Supporting Information

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SI Text

The EKEK Motif Introduces a Folding Barrier into a Canonical Coiled Coil. As shown in the section *Segment I Is a Nondividable Subdomain* in the main text, the introduction of the EKEK-motif into the canonical LZ26 coiled coil (Fig. 4*A*) leads to a local destabilization of the structure by 10.6 k_BT (Fig. 4*B*). Additionally, insertion of the EKEK sequence causes a second effect: It shifts the coiled-coil folding locally away from thermodynamic equilibrium. This can be seen in Fig. S2 *a* and *c*, where averaged forward (black) and backward (blue) force traces measured at two different pulling velocities are shown. Already at relatively slow pulling velocities of 150 nm/s (Fig. S2*a*) two hystereses (yellow areas) can be observed, one between the states [4] and [3] and one between states [3] and [2]. The hysteresis between states [4] and [3] results from the seed formation process of the LZ26 zipper and is discussed elsewhere (1), whereas insertion of the EKEK-motif close to position [3] causes a second hysteresis that grows with increasing pulling velocity (compare Fig. S2 *a* and *c*). The LZ26 coiled coil, without any modifications at this position, folds after nucleation seed formation in thermodynamic equilibrium without dissipating further energy to the heat bath. The hysteresis between states [3] and [2] allows us now to map the corresponding energy barrier. The energy landscape shown in Fig. S2*d* together with the described Monte Carlo simulation reproduces the experimental measured data (See red traces in Fig. S2 *a* and *c*). The energy landscape consists of energy parameters known from LZ26 unzipping experiments (1) and only the parameters associated with the transition [2] ⇄ [3] are adjusted to fit the EKEK-LZ26 data. The size and shape of the refolding barrier at position [3] is similar to the one associated with formation of the native segment I within the DmK-LZ10 construct (see Fig. 3*D*). Thus, the folding barrier within the native DmK coiled coil comprises of two different contributions: The formation of the C-terminal nucleation seed and slow folding kinetics of the noncanonical EKEK motif.

1. Bornschlögl T, Rief M (2008) Single-molecule dynamics of mechanical coiled-coil unzipping. *Langmuir* 24(4):1338–1342.

