Analysis of Experimental Data in Bacterial Adhesion

Albert Mach
Bioengineering, University of California Berkeley

NNIN REU Site: Center for Nanotechnology, University of Washington
NNIN REU Principal Investigator: Wendy Thomas, Bioengineering, University of Washington
NNIN REU Mentors: Brian Kidd and Olga Yakovenko, Bioengineering, University of Washington
Contact: amach@berkeley.edu, wendyt@u.washington.edu

Abstract:

The receptor-ligand interaction, as mediated by the adhesive protein FimH and mannose-covered surfaces, plays an important role in bacterial adhesion. It has been suggested that the FimH receptor forms allosteric catch bonds with ligand mannose by undergoing a force-induced conformational change. Here, we use an atomic force microscope (AFM) to measure the force response of single receptor-ligand bonds, and present a computational tool for automating the calculation and analysis of the data. A computer program scripted in Matlab calculates the rupture force of single bonds and produces a bimodal figure that is consistent with the concept of two distinct, allostery bond states.

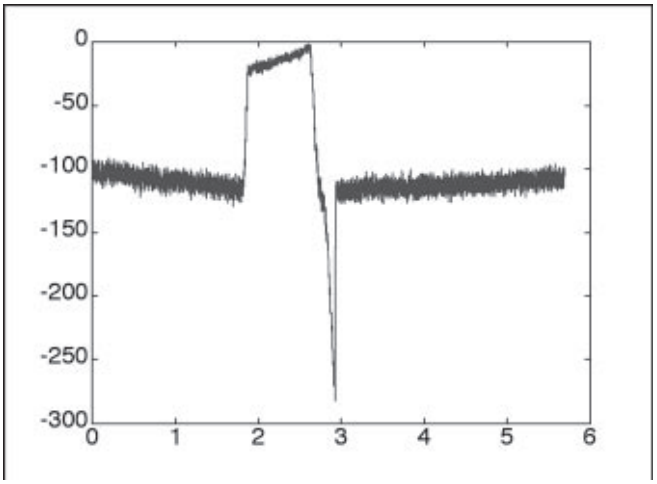

Figure 1: The V-shaped peak represents FimH-mannose interaction.

Introduction:

Understanding the molecular basis of bacterial adhesion creates opportunities for treating infectious diseases and developing novel adhesives for nanotechnology. In *Escherichia coli*, this adhesion is governed by a complex interplay among the receptor-ligand interaction as mediated by the adhesive protein FimH and carbohydrate mannose. Single FimH-mannose bonds were pulled apart with an AFM to calculate the force of the bond mediating this adhesion.

Calculating the rupture force and spring constant would help determine whether FimH forms allosteric catch bonds, which are longer lived bonds with increasing tensile mechanical force [1]. Current methods require days of manual hand calculations and are prone to error due to the difficulty of addressing noise in the readings. As a result, a Matlab program was written to automate the calculation and analysis of the data because it provided an efficient and accurate way to compute.

Methods:

Experimental:

To determine the force of the bond mediating bacterial adhesion, single FimH-mannose bonds were pulled apart with an AFM in contact mode. The AFM consists of a micro-scale cantilever tip coated with mannose and bovine serum albumin (BSA) as well as a sample surface covered with isolated FimH complexes at the tips.

In these experiments, a stiff cantilever is pressed on the surface for a second with 100 pN force to form a bond between the tip and sample. It is then retracted from the surface at different pulling velocities. The AFM outputs binary files comprising of the force response and position of the tip.

Computational:

The goal of the Matlab program was to compute the rupture force and spring constant of the molecule consisting of fimbriae and FimH. It first detected valid adhesion events between the man-BSA cantilever tip and the fimbriae-FimH surface. An event occurred when the force increased linearly past the baseline signifying an interaction between the tip and surface in the experiment as shown in Figure 1. The baseline was created so that the program automatically filters the noise level of about 10 pN by fitting a line through the raw data and finding the intersection of certain points.

