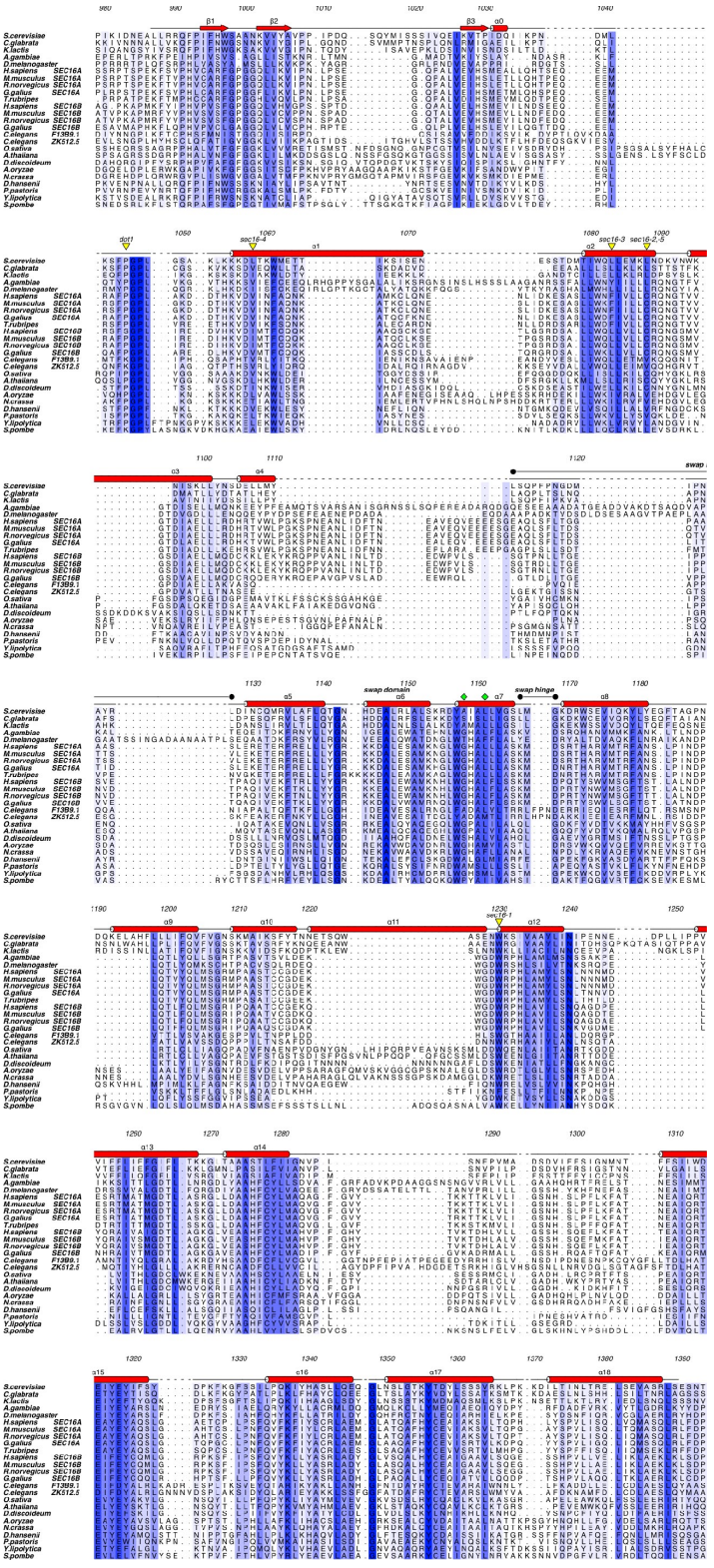

Figure S1. **Multiple sequence alignment of the CCD of Sec16 homologues.** Sequences were retrieved by PSI-BLAST, trimmed to contain only the CCD, and aligned by MAFFT. Columns are colored by sequence similarity from most (dark blue) to least (white) conserved. β-strands β1–β3 and α-helices α0–α18 are diagramed. The causative mutations for known temperature-sensitive alleles in *S. cerevisiae* (*sec16-1,-2,-3,-4/-5*) or *P. pastoris* (*dot1*) are marked with yellow triangles. Green diamonds mark alanine 1159 and leucine 1162, which were mutated to glutamic acid to generate the mutant Sec16EE. The swap domain, α-helices α5–α7, is labeled, as are the swap loop and swap hinge. Numbering is according to the sequence in *S. cerevisiae*.

