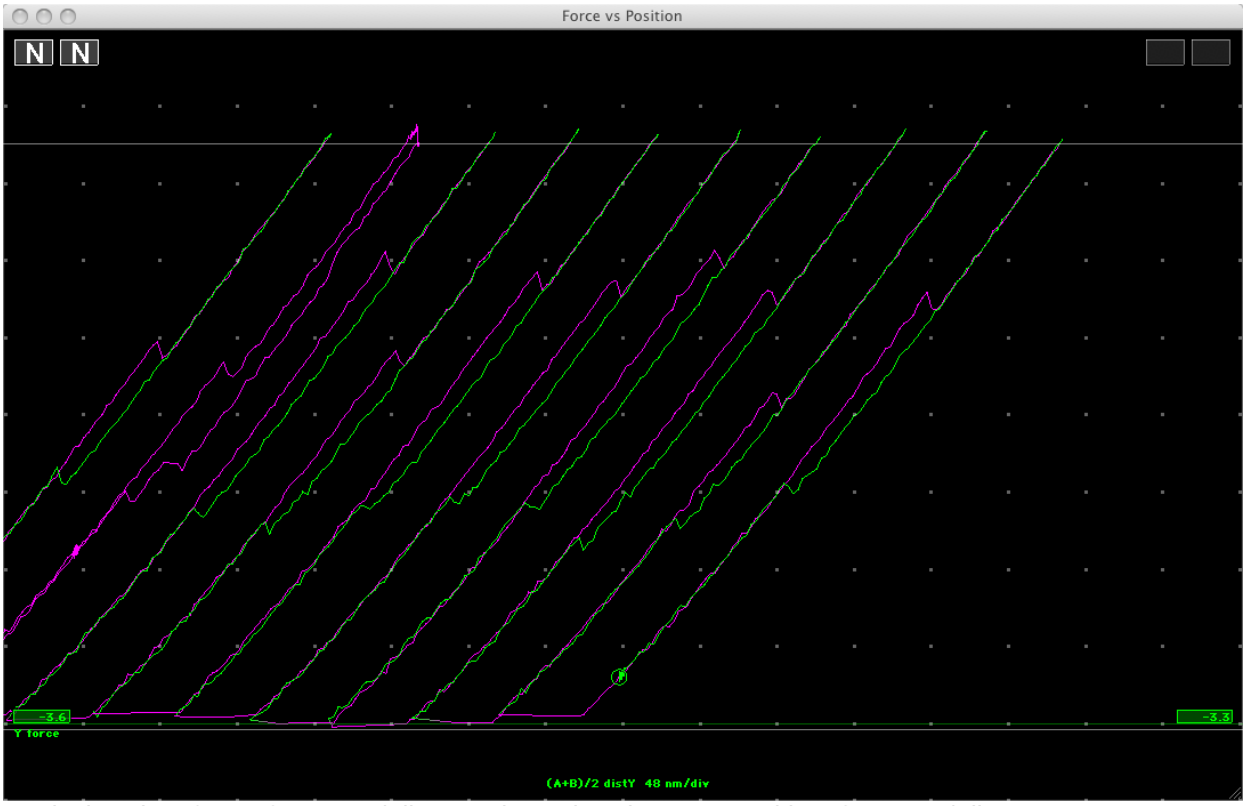
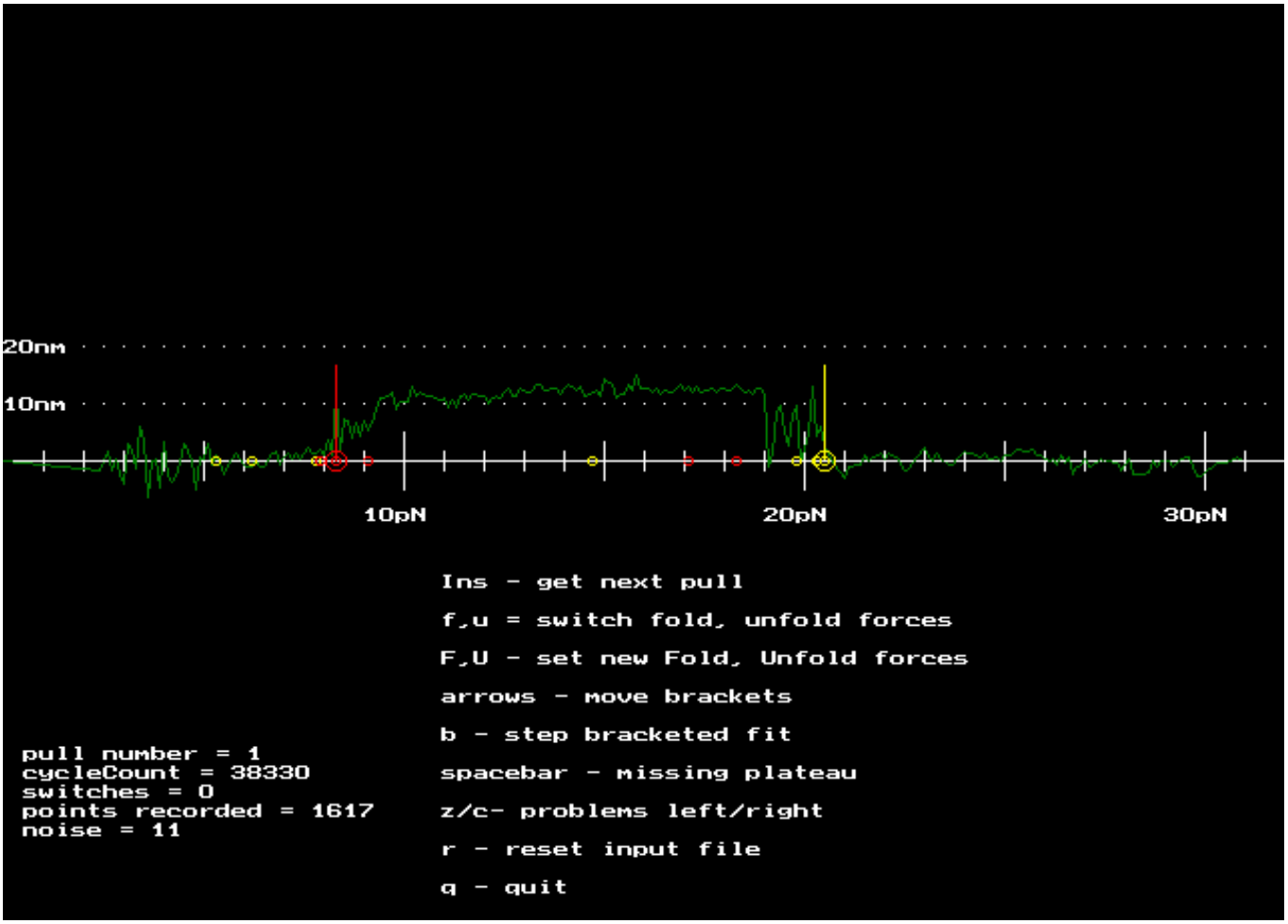
A procedure to extract contour length from unfold/refold trajectories

