Biophysical Journal, Volume 96

Supporting Material

Dynamics of Type IV Pili is Controlled by Switching Between Multiple States

Martin Clausen, Michael Koomey, and Berenike Maier

Supplementary Materials and Methods for

Dynamics of type IV pili is controlled by switching between multiple states

Martin Clausen*, Michael Koomey[§] and Berenike Maier*

Experimental setup

The optical tweezers setup consisted of a Nd:YVO_4 laser working at 1064nm (Spectra Physics), an inverted microscope (Axiovert 200, Zeiss), a piezo table (PI) and a personal computer. Positional data was acquired at 20kHz time resolution with a four quadrant photodiode (Hamamatsu Photonics) through a DSP card (Sheldon Instruments). The DSP processed incoming data and computed the position of the piezo in real time as well as streamed the data to the personal computer. Force feedback was only activated, after the force reached the preprogrammed level. Then the position of the piezo was adjusted such, that the displacement of the bead remained constant. Force feedback parameters were set to minimize noise while allowing for sufficiently good tracking. In this case the change in the force during directional reversal was set to approximately half the SD of the system noise.

All data acquisition software was written in Labview 8.21 (National Instruments). The trap stiffness was calibrated by the viscous drag method and verified with the power spectrum analysis of the bead's Brownian motion. For measurements with forces up to 60pN we used a stiffness of 0.2pN/nm and for forces exceeding 80pN we used a stiffness of 0.36pN/nm.

Data analysis. The data was analyzed with software written in Igor Pro 6.02a (Wavemetrics). Raw data was filtered three times with a notch filter (Q=3) at 42Hz and smoothed with a moving average filter over 20 data points. The initial direction of the pilus retraction was determined from the bead displacement. Then the piezo position was interpolated down to a resolution of 10ms. Depending on the direction between two successive data points with respect to the initial direction, the movement was classified as retraction, extension or invalid.

The duration of stretches of uniform motion were determined from dissected data. Durations were then plotted in a cumulative histogram and fitted with a double exponential. Velocities were determined by numerical differentiation.

Western blotting. PilT was detected with a polyclonal antibody raised in rabbit against the whole molecule. Quantification was done by scanning the ECL signal (GE Healthcare, Amersham ECL Western Blotting Analysis System). PilT Protein levels were determined as $MS11_{pilT\ oe}$: MS11 : $MS11_{pilT\ ind}$ to be 5 : 1 : 0.2 (see Supplementary Fig. 4). The amount of total protein was checked by Coomassie staining.

Supplementary text for

Dynamics of type IV pili is controlled by switching between multiple states

Martin Clausen, Michael Koomey and Berenike Maier

Effect of time resolution and velocity thresholding during data dissection

Automatic data dissection into the three modes of pilus dynamics (e.g. retraction, pausing, elongation) was not trivial, since temporal and spatial resolution of the obtained data did not resolve single subunits. Therefore the parameters of the algorithm had to be set carefully to obtain good spatial and temporal resolution and in the following, we discuss the influence of these parameters on the results.

Pilus retraction data from active bacteria were evaluated with different parameters for the curve dissection. Supplementary Fig. 1 shows the same pilus retraction track (MS11, 100pN) analyzed with interpolations to different time bases Δt (5, 10 and 20ms). Due to Brownian fluctuations we set a velocity threshold which separates pauses from pilus length change. The maximum velocity for pause detection was set to 150nm/s, except for the 5ms time base where 300nm/s due to the higher noise level was used. The time base Δt = 5ms leads to the assignment of elongation (which has a lower speed then retraction) and even short pieces of retraction to the pause state, since we had to set the threshold for pauses to 300nm/s due to the high noise level. The time base Δt = 20ms causes the algorithm to miss details of the track as well as to miss assignment of elongation and retraction to the pause state, due to the averaging over many data points. In comparison the Δt = 10 ms time base lead to the best agreement between algorithm based assignment and manual assignment.

