complexes assemble at or near membranes. We develop coarse-grained models and effective energy functions for simulating such large multi-protein complexes with low to intermediate binding affinities ($K_d > 1$ μM). The models are validated against structure and binding-affinity data for a broad range of binary protein complexes. Using replica exchange Monte Carlo simulation techniques, we apply our model to study the assembly, energetics, and dynamics of the complex between Vps27/Hse1 and membrane-tethered ubiquitin. The yeast Vps27/Hse1 complex and the homologous mammalian Hrs/STAM complex deliver ubiquitinated transmembrane proteins to the ESCRT endosomal protein-sorting pathway that is important in many biological processes. Vps27 and Hse1 contain several folded domains and flexible linkers. We find that the membrane-tethered ubiquitin binds preferentially to the UIM domains of Vps27. However, the simulations also show that ubiquitin interacts with other domains. The observed multiple specific and non-specific ubiquitin-Vps27 interactions greatly enhance the overall binding affinity. In the complex, the structure of Vps27/Hse1 is highly dynamic and flexible, reflecting the ability of Vps27/Hse1 to bind to a diverse set of ubiquitinated protein targets. The models developed here can easily incorporate additional experimental information (e.g., from fluorescence, scattering, electron microscopy), and hold promise for simulations of other large multi-protein complexes.

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Modulation of Membrane Mechanical Properties by Sar1, a Vesicle Trafficking Protein

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The trafficking of cargo in cells involves dramatic changes in membrane shape and topology. Though trafficking is widely studied and the identities and biochemical interactions of the responsible proteins are well mapped, remarkably little is known about the mechanics involved. We focus on Sar1, the key regulator of the coat protein complex II (COPII) family that ferries newly synthesized proteins from the ER to the Golgi. Sar1 is the only member of the COPII coat that interacts directly with the ER lipid bilayer membrane. It has an amphipathic N-terminal helix; when Sar1 is GTP-bound, the helix is exposed and the hydrophobic hemi-cylinder can insert into the bilayer. To investigate whether Sar1 has a physical role beyond merely localizing the other COPII proteins, we directly measure the force involved in membrane deformation as a function of its presence or absence, using optical traps and membrane-bound microspheres to pull tethers from lipid membranes. The lipid composition and large available surface area mimic the composition and geometry of the ER. Measurements of tether forces and radii allow extraction of the membrane bending modulus, the material parameter that dictates the energy required for deformation. We find that the bending modulus measured in the presence of Sar1 with a non-hydrolyzable GTP analogue, at concentrations sufficient for dense membrane coverage, is half that measured without Sar1 or with Sar1-GDP. These results reveal a paradigm-altering insight into COPII trafficking: Sar1 actively alters the material properties of the membranes it binds to, lowering the energetic cost of curvature generation.

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Blocking helix formation without blocking organellar localization in Plasmodium falciparum

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Transit peptide (TP) recognition in mitochondria and chloroplast localization is well described and requires a receptor to recognize the TP bound as an amphipathic alpha helix. This functional interaction leads to organellar import of the payload protein. *Plasmodium falciparum* (*Pf*), the causative agent of malaria, contains an organelle called the apicoplast. The apicoplast is evolutionarily related to the chloroplast, is essential to the metabolism of *Pf*, and contains numerous putative drug targets. As in chloroplasts, nuclear-encoded apicoplast proteins must be post-translationally targeted to the apicoplast. In contrast to chloroplast localization, molecular details of TP recognition in *Pf* are currently unknown. To assess if apicoplast TPs must form helical intermediates for proper organellar localization, we have examined the TP of *Pf* acyl carrier protein by circular dichroism (CD), nuclear magnetic resonance (NMR), and epifluorescent microscopy of mutant TP-GFP fusions. CD and NMR of acyl carrier protein with its TP in solution are consistent with the presence of a small population of helix in the TP. However, structure-disrupting proline mutations are correctly targeted to the apicoplast when observed *in vivo*. This observation contradicts the theory that apicoplast TP recognition occurs via a mechanism similar to chloroplast TPs, and instead suggests that the dominant population of disordered TP may be the active form and that *Pf* has evolved a distinct solution to the problem of organellar targeting.