During a pull-relax cycle, individual protein molecules usually unfold at a high force but refold at a low force. Such hysteresis is greater when the temperature is low and the pulling speed is high. The figure below superposes pull (magenta) and relax (green) force /position curves for protein MJ0366.

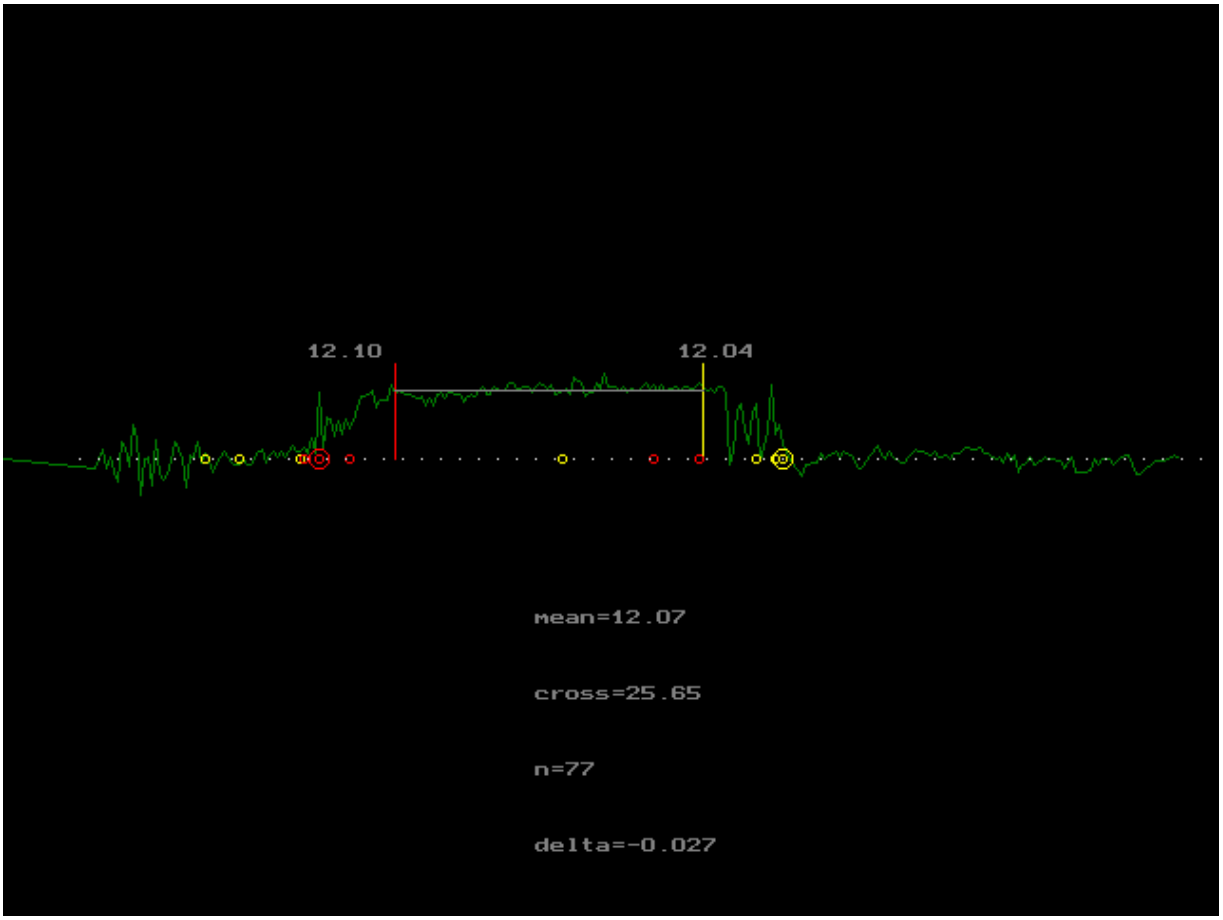


Vertical scale = force @ 3.6 pN / div. Horizontal scale = trap position @ 48 nm / div. Gaps seen between pull and relax curves represent the contour length of unfolded protein subject to the applied force versus entropic contraction due to temperature. To estimate contour length, such gaps must also be increased by the native folded length between attachment points (4.1 nm) in MJ0366.