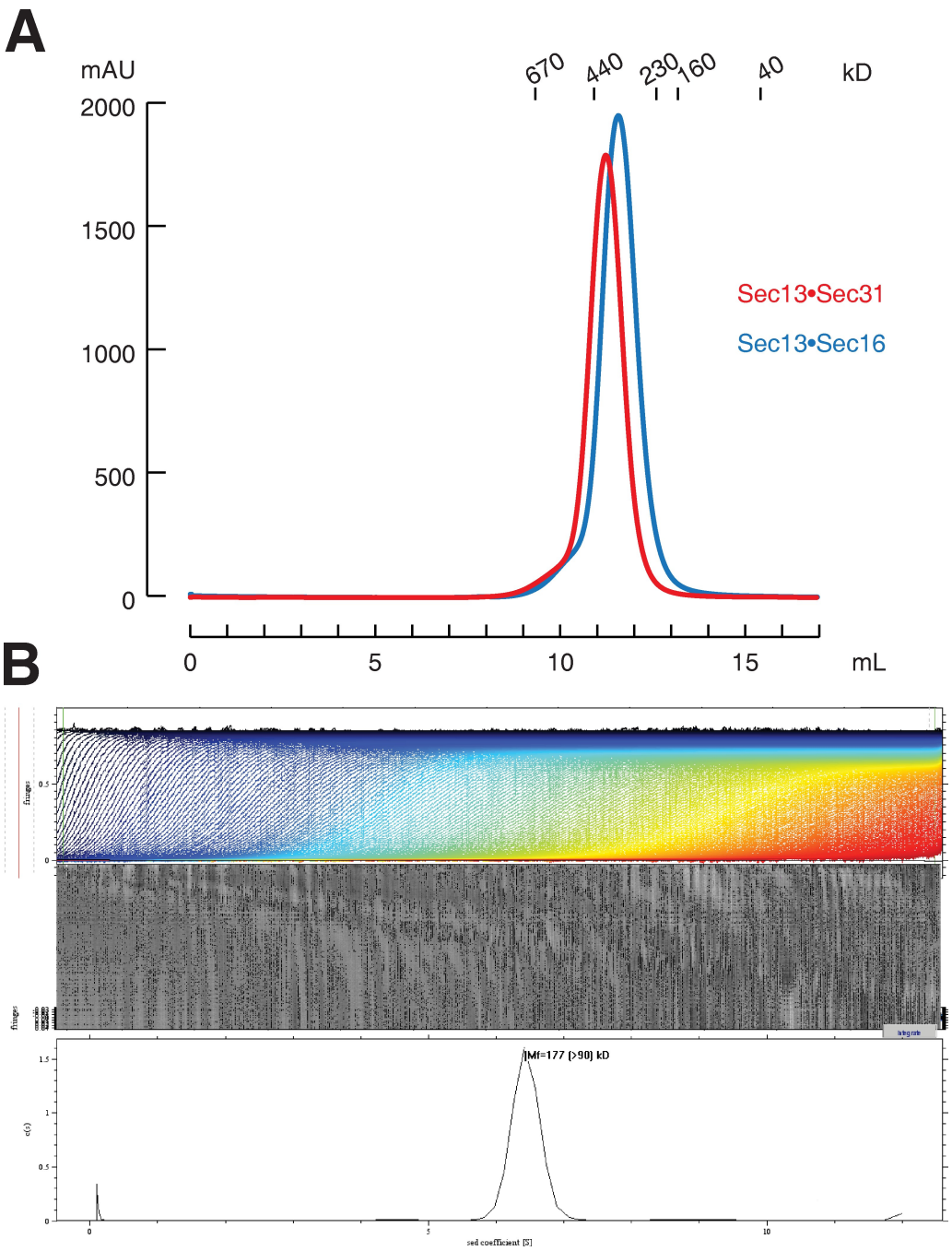


Figure S2. **Hydrodynamic characterization of Sec13–Sec16.** (A) Size exclusion chromatography of Sec13–Sec16 compared with Sec13–Sec31 on a Superdex 200 10/300 column. Absorption at λ = 280 nm is plotted against elution volume for each sample. (B) Sedimentation velocity analytical ultracentrifugation of Sec13–Sec16. Data collected in interference mode were analyzed with SEDFIT using the continuous sedimentation coefficient distribution, c(s), and an estimated molecular mass, $M_f$, was calculated. Fringe displacement versus radial distance is plotted as a function of time (blue to red). Fit residuals are shown as a grayscale bitmap. The distribution plot shows a single species with $M_f$ = 177 kD.

