

Supplemental Data

The SNARE Complex from Yeast Is Partially

Unstructured on the Membrane

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Supplemental Experimental Procedures

Ternary Complex Formation and EPR Data Collection

Three spin labeled mutants of SN2 (D599C, D620C and H641C) were mixed with reconstituted Sso1pHT and Snc2pS in a molar ratio of 1:1:1, 1:2:2, and 1:4:4 and the mixtures were left at room temperature to form the ternary complex for 60 min. The samples were then concentrated using a 100-kDa centrifugal filter (Millipore). The EPR spectra were collected at room temperature using the Bruker ESP 300 spectrometer (Bruker, Germany) equipped with a low noise microwave amplifier (Miteq, Hauppauge, New York) and a loop-gap resonator (Medical Advances, Milwaukee, Wisconsin).

Figure S1

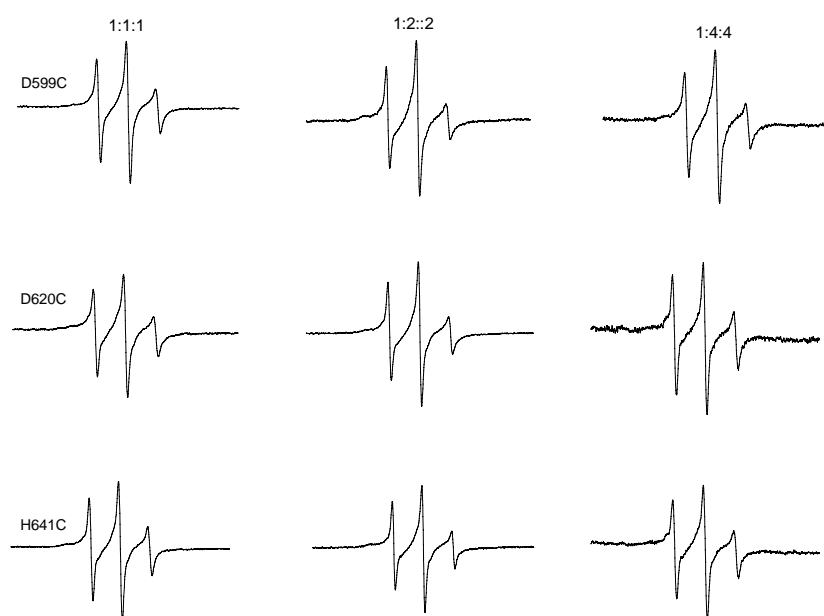


Figure S1. EPR Assay of SNARE Complex Formation

Spin labeled Sec9c mutants were mixed with one-fold (left row), two-fold (middle row) and four-fold (right row) molar excess of reconstituted Sso1pHT and Snc2pS. The fraction unstructured was constant over the molar ratios investigated in this work within experimental errors. The results supporting that the sharp component was due to the unstructured SN2 of Sec9c in the complex and it was neither due to insufficient number of the binding partner nor due to intermolecular equilibrium.