Protein folding modulates the chemical reactivity of a Gram-positive adhesin

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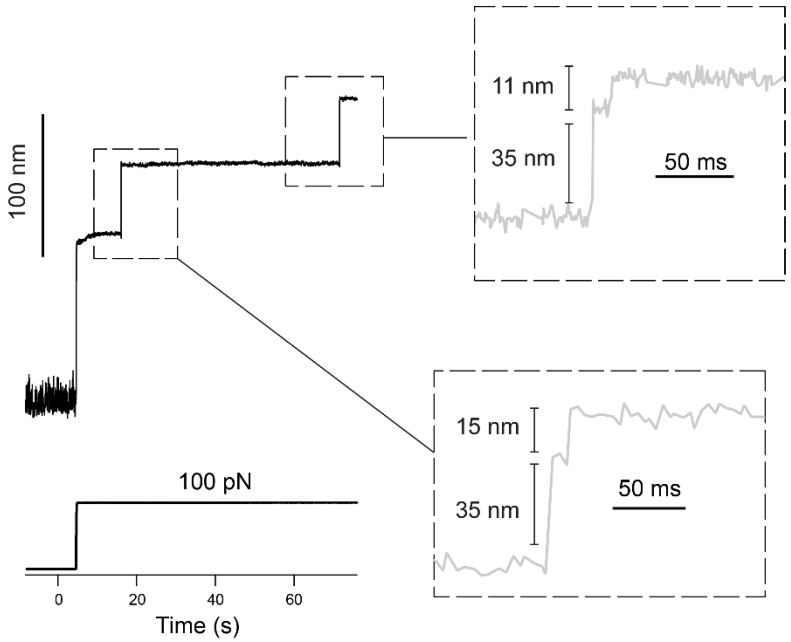
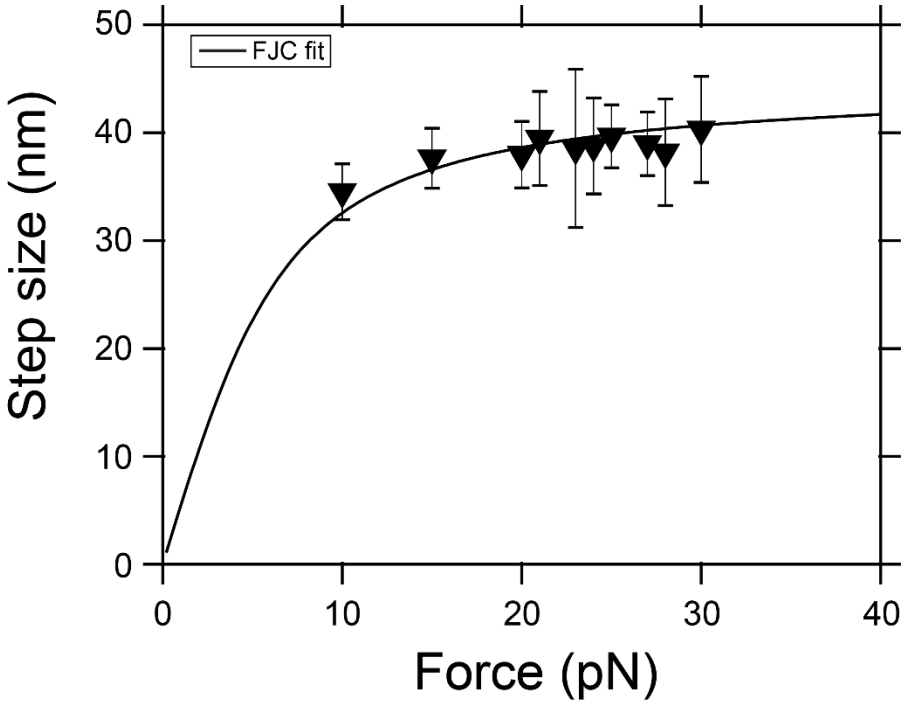
SUPPLEMENTARY INFORMATION

