

MODELING THE IMPACT OF SPATIAL LOCALIZATION OF PROTEIN FUNCTION IN THE ENDOPLASMIC RETICULUM OF YEAST

M. B. Griesemer^{1*}, C. L. Young³, D. Raden³, F. J. Doyle III², A.S. Robinson³, L.R. Petzold¹

¹Department of Computer Science
²Department of Chemical Engineering
University of California, Santa Barbara
Santa Barbara, CA 93106

³Department of Chemical Engineering
University of Delaware
Newark, DE 19716

Abstract

Protein-protein interactions underlie the fundamental processes of every cell. Certain proteins called chaperones specialize in protein folding, maturation, and degradation functions within a cell. BiP is the resident chaperone in the endoplasmic reticulum (ER) of eukaryotic cells. Following adverse cellular conditions, BiP acts as a mediator in returning the cell to homeostasis. Spatial localization of BiP in the ER is also thought to impact its function, as it can interact with proteins on the ER membrane and in the ER interior (lumen). Our focus is on the process of translocation, in which BiP assists in the transport of nascent proteins through membrane channels into the lumen. A deterministic model has been created to represent the system as a set of reaction-diffusion equations in order to explore the spatial distribution (and localization) of BiP under various experimental scenarios. Further analysis has underscored the need for stochastic modeling using methods such as Gillespie's Stochastic Simulation Algorithm (SSA). Comparisons of these deterministic and stochastic simulations are presented along with an investigation of the interplay of kinetic, diffusion, and geometric parameters of system behavior represented by these models.

Keywords

Chaperones, protein interactions, endoplasmic reticulum, membrane, *Saccharomyces cerevisiae*.

Introduction

Proteins play many roles in the cell, including as catalysts of biochemical reactions; gene regulators; molecular switches in signaling; and as physical components in cellular structures (Alberts, 1994). Many of a protein's

functions are performed through interactions with other proteins in compartments in the cell called organelles. The endoplasmic reticulum (ER) is one of the most important organelles for the staging of proteins destined for the

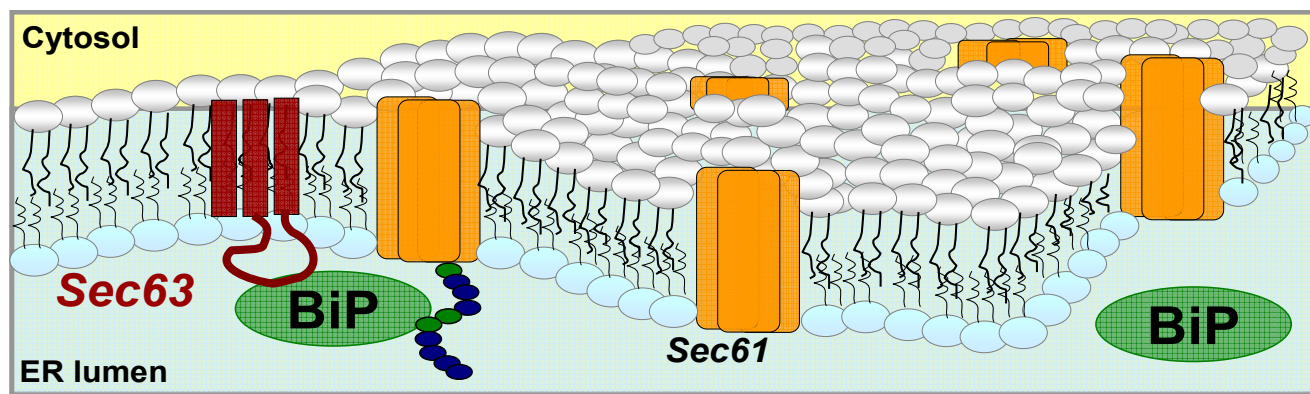


Figure 1: Key proteins (BiP, Sec63) present in the ER membrane and lumen that facilitate protein translocation at pores consisting of clusters of 3-4 Sec61s.

