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Supporting Material

Mechanical properties of a complete microtubule revealed through molecular dynamics simulation

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Supplemental Information for: Mechanical properties of a complete microtubule revealed through molecular dynamics simulation

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Water placement and histidine protonation.

To place water into the internal cavities of the mictorubule's protofilaments the dowserx variation of the program DOWSER was used (1). As a result of the iterative water placement procedure, 1272 and 1275 water molecules were embedded into the N and S protofilament system, respectively. The protonation states of the histidines were assigned based on the local environment of each histidine residue. Thus, residues 28, 61, 88, 393, and 406 of α-tubulin were assigned the HSE protonation state; 8, 107, 139, 192, 197, 283, and 309 were assigned the HSD state; and HIS:266 was assigned the HSP state. In β-tubulin, residues 6, 37, 139, 192, and 406 were identified as HSE; 28, 107, and 229 as HSD; and 266 and 309 as HSP. Using the SOLVATE program (2), a minimum 3 Å layer of water was created around each protofilament, adding 12,628 and 15,184 water molecules to the N and S system, respectively.

References

1. Zhang, L., and J. Hermans, 1996. Hydrophilicity of Cavities in Proteins. *Proteins: Struct., Func., Gen.* 24:433–438.

2. Grubmüller, H., B. Heymann, and P. Tavan, 1996. Ligand Binding: Molecular Mechanics Calculation of the Streptavidin-Biotin Rupture Force. *Science* 271:997–999.

3. Frishman, D., and P. Argos, 1995. Knowledge-based secondary structure assignment. *Proteins: Struct., Func., Gen.* 23:566–579.

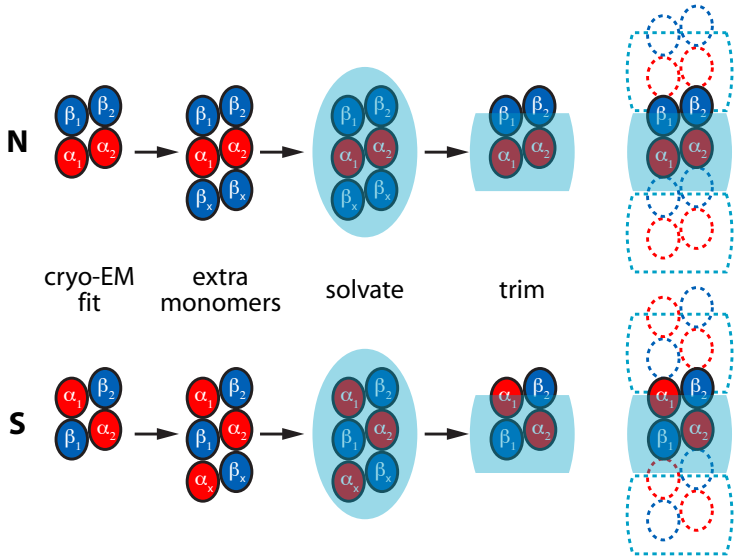

Figure S1. Graphical representation of solvation process for the N (*top row*) and S (*bottom row*) systems. α-tubulin is shown in red, β-tubulin in blue, and water as translucent light blue. Because DOWSER and SOLVATE do not support periodic boundary conditions, we built intermediate systems with extra monomers, labelled α_x and β_x. These systems were then solvated with DOWSER and SOLVATE. The resulting water shell was then trimmed to the periodic length of 81.2 Å in z, and the extra monomers removed, resulting in a system that can tile in the z-dimension without water or protein overlap, depicted on the right with periodic images shown as dashed outlines.

