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Perspective papers

A high throughput MATLAB program for automated force–curve processing using the AdG polymer model

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ABSTRACT

Research in understanding biofilm formation is dependent on accurate and representative measurements of the steric forces related to brush on bacterial surfaces. A MATLAB program to analyze force curves from an AFM efficiently, accurately, and with minimal user bias has been developed. The analysis is based on a modified version of the Alexander and de Gennes (AdG) polymer model, which is a function of equilibrium polymer brush length, probe radius, temperature, separation distance, and a density variable. Automating the analysis reduces the amount of time required to process 100 force curves from several days to less than 2 min. The use of this program to crop and fit force curves to the AdG model will allow researchers to ensure proper processing of large amounts of experimental data and reduce the time required for analysis and comparison of data, thereby enabling higher quality results in a shorter period of time.

1. Introduction

Force curves measured with an atomic force microscope (AFM) have become a fundamental tool in several fields of research (Dupres et al., 2010; Burnham and Colton, 1989). In all applications, the AFM must be properly calibrated to yield accurate results. However, the quality of the results can be overshadowed by a lack of thorough analysis methods. The traditional method of a researcher fitting each experimental force curve individually to a model is time-consuming. This drawback has led researchers to either use small data sets or otherwise compromise the accuracy of their results by averaging measured force curves and fitting the average (Camesano and Logan, 2000; Taylor and Lower, 2008; Block and Helm, 2008; Gaboriaud et al., 2005; Chandraprabha et al., 2010; Knittel et al., 2014). The traditional method also yields subjective results by requiring the researcher to influence the cropping and fitting procedure. The use of a computer program to analyze force curves would reduce processing time and remove user bias. Shorter processing time would enable researchers to examine greater numbers of force curves in their experiments, thereby yielding more precise results. The program presented here uses the Alexander and de Gennes (AdG) polymer model to analyze force–curve data. The AdG model is well-documented and used in many polymer brush studies (Taylor and Lower, 2008; Boyd et al., 2002; Whitehead et al., 2006; Ivanov et al., 2011; DeGennes, 1987; Butt et al., 1999, 2005), though few, if any, have considered analyzing quantities of data as large as are presented in this paper.

The data presented in this paper reflect force curves measured on *Pseudomonas aeruginosa*, a gram-negative aquatic bacterium that is capable of forming biofilms. *P. aeruginosa* was chosen as a representative organism to demonstrate the functionality of this program and could be substituted with any other organism. The lipopolysaccharides (LPS) attached to the cell wall have been linked to the bacteria's adhesive properties and, therefore, its ability to form biofilms (Ivanov et al., 2011). Understanding the forces that aid bacteria in adhering to surfaces could lead to advances in the prevention of biofilm formation. Force curves from an AFM graphically indicate how a cantilever applies force to a sample, or in the case of the data discussed in this paper, how a cantilever applies force to the LPS polymers on the surface of *P. aeruginosa* cultures. When successfully applied, the AdG polymer model describes the shape of a force curve for bacterial polymers and can give information about the physical characteristics (i.e. polymer layer thickness, density, etc.) of the polymers on the surface of the sample. The AdG model is applicable between well-defined boundaries (DeGennes, 1987). The model is only valid while the cantilever is in contact with the polymer brush, and is no longer valid once the logarithmic slope of the force curve exceeds a defined value or once the cantilever has compressed the sample by a distance equal to the cantilever tip radius. Therefore, before the polymer model can be fit, the data must be cropped to the region of applicability of the model. This manuscript outlines the MATLAB program developed for cropping the force–curve data, fitting the AdG model, and interpreting the results.

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2. Atomic force microscopy

The atomic force microscope is the tool used to measure steric forces from the LPS on the surfaces of bacteria from the bacterial polymers in water. The AFM cantilever, or probe, approaches a sample from a few micrometers above, makes contact with the sample, indents the sample until the cantilever is deflected a predetermined amount, and then withdraws from the sample. The vertical movement of the cantilever towards and away from the sample is measured by an internal position sensor. Any recorded deflection is proportional to the force applied to the sample and can be measured by reflecting a laser off the back of the cantilever and onto a position-sensitive photodiode. Since the deflection, and therefore force, can be recorded throughout the whole cycle, the process of approaching, contacting, and withdrawing from a sample creates a force curve describing the forces applied to the sample with respect to the separation between the cantilever and the sample. Initially, deflection and z-sensor positioning of the cantilever are recorded by the AFM; force and separation are calculated later. In an effort to facilitate this calculation and the analysis which follows, the AFM software also saves a 7-th order polynomial fit to the z-sensor data (Asylum, 2008). This MATLAB program takes the vectors of z-sensor, deflection, and fitted z-sensor data as its inputs for force–curve processing as these are the default vectors saved by the AFM software, but it only uses the raw z-sensor and deflection data for processing.