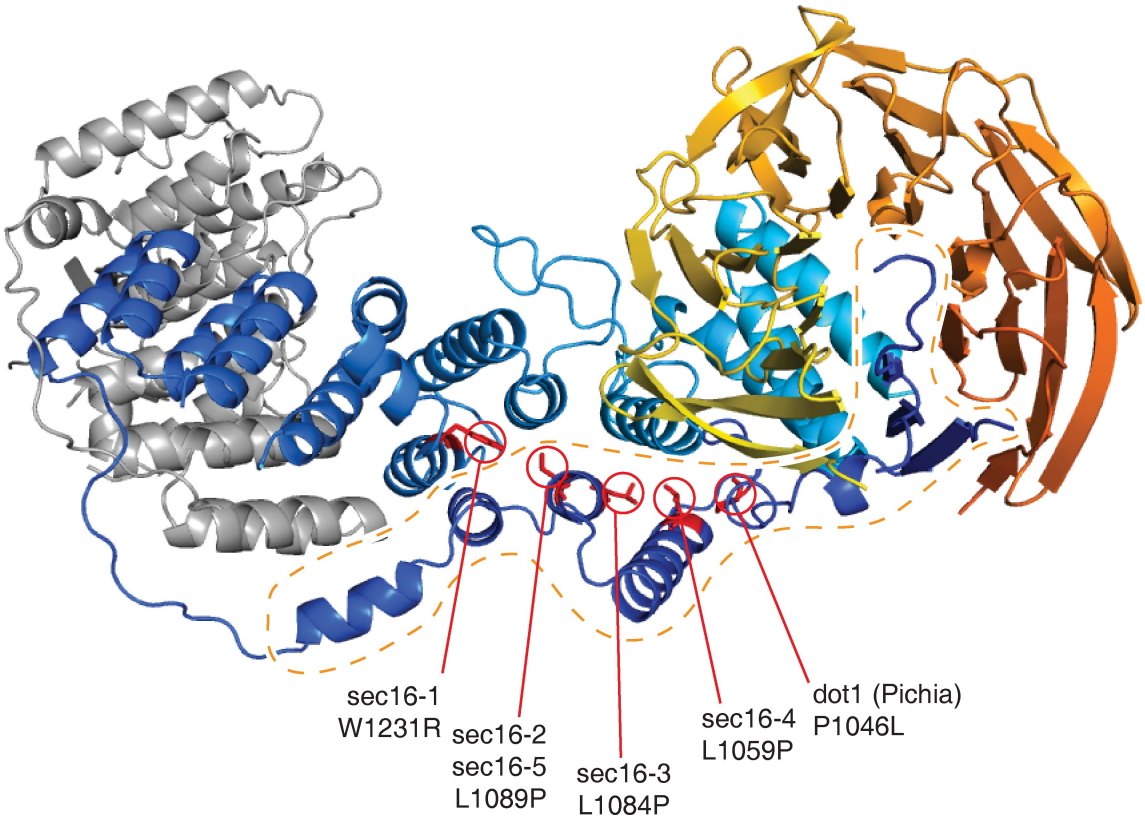


Figure S3. **Structural consequences of temperature-sensitive alleles of Sec16.** The known temperature-sensitive alleles of Sec16 in *S. cerevisiae* (Espenshade et al., 1995) and *P. pastoris* (Connerly et al., 2005) are shown mapped to the structure of Sec13–Sec16. Sec16 is colored blue to cyan and Sec13 is colored orange to yellow from the N to C terminus. Residues mutated in each temperature-sensitive allele are shown in red and labeled. The dashed line encircles the N-terminal portion of the Sec16 central domain.