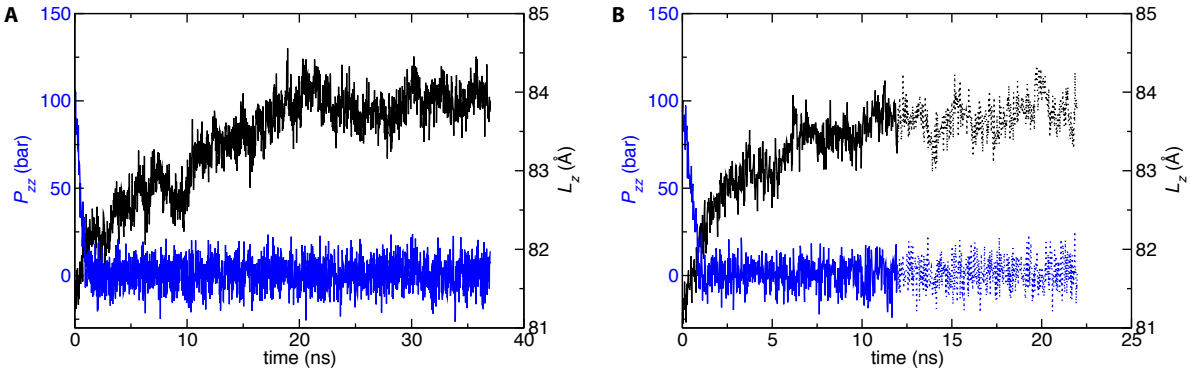

Figure S2. Pressure adjustment simulations of the N and S protofilament systems. (*A, B*) The length of the periodic cell along the protofilament L_z (*black*) and the P_{zz} (*blue*) component of the stress tensor for the N (*A*) and S (*B*) systems. Scales for P_{zz} and L_z are shown on the left and right vertical axes, respectively. Dotted lines indicate additional equilibration data. The latter were not used for subsequent runs, but demonstrate that L_z has indeed reached a steady state.