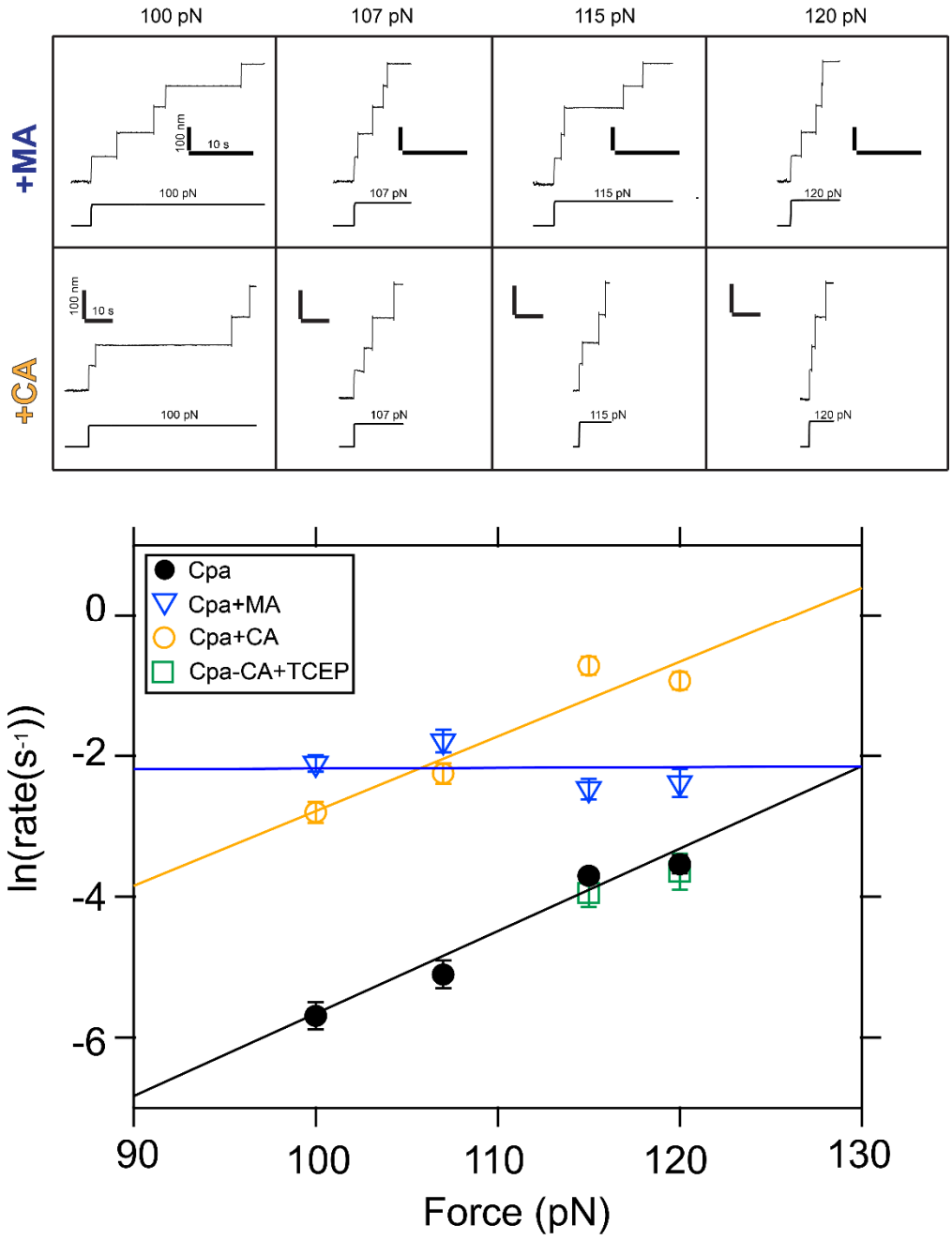
Supplementary Figures



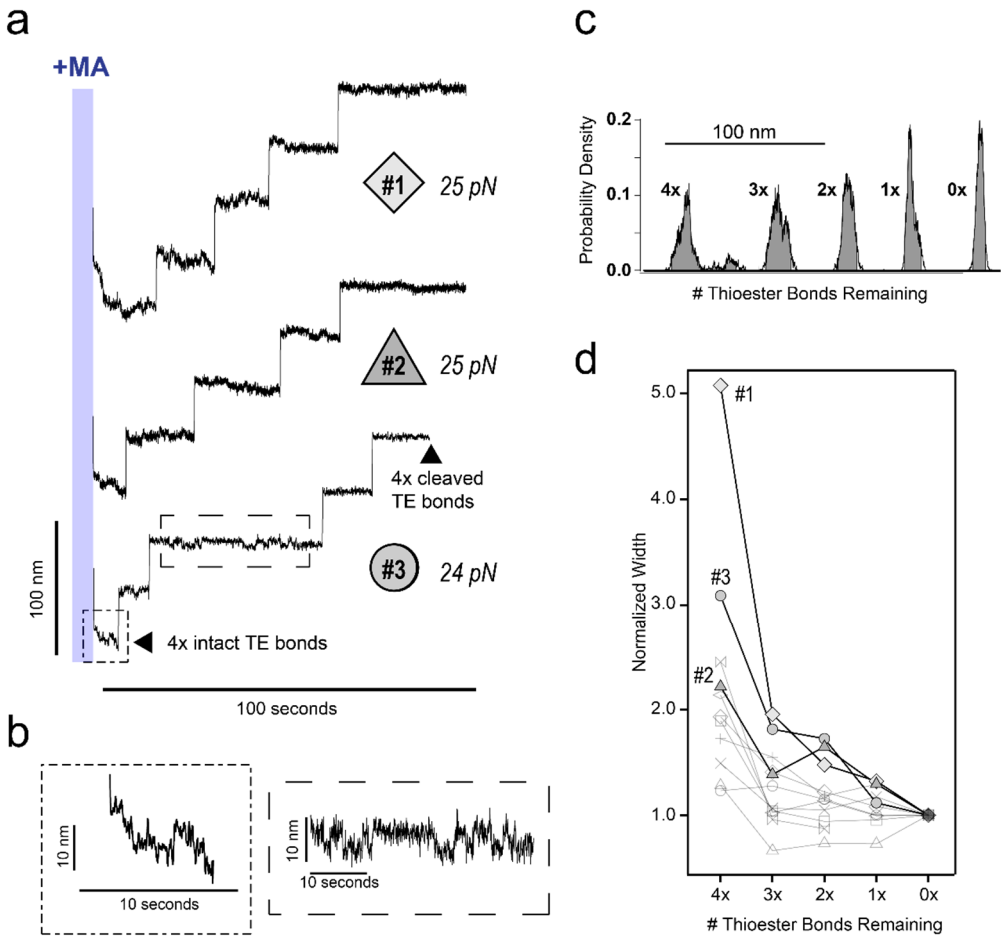
Supplementary Figure 1. Cpa intermediate. At forces below 115 pN, it is possible to detect the unfolding of thioester-intact Cpa proteins with an intermediate state. After the polypeptide extension of the CnaB domain (~35 nm), the extension of the sequence non-sequestered by the thioester bond (A393-C426) is rapidly released in a few ms (~13 nm). Insets show the unfiltered raw trajectory.



