Supplementary Information
Stepwise reversible nanomechanical buckling in a viral capsid

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1. Analysis of buckling step size

To minimize preconception in the analysis of buckling step size, we employed the following procedure. Raw data of step sizes were first obtained from force *versus* distance curves collected during the nanoindentation of T7 capsids where maximal indentation force was limited to 1.5 nN. Step size was measured as the distance gain during a buckling step at the instantaneous force of the buckling transition. The raw step-size data were then sorted in increasing order, and a monotonously increasing sequential number *versus* distance curve was generated. From this curve, after normalization by dividing each sequential number with the maximal sequential number, the cumulative distribution function of the dataset was computed (**Figs. S1.a and c**). The smoothed derivative of the cumulative distribution function yielded the density function of the distribution which was fitted by five or three Gaussians without any preconception for the room-temperature (**Fig. S1.b**) or the 65-degree data (**Fig. S1.d**), respectively. Following numerical integration of all the fitted Gaussians we obtained the calculated cumulative distribution function (**Figs. S1.a and c**). Subtracting the cumulative distribution function of the dataset from the calculated one, we computed the residue which slightly fluctuated around zero, proving the goodness of fit.

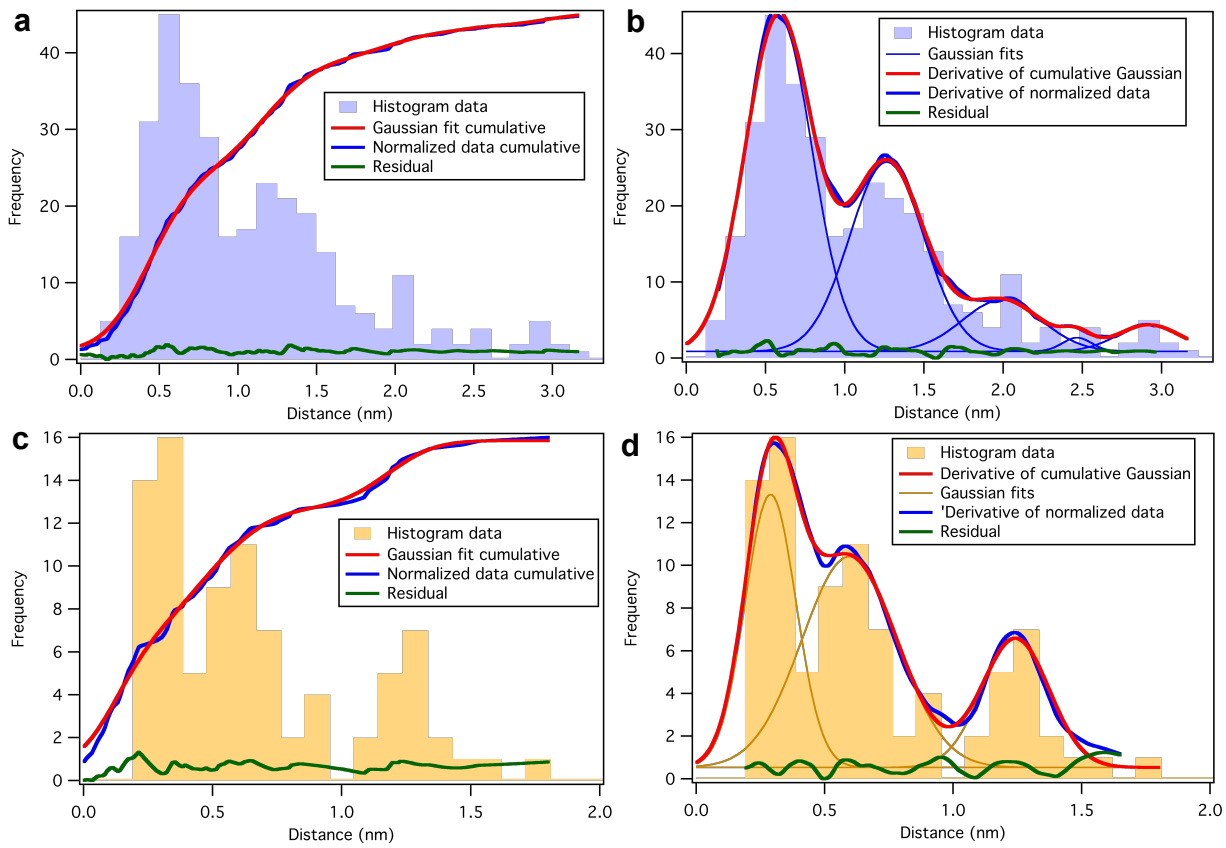

Figure S1. Analysis of the buckling step size data. **a.** The integrated Gaussian-fit data (red) overlaid on the normalized cumulative step-size dataset (blue) for room-temperature measurements.