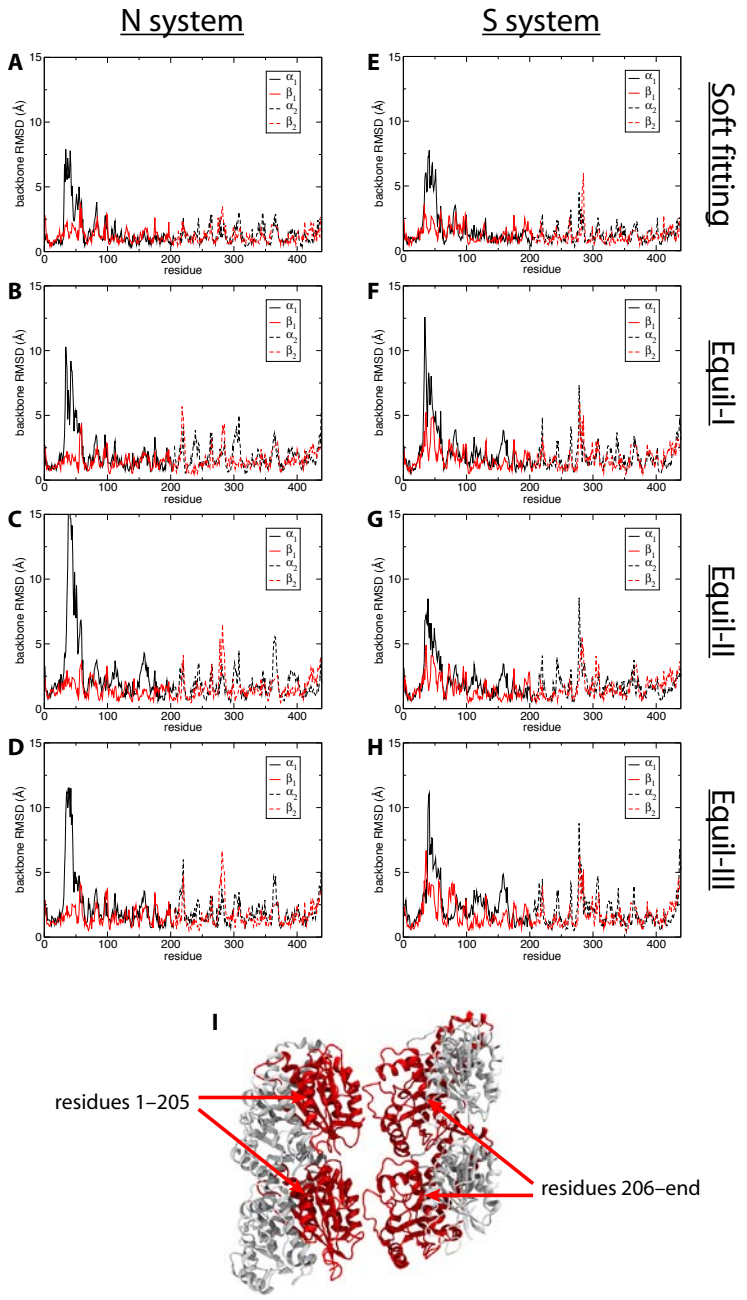

Figure S3. Per residue RMSD of the N and S protofilaments' backbone atoms from the respective initial structures. The data for the N and S systems are shown in the left and right columns, respectively. The RMSD after each stage of the simulation is shown. The data is shown for residues 1–205 of α_1 (*solid black line*) and β_1 (*solid red line*), and 206–439 of α_2 (*dashed black line*) and 206–437 of β_2 (*dashed red line*). This set of residues is shown in red in panel *I*. To compute the RMSD, the structure of each monomer was first aligned with the reference structure. For each residue, the RMSD was then calculated for backbone atoms averaged over the last ns of the trajectory.

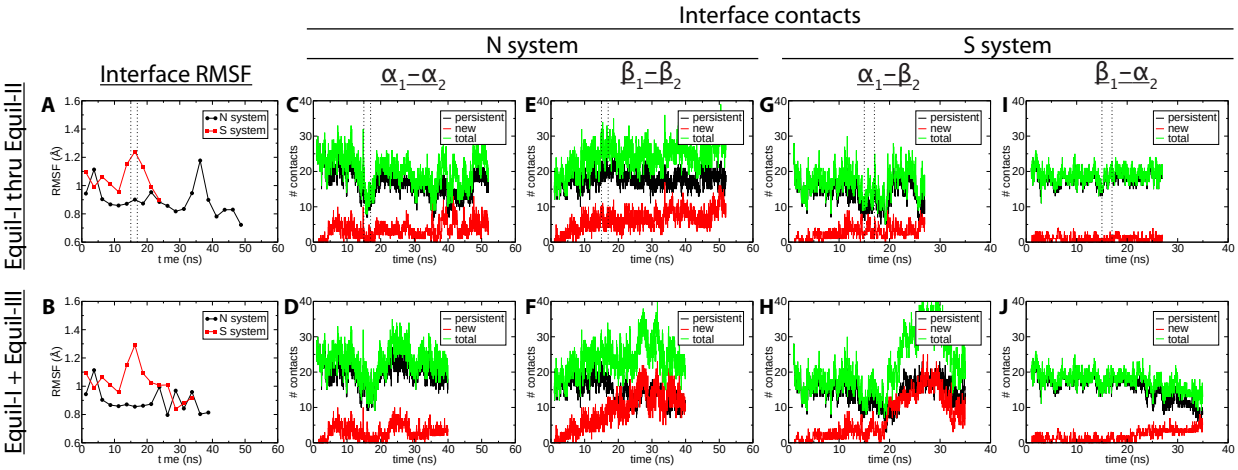

Figure S4. Properties of the protofilament interface in the N and S systems. (*A, B*) Interface RMSF, calculated using C^{α} atoms of the interface residues for the N (*black*) and S (*red*) systems. To compute RMSF, a reference structure was determined for each 2.5-ns window as the time average after structural alignment. The RMSF within the window is then the RMSD of the C^{α} coordinates from that window's reference structure averaged over the window. For these calculations, the interface residues were defined as those whose heavy atoms resided within 5 Å of the adjacent monomer. (*C–J*) Number of unique residue-residue contact pairs between α_1-α_2 (*C, D*) and β_1-β_2 (*E, F*) in the N system, and α_1-β_2 (*G, H*) and β_1-α_2 (*I, J*) in the S system. Two residues with heavy atoms within 5 Å of each other were considered to be in contact. Each contact plot shows the number of persistent (*black*), new (*red*), and total (*green*) number of contacts. The persistent contacts are defined as those present in the first 1 ns of equilibration-I. Vertical dotted lines indicate the beginning and end of pressure adjustment.

