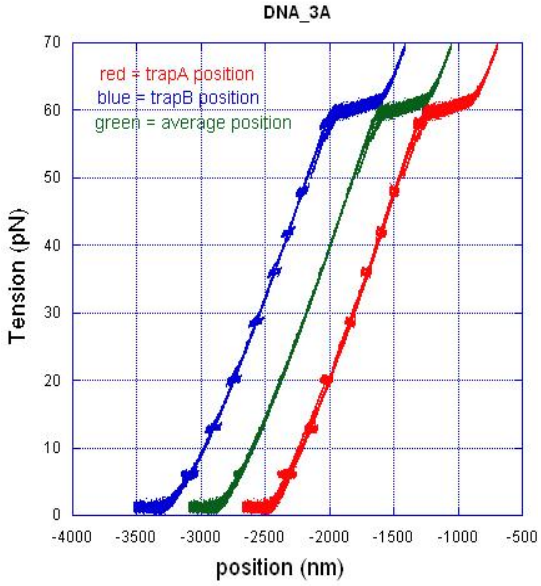
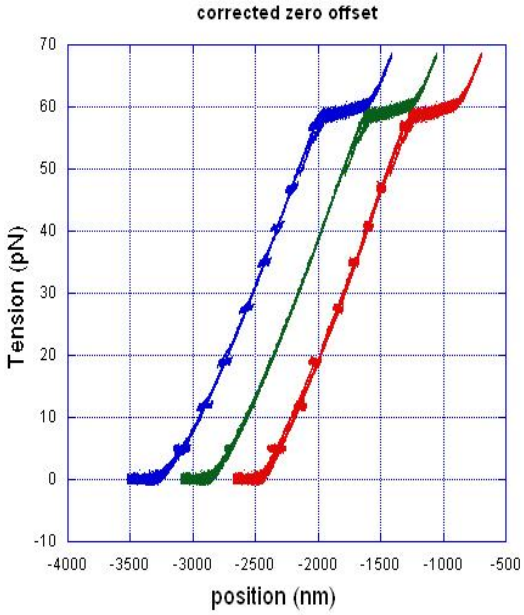
How to use data from the Stiffness Test to extract force-extension curves for a short molecule.

(1) In the miniTweezers PREF file, set flags to record the following set of variables: "cycleCount/n", "yForce" , "Tension" , "A_distY" , "B_distY" and "Status". Other variables may also be recorded as needed for your experiment.
(2) Catch a bead and zero the force when bead is near to the pipette.
(3) Fish molecule and make force-position curves using P1 or P2 pulling protocols.
(4) Click the "Stiffness Test" protocol button at some point along the pulling curve. Observe that the force ramp stops and the light-lever distance hops back and forth between nearby points. Allow this hopping to continue at least 10 cycles before pushing P1 or P2 to continue the pulling protocol. Repeat the stiffness test at several forces along the pulling curve. Avoid regions with force plateaus or rips because the molecule has no definite length at these forces and the stiffness test will not work there.
(5) Export data file to PC computer with 32-bit operating system. The Pascal program only runs on older systems such as Windows –XP or earlier. Open data file with WordPad and save it immediately to fix a problem with CR/LF characters. Rename text file with 8 characters or fewer. Otherwise it is too long for Pascal program to recognize.
(6) Plot yForce versus trap positions. Compare the curves using A_distY and B_distY. If the curves look similar, make a new data column which is the average position avDist = (A_distY + B_distY) / 2 and plot force vs. avDist. (green curve). If the molecule goes slack but the plateau force is not zero, then correct the force column by adding an offset.





Here we subtracted 1.1 pN from yForce to make the force zero when the molecule is slack. Next, save corrected data as tab-delineated text file.

(7) Download program code STIFTST5.pas and STFTST5.exe from the Software section of TweezersLab web site. The default constant in the Pascal program "riseTime = 5" is appropriate when recording data at 200 Hz (*samples/line_LO = 20* in PREF file). If the

recording was done at 1000 Hz, then edit the Pascal file to read “riseTime = 25” and re-compile the code.

(8) Place the zero-corrected data.txt file in same folder as program files and run the STFTST5.exe program. Enter name of data.txt file (must be 8 characters or fewer). Output will appear with same filename but with “.out” extension. Typical output follows:

