Electronic Supplementary Information for: Microtubule self-healing and defect creation investigated by in-line force measurements during high-speed atomic force microscopy

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Time distribution of self-healing events

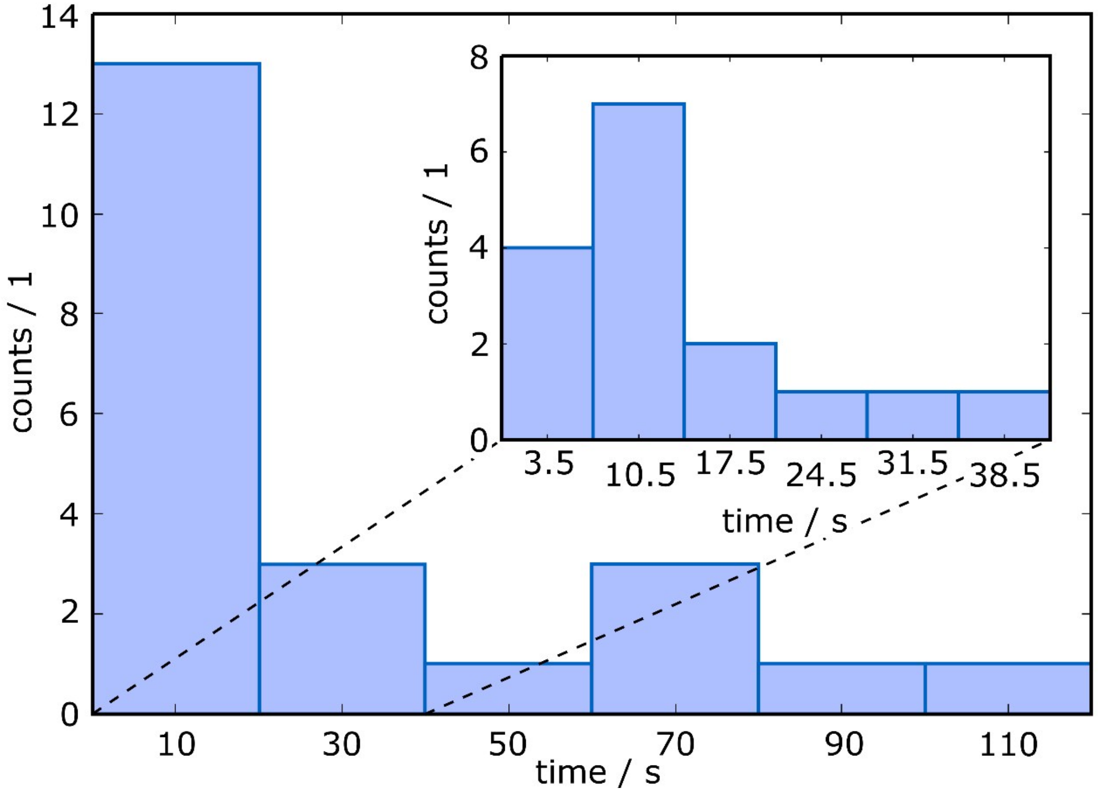

Figure S1: Histogram of the time between defect creation and a single self-healing event. The histogram was constructed of altogether 22 measurements. In the inset the distribution of healing time < 42 s is shown, which consists of the majority of data (16 measurements). Note that in the main article, 466 defects were created and only 9 self-healing events were observed. Additional data were taken from other experiments, where no force curves were recorded.

