Purifying two SNARE proteins: Nyv1 and Vam3

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Introduction

The fusion of biological membranes in eukaryotes requires a family of proteins known as SNAREs, which are responsible for docking of cargo-containing vesicles, tethering the membranes, and providing the force necessary for fusion. The structure and function of these proteins is highly conserved across species.

Each SNARE protein has at least one SNARE motif, which is the region of the protein which binds to the other SNARE proteins at their motifs. During fusion, the SNARE protein bound to the cargo-containing vesicle membrane associates through its SNARE motif with the two SNARE proteins on the surface of the target membrane. This yields a coiled-coil structure consisting of three proteins and four motifs.

Binding and specificity are facilitated by four "zero layer" residues—an arginine on one motif, and a glutamine on each of the others—which form strong electrostatic interactions at the core of the SNARE bundle. This is in contrast to the other residues at the inside of the bundle, which are predominantly hydrophobic. However, spontaneous assembly of the SNARE complex under physiologic conditions is inefficient. This conundrum has prompted a search for other proteins that may be involved in SNARE complex assembly.

A strong candidate has been identified in the Sec1/Munc18-like (SM) protein family. It is hypothesized that SM proteins associate with the R- and Qa-SNARE motifs, forming a template for subsequent binding of the Qb- and Qc-SNAREs which complete the bundle.

While the long-term goal of my research is to investigate the binding relationships of these proteins, they must first be purified for any binding assays to be performed. This summer I have therefore been purifying the variants of these three proteins found in yeast vacuole fusion complexes—Vps33 (SM), Nyv1 (R), and Vam3 (Qa).

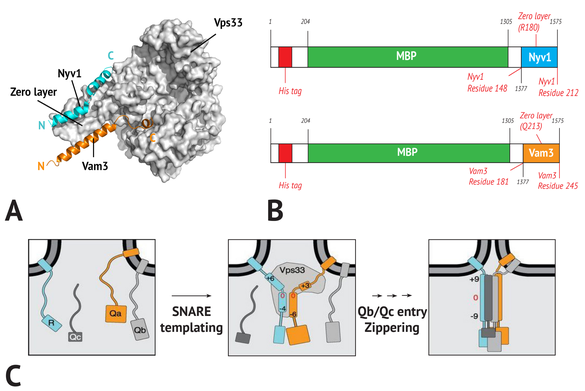
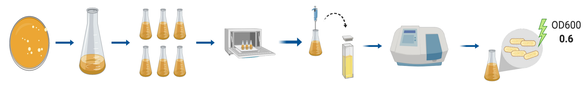


Figure 1: a. *Vps33 (gray) bound to the SNARE motifs of Nyv1 (blue) and Vam3 (orange), adapted from Baker et. al. (2015);* ***b.*** *Schematics of the Nyv1-MBP (top) and Vam3-MBP (bottom) fusion proteins which were purified;* ***c.*** *Steps of SNARE bundle assembly, including intermediate template step facilitated by Vps33 (Sm protein), adapted from Baker et. al. (2015)*

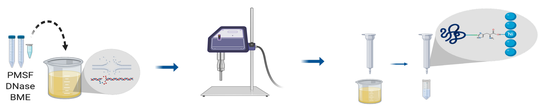
Materials and Methods

Transformation and Expression



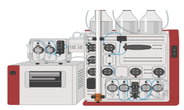
BL21 CodonPlus *E. coli* competent cells were transformed with Nyv1 or Vam3 DNA and expression was induced with IPTG after growth had reached OD600 0.6.

Purification by Chelating Chromatography



Cells were treated with BME, DNase, and PMSF. They were homogenized and the lysate was run through a nickel sulfate column (proteins were His-tagged). Elution was carried out using imidazole.

Purification by MonoQ (anion exchange) chromatography, then size-exclusion (gel filtration) chromatography



Purification was carried out using column chromatography on an AKTA Pure unit, first on a MonoQ (anion-exchange) and then on a gel (size-exclusion) column. Elution on MonoQ was carried out using increasing buffer gradient (50 mM to 500 mM).

Discussion

The purity of protein obtained in both cases was high, as indicated in the rightmost SEC lanes of the SDS-PAGE gels shown in **Figures 2 and 5**. The concentrations of each were also relatively high, although Nyv1-MBP had a significantly higher yield than Vam3.

Each of the proteins was successfully eluted from the size-exclusion column in the final step, as shown by the curves in **Figures 4 and 7**. Vam3 eluted off the pH 8.0 MonoQ column (**Figure 6**), as its isoelectric point (pI) is 5.21. Conversely, Nyv1 did not bind to the pH 8.0 MonoQ column (**Figure 3**), despite having a theoretical isoelectric point (pI) of 6.01; at pH 8.0 it should be negatively charged and therefore have a high affinity for the anion-exchange column. However, the Nyv1 domain of the fusion protein in its own right has a much higher pI (9.67) than the Vam3 domain of its fusion protein (4.28), which may explain this discrepancy.

The future direction of my research will be to purify the SM protein (Vps33) and perform binding assays on the three (such as isothermal titration calorimetry).

