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APPLICATION NOTE

AN1619: Binding Stoichiometry of an Antibody Fragment

Introduction

Antibody fragments, especially Fab-fragments, have emerged as a tool to facilitate the crystal growth and improve crystal quality of membrane proteins. We have selected a recombinant antibody Fab-fragment from a synthetic library against a detergent-solubilized membrane protein using phage display.

Materials and Methods

To determine the binding stoichiometry and the oligomeric state of the selected FAB fragment in complex with its target, we have used the powerful method of multi-angle light scattering in combination with size exclusion chromatography (SEC-MALS). This information is *instrumental* in further biophysical studies and co-crystallization experiments. Additional information such as weight fraction of protein and detergent in the protein-detergent micelle are obtained *in the same experiment* when using both UV and RI-detectors.

Results and Discussion

In Figure 1, the peak at 16.1 ml is the membrane protein-detergent complex. The protein fraction (red) is around 47%. The molar mass of the protein fraction is 100 kD to 110 kD, which corresponds to the dimer.

In Figure 2, the Fab fragment in complex with the membrane protein elutes at 15.6 ml and the peak is shifted by about 0.5 ml compared to the membrane protein alone.

The molar mass of the protein fraction lies between 150 kD and 165 kD (dark red). This shows that only one Fab fragment (50 kD) is bound to the homodimer of the membrane protein. At around 18.2 ml the detergent micelles and the excess of unbound Fab fragments co-elute.

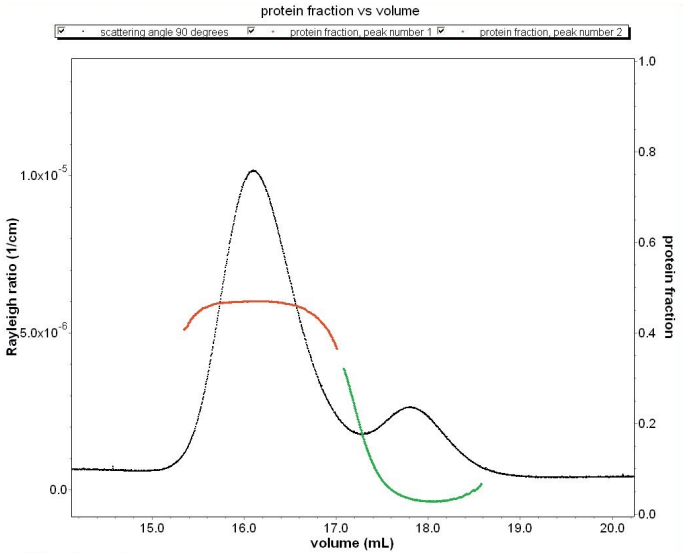

Figure 1: Plot of protein fraction vs. elution volume. The Rayleigh-ratio is indicated in black.

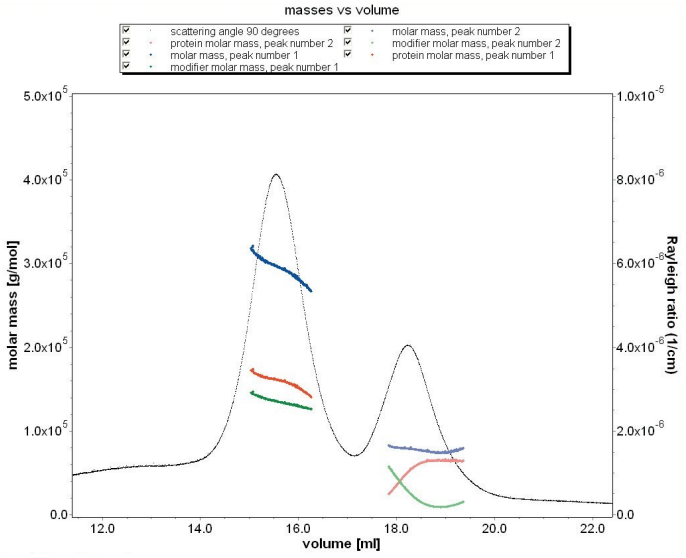

Figure 2: Plot of molar masses of the core protein (red), the detergent fraction (green) and the total protein-detergent complex (blue) vs. elution volume. The Rayleigh-ratio is indicated in black.

Conclusions

SEC-MALS shows clearly that the membrane protein *alone* is dimeric *in solution* and that one Fab-fragment binds to the dimer of the membrane protein. Furthermore, it can be nicely shown that the protein fraction of the membrane protein-detergent complex is about 47%, and about 55% for the complex of Fab fragment and membrane protein.

Acknowledgement

This note was graciously submitted by Thomas Huber, University of Zurich, Department of Biochemistry, Laboratory of Prof. A. Plückthun, Winterthurer. (2005).

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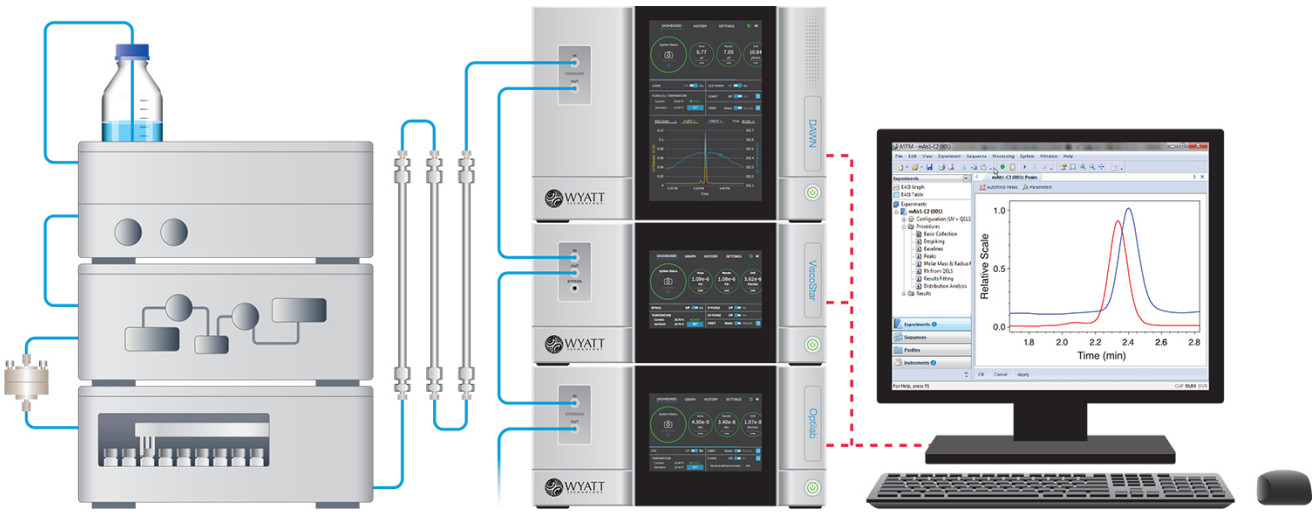

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