Note that due to averaging short pieces of elongation in a long stretch of extension (and vice versa) may not be detected as a movement, but as a pause. This still leads to a disruption and therefore even events shorter than the time base can be detected. However, the time base presents a time scale lower limit for events to occur within the histograms.

To test the dependence of our results on the dissection parameters, we evaluated all pilus retraction data for all three time bases Δt. Velocity histograms showed more pronounced peaks for longer time bases, because of reduced noise levels (data not shown). The

probabilities for pilus elongation, pausing and pilus retraction changed in the following manner (data not shown): 5ms dissected data showed an increase in pauses to 19% over 7% (average over all data) for the 10ms data, which can be attributed to the higher velocity threshold and the higher noise level due to less averaging. Data with a 20ms time base showed slightly lower levels of pauses and polymerization with respect to the 10ms interpolation, because the algorithm missed the details of the retraction curves. Although the values depend quantitatively on the dissection parameters, the fact that directional switching occurred at two distinct time scales and the order of magnitudes of elongation and retraction intervals are valid for all parameters except for 20ms and 8pN where a fit failed, because the elongation and retraction intervals became very long and therefore very few in number (Supplementary Fig. 2). The duration of elongation and retraction intervals extended as expected with increasing time base, since due to the increased averaging the noise level is reduced and shorted lived features of the dynamics are ignored. However, the longer time scale remained always approximately one order of magnitude separated from the shorter time scale.

Since a time base to 10ms and a velocity cut-off for pausing at 150nm/sec yielded best results for curve dissection, we evaluated whether directional switching was caused by the bacterial motor and not an artefact of the setup, we bound a bead to the pilus of a MS11$_{pilT-}$ bacterium (a mutant which does not perform pilus retraction) and the bacterium was moved with the motorized microscope table at a speed of ca. 2000nm/s. Under these conditions the dissection algorithm did not detect directional changes.

Error estimation for retraction, pause and elongation probablities

For the determination of the standard error of retraction, pause and elongation probabilities the statistical resampling method jackknife was used [1]. The data containing *N* data points was evaluated *N* times while leaving out one different data point each time. From the average of each of these datasets the standard deviation as well as the mean was determined.

Elongation velocity as a function of external force

The elongation velocity tended to increase with increasing force as expected for an energy requiring process (Supplementary Fig. 3). For other forces and PilT concentrations the speed could not be determined, since the stretches of elongation were to short, for the same reason there is no error bar for the WT at 60pN. Please note that velocities below the pausing velocity threshold are missing. The distributions are extending into that region and therefore the velocity values are more uncertain than those for the retraction, which occurs at higher

speeds. Furthermore, our measurements may be biased by the fact that events are exclusively triggered by retraction but never by elongation. Although our individual events include enough switching events it is likely that we do not characterize the major elongation mode.

