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Supporting Information

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for

Hierarchical Mechanochemical Switches in Angiostatin

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Amount of analyzed data: The number of analyzed data (individual peaks) for each reduction state (I-II-III, see text for explanation) and pulling speed (v) is as following:

v = 100 nm/s	State I = 38	State II = 12	State III = 11
v = 900 nm/s	State I = 140	State II = 60	State III = 54
v = 1900 nm/s	State I = 120	State II = 86	State III = 70
v = 2700 nm/s	State I = 107	State II = 74	State III = 61
v = 5600 nm/s	State I = 48	State II = 42	State III = 20

Monte Carlo simulations: Monte Carlo simulations of the stretching experiment were performed on the basis of ref. [23] (see text) with a time step of 10^{-5} s and a protein composed of two modules (the case of three unfolding modules, although observed, is very rare in our experiments). Three different contour length were used, corresponding to the three levels of reduction.The dependence of the unfolding force on the pulling speed was simulated at the same five different pulling speeds used in the experiment. The single stretching cycle was repeated 500 times for each set of conditions.

The meeting between simulated and experimental speed dependence was obtained in a two-step procedure. First, the barrier position leading to the same slope in the linear dpendence was identified. This is possible since the slope only depends from the barrier position. Second, once identified the correct slope, the intercept ensuring the best fit of the data was identified.

The Monte Carlo analysis has been used for fitting of the linear force dependence, but it was impossible to fit the single experimental force histograms with that obtained by mean of Monte Carlo. In Figure S1 (see below) it is possible to see that their shapes cannot be superimposed. Forces lower than 50 pN are most likely under represented because they can be hidden in the force curve thermal noise. It is possible that multiple detachment peaks that could have not been distinguished from genuine unfolding peaks might have been included in the tail of forces higher than the most probable one. Moreover the dishomogeneity due to the nonidentical structures of the domains can have altered the width of the distribution. For these reasons one cannot get safe informations on the energy landscape from the shape of the unfolding forces distributions only.

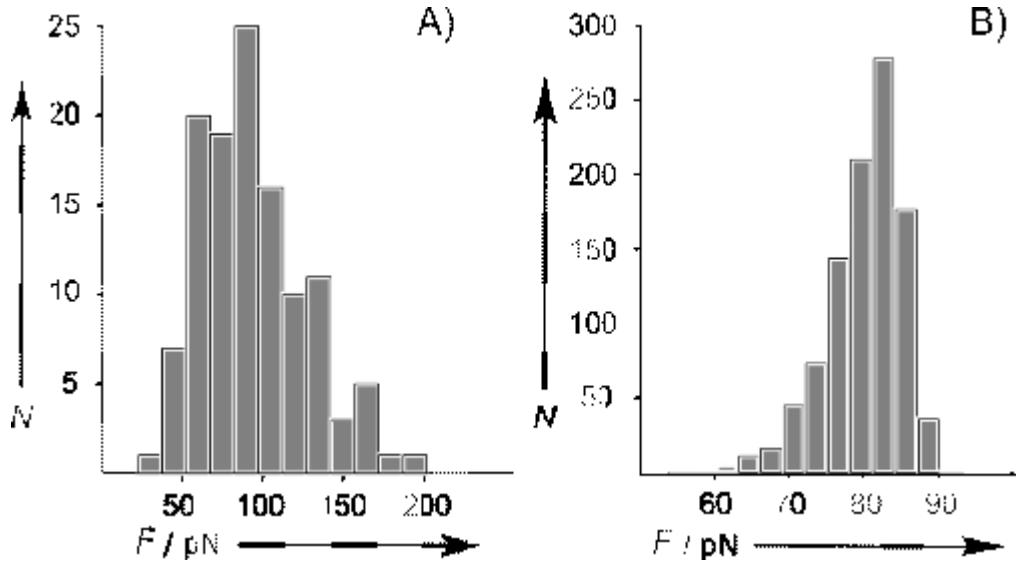

Figure S1. One example of the distributions of the unfolding forces obtained from the unfolding of K1 5 (A), compared with the distribution one should expect from a typical force spectroscopy experiment (B), obtained by Monte Carlo simulation. The distribution of the experimental data appears to be wider than that simulated and it also shows a different shape.