* To whom all correspondence should be addressed: marcgri@cs.ucsb.edu.

membrane or secretion. BiP is a specialized protein called a chaperone that is involved in protein folding, maturation, and transport in the ER of eukaryotic cells (Ghaemmaghami, 2003). BiP is one player in the cell's response to stressful conditions such as elevated temperatures, imbalance in pH, or lack of nutrients, which can damage protein folding or function (Schroder, 2005). Spatial localization of BiP in the ER is also thought to impact its function, as it can interact with other proteins present on the ER membrane and in the interior lumen (Nishikawa, 1994). Our focus is on the process of translocation in which BiP assists in the transport of unfolded proteins through membrane pore channels into the lumen. A deterministic model has been created to represent the system as a set of reaction-diffusion equations in order to explore the distribution (and localization) of BiP under various experimental scenarios. Further analysis has underscored the need for stochastic modeling using methods such as Gillespie's Stochastic Simulation Algorithm (SSA) (Gillespie, 1976). Comparisons of these deterministic and stochastic simulations are presented along with an initial investigation into the dynamics of proteins in the ER.

Our goals in this computational effort are: (1) to model protein interactions under normal and stress-induced conditions to examine the distribution of BiP in the ER, and (2) to create a simplified spatial model of the ER with a two-dimensional membrane and an interior lumen to examine the localized nature of interactions.

Background

Experimental work has determined that BiP interacts with various co-chaperones in the ER (Hennessy, 2005); there is a one-to-one correspondence between a certain co-chaperones and BiP function. One of these processes, translocation, occurs when BiP assists nascent proteins to move through channels in the ER membrane. The presence of the membrane-bound co-chaperone Sec63 enhances the translocation rate by causing a conformational change in BiP (Corsi, 1997). Translocation occurs through pores comprised of Sec61 proteins (Romisch, 1999) arranged in a cluster that also can diffuse across the membrane.

The ER is an irregularly shaped organelle surrounding the nucleus. One consequence of this shape is that it has roughly ten times the surface area compared to a sphere of the same volume (Means, 2006). This means that the large surface area impacts the reactions that occur there. In addition, other proteins present in the membrane contribute to the heterogeneous character of the ER membrane. The lipid bilayer of the membrane contains lipid rafts, an actin cytoskeleton, multiple families of proteins, and translocation pores in a dynamic mosaic (Vereb, 2003). Proteins on the ER membrane diffuse very slowly compared to proteins in the lumen. The diffusion is constrained by the presence of these molecules embedded in the membrane. A fast interaction near the membrane

surface between BiP and unfolded proteins (U) will be diffusion-limited, as the mean reaction timescale is much less than the diffusion timescale. Our experimental confocal microscopy data support the existence of spatial heterogeneity of BiP compared to the ER membrane proteins Sec63 and Sec61.

Model Descriptions

Our ordinary differential equations (ODE) model (Figure 2) of the reaction mechanism consists of seven states, described by interactions between BiP, Sec63, and unfolded proteins (U). (We have incorporated a nucleotide exchange factor (NEF) that facilitates the substitution of ADP for ATP.) Two main pathways exist that account for co-chaperone dependent and independent routes with ATP hydrolysis occurring from X_3 to X_4 and X_6 to X_7 . Simulations determined that states X_1 (BiP-ATP) and X_6 (BiP-U-ATP) have the highest concentrations by an order of magnitude. We believe that this is primarily due to the higher concentrations of constituent molecules (BiP and U) compared to Sec63, as well as to the fast rates between BiP and U ($k_7 = 4.5 \cdot 10^5 \text{ M}^{-1} \text{ s}^{-1}$). Kinetic constants in our model are currently determined from *E. coli* homologs (Hu, 2006) while our ongoing experiments will determine these parameters for the yeast homologs.

The spatial dependence in our model is represented by the ER membrane and three (non-spatial) pools including nascent protein (cytosolic), unfolded protein in the ER lumen (U_{lumen}), and degradation products (degradation) which are removed through ERAD. The membrane is modeled as a two-dimensional grid with periodic boundary conditions (Figure 3). Two diffusion rates exist: $D_{lumen} = 0.45 \text{ } \mu\text{m}^2/\text{s}$ (Nehls, 2000) which represents free diffusion in the lumen and a slower rate, $D_{membrane} = 0.04 \text{ } \mu\text{m}^2/\text{s}$ (Nikonov, 2002), at the membrane interface. Diffusion of the pores takes place at one-hundredth of the membrane protein rate. These values are estimates because actual membranes are very disordered media, and diffusion can be anomalous based on spatial location.