Comparison of simulated defects with experiments

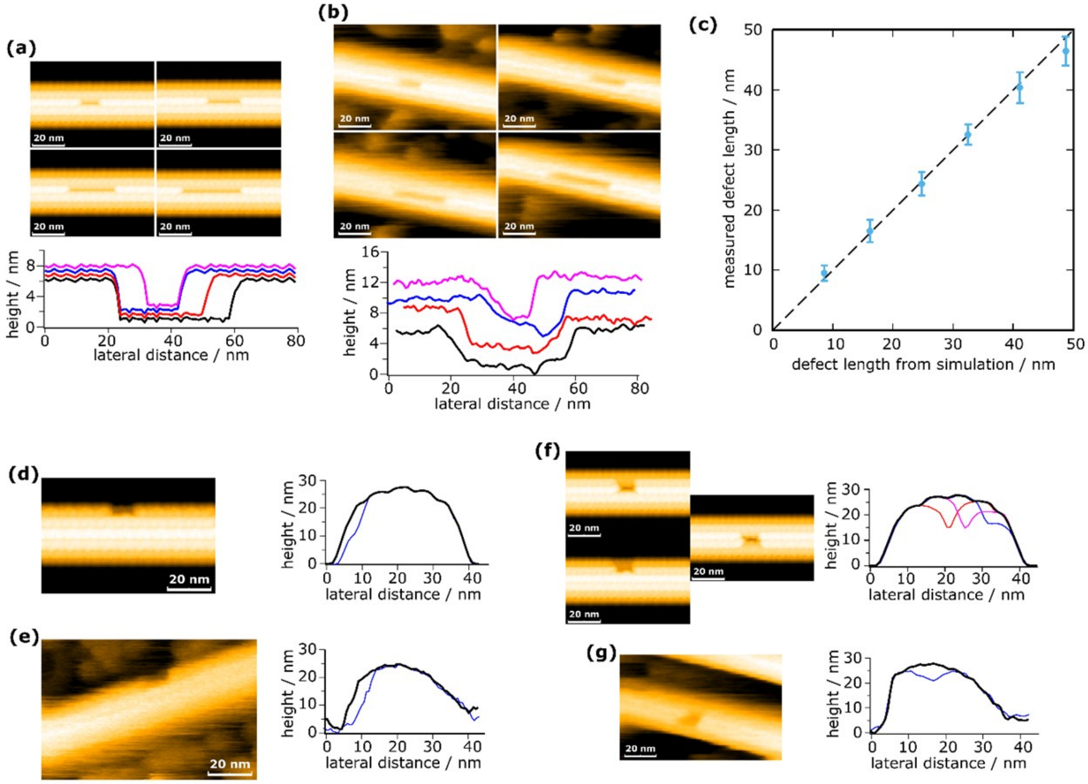

Figure S2: Comparison of simulated and experimental AFM images and line profiles. Simulations (a,d,f) and experiments (b,e,g) compare well. (c) Defect length measured from HS-AFM images plotted against the defect length of simulated AFM images. The dashed line indicates the case where simulations and experiments are identical. The error bars are the standard deviations.

Exemplary force curves

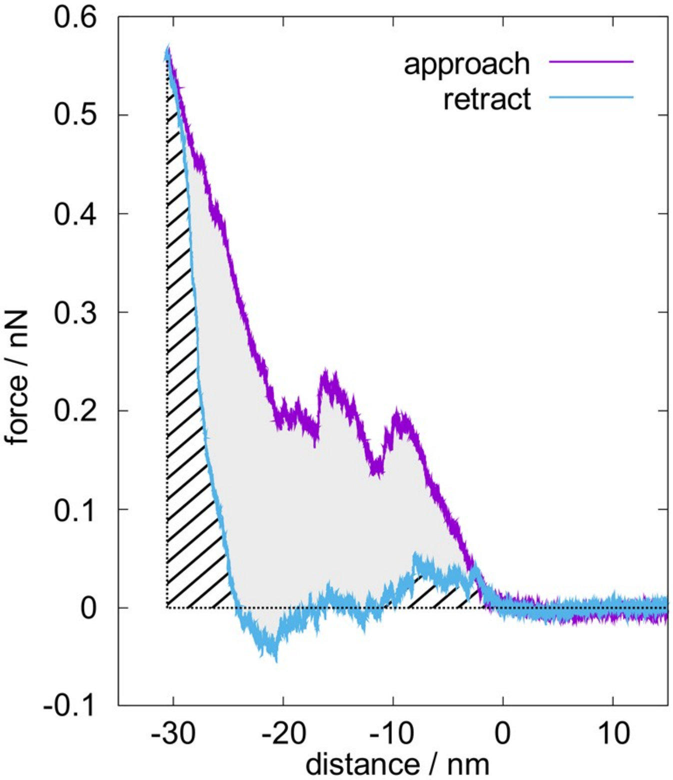

Figure S3: Exemplary force curve performed on a microtubule. The hatched area marks the energy restored during unloading of the microtubule. The solid gray area identifies the energy that was not recovered upon unloading.