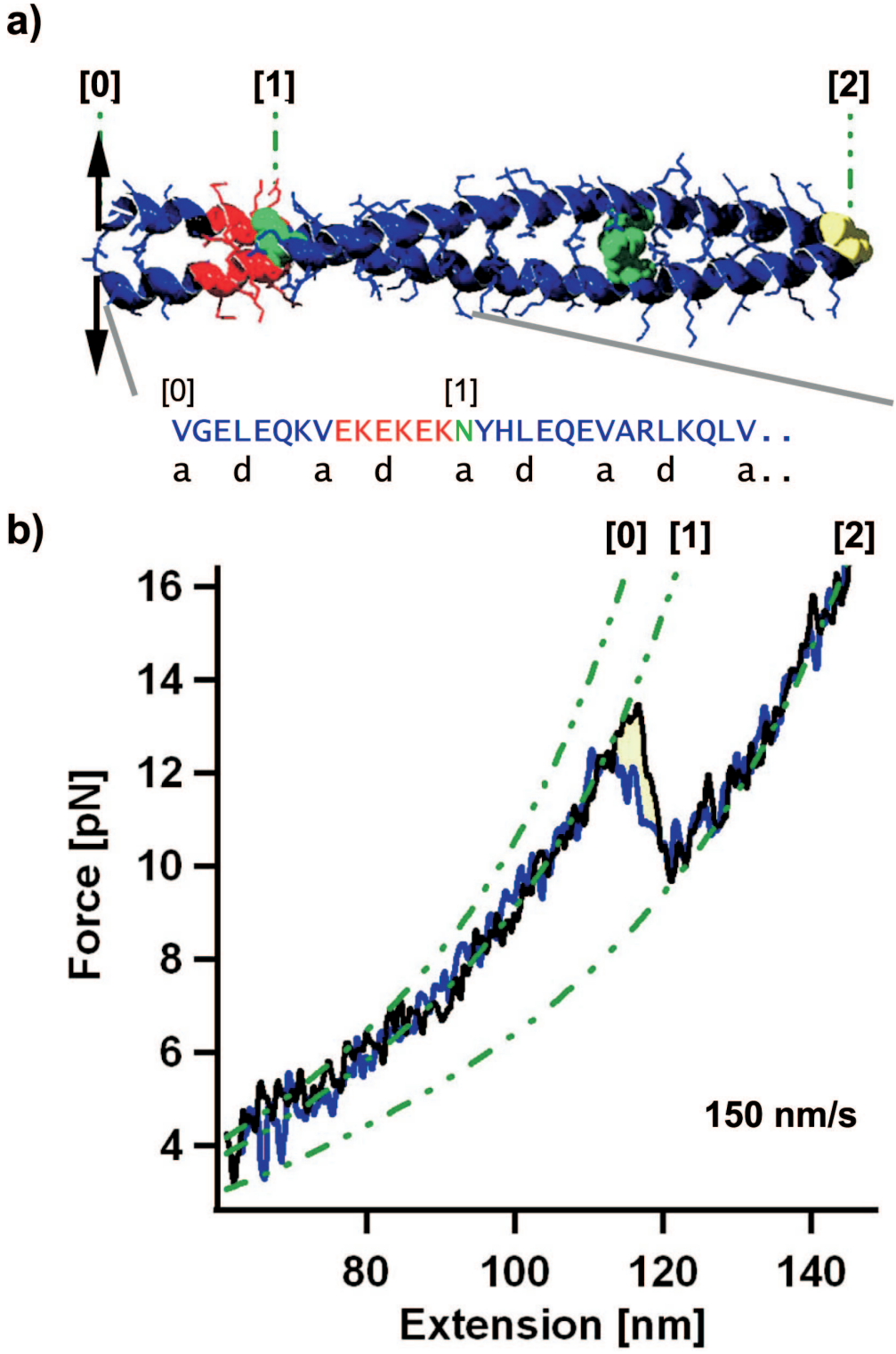

Fig. S1. N-terminal addition of two coiled-coil turns onto the EKEK sequence does not lead to a stable structure. (*a*) Schematic structure for the designed coiled coil if it is fully folded. At the N terminus of the EKEK sequence 8 amino acids from a canonical coiled coil are added. The complete sequence of the resulting structure is: VGELEQKVEKEKEKNYHLEQEVARLKQLVGELEQKVEELLQKNYHLEQEVARLKQLVGEC. (*b*) Averaged force traces at a velocity of 150 nm/s. The green dotted line [0] denotes the expected WLC elasticity trace for a totally folded coiled coil. The measured contour length increase between the traces [1] and [2] is connected to the unfolding of only the C-terminal part of the coiled coil following the EKEK sequence. Thus the N terminal part before the EKEK sequence is not stable at forces higher than 5 pN.

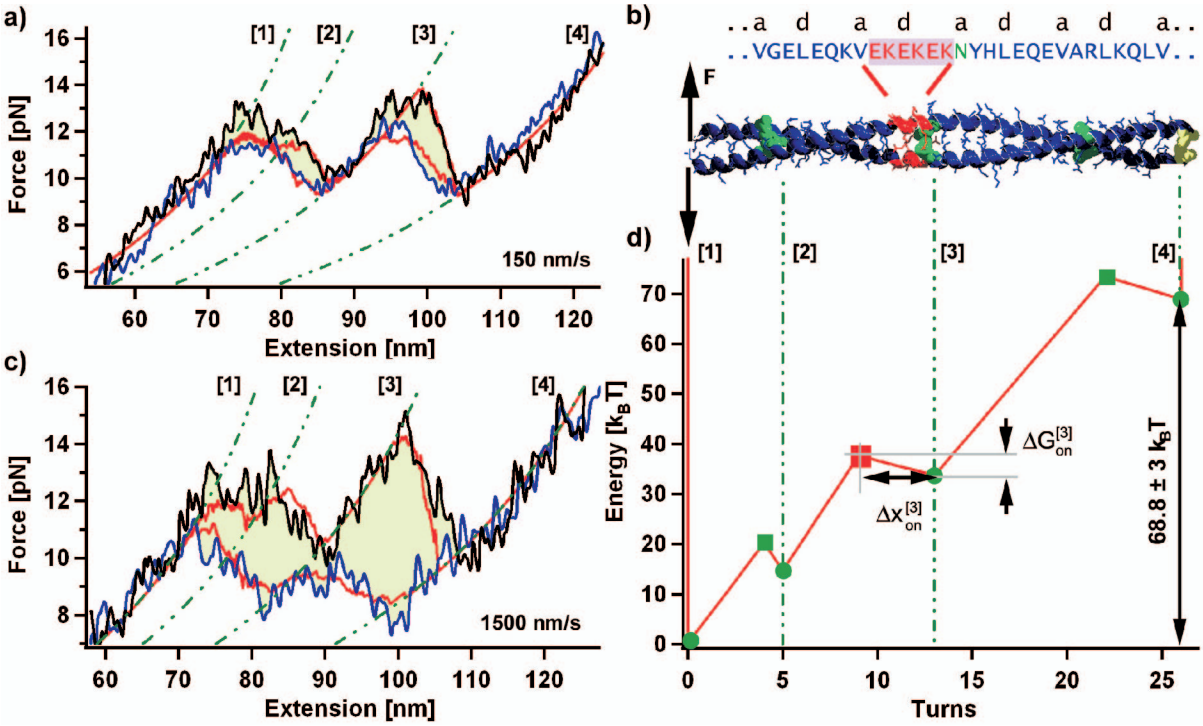

Fig. S2. Mapping the folding barrier associated with the insertion of the EKEK motif into a canonical coiled coil. (*a* and *c*) Averaged forward (black) and backward (blue) force traces from the EKEK-LZ26 coiled coil measured at pulling velocities of 150 nm/s (*a*) and 1500 nm/s (*c*). (*b*) Schematic structure of the EKEK-LZ26 construct with the surrounding amino acid sequence. (*d*) Energy landscape that reproduces via the Monte Carlo simulation the experimental force traces. The folding barrier between states [3] and [2] determines the force dependence of the hysteresis within the force traces between these states and can thus be mapped to be $\Delta G_{on}^{[3]} = 4 \pm 1\ k_BT$ and $\Delta X_{on}^{[3]} = 4 \pm 1$ turns.