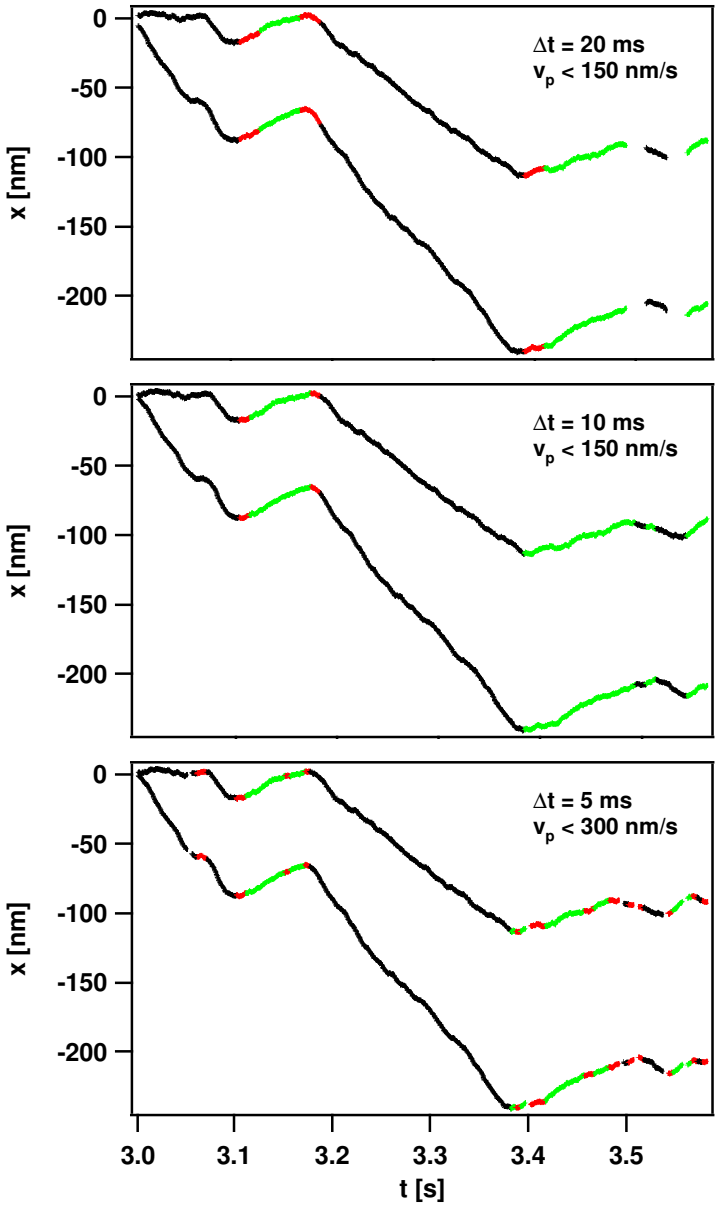
References

1. Bradley, E. 1981. Nonparametric estimates of standard error: The jackknife, the bootstrap and other methods. Biometrika 68: 589-599