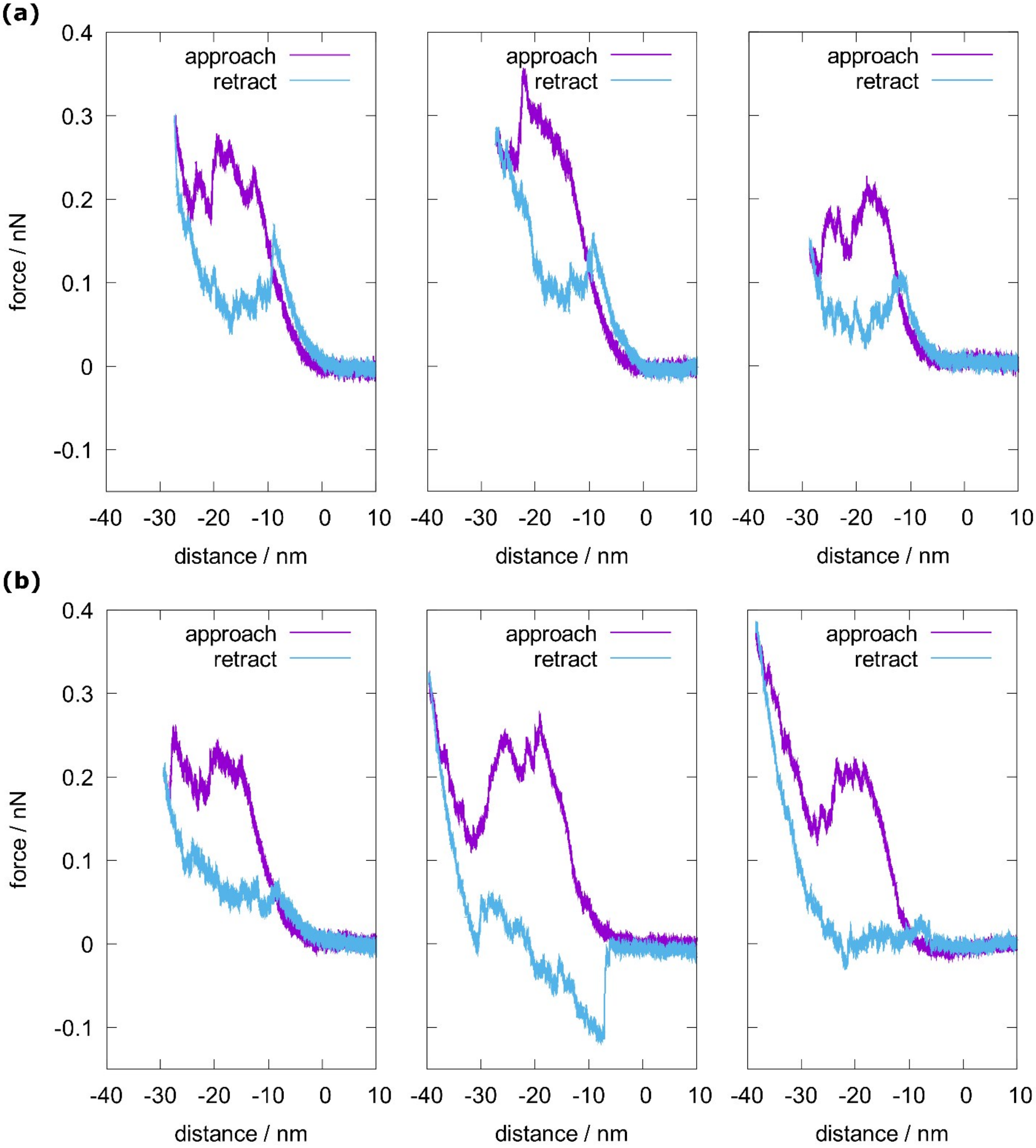

Figure S4: Exemplary force curves. Force curves for whose (a) reversible and (b) irreversible deformation is observed.