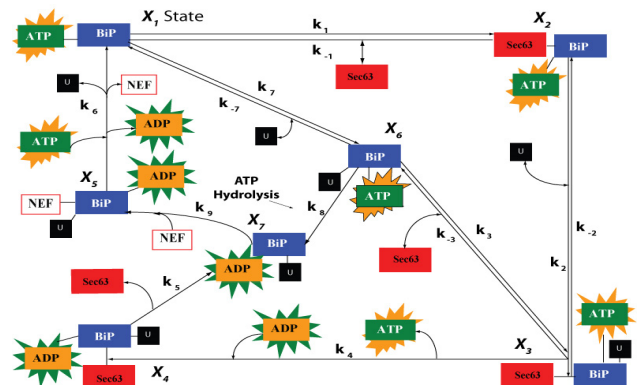


Figure 2: Schematic of the ODE model

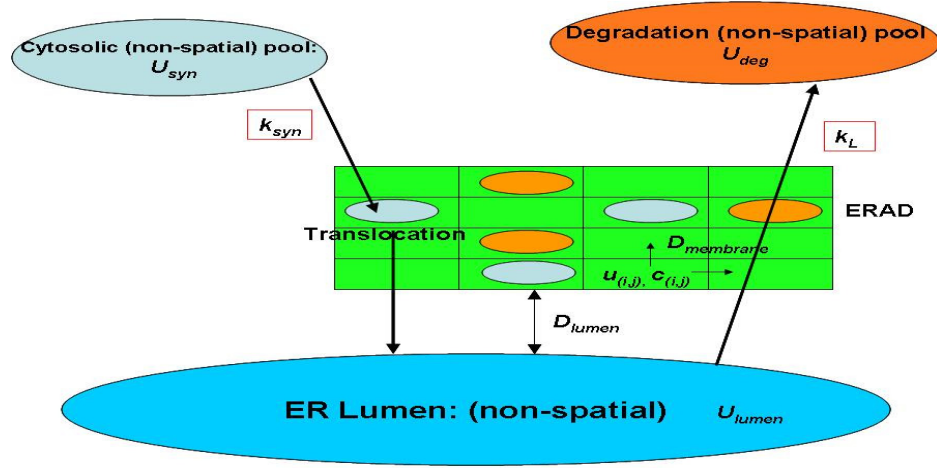


Figure 3: Spatial model of protein interactions in the ER representing the membrane and lumen.

Pores that enable protein translocation through the membrane are represented by sources and sinks. Nascent proteins entering the translocon are described by a creation reaction from the cytoplasmic pool. The process of translocation (source) is described by the reaction path $X_2 \rightarrow X_3 \rightarrow X_4 \rightarrow X_7 \rightarrow X_5$ at the pore. Proteins leaving

the lumen through ER associated degradation (ERAD) are treated as a reaction to the cytosol (sink). The degradation pool U_{deg} catalogues the amount of protein that has left the ER through ERAD. These sources and sinks are best modeled as a continuous pore density in molecules/ μm^2 . The lumen pool consists of those species that can diffuse freely. We set the translocation rate proportional to the source pore density and set the level of the cytosolic pool of nascent protein U_{syn} such that it starts at a significant level as not to be depleted during the simulation.

Comparison of Stochastic and Deterministic (Reactions-Only) Model Results

We took the ODE model of the reaction mechanism and converted it into a stochastic model using a membrane volume calculated from a spherical annular thickness of 35 nm based on measurements from our confocal data. We then ran simulations using Gillespie's SSA algorithm for ten, a hundred, and a thousand realizations with measurement of means and variances. We verified that as we increased the volume to infinity, the stochastic results approached the deterministic ones. Then we compared the stochastic model to the ODE model (corresponding to Figure 2) by subtracting the deterministic result from the stochastic ensemble. Three types of behavior are observed in the simulations. For states such as X_1 (BiP-ATP) and X_6 (BiP-U-ATP) that have high concentrations, the stochastic results are very close to the deterministic results. Other states such as X_5 (BiP-U-NEF-ATP) have noisy stochastic trajectories (Figure 4). States with small molecule counts such as X_7 (BiP-U-ADP) have very small constant concentration for the deterministic case but the stochastic model oscillates between 0 and 1 molecules (Figure 4).

