DEPOLYMERIZATION OF FtsZ AT HIGH PRESSURE AND ITS EFFECT ON CELL DIVISON. S. M. Dicken[1], L. Fakhraei[2], and P. Kumar[2], [1]Virginia Commonwealth University, Richmond, VA, [2]Department of Physics, University of Arkansas, Fayetteville, AR.

Introduction: When bacteria such as Escherichia coli are grown under high pressure conditions, they exhibit clear morphological differences from those bacteria grown at standard pressure [1]. This morphological development is thought to be attributable to effects on the cell-division process [2]. The prokaryotic protein FtsZ plays an important but poorly-understood role in bacterial cell division [3]. Prior research has shown that GTP-bound FtsZ polymerizes in a ring-like structure called a Z-ring at the cell-septum site, where division occurs [4]. The depolymerization of the Z-ring upon hydrolysis of GTP provides the mechanical forces needed for cell division [3]. The present study explored the hypothesis that polymerization of GTP-FtsZ is inhibited at high pressures—preventing bacteria from dividing—and thus explains the observed morphological disparity.

Methods: This study had two distinct components: live growth of E.coli in the laboratory at standard and high pressures and a molecular dynamics simulation.

Bacteria Growth: Two different samples of E. coli were grown in Luria broth with ampicillin (LB-AMP) at 37°C. The first sample consisted of 8 µL of bacteria and 10 mL LB-AMP. Bacteria were placed in a temperature-controlled shaker at 1 atm pressure for 230 minutes until it reached an optical density (OD) of 0.740. The second sample consisted of 20 µL of bacteria and 10 mL LB-AMP. Bacteria were placed in a temperature-controlled shaker at 1 atm pressure for 135 minutes until it reached an OD of 0.182. It was then diluted to an OD of 0.050, inserted into a high-pressure growth chamber, and left for 300 minutes at 400 atm. Approximately 50 images of each of the samples were taken using a light microscope with oil immersion objective (Nikon) at 100x magnification. The images were then analyzed in MATLAB.

Molecular Dynamics: Molecular dynamics simulations were carried out to calculate free energy difference between dimer and monomer states of GTP-bound FtsZ from a thermophilic archaea M. janaschii. Simulations were performed in the Groningen Machine for Chemical Simulations (GROMACS). The simulation separated a dimer of GTP-FtsZ to a distance of 4 nm and measured the free energy every 0.2 nm. The simulation was run at 1 atm, 200 atm, 400 atm, 600 atm, and 1000 atm. Using the techniques of free energy perturbation [5], the change in Gibbs free energy (ΔG) between the dimer and monomer forms of GTP-FtsZ was then calculated for all six pressures.

Results: For clarity, we treat the results of our experimental and computational component separately.

Bacteria Growth: The experimental component of our study produced results similar to those of earlier work. The two samples of E. coli grown in the lab showed obvious morphological differences. On average, E. coli grown at 1 atm had a length that fell between 1.3 µm and 4.6 µm (Fig. 1-A). E. coli are normally about 2 microns long at standard pressure; our sample included several bacteria that were close to dividing at the time of measurement, which explains the large number of bacteria between 3 µm and 4.6 µm. 0.9% of the bacteria were longer than 4.6 µm, which is to be expected since cell growth and division is a stochastic process.

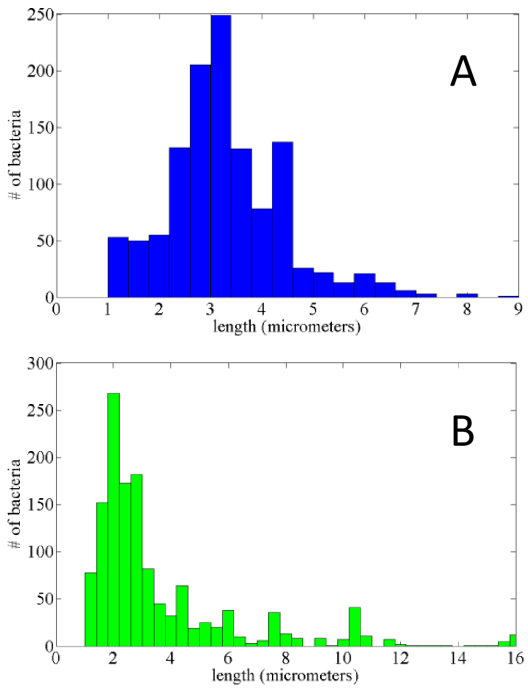

Figure 1: E. coli length after growth at 1 atm (A) and 400 atm (B)

In contrast, E. coli grown at 400 atm showed a much greater incidence of increased length (Fig. 1-B); 19.9% of the bacteria in the second sample measured longer than 4.6 µm, a 20-fold increase over the sample exposed to standard pressure. Even so, the majority of the E. coli grown at 400 atm displayed a "normal" morphology, as evidenced by the large peak near 2 microns in Fig. 1-B above.

