

Ribosomal Components Involved in Protein Biosynthesis

The L7/L12 stalk of the large ribosomal subunit plays crucial roles in protein biosynthesis, contacting most of the translation factors and stimulating their GTP hydrolysis. In *Escherichia coli*, this stalk encompasses protein L10 and four copies of protein L12 (Subramanian, 1975). To elucidate its mechanism of action on the ribosome, we have determined the crystal structure of the protein L10 in complex with L12 N-terminal domain (NTD) from the hyperthermophilic bacterium *Thermotoga maritima*.

The crystal structure revealed an unexpected stoichiometry of the stalk proteins, where protein L10 contacts 6 copies of the L12 NTD. In order to confirm the L12 copy number in *T. maritima*, we have used multi-angle laser light scattering approach for molecular mass determination in different bacteria.

We purified full-length L10-L12 complexes in *T. maritima* and *E. coli*. 200 µl from each sample (2 mg/ml) were analyzed by flow field-flow fractionation using an Eclipse particle sizing system, operated in PBS, pH 7.4, at room temperature. The complexes eluted as single peaks, which were analyzed by MALS on an 18-angle DAWN light-scattering detector equipped with a linearly polarized 30 mW gallium-arsenide 690 nm laser light source and an Optilab refractometer. Data were processed using the ASTRA software.

MALS measurements indicated a molecular mass of 101 ± 2 kD for the L10-L12 complex in *T. maritima*, in excellent agreement with the predicted mass of 102.6 kD for a L10-(L12)₆ (Fig 1 and 2). A recombinant full-length complex from the *E. coli* showed a mass of 68 ± 2 kD as compared to 66.8 kD predicted for a L10-(L12)₄ composition.

The *T. maritima* L7/L12 stalk contains six copies of protein L12 linked to protein L10, which may augment the activities of L12 in certain environments or even carry out additional functions in translation.

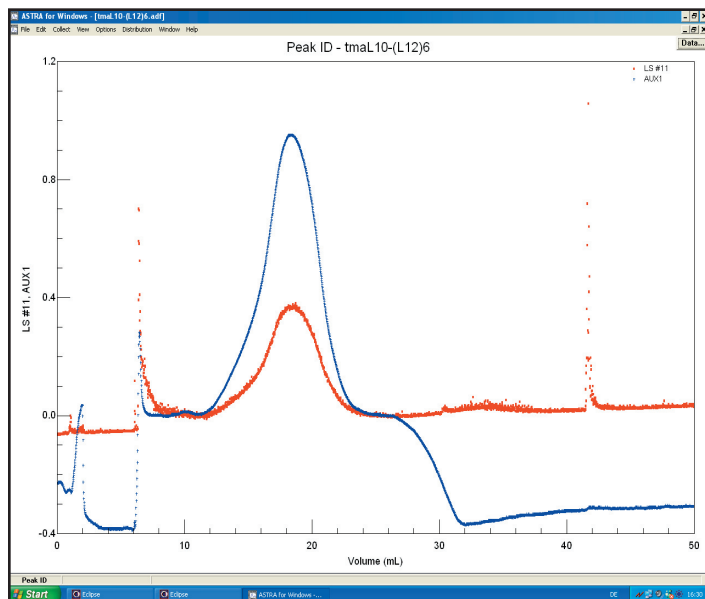


Figure 1. Molecular mass determination of the L10-(L12)₆ complex.

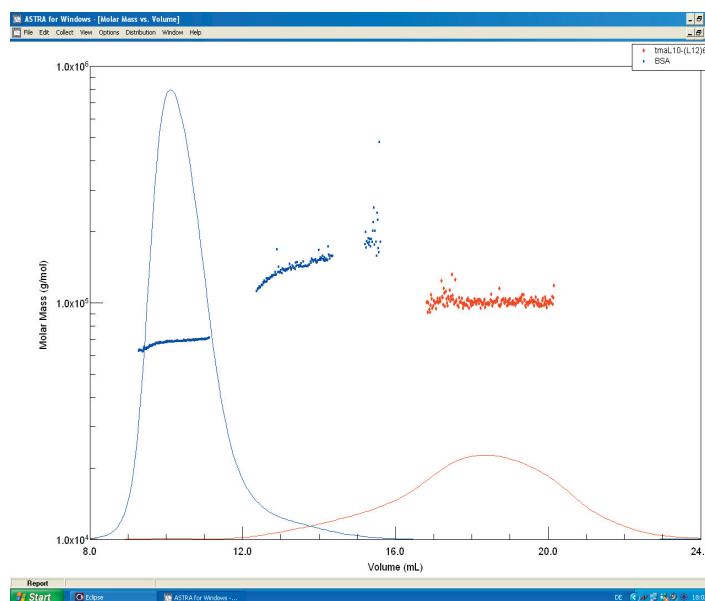
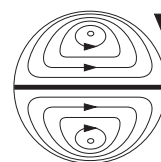


Figure 2. Molecular mass (g/mol) distribution versus volume (ml) of the L10-(L12)₆ complex and BSA, respectively.

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