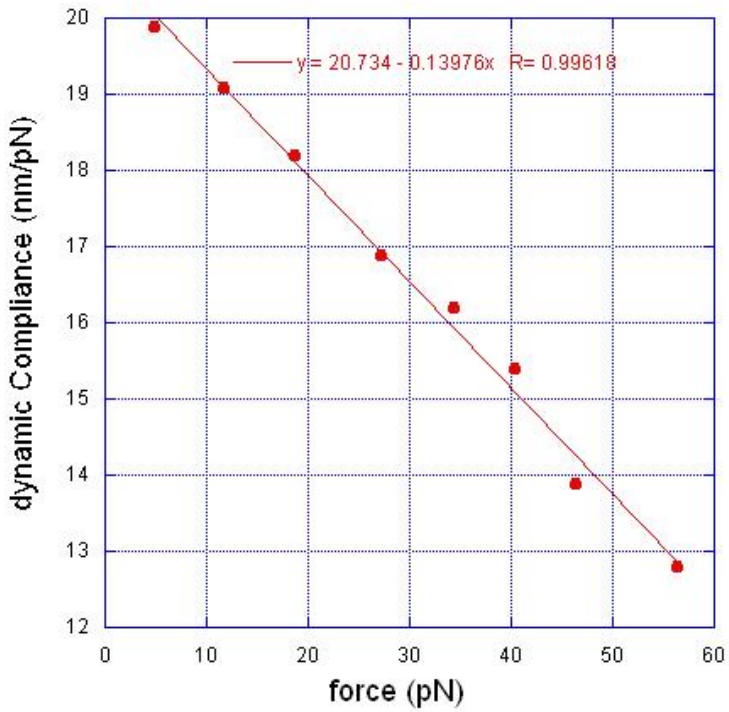
CycleCount	yForce(pN)	distA(nm)	distB(nm)	HiPulses	LoPulses
12788	4.9	68.8	94.4	21	21
25651	18.6	71.1	74.5	30	31
36773	34.4	65.6	64.4	15	16
44682	46.3	56.2	55.3	18	18
53798	56.3	52.4	50.2	19	20
75133	40.3	62.4	60.7	23	23
85183	27.1	67.5	67.4	20	21
96145	11.6	72.9	80.3	21	20

The first column gives the time code in the data file where a particular test occured. The second column gives the force used in that test. The third and fourth columns give the average distances moved by trapA and trapB respectively as they change their constant-force setpoints by +/- 1 pN. Note that the force changes in each trap by 2 pN during a pulse, but the total force on the molecule remains constant. The last 2 columns give the number of pulses that were analyzed to produce the average distance moved at a given force. Pulse numbers greater than 10 ensure accurate readings.

(9) Compute a new column “ dComp” which stands for dynamic trap compliance. That is the mean distance both traps move for a tension change of 1 pN as a function of the total force. To obtain that number, compute the separate trap stiffness values, add the 2 stiffness values and take the inverse of the sum. Since the distA and distB steps are for a 2-pN change, the formula is as follows:
dComp = 1 / [(2 /distA) + (2 /distB)]

yForce	distA(nm)	distB(nm)	dCompl(nm/pN)
4.9	68.8	94.4	19.9
18.6	71.1	74.5	18.2
34.4	65.6	64.4	16.2
46.3	56.2	55.3	13.9
56.3	52.4	50.2	12.8
40.3	62.4	60.7	15.4
27.1	67.5	67.4	16.9
11.6	72.9	80.3	19.1

(10) Plot dynamic compliance versus force and make a linear fit to the data.



(11) Consider that the total bead compliance (its position relative to the trap) can be obtained as the summation of dynamic compliance over incremental force steps from zero to the test force. Therefore, integrate the dynamic compliance versus force from zero up to the test force. Our example gives the following values:

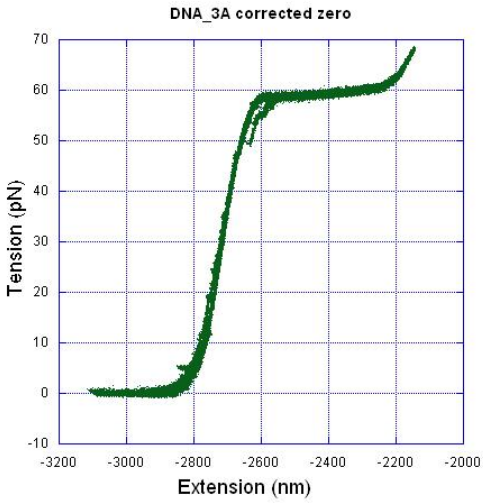
dComp/dForce = 20.734 – 0.13976 * force

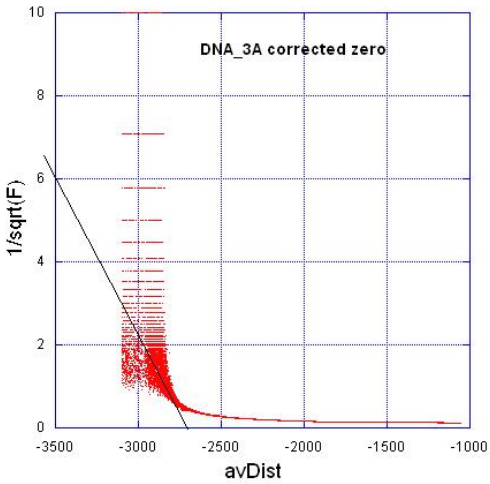
Compliance = (20.734 *force) – 0.13976 / 2 * force^2

(12) Create "Compliance" column in data file by operating on force = "Tension" column with equation above.

(13) Subtract "Compliance" column from "avDist" column to give "Extension" column

(14) Plot Tension versus Extension. Compare with WLC model using 1/Sqrt(F) plot. See Steve's group meeting of August 15, 2008 for graph of ideal WLC in 1/Sqrt(F) plot.





Looks like the compliance was not big enough because the slope at Ext = -2700 is not high enough. The stretch coefficient of dsDNA is ~1000 pN which means pulling with 60 pN increases the countour length by 6%. This sample seems to stretch by 150nm/570 nm = 26%.

The overstretch plateau is short (400 nm) so I assume the B-form length is 570nm and the number of base pairs is only 1700. Beads must have attached somewhere inside the 2340 bp molecule, not at the ends.