Residual (green) is the difference of the two curves. Histogram data is highlighted in the background for reference. **b.** Gaussian fits (thin blue lines) and their sum (red) overlaid on the derivative of the normalized cumulative dataset (blue) for room-temperature measurements. Residual (green) is the difference of the two curves. **c.** The integrated Gaussian-fit data (red) overlaid on the normalized cumulative step-size dataset (blue) for 65-degree measurements. **d.** Gaussian fits (thin blue lines) and the derivative of their integral (red) overlaid on the derivative of the normalized cumulative dataset (blue) for 65-degree measurements.

2. Measurement of loading rates

Loading rate (r) is the instantaneous change in the loading force on the T7 capsids in the moment of the induced structural transition. We calculated the loading rate from the experimental reversible nanoindentation data by first plotting force (F) as a function of time (t), then finding the slope ($r=\Delta F/\Delta t$) of the curve just prior the buckling transition either in the forward (indentation) or reverse (relaxation) direction (**Fig. S2**).

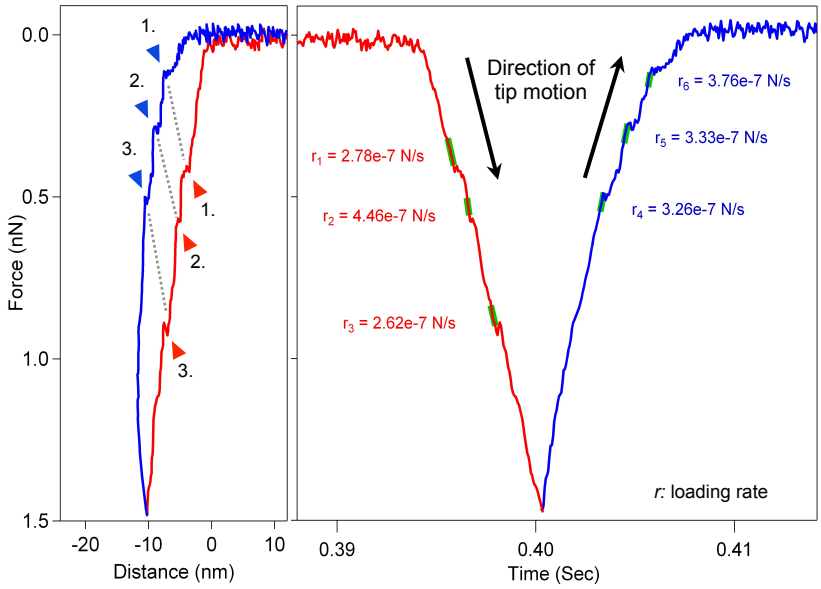

Figure S2. Measurement of of loading rate. **Left**, the first, second and third buckling transitions (labeled 1, 2 and 3) identified on the indentation force trace were matched with their corresponding reverse transitions on the retraction curve. **Right**, loading rates were measured on the force *versus* time traces independently for both the indentation and retraction traces as the slope of trace just prior the transition (green lines).

3. Dynamic force spectroscopy

By measuring the instantaneous forces of the buckling transition as a function of loading rate we carried out a dynamic force spectroscopy analysis. We analyzed the first three buckling transitions in both the forward and reverse directions. The transition force versus loading rate data (**Fig. S3**) were smoothed with the Savitzky-Golay filter (35-point window), then fitted with **equation 2** (see Materials and Methods in the main manuscript text). Although fitting could be carried out for the first two transitions, the smaller sample size and the large scatter in the data of the third transition prevented a reliable analysis. Coefficients (transition distance x_β and spontaneous rate k_{off}) of the fit are listed in **Table S1**. We note that fitting the reverse direction dataset requires a negative slope, which is only possible, with the logarithmic function of **equation 2**, if both x_β and k_{off} are negative.