Results: Nyv1

Nyv1-MBP

Concentration: 35.9 mg/mL
Yield: 2.47 mg/L [of culture]

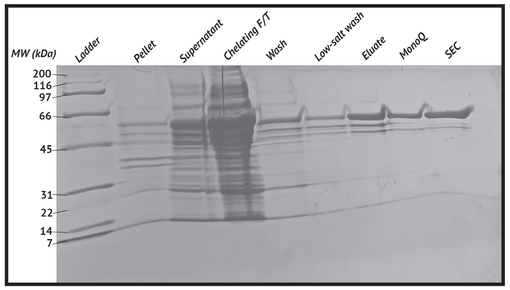


Figure 2: *SDS-PAGE gel showing fractions from each stage of Nyv1 expression/purification, beginning with the pellet and concluding with the purified SEC eluate.*

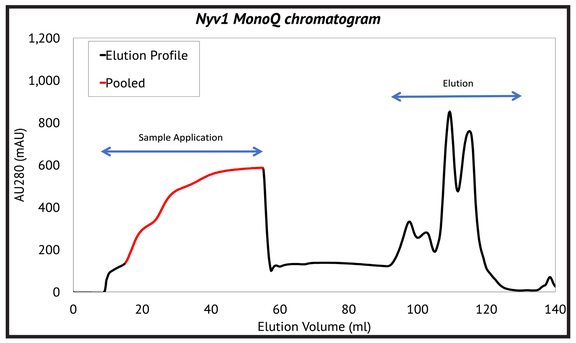


Figure 3: *MonoQ (anion-exchange) chromatogram, showing the sample application and elution. The red portion of the curve indicates the fractions which were pooled for application to the SEC column. Nyv1 did not bind to the column.*

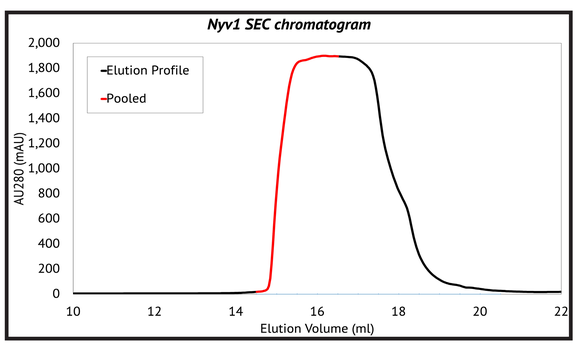


Figure 4: *Size-exclusion (gel filtration) chromatogram showing the elution of purified Nyv1-MBP.*

Results: Vam3

Vam3-MBP

Concentration: 8.04 mg/mL
Yield: 0.55 mg/L [of culture]

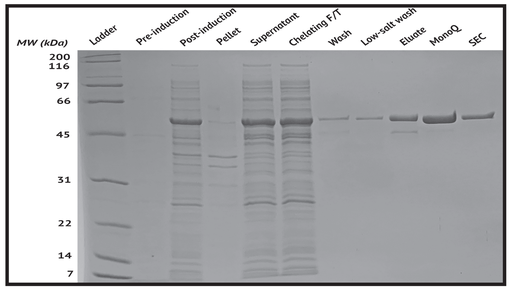


Figure 5: *SDS-PAGE gel showing fractions from each stage of Vam3 expression/purification, beginning with the pre-induction culture and concluding with the purified SEC eluate.*

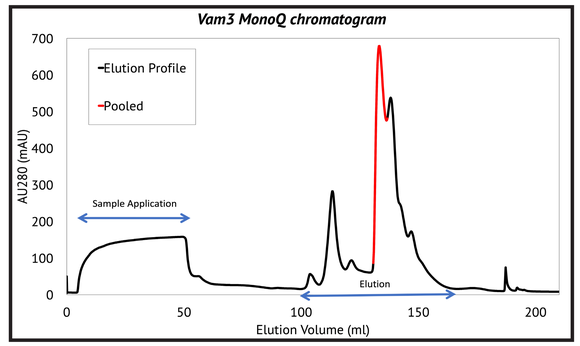


Figure 6: *MonoQ (anion-exchange) chromatogram, showing the sample application and elution. Red indicates fractions which were pooled for concentration. Vam3 bound to column and was eluted at around 130 mL.*

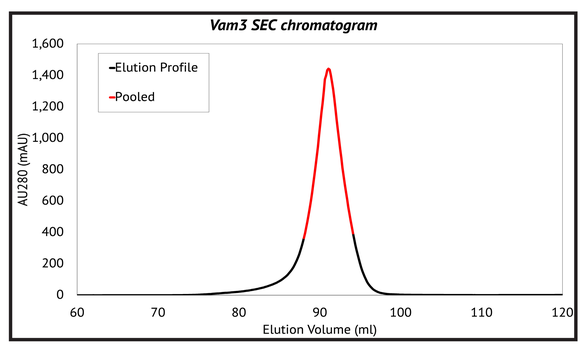


Figure 7: *Size-exclusion (gel) chromatogram, showing the elution fractions.*

Acknowledgements

Materials and Methods graphics courtesy of BioRender. Schematics in **Figure 1** adapted from Baker et. al. (2015), PubMed ID 26339030. With thanks to Travis Eisemann and Fred Hughson.