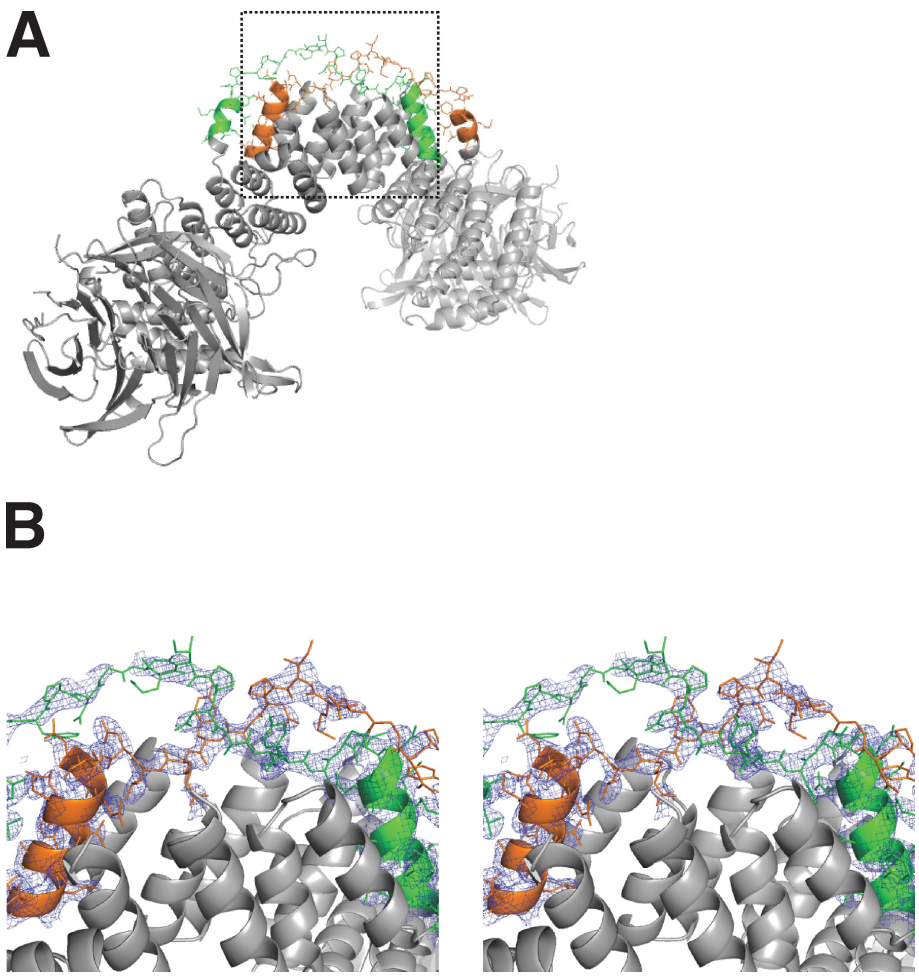


Figure S4. **Simulated annealing omit map of Sec16 swap loop.** (A) Crystal structure of Sec13–Sec16. Helix α4, the swap loop, and helix α5 are colored green. The corresponding portion of the other Sec16 molecule is orange. The region enlarged in B is boxed. (B) Stereogram of the simulated annealing omit map of the Sec16 swap loop. The $2F_O$-$F_C$ simulated annealing omit map is contoured at 1 σ. Although the loops are adjacent in space, they touch and then continue over the opposite swap loop rather than interlock (by passing underneath the opposite swap loop).