Supplementary Figure 2. Force-dependent thioester bond cleavage step size. Cpa thioester bond cleavage step size as a function of force. Data points are the mean±SD of the step size measured at each force (n=33 at 10 pN; n=37 at 15 pN, n=21 at 20 pN, n=27 at 21 pN, n=38 at 23 pN, n=25 at 24 pN, n=36 at 25 pN, n=21 at 27 pN, n=12 at 28 pN, n=18 at 30 pN). The line is the freely jointed chain (FJC) fit for polymer elasticity to the Cpa thioester bond-cleavage extensions. The predicted contour length L_c was determined subtracting the value for thioester-intact Cpa (49 nm) to thioester-cleaved Cpa (95 nm): L_c=95-49=46 nm. From the fit of the FJC to the data we obtain a L_c=44.8±3.0 nm, and a Kuhn length value of l_k=1.5±0.5 nm.



Supplementary Figure 3. Unfolding kinetics of Cpa. Characterization of the unfolding kinetics of Cpa at 100, 107, 115, and 120 pN under different conditions: Cpa (circles), Cpa with methylamine (Cpa+MA, blue triangles), Cpa with cystamine (Cpa+CA, orange circles), and Cpa after the treatment with CA followed by TCEP (Cpa-CA+TCEP, green squares). When Cpa thioester bond is broken and either MA or CA are bound, the unfolding kinetics of the protein increase demonstrating the lower mechanical stability of the adhesin in the presence of these nucleophiles. Cpa+CA molecules ($x^{\dagger}$=0.44 nm) can be treated with TCEP to trigger thioester bond reformation, which reestablishes the mechanical stability of the protein, as it can be seen at the forces of 115 and 120 pN where the rates of unfolding of the protein after the treatment with TCEP are identical to the ones exhibited by the untreated Cpa ($x^{\dagger}$=0.48 nm). Interestingly, while all the conditions studied show an exponential increase trend of the unfolding rate with the force, the Cpa+MA condition show insensitivity to the force (Cpa: n=40 at 100 pN, n=35 at 107 pN, n=251 at 115 pN, n=93 at 120 pN; Cpa+MA: n=70 at 100 pN, n=56 at 107 pN, n=101 at 115 pN, n=98 at 120 pN; Cpa+CA: n=56 at 100 pN, n=59 at 107 pN, n=71 at 115 pN, n=75 at 120 pN; Cpa-CA+TCEP: n=32 at 115 pN, n=9 at 120 pN).



Supplementary Figure 4. Thioester cleavage follows short lengthwise fluctuations. a) Representative recordings of Cpa polyprotein after the addition of methylamine and relaxation from 115 pN to 24 and 25 pN. Four thioester bonds are intact at the beginning of each recording and four subsequent thioester cleavage steps follow. **b)** Expansions of the initial collapse trajectory and the equilibrium fluctuations from trajectory #3. **c)** The normalized probability density of each step level in trajectory #1 is shown with the 95% probability width shaded in grey and **d)** plotted as a function of the number of intact thioester bonds remaining for each of 11 single molecule recordings.