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Mapping of the Signal Peptide-binding Domain of Escherichia coli SecA Using Förster Resonance Energy Transfer

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Identification of the signal peptide-binding domain within SecA ATPase is an important goal for understanding the molecular basis of SecA preprotein recognition as well as elucidating the chemo-mechanical cycle of this nanomotor during protein translocation. While recent studies have addressed this topic, the precise signal-peptide binding site on SecA remains controversial. The aim of the present study was to identify the SecA signal peptide-binding site using Förster Resonance Energy Transfer (FRET). FRET provides a more global view of the binding site and circumvents the common limitations of more genetic approaches where deletion and substitution mutagenesis can confound the correct interpretation of protein structure-function analysis. This study employs a collection of functional, monocysteine SecA mutants labeled with a donor fluorophore along with cysteine-containing, acceptor fluorophore-carrying PhoA signal peptides. Fluorescence anisotropy was utilized to determine equilibrium binding constants of 1.4 μM or 10.7 μM for the alkaline phosphatase signal peptide labeled at residue 22 or 2, respectively, for SecA, with a binding stoichiometry of one signal peptide bound per SecA protomer. Distance measurements determined for nine SecA mutants indicate that the signal peptide-binding domain encompasses a region proximal to residues 225–228, 371–375, 652–657, and 771–780 when mapped onto the recent NMR structure of SecA (Gelis, I., Bonvin, A., Keramisanou, D., Koukaki, M., Gouridis, G., Karamanou, S., Economou, A., and Kalodimos, C. (2007) Cell 131, 756–769). This places the signal peptide-binding domain within the heart of SecA, surrounded by and potentially responsive to domains important for binding nucleotide, mature portions of the preprotein, and the SecYEG channel component.

Funding provided by National Institute of Health

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Evaluating Protein Interactions & Organelle Dynamics in Saccharomyces cerevisiae: Spatial Distribution of Molecular Chaperone/Co-Chaperones Evident at a Sub-organelle Level in the Endoplasmic Reticulum

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BiP/Kar2 is a member of the Hsp70 family of chaperones that resides in the endoplasmic reticulum (ER) of *S. cerevisiae*. Biochemical and genetics experiments have demonstrated BiP's association with selective co-chaperones in multiple critical processes of the cell including translocation of protein into the ER, protein folding/maturation, and ER-associated degradation (ERAD). BiP's relative high cellular concentration combined with the low resolution of traditional immunofluorescence techniques has hindered the determination of protein localization effects. We hypothesize that the spatial heterogeneity of chaperones is regulated by co-chaperones, and this heterogeneity serves as a means of dictating cellular functions. To generate physiologically-relevant data of protein dynamics in *S. cerevisiae*, variants of green fluorescent protein (GFP) coupled with advances in confocal light microscopy techniques have allowed us to track multiple fluorescently-tagged proteins *in vivo.*

Dual expression strains composed of fusion proteins, BiP and co-chaperone Sec63, reveal that a heterogeneous spatial distribution is evident at the sub-organelle level. Secondary confirmation of our results has been performed using immunofluorescence techniques in multiple *S. cerevisiae* strains. Deconvolution of fixed cell images has allowed us to reconstruct and quantify the co-localization of BiP and Sec63 in three dimensions. We have captured the spatiotemporal effects of protein dynamics in live cells by monitoring ER membrane and luminal proteins, in addition to the nuclear pore complex; confirmed that the ER is continuous through Fluorescence Loss in Photobleaching (FLiP) experiments; and captured rapid diffusion of ER resident proteins. Integration of experimental data and computational design enables us to develop stochastic models of biological systems that accurately reflect spatiotemporal effects of molecular chaperone/co-chaperone interactions.

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Microtubule Network is Necessary to Direct and Maintain The Apical Localization of Slo1 Channels in Epithelial Cells

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The cytoskeleton plays a key role in different cellular processes such as cell motility, muscle contraction, mitosis and maintenance of cell shape. In polarized cells, microtubules are involved mainly in the apical targeting of proteins.