Although fixing the coefficients as negative values allowed curve fitting, the negative signs carry no physical meaning other than the reverse direction of the reaction.

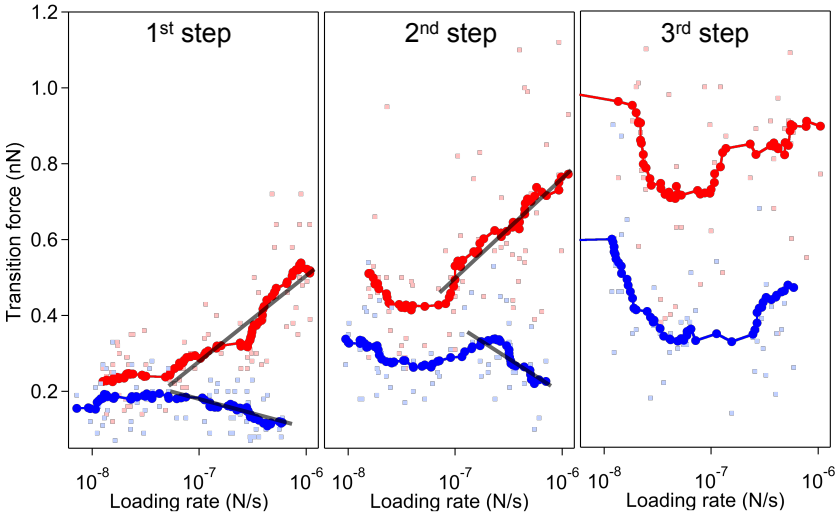

Figure S3. Dynamic force spectroscopy of the T7 capsid at room temperature. Transition forces as a function of loading rate during the forward (buckling, red) and reverse (relaxation, blue) transitions are shown for first, second and third steps.

	Transition distance (x_β)	Spontaneous rate (k_{off})
First step forward (buckling)	0.4 Å	52 s^{-1}
First step reverse (relaxation)	1 Å	7×10^5 s^{-1}
Second step forward (buckling)	0.4 Å	10 s^{-1}
Second step reverse (relaxation)	0.5 Å	2×10^5 s^{-1}

Table S1. Coefficients obtained by fitting the dynamic force spectroscopy data.

4. Geometrical estimation of force-driven T7 capsid buckling step size

Our data suggest that the nanomechanical buckling is a transition related to the pentameric unit (**Fig. S4.a**) of the T7 capsid. We speculate that buckling is initiated at or near one of the corners of the pentamer (**Fig. S4.b**), given that this is a protrusion on the capsid surface. This corner is bounded by the pentamer and two neighboring hexamers. Let us consider this convex triangular pyramid demarkated with blue contour in **Fig. S4.b top**. Because of buckling the pyramid inverses and becomes concave by chiral symmetry (**Fig. S4.b bottom**). The double of the height of the pyramid provides a geometrical estimation of the buckling step size. Using simple trigonometry (**Fig. S4.c**) this height is about 0.2 times the edge length. As the average capsid diameter is ~60 nm, the edge length is 7-8 nm. The estimated step size is thus ~3 nm, which is five times greater than the measured one (0.6 nm). Accordingly, the unit buckling step involves a structural transition that occurs along a fraction of the edge (**Fig. S4.d**).