Healing of defects

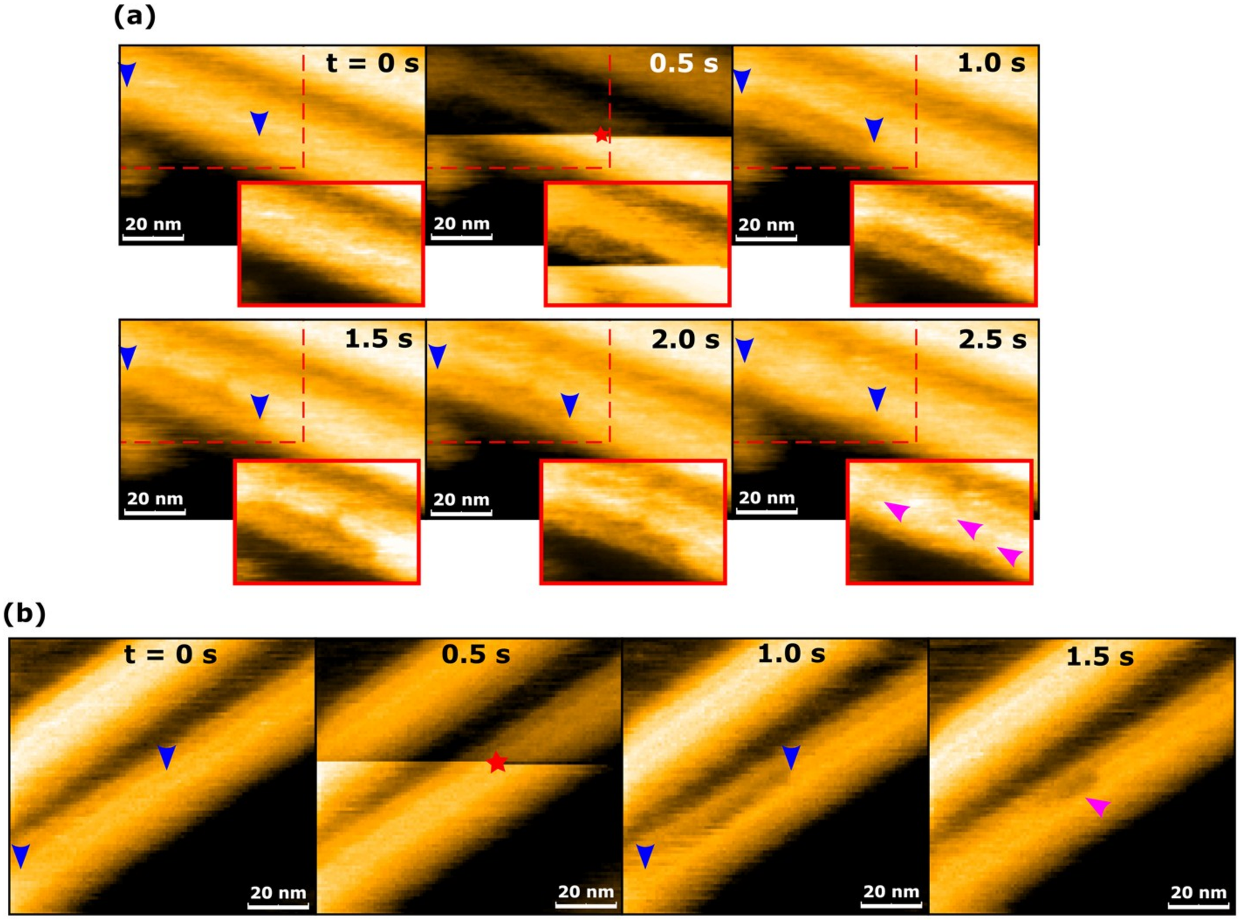

Figure S5: Removal of protofilament segments. A protofilament segment (indicated by blue arrows) is removed at 0.5s (red star) (a,b) and is healed at 2.5 s(a) and 1.5 s (b). The protofilament segments are healed, but unconnected defects remain (magenta arrows). The insets show the areas marked with a dashed rectangle with changed contrast to enhance the created defects.

