Supplementary Materials for Viscoelasticity of a single poly-protein probed step-by-step during its mechanical unfolding and refolding under the force-clamp conditions

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Supplementary Text

Estimations of global friction coefficients for I27

A recently proposed equation for calculating the global friction coefficient γ_g in $\alpha-$helical peptides is: (*21, 22*)

$$\gamma_g = \frac{k_B T}{\sum_i \Delta x_i^2} \left(\frac{1}{k_{\text{fold}}} + \frac{1}{k_{\text{unfold}}} \right) \tag{S1}$$

where k_{unfold}, k_{fold} are unfolding and folding rate constants, respectively, and Δx_i is the ensemble-averaged change in the end-to-end distance of the peptide after passing from i to $i+1$ hydrogen bonds along its folding trajectory. The global friction coefficient corresponds to a major conformational transition, such as complete (un)folding.

We apply Eq. 1 to calculate γ_g in the case of I27 protein. Its unfolding and folding rate constants have been already measured and estimated in variety of experimental studies. Taniguchi et al. reported the largest unfolding constant of k_{unfold} of $2 \times 10^{-3} s^{-1}$, (*44*) while earlier studies found smaller unfolding rates of $4.9 \times 10^{-4} s^{-1}$ through the chemical method extrapolated to 0M denaturant, (*15*) and of $3.3 \times 10^{-4} s^{-1}$ from AFM data extrapolated to zero force. (*45*) Recent FX-AFM studies using the $I27_8$ construct in PBS obtained k_{unfold}, at zero force, as $3.0 \times 10^{-4} s^{-1}$. (*28*) Recent unfolding kinetics studies at ambient pressure via NMR obtained k_{unfold} of 2.97 ± 0.48 and $2.13 \pm 0.19 \times 10^{-4} s^{-1}$ for single and two I27 modules in tandem, respectively. (*31*) Reported values of k_{fold} are 1.2 s^{-1} or more, (*45, 46*) which means that global friction is dominated by the unfolding rate, due to its much smaller value.

Thus, we estimate a lower bound on γ_g by using the largest reported k_{unfold} and Δx of 24 nm. The latter is obtained as difference between an end-to-end distance for unfolded and stretched I27 monomer (d_u = 28.5 nm at 190 pN stretching force) and an end-to-end distance for folded I27 (d_f = 4.5 nm). Consequently, we obtain $\gamma_g = 3.56 \times 10^{-3}$ kg/s for a monomer of I27, $\gamma_g = 8.91 \times 10^{-4}$ kg/s for a dimer $I27_2$ with Δx of 48 nm, and $\gamma_g = 2.23 \times 10^{-4}$ kg/s for $I27_4$ with Δx of 96 nm. The last value is still three orders of magnitudes higher than experimental data reported in the main paper. Thus, while the model is applicable to α-helical peptides, where HBs were good predictors of the peptide state, it overestimates internal friction in the case of I27. This is likely due to the fact that I27 is larger than peptides and also $\beta-$type, so that formation/rupture of its hydrogen bonds do

not have as direct effects on the end-to-end length as in the case of $\alpha-$type peptides.

As a corollary, local unfolding of a short N-terminal $\beta-$sheet has been proposed (*26*) and observed (*15, 47*) as the starting point for mechanical force initiated I27 unfolding. This is an intermediate state (I) along the unfolding pathway. From high-speed FX-SMFS with AFM Rico et al. (*48*) has estimated unfolding and folding rate constants for this intermediate as $k_{\mathrm{I,fold}} = 7 \times 10^3$ and $k_{\mathrm{I,fold}} = 4 \times 10^5\ s^{-1}$, respectively. Using these values and an end-to-end extension of $\delta x = 0.6$ nm, (*31*) one obtains $\gamma_g = 1.66 \times 10^{-6}$ kg/s, which is still one order of magnitude higher than any fluctuations in the friction coefficients observed within our FQ-AFM experiments.

Supplementary figure: power spectra

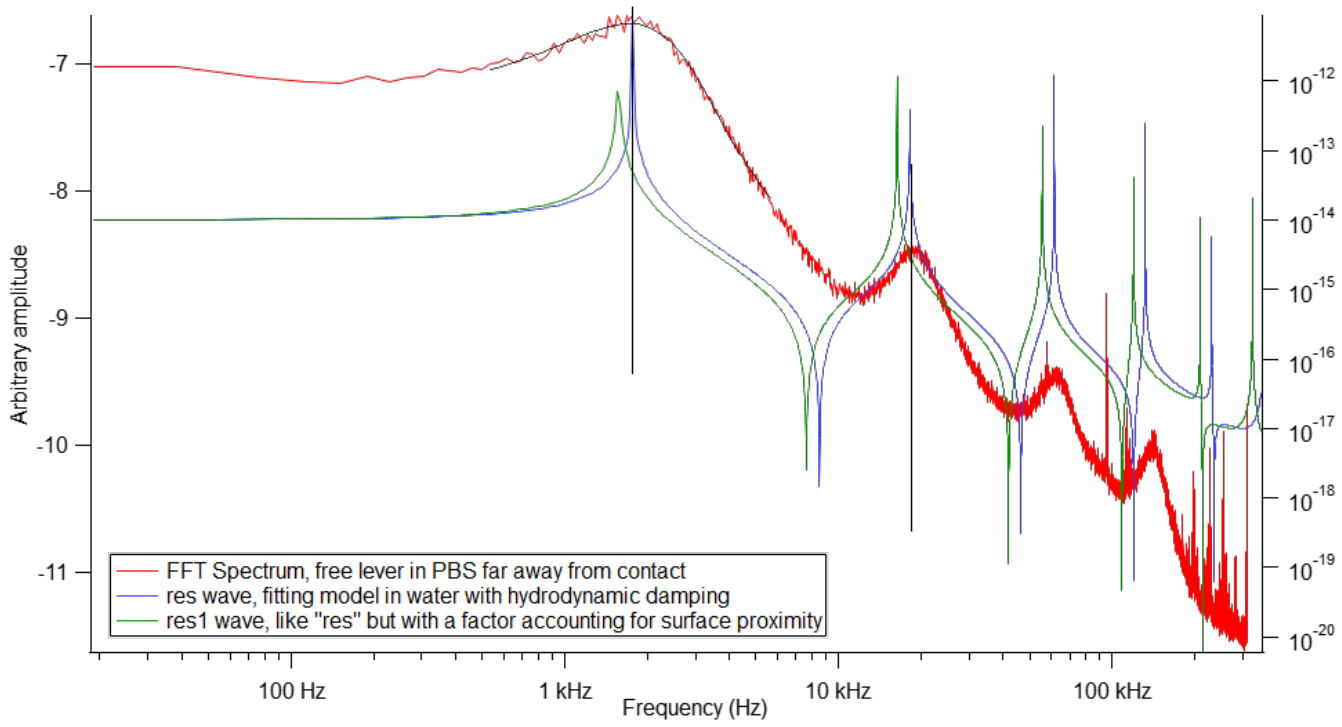

Figure S1: **Some related power spectra**.