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Supporting Material

An Unusual Hydrophobic Core Confers Extreme Reversible Flexibility To HEAT Repeat Proteins

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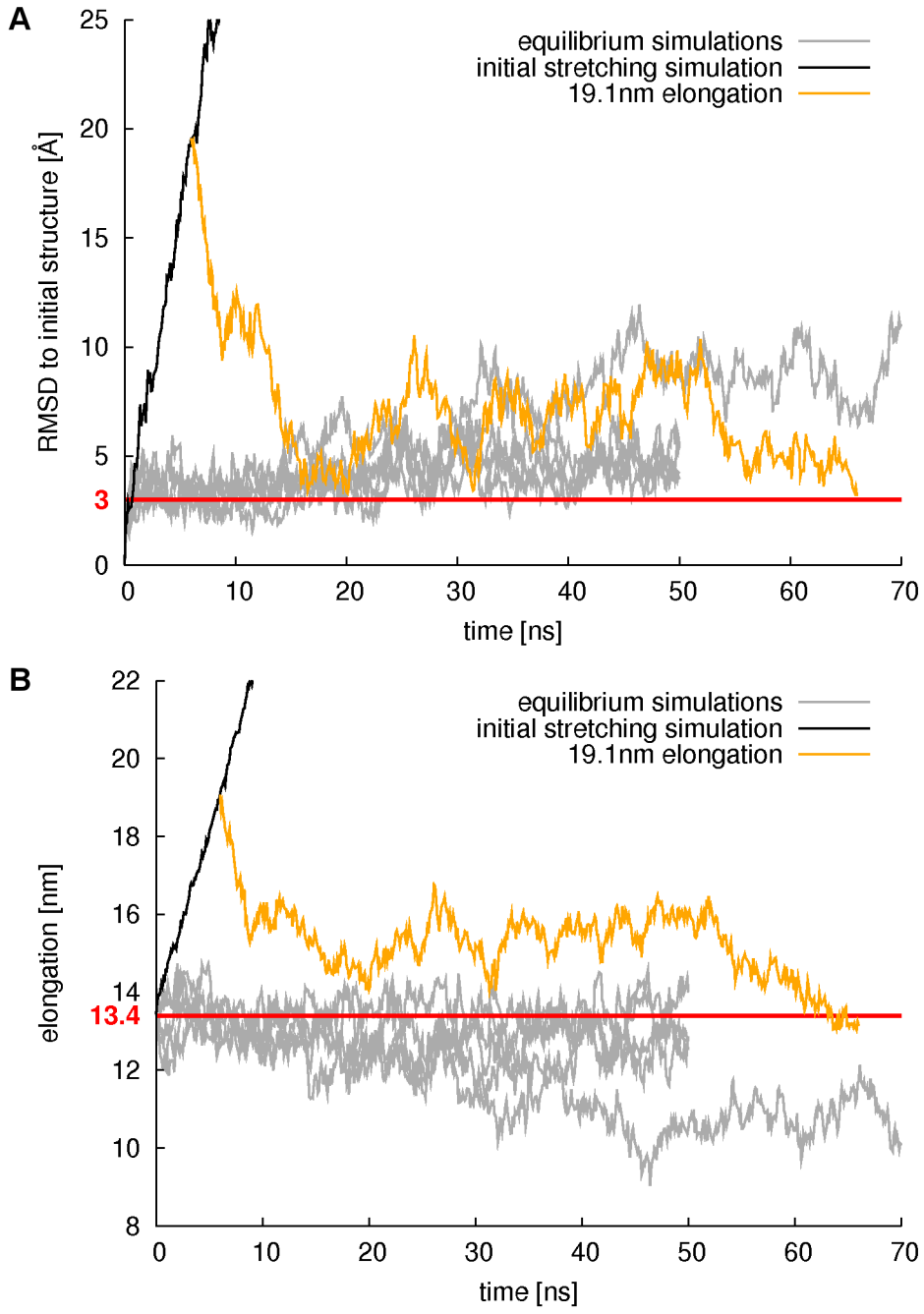

Figure S1: Longest observed relaxation process of yImpβ.
(A) Root-mean square deviation (RMSD) of backbone atoms from the initial pulling simulation (black line) and from one subsequent relaxation simulation (orange line). Gray lines show RMSD curves from five equilibrium simulations.
(B) End-to-end distance of yImpβ during stretching and subsequent relaxation together with end-to-end distances of yImpβ from equilibrium simulations. The same colors are used as in panel (A). The red line at an elongation of 13.4nm denotes the initial elongation of the free unbound protein.