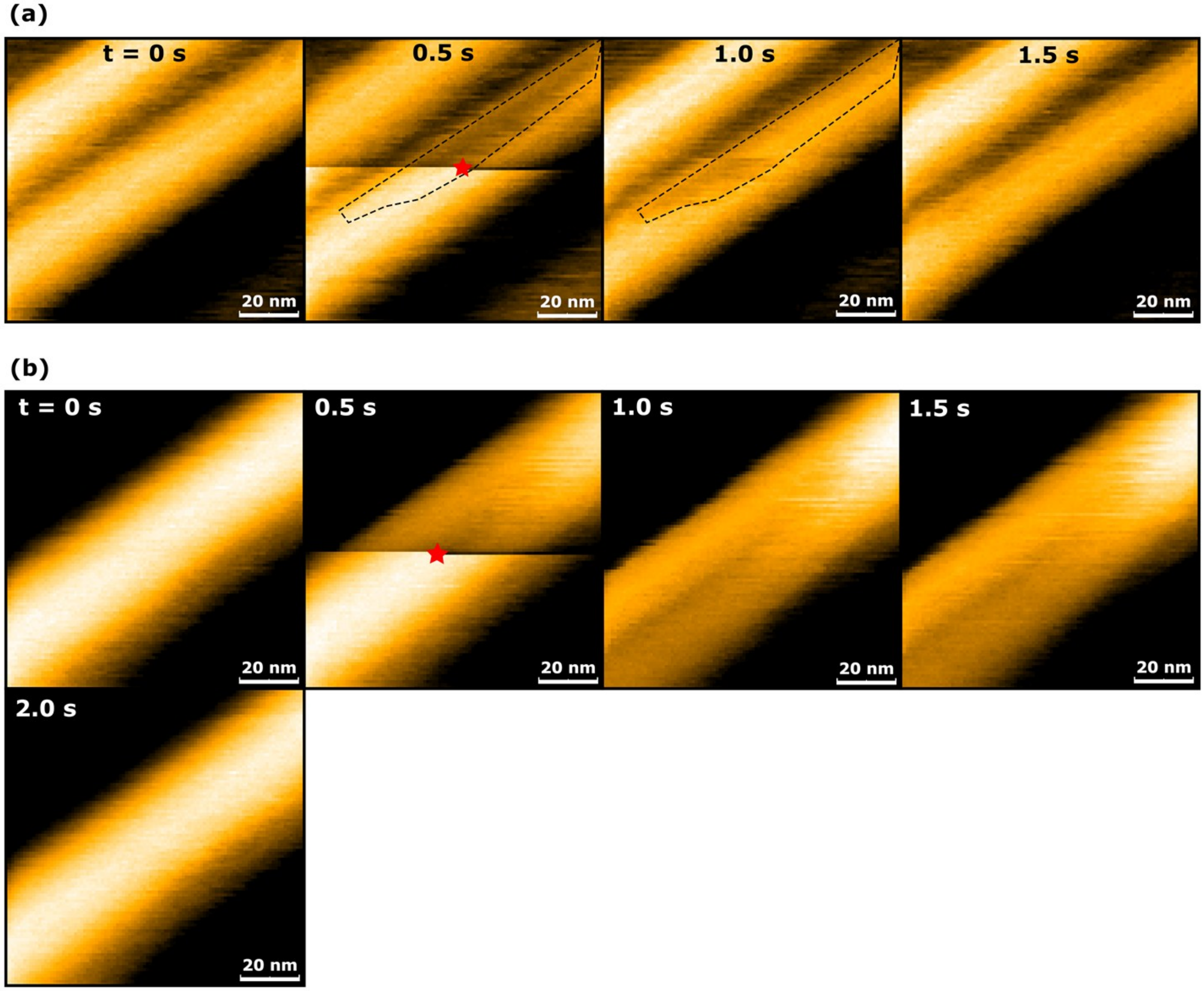

Figure S6: Healing of microtubule chunks. (a) A chunk spanning two protofilaments is removed (12 dimers in one protofilament and 9 dimers in the other, indicated by the dotted line) at 0.5 s. The fuzzy could mean that the chunk is still partly attached. At 1.0 s the chunk defect is healed, as is evident by 1.5 s. Note that the HS-AFM scan is always performed bottom to top. The force was applied at 0.5 s, marked by the red star. (b) A large chunk is removed from the MT at 0.5 s (marked by the red star). The fuzzy edges at the top right side indicate that the chunk is not fully removed, but still attached to the MT. At 2 s, the chunk defect has healed completely.