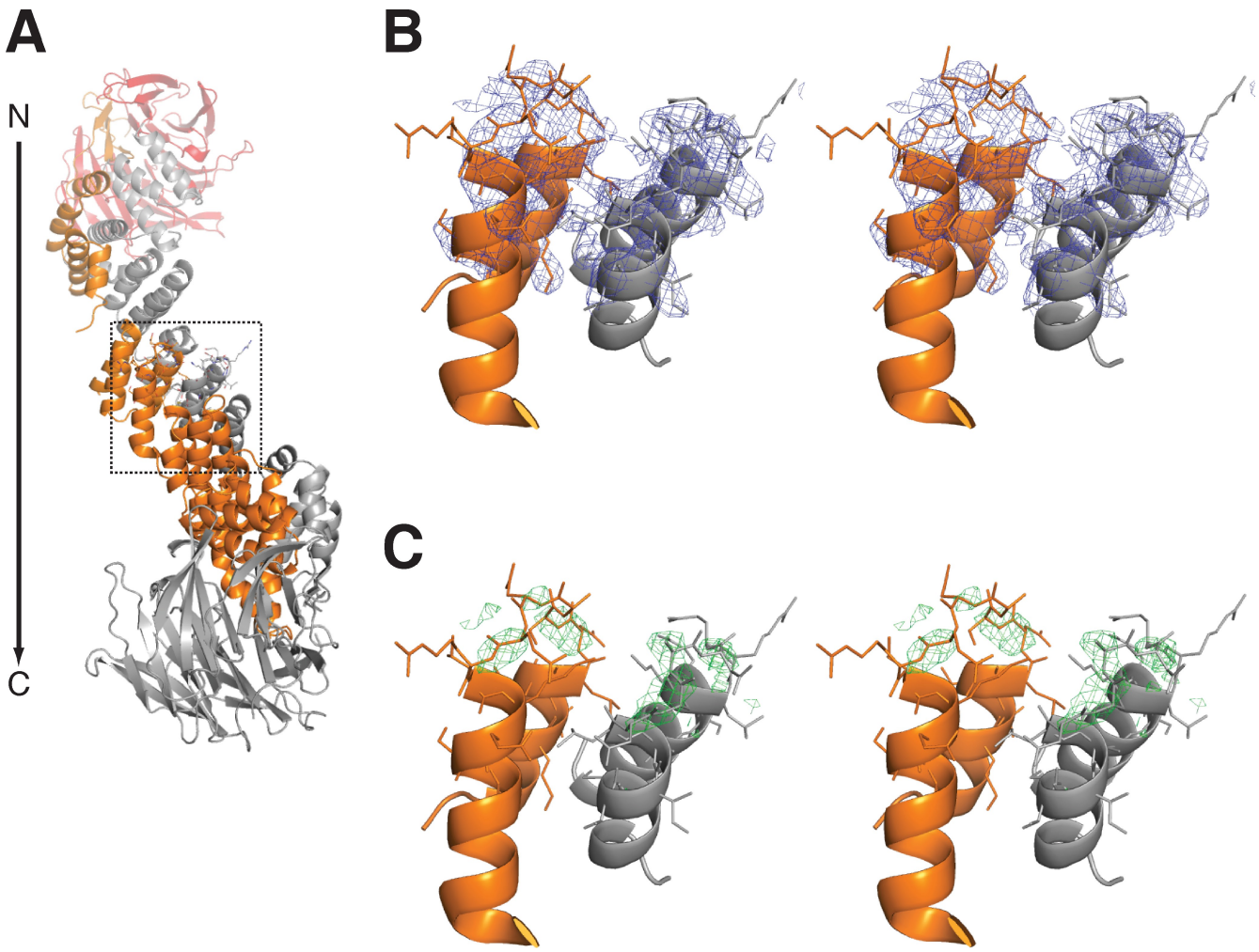


Figure S5. **Electron density at Sec31ΔL swap hinge.** (A) Crystal structure of the Sec13–Sec31ΔL. Sec13 is colored red or gray. Sec31ΔL is colored orange or gray. The orange Sec31ΔL runs top to bottom from the N to C terminus, as labeled. The region enlarged in B and C is boxed. (B) Stereogram of swap hinges, connecting helices α7–α8 (orange) or connecting helices α7′–α8′ (gray) of Sec31ΔL. An electron density map ($2F_O$-$F_C$, contoured at 1 σ) calculated before modeling the hinge is shown. (C) Stereogram as in B. Difference density ($F_O$-$F_C$, contoured at 3 σ) is shown. The observed connectivity proves lamination of the Sec13–Sec31ΔL tetramer. If Sec31ΔL were not laminated, the swap hinge would connect helix α7 to α8′ and helix α7′ to α8.

## References

Connerly, P.L., M. Esaki, E.A. Montegna, D.E. Strongin, S. Levi, J. Soderholm, and B.S. Glick. 2005. Sec16 is a determinant of transitional ER organization. *Curr. Biol.* 15:1439–1447. doi:10.1016/j.cub.2005.06.065

Espenshade, P., R.E. Gimeno, E. Holzmacher, P. Teung, and C.A. Kaiser. 1995. Yeast SEC16 gene encodes a multidomain vesicle coat protein that interacts with Sec23p. *J. Cell Biol.* 131:311–324. doi:10.1083/jcb.131.2.311