TREOS, and an Optilab rEX (Wyatt Technology), respectively.

Because of the ATPase activity, we performed these experiments in the absence and presence of ATP, to test whether the nucleotide can stabilise the oligomeric state of the protein, as is often found for other ATPases of the AAA family. Different protein concentrations were also tested to check whether concentration is a factor affecting the protein oligomerization state. We used a KW-804 column (Shodex) run at 0.5 ml min^{-1} on an Alliance HPLC 2695 system (Waters).

Our results confirm that ATP indeed enhances the hexamer formation. However, other higher-order oligomers are present in solution. We presume that they are a consequence of possibly non-specific interactions established by the C-terminal coiled-coil domain.

Sequence analysis classifies ORF618 in the MoxR subfamily of AAA ATPases [1]. As such, it is expected to interact with other proteins from the virus or the host. MALS/SEC/QELS/RI will be a powerful tool in studying the oligomerization and the stoichiometry of these complexes, as well as the effect upon them of the presence of ATP.

This note graciously submitted by Catarina Rodrigues, AFMB, Marseille, FRANCE.

[1] U. Scheele, S. Erdmann, E. J. Ungewickell, C. Felisberto-Rodrigues, M. Ortiz-Lombardía, and R. A. Garrett. Chaperone role for proteins p618 and p892 in the extracellular tail development of acidianus two-tailed virus. (2011) *J Virol*, **85**:4812–4821.

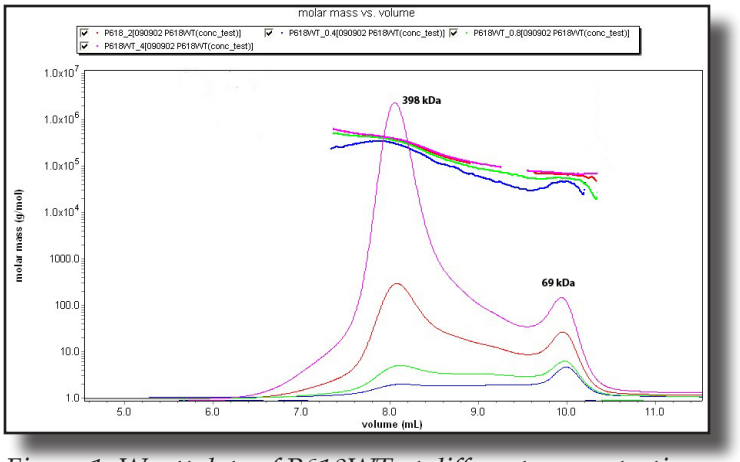

Figure 1. Wyatt data of P618WT at different concentrations (0.4, 0.8, 2 and 4 mg/ml).

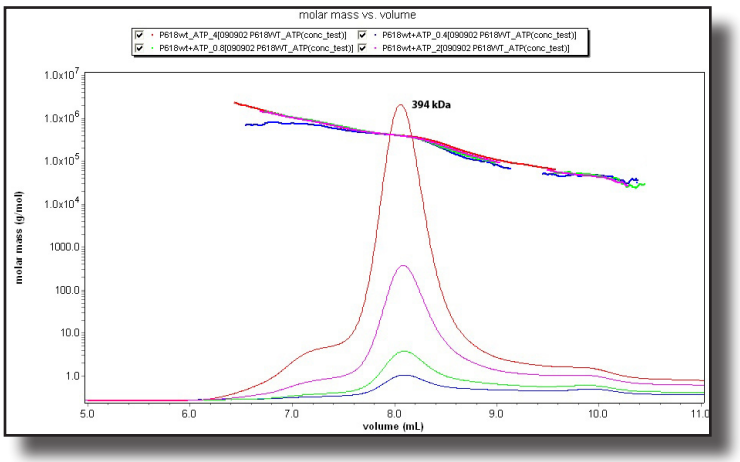

Figure 2. Wyatt data of P618WT + 0.2 mM ATP + 2mM $MgCl_2$ at different concentrations (0.4, 0.8, 2 and 4 mg/ml).

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