Protein Solutions™

Membrane Protein Quaternary Structure Analysis and Detergent Selection

Membrane proteins are key components of living cells. They constitute one third of the ORFs of virtually all genomes, perform crucial roles and are targeted by a huge number of drugs. When present in their native environment, transmembrane proteins are inserted via hydrophobic segments in a lipidic bilayer. Their *in vitro* characterization requires us to extract proteins from the membrane and to maintain them in a soluble and native state. This is generally achieved using amphiphilic compounds, termed detergents, yielding so-called protein-detergent complexes (pdc). Several membrane proteins are naturally found as oligomers and maintaining this precise macromolecular assembly is a crucial issue for further studies.

We describe here a method to determine the quaternary structure of membrane proteins as well as to follow their retention during protein handling. It is well known that classical SEC column calibration does not apply in the case of pdc, whose volume and shape also depend on the detergent fraction.

An illustration of this method is given by the quaternary structure study of the *Methanosarcina mazei* CorA transporter in two detergents. Crystallographic studies of a homologous CorA revealed that the functional protein works as a pentamer to conduct ions across the membrane. The SEC elution profile of this protein purified in LDAO ($CorA_{LDAO}$, Fig 1) was sharper and more symmetrical than the one obtained in DDM ($CorA_{DDM}$, Fig 2).

Combining measurements from MALS, refractometry and UV_{280nm} absorbance, we were able to solve a "two equations with two unknown parameters" system providing masses of protein and detergent in each pdc. Surprisingly, despite its nicer elution aspect, $CorA_{LDAO}$ was in a non-native monomeric state whereas $CorA_{DDM}$ retained its pentameric physiological quaternary structure.

This elegant approach provides hints about the retention of the native, and thus *active*, membrane protein quaternary structure that is a crucial issue for crystallization or other biochemical studies, *without* performing laborious activity tests.

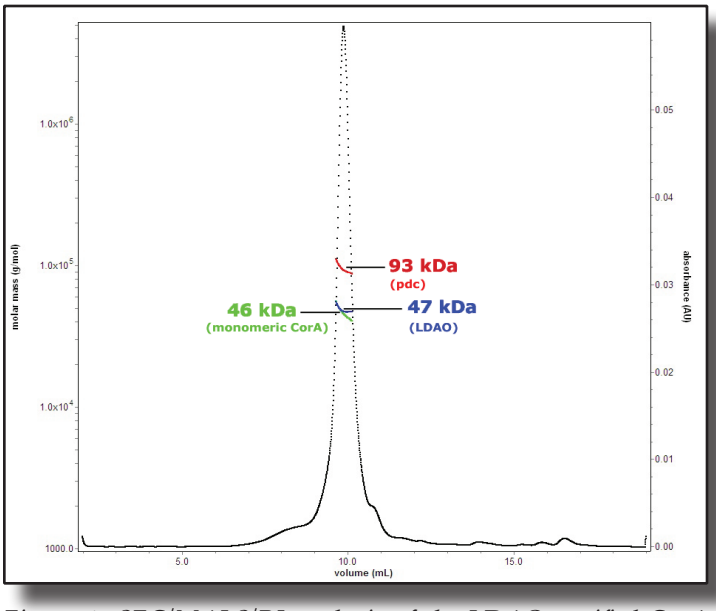

Figure 1. SEC/MALS/RI analysis of the LDAO-purified-CorA using a 15 mL Shodex KW804 column. Only the monomeric form of CorA was obtained in this detergent.

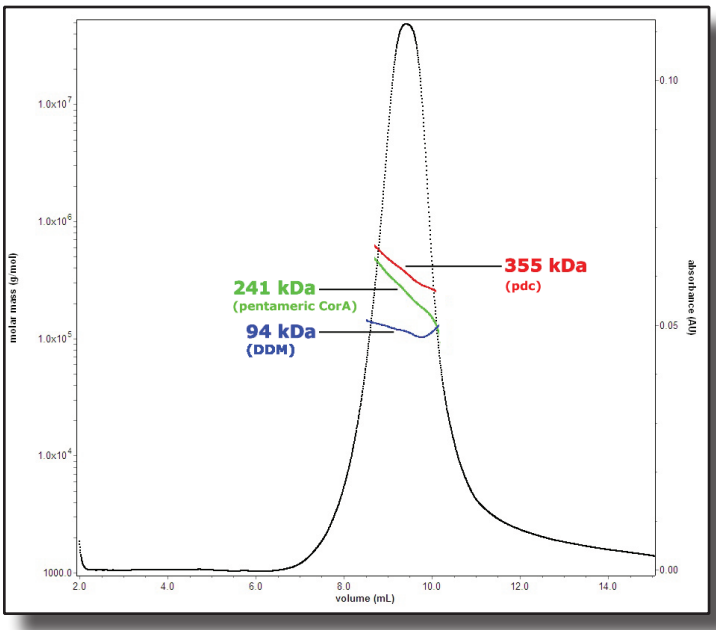

Figure 2. SEC/MALS/RI analysis of the DDM-purified-CorA using a 15 mL Shodex KW804 column. In this case, the pentameric form of CorA was observed.

This note graciously submitted by David Veesler, Architecture et Fonction des Macromolécules Biologiques, Unité Mixte de Recherches UMR6098, CNRS,Université de Provence Université de la Méditerranée, Marseille France.

Wyatt Technology CORPORATION
6300 Hollister Avenue • Santa Barbara, CA 93117
TEL (805) 681-9009 • FAX (805) 681-0123
E-mail: info@wyatt.com • URL http://www.wyatt.com