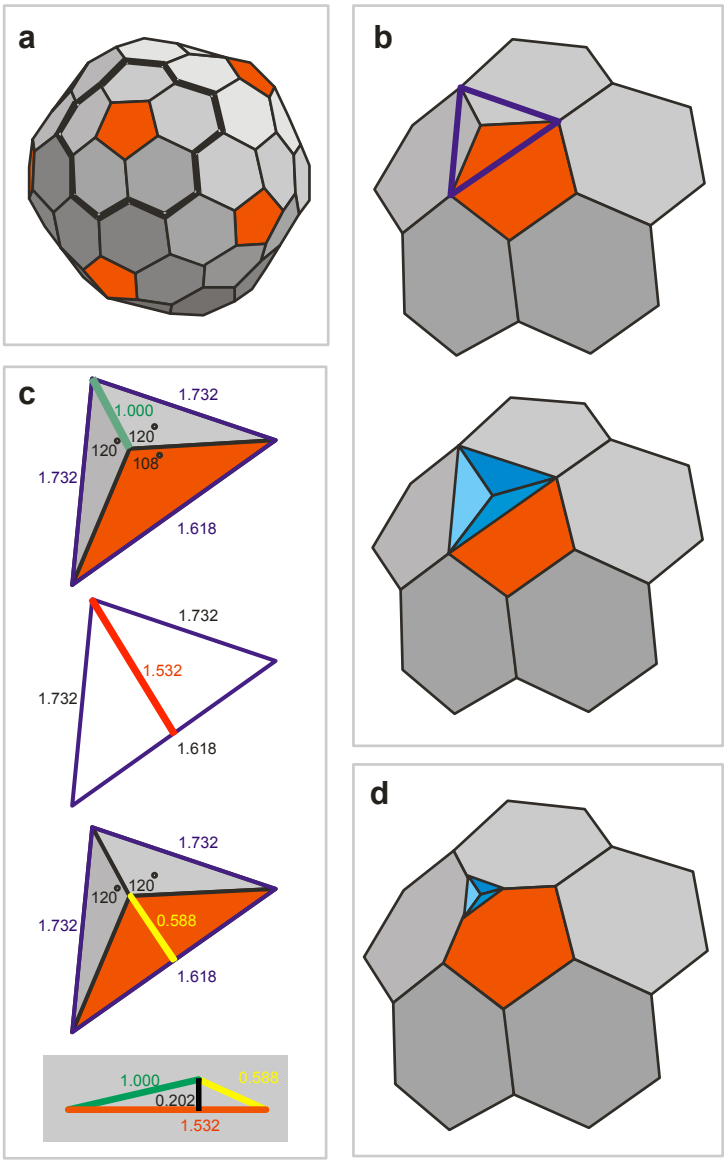

Figure S4. Geometrical estimation of the buckling step size. **a.** The icosahedral symmetry of the T7 phage. The pentameric unit with its associated hexameric capsomers is highlighted with thick black line. **b.** Outline of the protruding pyramid on the capsid surface in the relaxed (top) and buckled (bottom) configuration. **c.** Trigonometrical analysis of the buckling step size. The height of the convex pyramid is 0.2 times the length of the edge (bottom figure) according to simple triangle height calculations. **d.** Buckling by inversion of only a fraction of the entire convex pyramid.

5. Testing the effect of heat treatment on the nanomechanics of capsid proteins

The effect of 65 ˚C heat treatment on the nanomechanical behavior of the capsomeric proteins was inferred from a subset of room-temperature capsids which displayed a force response remarkably similar to heat-treated ones (**Fig. S5**). This subset of room-temperature virions have most likely lost their DNA due to spontaneous ejection. The fortuitous data subset allowed us to compare DNA-free capsids exposed to 65 ˚C heat treatment with ones that stayed at room temperature throughout the experiment. The overall similarity of the force curves collected on the DNA-free capsids exposed to the different temperatures indicates that heat treatmend at 65 ˚C did not cause a detectable change in the nanomechanical properties of the capsid proteins.

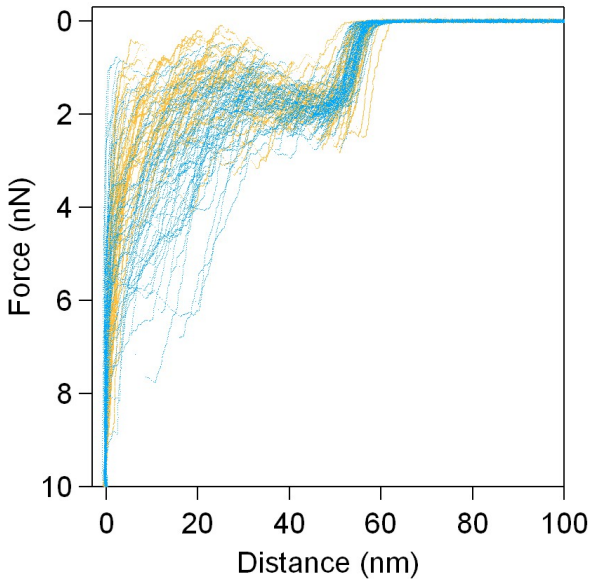

Figure S5. Force versus indentation distance curves obtained on a subset of room-temperature capsids (blue, n=55) and heat-treated capsids (65 ˚C, yellow, n=40).