Because we would like to calculate the rupture force and molecule spring constant of single bonds, two distinct double peaks as a result of multiple bonds and null event

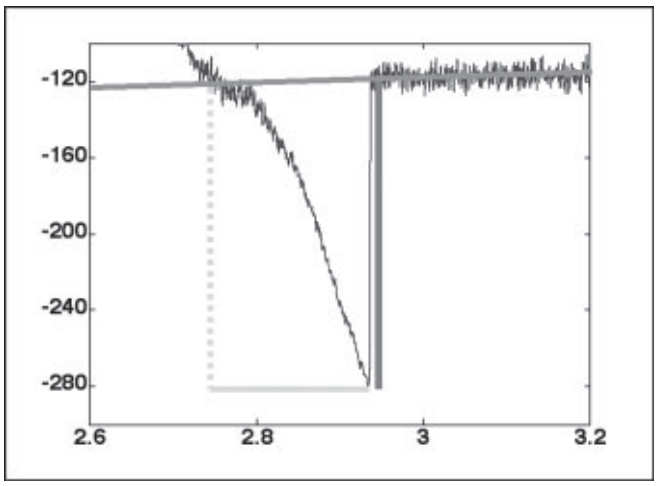

Figure 2: Baseline = upper horizontal line. Rupture force = solid vertical line. Distance = lower horizontal line.

datasets were removed from further analysis.

For single events or peaks, the rupture force and distance were measured as shown in Figure 2. The rupture force is calculated as the height of the peak, or the difference between the tensile force peak and the baseline. The distance is calculated as the difference between the position of initial bond formation and the position of bond rupture.

Since pulling the cantilever and molecule acted like springs in series, we can use Hooke's Law to compute the total spring constant, comprising of the cantilever spring constant and molecule spring constant, and then calculating the molecule spring constant using the following equations, respectively:

$$F_{Rupture} = k_{Total} * x$$

$$k_{Molecule} = \frac{k_{Total} * k_{Cantilever}}{k_{Total} - k_{Cantilever}}$$

The molecule spring constant provides information on whether the binding adhesion is specific or non-specific, representing strong and weak forces, respectively. Non-specific adhesions are a result of low or no interaction between the tip and sample surface.

In order to run the program, the user simply inputted an initial interval for the baseline and the cantilever spring constant and chose which dataset to analyze. The Matlab program outputs a matrix containing the rupture force, total spring constant, and molecule spring constant.

Results and Conclusion:

In these experiments, a histogram of the rupture forces produced a bimodal distribution with a low force peak of 20 to 60 pN and a high force peak of 120 to 160 pN as shown in Figure 3. The different pulling velocities suggest how the rate at which the force was increased varied. A slower pulling velocity had a higher number of low force peaks with a lower number of high force peaks while a faster pulling velocity had a lower number of low force peaks with a higher number of high force peaks.

As a result, the bimodal distribution suggests that FimH forms an allosteric catch bond. The two force peaks is consistent with the concept of two distinct bond shapes, in which FimH undergoes a conformational change for binding at a higher affinity.

However, additional calculations and further analysis are required for producing meaningful data of the molecule spring constants. Solving for the spring constant would help distinguish specific from non-specific adhesion events, which ultimately shows whether FimH forms allosteric catch bonds.

The Matlab program was fast and accurate while removing user bias. It calculated thousands of files in a few minutes and yielded similar solutions compared to manual calculations. Lastly, it offered greater insight into the FimH-mannose interaction by providing a tool to guide future experimental work.

Acknowledgements:

Many thanks to Dr. Wendy Thomas and Brian Kidd for providing an invaluable opportunity, Dr. Olga Yakovenko for gathering AFM data, Tia Ghose and Thomas lab, as well as NTUF, National Nanotechnology Infrastructure Network Research Experience for Undergraduates Program and NSF.

References:

[1] W. Thomas, et. al., "Catch-Bond Model Derived from Allostery Explains Force-Activated Bacterial Adhesion," Biophysical Journal, 2006, 90, 753-764.

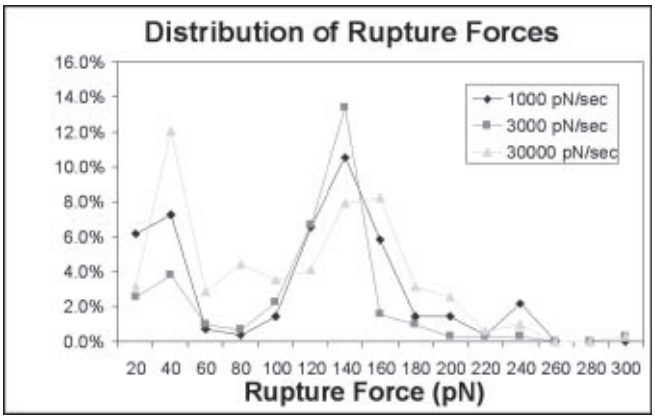

Figure 3: Rupture force histogram.