3. Data processing

This section describes the order of data processing executed in the MATLAB program in sequential subsections. The program, outlined in Fig. 1, first prompts the user to upload an experiment file of force curves. The formatting of the force–curve file is specified in Section 5.1.

3.1. Pre-processing

The selected force curves are imported from Excel and separated from their current format into a matrix of column pairs: the z-sensor and deflection data are saved in sequential adjoining columns as a matrix in MATLAB. The program then prompts the user to indicate whether to process all of the force curves in the file or only a range of force curves. This option is useful if the researcher knows that a portion of his measurements were not ideal, if there was an anomaly in some part of the experiment, or if he only wants to analyze a few force curves as proof of concept. Nothing about the analysis changes except for the size of the matrix imported from Excel. Once the relevant Excel data has been read and stored, the program prompts the user for the following experimental constants: temperature in Celsius, tip geometry (spherical, conical, or pyramidal) and associated tip size, and cantilever stiffness. Each of these parameters becomes a constant in the AdG fit and is necessary to accurately process the data. Next, the program asks the user whether to use default cropping and fitting parameters for processing or to allow the user to specify values. When processing a data set for the first time, it is recommended that the user select the default parameters. However, if a crop with the default parameters is suspect or the researchers wish to manually specify their own parameters, they are given the option to specify the following: the logarithmic slope beyond which the upper crop should occur, the amount of approach data to remove, the amount of deviation above the noise floor to indicate contact with the sample, the acceptable R^2 value to statistically determine a good fit, and the number of points to smooth over when performing a moving average for the purposes of noise reduction.

The data are then separated from the MATLAB matrix into pairs of column vectors, with one column for z-sensor data and one for the corresponding deflection data. Any baseline slope in the approach data is removed such that the approach data points lie flat along the x-axis. This step is included to remedy any error in AFM calibration that would result in a changing deflection before the cantilever has made contact with the sample. Finally, separation and force are calculated from the z-sensor and deflection data. The tip–sample separation is determined by resolving the difference between the z-sensor position of the base of the cantilever relative to the position of the bacterial cell wall and the amount of deflection of the cantilever tip. The magnitude of the force is the product of the cantilever spring constant and the measured cantilever deflection.

The amount of approach data specified by the user (or the default value discussed above) is removed from each force curve to reduce the amount of data carried through the rest of the program and, therefore, to reduce processing time.

3.2. Cropping

The AdG model is only applicable in a bounded region, hereafter referred to as the fitting window since the part of the curve present here will be fit to the model. The lower bound of the fitting window occurs when the cantilever makes contact with the sample and the curve rises above the noise of the approach data. To determine where the fitting window begins, the data are smoothed using a moving average over a number of points specified by the user to reduce the influence of the noise and focus on the trend of the curve. Determination of the lower bound occurs when the smoothed data deviate from the noise by a default value of 10 pN (this can be adjusted by the user). All approach data prior to this point are removed. This concept is illustrated in Fig. 2, where the lower bound of the fitting window is located at the intersection of the red and blue data, and the red data corresponding to the cantilever approaching the data would be removed.

The upper bound occurs either when the logarithmic slope of the deflection data exceeds a particular slope (depending on the geometry of the cantilever tip) or when the cantilever has compressed a distance equal to its tip radius into the brush, whichever comes first. The log of both separation and force is taken and smoothed in the same manner described above. Then, the slope is incrementally measured from the lower bound through the rest of the data. If a slope is measured that is smaller in magnitude (or less negative) than ($-5/4$) for a spherical tip or ($-1/4$) for a conical or pyramidal tip, this is the upper bound and all data beyond this point are removed. Then, the separation between the upper and lower crops is calculated; if the separation is greater than the tip radius, the new upper crop becomes the point that is a distance equal to the tip radius from the lower crop. If the appropriate slope was not found, the upper crop is simply the point separated from the lower crop by the tip radius. The remaining region is the area of applicability for the AdG model (Anderson, 2012). This process is illustrated in Fig. 3 below.

This process is completed for every force curve that the user wishes to analyze. Once all curves have been cropped, the original data and the cropped data are plotted so the user can visually inspect the cropped

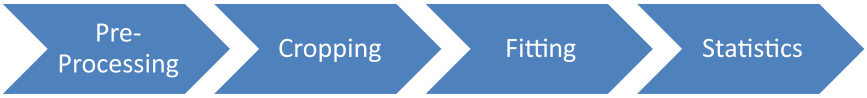

Fig. 1. The MATLAB program applies the AdG process to force–curve data and can use statistics to remove outliers. The data must first be formatted and cropped before being fit to the AdG model. Then, statistics are applied to remove outliers and poorly-fitted data.