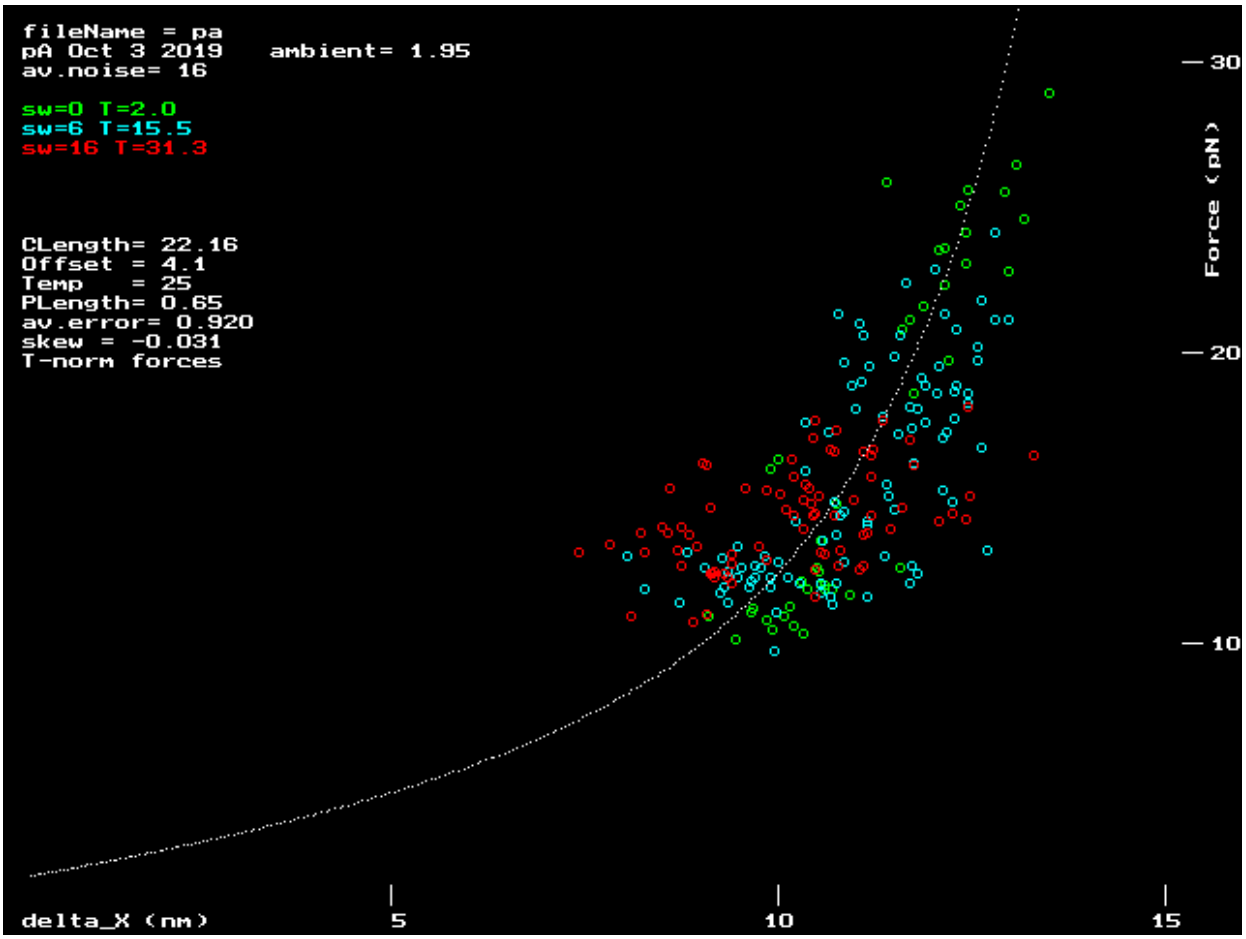
A semi-automated method was developed for displaying, sorting and measuring hysteresis gap data. Source code presented here was edited and compiled using Borland Turbo Pascal 7.0 running on a 32-bit computer and XP operating system. Only molecules with a large number of pull/relax cycles (>20) were chosen for analysis. A sample data set, pA.TXT, is included here where raw data from an Apple computer was opened by WordPad in a Windows computer and then re-saved in order to add carriage returns and line feeds as required by Pascal procedures. Next, an operator opened the modified data set in the program FINDBLG4.PAS and was presented with a graph of gap width vs. force for the first pull/relax cycle.



Hysteresis gap distance plotted vertical (10 nm / div.) and force horizontal. Keystroke operations available to the operator are listed on the screen. Experimental data from pull #1 are displayed at lower left. The line “switches = 0” indicates that no extra heat above ambient was acting on the molecule for that cycle. The yellow bracket line automatically appears slightly above the unfolding force and the red bracket appears below the refolding force.



Next, the operator uses keystrokes to move the brackets inside the relatively flat plateau region. With a certain keystroke, the program makes a linear fit to the bracketed region and records both intercepts into a file such as pA.FIT. This process is repeated for many pull/relax cycles. Finally the FIT file is edited manually to include the ambient chamber temperature (tempB from comments file pCOM.TXT) in the file header.



Any FIT file can be opened by the program PLOTBLG8.PAS which then displays a plot of the two intercept distances (horizontal) versus the intercept forces (vertical). Dot color indicates the local buffer temperature due to chamber ambient plus the 1435nm heating laser whose effect had been calibrated by water viscosity using the program SALTVISC.PAS. A summary of temperature rise vs. laser switch position (SW =) was stored in a local file named TEMPS.TXT.

The program PROTEIN.PAS had produced WLC fractional extensions vs log-spaced forces and recorded those values in the WLCBB25.TXT file. Those data were then used to draw the while dotted line; however the horizontal scale of the line depends on choosing a contour length as a free parameter which is adjustable by keystrokes. The operator adjusts the model contour length while observing the fit parameter " av.error " which is the mean square horizontal distance error for all dots from the WLC line. The estimated contour length (CLength =) for that molecule is obtained when the average error reaches a minimum. The notification line "T-norm forces" says the force coordinate for each colored dot had been normalized to a temperature of 25 deg.C by multiplying the factor $[298/(T+273)]^2$