Derivation of defect energy

Each dimer is simplified to have 4 bonds: 2 longitudinal ones and 2 lateral ones. Longitudinal bonds are bonds along the protofilament while lateral ones are then bonds between the protofilaments. It is further assumed that each lateral bond is exactly the same. The longitudinal bond energy is ΔG_{lo} and the lateral one is ΔG_{la}. A microtubule is made of $M - 1$ protofilaments and at each protofilament n_j dimers are removed. n_M is always 0. The overall number of removed dimers is then:

$$N = \sum_{i=1}^{M} n_i \#(1)$$

Dimer by dimer

First, we consider creating the defect dimer by dimer. Obviously, the energy to remove the first dimer is

$$\Delta G_{11} = 2\Delta G_{lo} + 2\Delta G_{la} \#(2)$$

The energy to remove the remaining dimers from the first protofilament is

$$\Delta G_{i1} = \Delta G_{lo} + 2\Delta G_{la},\ i > 1 \ \#(3)$$

To remove the first dimer from any following protofilament, the energy needed is

$$\Delta G_{1j} = \left(2\Delta G_{lo} + \Delta G_{la}\right) \cdot H\left(n_j\right),\ j > 1 \ \#(4)$$

$H(n_j)$ is the Heaviside step function with $H(x) = 0$ if $x \leq 0$ and $H(x) = 1$ otherwise.
The energy to remove any other dimers is

$$\Delta G_{ij} = \left(\Delta G_{lo} + \Delta G_{la}\right) \cdot H\left(n_j\right),\ i > 1\ and\ j > 1 \ \#(5)$$

The sum of all energies is

$$\Delta G_{sum} = \Delta G_{11} + \sum_{j=2}^{M} \Delta G_{1j} + \sum_{j=2}^{M} \sum_{i=2}^{n_j} \Delta G_{ij} \ \#(6)$$

$$\Delta G_{sum} = 2\Delta G_{lo} + 2\Delta G_{la} + (n_1 - 1)(\Delta G_{lo} + 2\Delta G_{la}) + (m - 1)(2\Delta G_{lo} + \Delta G_{la}) +$$
$$+ (N - n_1 - m + 1)(\Delta G_{lo} + \Delta G_{la})\#(7)$$

In the above equation 7, $m = \sum_{j=1}^{M} H(n_j)$ and describes the number of protofilament segments that have been removed to create the defect. Rearranging equation 7 yields:

$$\Delta G_{sum} = (N + m)\Delta G_{lo} + (N + n_1)\Delta G_{la}\#(8)$$

Starting with protofilament $j > 1$ the adjacent protofilament might have more or less dimers removed. This fact has to be considered when calculating the number of broken bonds. To do this, one calculates the difference $\Delta_j = n_j - n_{j-1}$. If Δ_j is less than zero, no amendment has to be made, but if Δ_j is larger than zero, the number of lateral bonds broken has to be increased by Δ_j (see Fig. S6 for an illustration). For the whole defect, this additional bond energy ΔG_{add} is then:

$$\Delta G_{add} = \Delta G_{la}\sum_{j=2}^{M}(n_j - n_{j-1}) \cdot H(n_j - n_{j-1})\#(9)$$

The total energy is then gained by adding equations 8 and 9:

$$\Delta G = \Delta G_{lo}(m + N) + \Delta G_{la}\left[n_1 + N + \sum_{j=2}^{M}(n_j - n_{j-1}) \cdot H(n_j - n_{j-1})\right]\#(10)$$

Protofilament by protofilament

Now, we consider creating a defect by removing protofilament segments. To remove the first protofilament, the energy needed is

$$\Delta G_1 = 2\Delta G_{lo} + 2n_1\Delta G_{la}\#(11)$$

Removing any following protofilaments, need the energy

$$\Delta G_j = \left(2\Delta G_{lo} + 2n_j\Delta G_{la}\right) \cdot H\left(n_j\right)\#(12)$$

The sum of these two energies is then

$$E\Delta G_{sum} = \Delta G_1 + \sum_{j=2}^{M} \Delta G_j \#(13)$$

$$\Delta G_{sum} = 2\Delta G_{lo} + 2n_1\Delta G_{la} + 2(m-1)\Delta G_{lo} + \left(N - n_1\right)\Delta G_{la}\#(14)$$

$$\Delta G_{sum} = 2m\Delta G_{lo} + \left(N + n_1\right)\Delta G_{la}\#(15)$$

Accounting for differences in the number of dimers in each protofilament, is done by equation 9, as before. The total energy is then

$$\Delta G = 2\Delta G_{lo}m + \Delta G_{la}\left[n_1 + N + \sum_{j=2}^{M}\left(n_j - n_{j-1}\right) \cdot H\left(n_j - n_{j-1}\right)\right]\#(16)$$