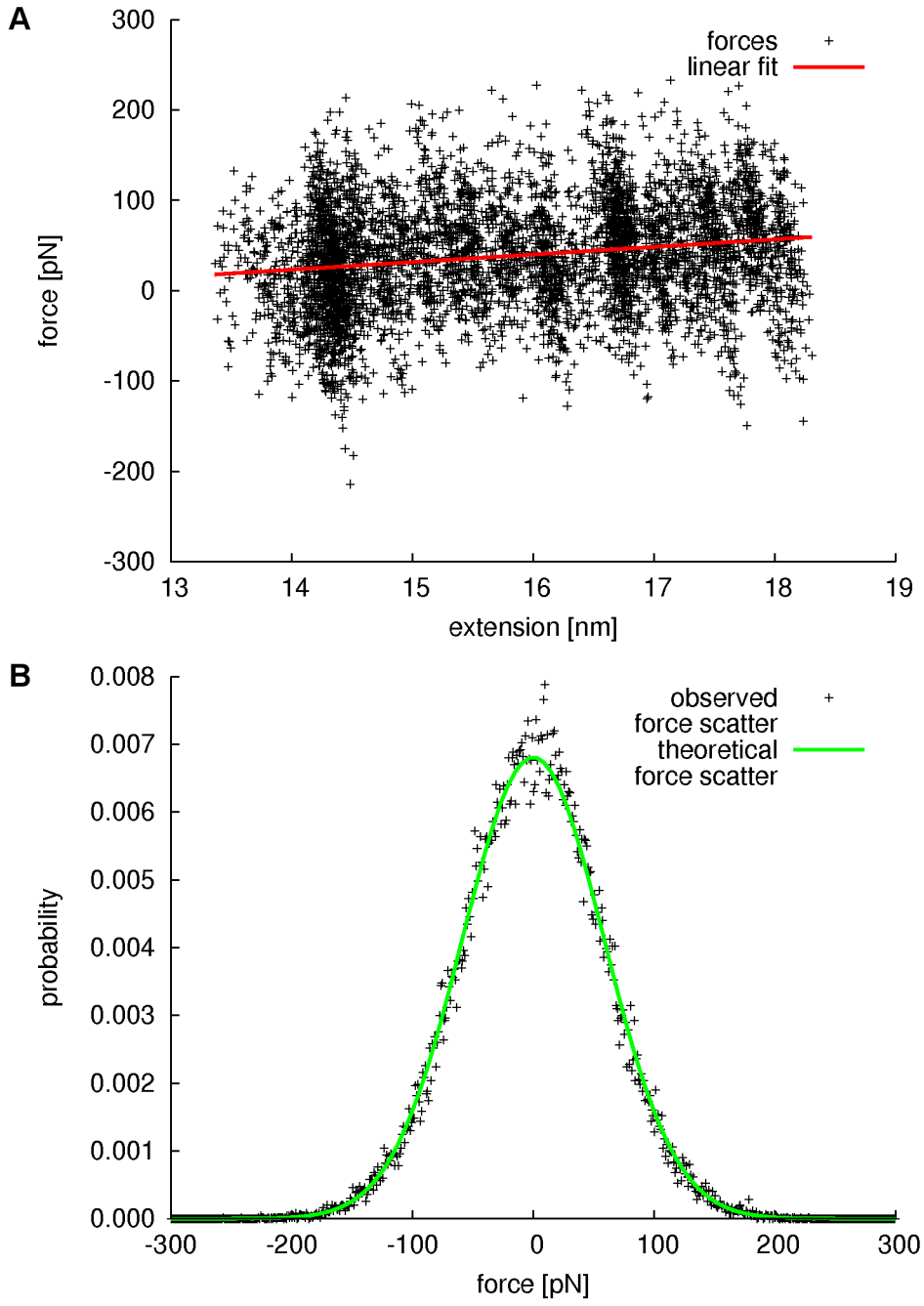

Figure S2: Analysis of scatter from stretching forces.
(A) Typical force profile obtained in stretching simulations of yImpβ (+, black) with linear fit (red line).
(B) Histogram of scatter around the linear fit of the forces shown in (A) (+, black) . The green line shows the expected Boltzmann distribution of forces according to $\frac{1}{\sqrt{2\pi}\,\sigma}\exp\left(\frac{-F^2}{2\sigma}\right)$ with $\sigma=\sqrt{k_B T k}$ for a spring constant of k=0.83N/m as used in the simulations.