Molecular Dynamics: Fig. 2 shows the results of our computational study. From Fig. 2-A, it is clear that

increasing pressure decreased ΔG at large distances. 8-9 nm is the approximate distance at which the real dimer will have depolymerized into the two monomers. At 1 atm, ΔG between the dimer and monomer forms was nearly 20 kcal mol^{-1}, while ΔG at pressures of 200 atm and above ranged from approximately 12.5 kcal mol^{-1} at 200 atm to 10 kcal mol^{-1} at 1000 atm. Fig. 2-B gives a clearer indication of the effect pressure had on free energy in the simulation. The change in ΔG between 1 atm and 200 atm was around 7.5 kcal mol^{-1}, while the corresponding change in ΔG between 200 atm and 1000 atm was only 2.5 kcal mol^{-1}.

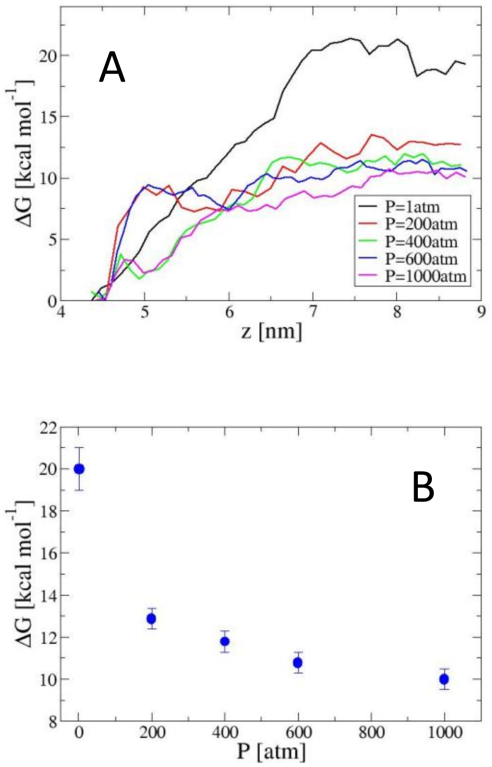

Figure 2: Results of the simulation: the top panel (A) shows the change in free energy as the GTP-FtsZ dimer is pulled apart at various pressures; the bottom panel (B) plots the difference in free energy between the dimer and monomer forms as a function of pressure.

Discussion: The difference in morphology between the E. coli that were grown at 1 atm and those grown at 400 atm suggests that high pressure has an effect on bacterial cell division. However, as evidenced by Fig. 1, this effect is stochastic in nature; many of the E. coli that were grown at 400 atm divided normally. Likewise, some of the E. coli that did not divide normally show regions where pinching of the cell-septum site started to occur but did not complete. We propose that the stochasticity of the cell division process is responsible for these observations; work done by members of this group as well as in other laboratories posited that formation of filamental FtsZ found in the Z-ring is itself stochastic, which means that the Z-ring does not always form. Moreover, it has been shown that high pressure can prevent FtsZ from assembling into a ring [6]. Our molecular dynamics simulation supports this finding, as the simulations data confirmed the expectation that the binding behavior of GTP-FtsZ is affected by high pressure conditions.

However, it is important to note that, while ΔG between the dimer and monomer forms of GTP-FtsZ is reduced by 35% or more at pressures of 200+ atm, the dimer form is still more energetically favorable as compared to the monomer form. This agrees with previous experimental results that determined that the majority of bacterial cells replicate normally at high pressure [1]. Nevertheless, we assert that the drastic reduction in ΔG between 1 atm and 200 atm in our simulation hints at a critical pressure value or range, P_{crit}, beyond which depolymerization of FtsZ is significantly more likely. This range is lower than we were expecting given the extreme conditions at submarine hydrothermal vents near which M. jannaschii lives [7]. We need further investigation in this range of pressures to narrow down a more specific value or range for P_{crit}.

Conclusion: The simulation data are consistent with the hypothesis that depolymerization of FtsZ at high pressure accounts for the observed morphological differences in bacterial cell division. Further work is necessary to improve our simulation. A good convergence of free energy requires that simulations are carried out for a long time. We hope to improve accuracy here by doubling the simulation period at each distance interval. Likewise, in calculating ΔG for several pressures between 1 atm and 200 atm—consequently improving our knowledge of P_{crit}—we seek to gain a better understanding of the behavior of FtsZ in bacteria exposed to non-standard pressure.

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Acknowledgments: The authors would like to thank the Arkansas Center for Space and Planetary Sciences for conducting the REU program in Summer 2014. Special thanks to the Arkansas High Performance Computing Center at the University of Arkansas. This project has been funded by NSF 1157002.