As a chunk

If the defect is created by removing all directly adjacent dimers as one part, the lowest number of bonds has to be broken. To derive an expression to calculate the total energy necessary to create the defect, consider figure S6. Obviously, $2m$ longitudinal bonds need to be broken (2 for each protofilament segment). Further, the number of later bonds broken is n_1 plus the sum $\sum_{j=2}^{M}\left|\Delta_j\right|$, as is directly evident from figure S6:

$$\Delta G_{add} = \Delta G_{la}\left[n_1 + \sum_{j=2}^{M}\left|n_j - n_{j-1}\right|\right]\#(17)$$

The total energy is then

$$\Delta G = 2\Delta G_{lo}m + \Delta G_{la}\left[n_1 + \sum_{j=2}^{M}\left|n_j - n_{j-1}\right|\right]\#(18)$$

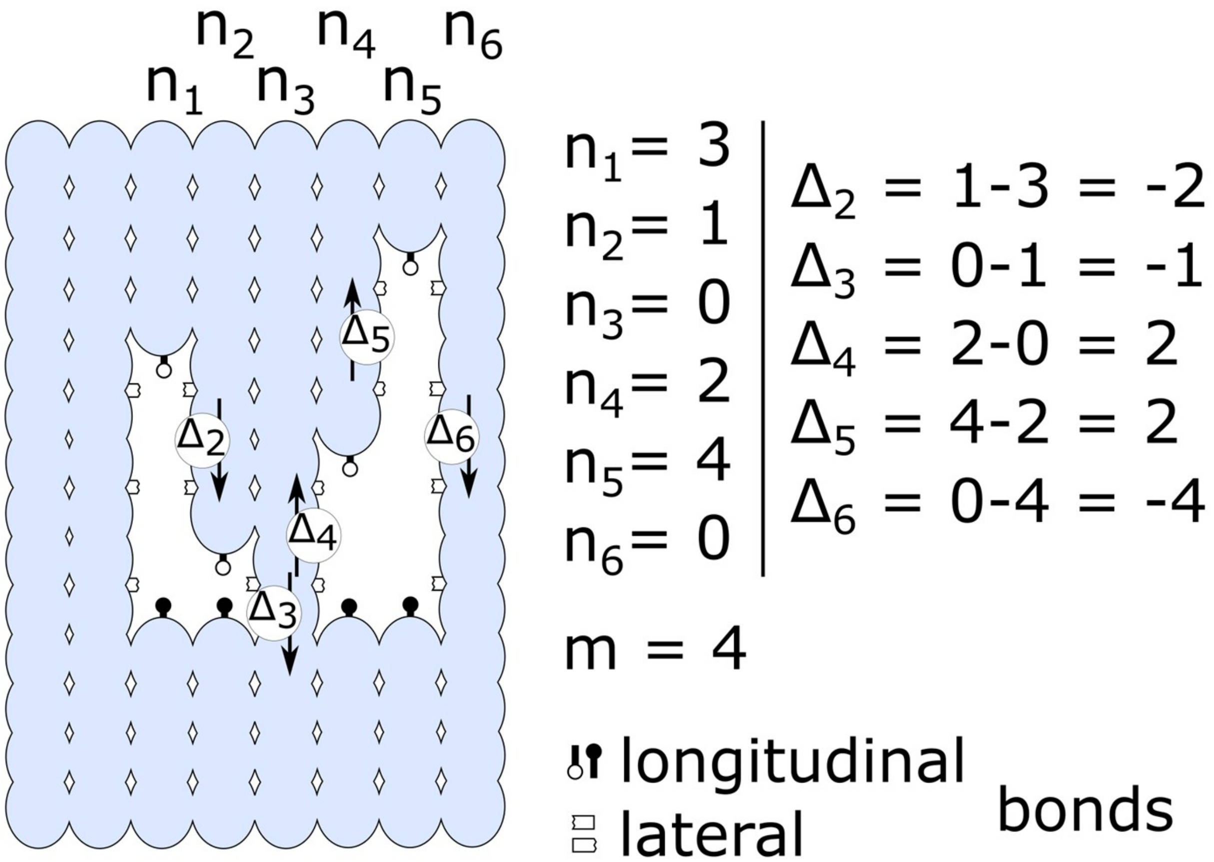

Figure S7: Schematic drawing of a discontinuous defect spanning 5 protofilaments to indicate the number of broken bonds. The discontinuity is caused by $n_3 = 0$.