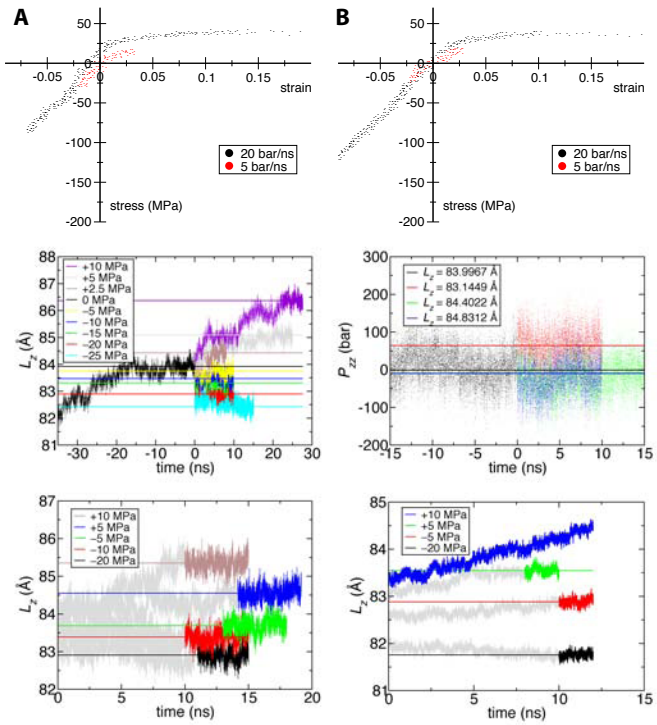

Figure S5. (*A*, *B*) Stress-strain curves from dynamic stress-controlled simulations of the N (*A*) and S (*B*) systems. For each system, the stress was changed at two different rates, 20 bar/ns (*black*) and 5 bar/ns (*red*). The 20 bar/ns simulation of the N system was performed starting from the protofilament structure that had not reached the equilibrium extension of 83.9 Å, hence the 20 bar/ns curve does not pass through the origin. (*C*–*F*) Raw data from the stress- and strain-controlled simulations of the N and S systems. (*C*) The L_z length of the N system versus time at various applied stresses. The black curve corresponds to equilibration, i.e. no applied stress. Horizontal lines indicate the mean value attained at the end of each trajectory. (*D*) The P_{zz} component of the stress tensor in the simulations of the N system versus time at several fixed values of L_z. Horizontal lines indicate the mean value attained at the end of each trajectory. (*E*) The L_z length of the S system versus time at various applied stresses. Horizontal lines indicate the mean value attained at the end of each trajectory. (*F*) The L_z length of the complete, infinite MT system versus time at various applied stresses. Horizontal lines indicate the mean value attained at the end of each trajectory. In the case of the +10 MPa simulation (*blue*), equilibrium was not reached within the simulation time scale.