Development of the spatial model and the investigation of its properties is still in its early stages. We have conducted simulations with the spatial model using basal concentrations of BiP, Sec63, and U that correspond

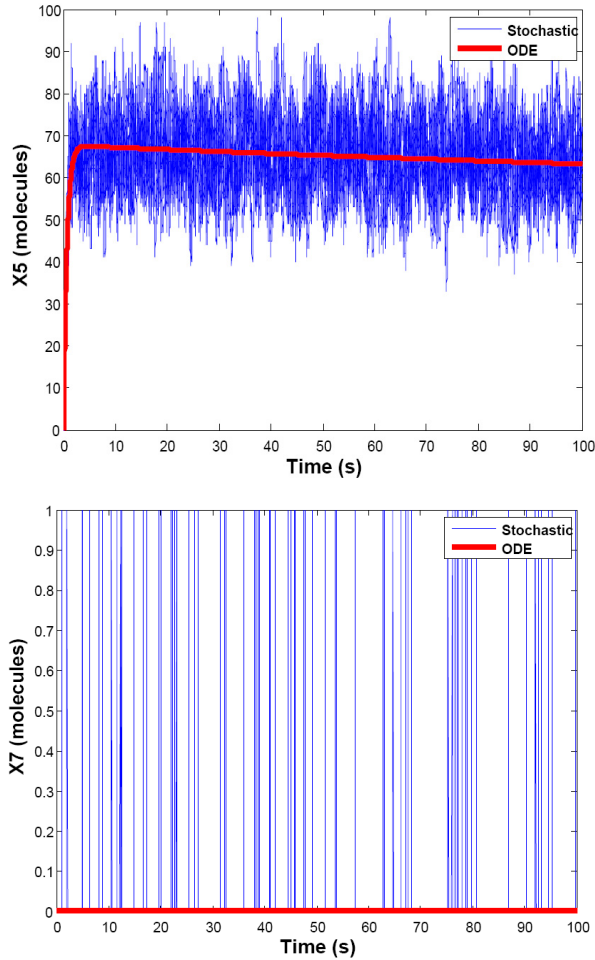


Figure 3: Comparison of the ODE model and the stochastic model for states X_5 (upper) and X_7 (lower).

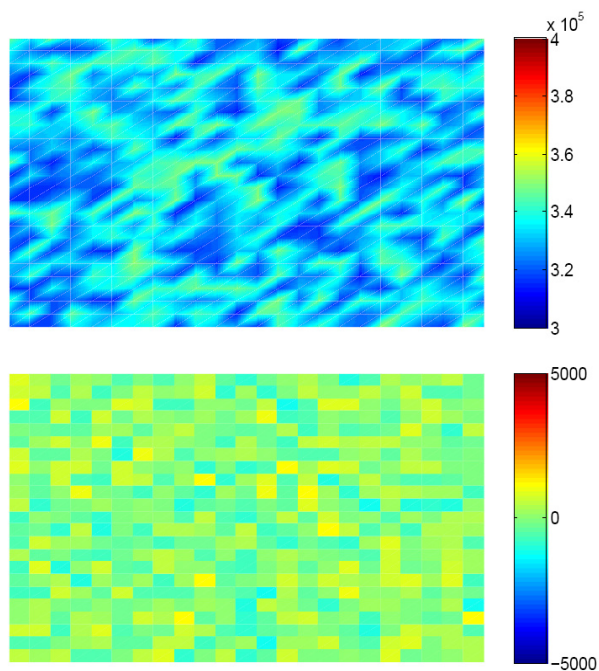


Figure 5: Snapshot of a membrane simulation of the spatial model with total BiP concentration (upper, in molecules/ μm^2) and net pore density (lower, difference of sources and sinks) on a 24 by 24 grid.

to molecule counts from the Yeast Genome Database ($3.37 \cdot 10^5$, $1.77 \cdot 10^4$, and 10^5 molecules, respectively) (Ghaemmaghani, 2003). The initial protein concentrations at each grid point were randomly set within 10 percent of these values. Furthermore, the density of pores acting as sources and sinks was varied 50 percent. The system was simulated to 100 seconds with diffusion across the membrane and into the lumen pool. After roughly $t=60$ seconds, patches of lower protein concentration occurred at locations corresponding to higher source density as translocation into the lumen took place. Further studies will examine how pattern formation is influenced by variation in reaction and diffusion rates as well as different geometries.