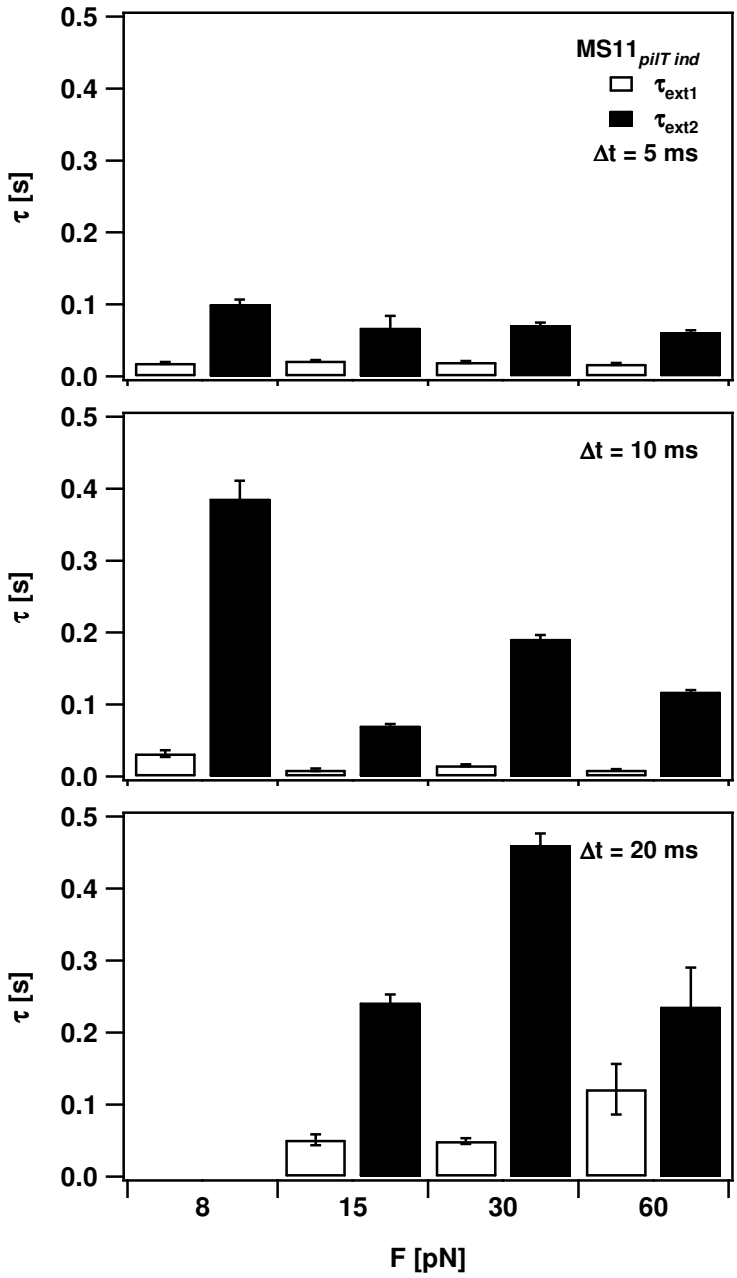
Supplementary figures for

Dynamics of type IV pili is controlled by switching between multiple states

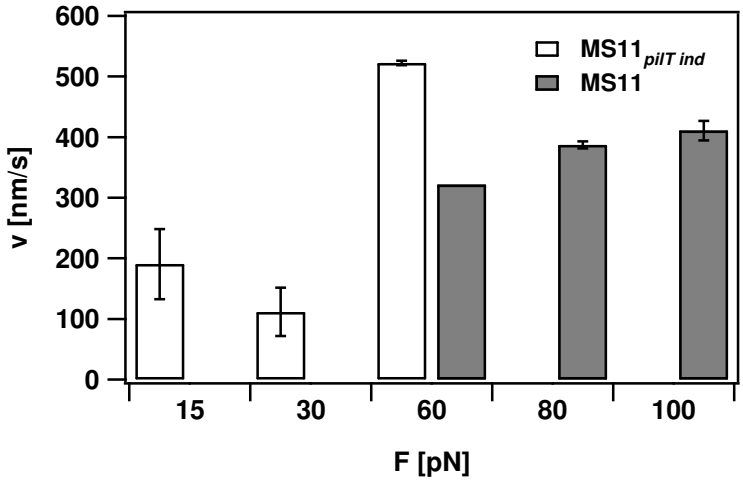
Martin Clausen*, Michael Koomey[§] and Berenike Maier*



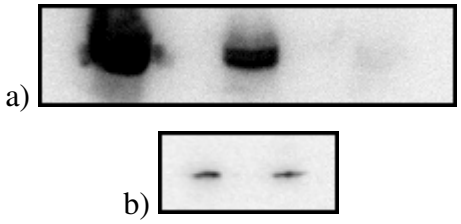
Supplementary Fig. 1. Dissection of pilus length change x with different time bases Δt and velocity thresholds v_p. A time base of 10ms leads to best results with respect to temporal and spatial resolution and recognition quality. black: retraction, red: pause, green: elongation.



Supplementary Fig. 2. Absolute values for the duration change depended on the dissection algorithm time base *Δt*.



Supplementary Fig. 3. Elongation velocity versus force.



Supplementary Fig. 4. a) Western blot of PilT protein in MS11$_{pilT\ oe}$, MS11 and MS11$_{pilT\ ind}$ form left to right. The ratio between the middle and the right lane was 1:0.2. The left lane was not quantified due to overloading. b) The ratio between MS11$_{pilT\ oe}$ (left) and MS11 (right) was determined by diluting the MS11$_{pilT\ oe}$ lysate 5 fold to obtain equal signals from both samples.