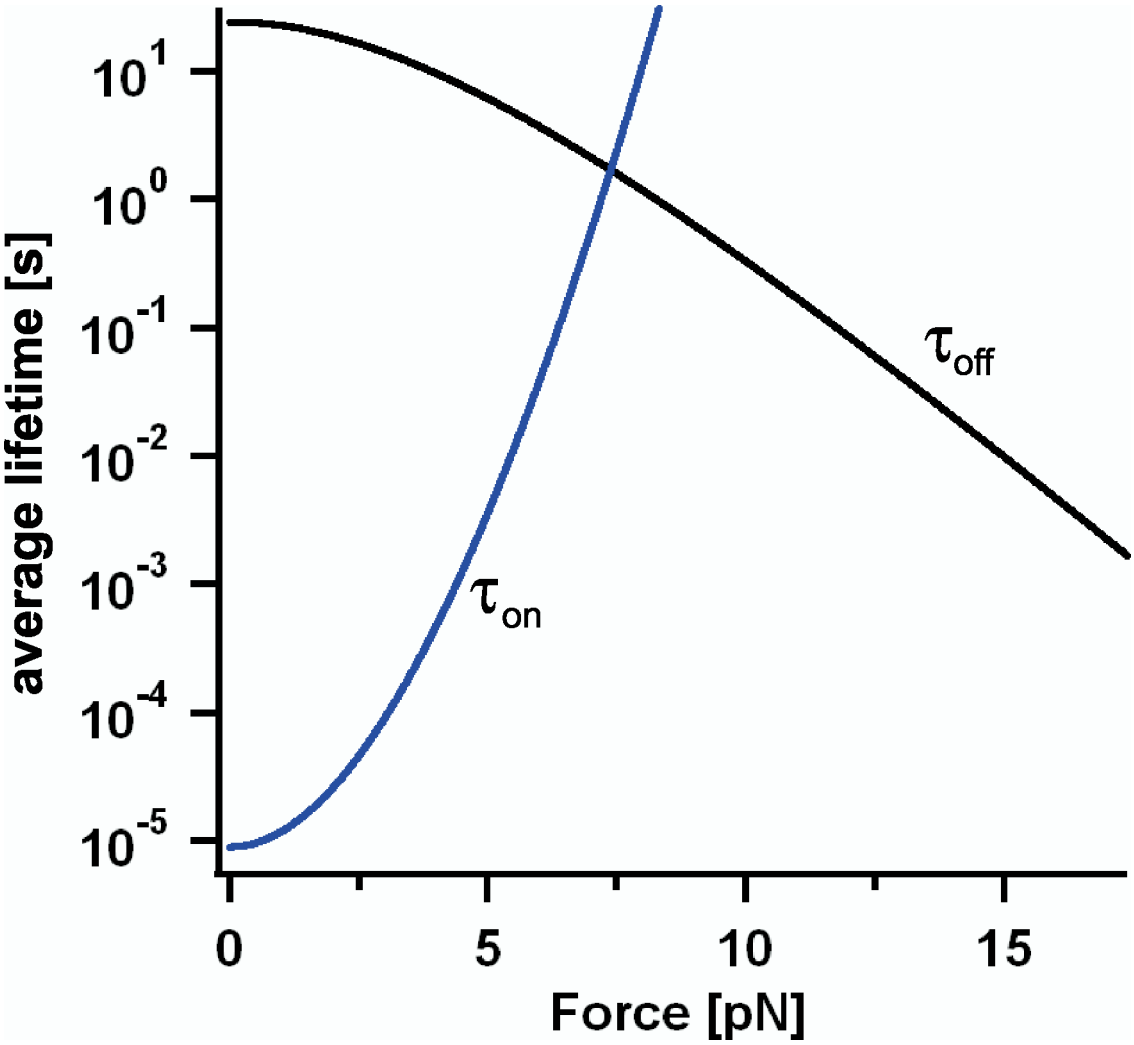

Fig. S3. Average folding times τ_{on} of the unfolded DmK neck (blue) and average unfolding times τ_{off} of the folded DmK neck (black) in dependence of the applied force. With increasing force, the folding times increase while unfolding times decrease. The deviations from simple exponential behavior at low forces is due to a force-dependent shift of the transition state position due to the elastic properties of the polypeptide. The intersection of both graphs gives the equilibrium unfolding force of the neck, if pulled infinitely slowly.