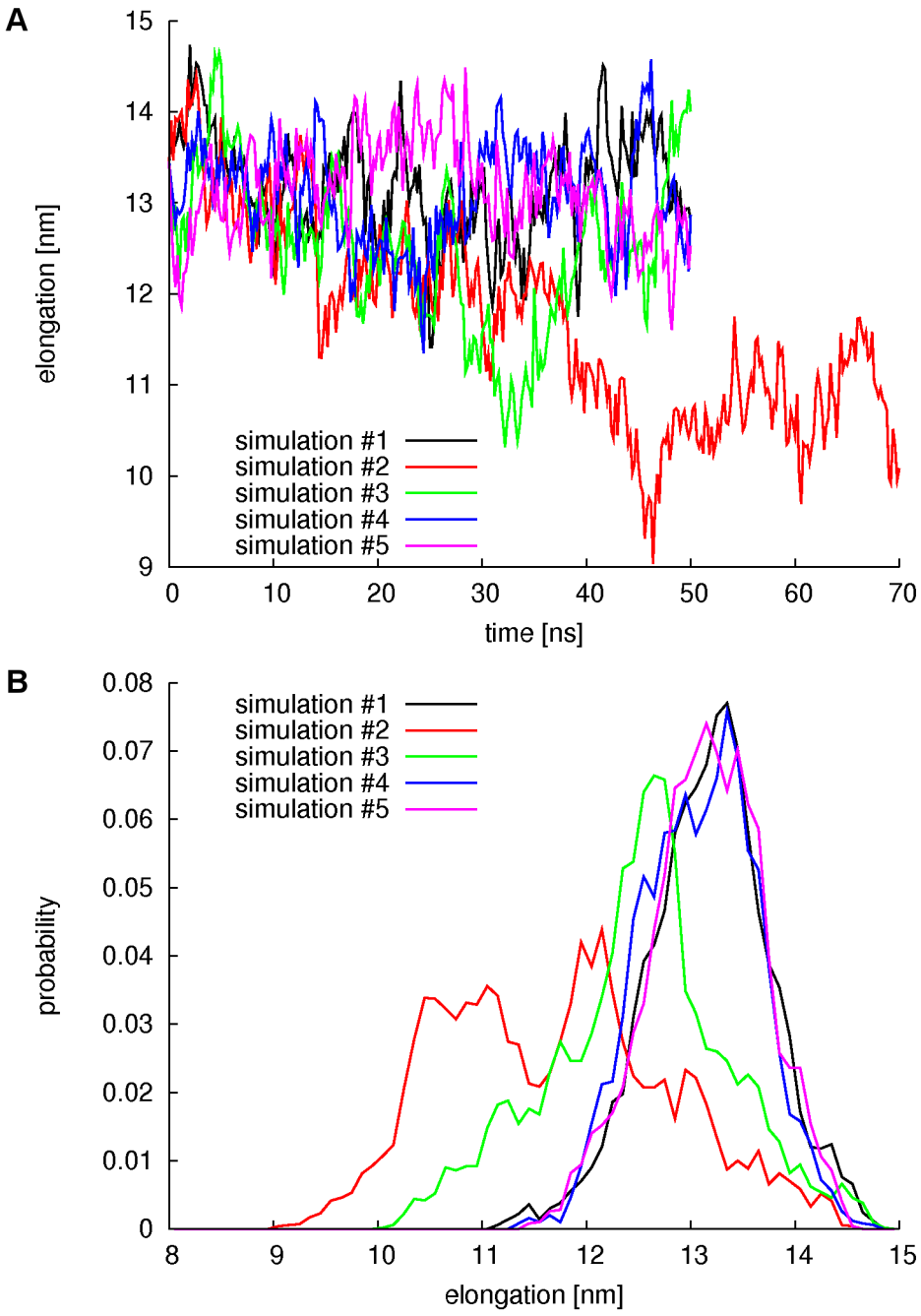

Figure S3: Fluctuations of elongation observed in five equilibrium simulations and their distributions.
(A) Elongation as a function of time for five equilibrium simulations. Simulation #2 (red line) was extended, demonstrating that the low value around 50ns corresponds to a fluctuation rather than a transition to a state with lower elongation. Fluctuations in all four other simulations remain closer to the equilibrium elongation.
(B) Histogram of equilibrium elongations of yImpβ. Colors are the same as in panel (A).

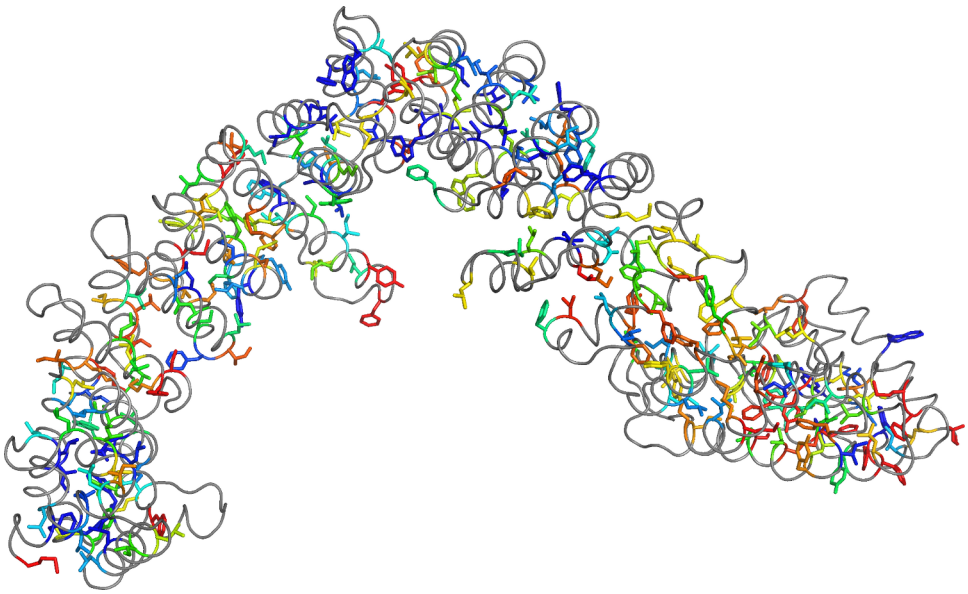

Figure S4: Side chain flexibility of all hydrophobic residues in yImpβ. The color code is the same as in fig. 4 in the main paper.

Table S1

Table of stretching simulations:

stretching velocity [m/s]	simulation lengths [ns]
0.08	**3x62.5**
0.1	**3x50**
0.2	**3x25**
0.3	**3x17**
0.4	**3x12.5**
0.5	**1x10 + 3x30**
0.8	**1x6.25 + 2x18.75**
1.0	**3x15**
1.5	**3x10**
2	**3x7.5**
3	**3x5**
5	**1x1 + 2x3**
10	**1x0.5 + 2x1.5**
20	**1x0.25 + 2x0.75**

total simulation time (stretching): **739.5ns**

Relaxation simulations:
from v=0.1m/s, d=15.1nm: **3x20ns**

from v=0.1m/s, d=17.3nm: **20ns + 30ns + 40ns**

from v=1m/s, d=15.3nm: **10ns + 2x20ns**

from v=1m/s, d=17.1nm: **10ns + 2x20ns**

from v=1m/s, d=19.1nm: **20ns + 40ns + 60ns**

from v=1m/s, d=21.0nm: **30ns + 2x40ns**

total simulation time (relaxation): **480ns**

Equilibrium simulations:

4x50ns + 1x70ns

total simulation time (equilibrium): **270ns**

Restrained elongation simulations (entropy estimation):

7x50ns

total simulation time (restrained elongation): **350ns**

Simulation time overall : **1839.5ns**