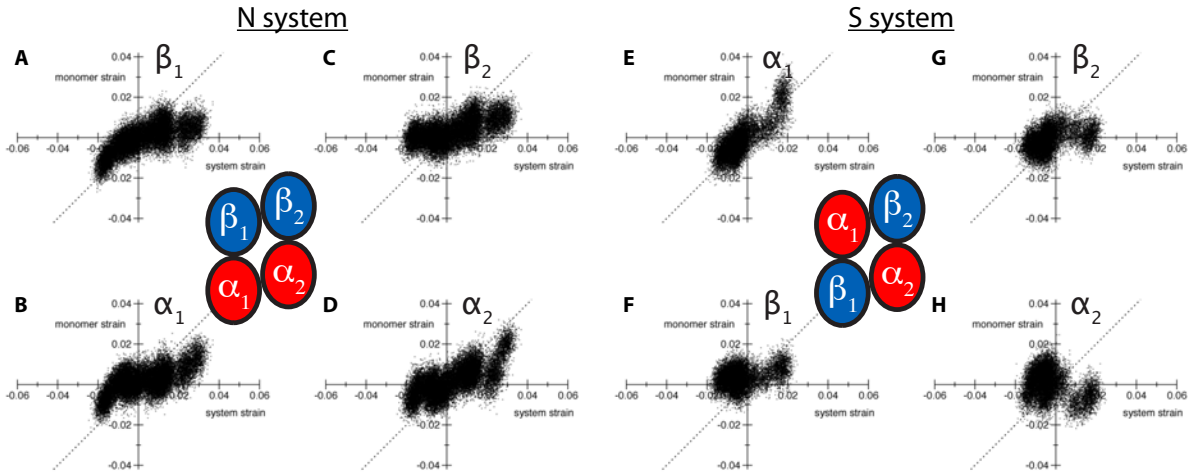

Figure S6. Monomer strain versus system strain for the N (*A*–*D*) and S (*E*–*H*) systems. Data were taken from the stress-controlled simulations. The dashed lines in each plot are of slope 1. If the data points lie along this line, then the individual monomers are stretching and compressing to the same degree as the filaments as a whole, implying that the strain of the system is entirely due to strain of the monomers. On the other hand, if the data points fall on the x-axis, then the monomers are not stretching or compressing at all, implying that the strain of the system is due to relative motion of the unstrained monomers, e.g. separation of monomers during stretching. Monomer strain was determined by first fitting select C^{α} atoms to a reference structure, then calculating the linear regression of the scatter plot $\Delta z_i = z_i - z_i^{\text{ref}}$ versus z_i^{ref}, where z_i and z_i^{ref} are the z-coordinates of the current and reference atom i, respectively. The slope calculated is then the strain of the individual monomer. Reference structures were taken as the average of the last 5 ns of equilibration. The residues selected for these calculations had either alpha-helical or beta-sheet secondary structure in the reference coordinates as determined by STRIDE (3).

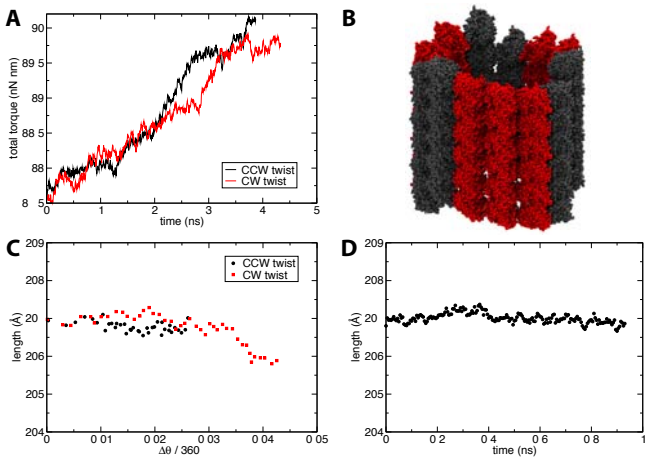

Figure S7. Torque and length of the MT during the twist simulations. (*A*) Total torque applied during the simulation of CCW (*black*) and CW (*red*) twist. (*B*) Microtubule system used for twist simulations. While most protofilaments have six lateral monomer contacts on both sides, some have only five on one side due to the finiteness of the system. Protofilaments having fewer than six lateral contacts are shown in gray and were not used to compute the average lengths shown in *C* and *D*. (*C*) Distance along the z-axis between the centers of mass of the two end tubulin monomers in the twist simulations, averaged over protofilaments that had six lateral contacts on each side, shown in red in *B*. (*D*) Distance along the z-axis between the centers of mass of the two end tubulin monomers of each protofilament during equilibration, averaged over the same protofilaments.