Summary

We have constructed a spatial ER model with a membrane grid describing the chaperone activity of *Saccharomyces cerevisiae* through reaction-diffusion equations. We compared the ODE model of the reaction mechanism with its discrete stochastic counterpart and found that while the results were very close for states with high concentrations, there was a divergence for low concentrations, highlighting the importance of using the stochastic simulation. Initial simulations of the spatial model demonstrate some heterogeneity of protein concentration on the ER membrane. Further studies need to be examined, including how changes in kinetic,

diffusion, and geometric parameters influence the evolution of the spatial system.

References

- Alberts, B., Bray, D., Lewis, J., Raff, M., Lewis, K., Watson, J.D. (1994). *Molecular Biology of the Cell*, Garland Publishing, New York.
- Burrage, K., Hancock, J., Leier, A., Nicolau, D.V. (2007). Modelling and Simulation Techniques in Membrane Biology. *Briefings in Bioinformatics* 8(4):234-244.
- Corsi, A. K., Schekman, R. (1997). The Luminal Domain of Sec63p Stimulates the ATPase activity of BiP and Mediates BiP Recruitment to the Translocon in *Saccharomyces cerevisiae*. *J. Cell Biol.* 137:1483-93.
- Ghaemmaghani, S., Huh, W., Bower, K., Howson, R., Belle, A., Dephoure, N., O'Shea, E., Weissman, J. (2003). Global Analysis of Protein Expression in Yeast. *Nature* 425(6959):737-41.
- Gillespie, D.T. (1976). A General Method for Numerically Simulating the Stochastic Evolution of Coupled Chemical Reactions. *J. Comp. Phys.* 22: 403-434.
- Hennessy, F., Nicoll, W., Zimmermann, R., Cheetham, M., Blatch, G. (2005). Not All J Domains are Created Equal: Implications for the Specificity of Hsp40-70 interactions. *Prot. Sci.* 14:1697-1709.
- Hu, B., Mayer, M., Tomita, M. (2006). Modeling Hsp70-Mediated Protein Folding. *Biophys. J.* 91 (2):496-507.
- Means S., Shepherd J., Shadid J., Fowler J., Mazel T., Wilson B.S. (2006). Reaction-diffusion Modeling of Calcium Dynamics with Realistic ER Geometry. *Biophys. J.* 91(2):537-557.
- Nehls, S., Snapp, E., Cole, N., Zaal, K., Roberts, T., Ellenberg, J., Presley, J., Siggia, E., Lippincott-Schwartz, J. (2000). Dynamics and Retention of Misfolded Proteins in Native ER Membranes. *Nature Cell Bio.* 2:288-295.
- Nikonov, A., Snapp, E., Lippincott-Schwartz, J., Kreibich, G. (2002). Active Translocon Complexes Labeled with GFP-Dad1 Diffuse Slowly as Large Polysome Arrays in the Endoplasmic Reticulum. *J. Cell Bio.* 158:497-506.
- Nishikawa, S., Hirata, A., Nakano, A. (1994). Inhibition of Endoplasmic Reticulum (ER)-to-Golgi Transport Induces Relocalization of BiP within the ER to Form the BiP Bodies. *Mol. Biol. Cell.* 5:1129-1143.
- Romisch, K. (1999). Surfing the Sec61 Channel: Bidirectional Protein Translocation across the ER Membrane. *J. Cell Sci.* 112(23):4185-91.
- Schroder, M and Kaufman, R.(2005).ER Stress and the Unfolded Protein Response. *Mutation Research* 569:29-63.
- Vereb, G., Szollosi, J., Matko, J., Nagy, P., Vigh, L., Matyus, L., Waldmann, T., Damjanovich, S. (2003). Dynamic, yet Structured: The Cell Membrane Three Decades after the Singer-Nicolson Model. *PNAS USA* 100:8053-8058.