Electronic Supplementary Material (ESI) for Soft Matter.

Supplemental Information

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This document describes attempts made to bolster the shear strength of the hexagonal protein lattice by mutating select residues to cysteines in order to form disulfide bridges across the lattice vertices. The mutated residues were selected by their proximity to the lattice vertex and their chemical similarity to cysteine - namely, possessing polar uncharged functional groups. Figure 1 illustrates serine, threonine, asparagine, and glutamine residues in the three turns of each beta-solenoid proximal to the lattice vertex. Two residues on each beta-solenoid protein were selected to be mutated to cysteine; these mutations were performed using VMD's mutator plugin.[1] The long bonds that were constructed as a result of these mutations were relaxed using steepest descent minimization in YASARA.[2]

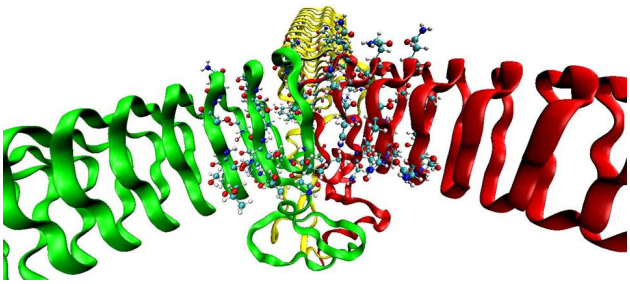

Figure 1: Cartoon representation of symmetric trimer and three beta-solenoid proteins; residues that were identified as candidates for mutating to cysteine are shown in atomistic detail.

The comparative strength of this modified structure versus the original was measured using center-of-mass pulling simulations in GROMACS.[3] In these simulations, a "reference"

group of residues near the distal end of one beta-solenoid protein was restrained to retain its original position. A "pull" group of residues near the distal end of the adjacent beta-solenoid protein was prescribed to move at a set speed under a harmonic biasing potential towards the reference group (see Figure 2). The applied force is measured throughout this

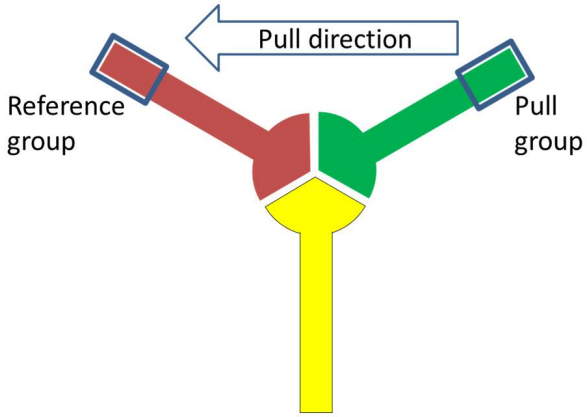

Figure 2: Center-of-mass pulling simulation setup. A reference group of residues is defined and restrained to stay in place; force is applied to a pull group in the direction indicated.

simulation and plotted against the displacement of the pull group in Figure 3 as a measure of the resistance to angular movement, which determines shear stiffness. This figure shows that there is virtually no difference in strength between these two structures. One possible reason for this result is that the mutations disrupted the hydrogen bonding network between sheets of the beta-solenoid protein, weakening the structure and canceling out any strengthening effect the disulfide bridges may have added.

References

(1) Humphrey, W.; Dalke, A.; Schulten, K. *Journal of Molecular Graphics* **1996**, *14*, 33–38.

(2) Krieger, E.; Vriend, G. *Bioinformatics (Oxford, England)* **2014**, *30*, 2981–2982.

(3) Hess, B.; Kutzner, C.; van der Spoel, D.; Lindahl, E. *J. Chem. Theory comput.* **2008**, 435–447.

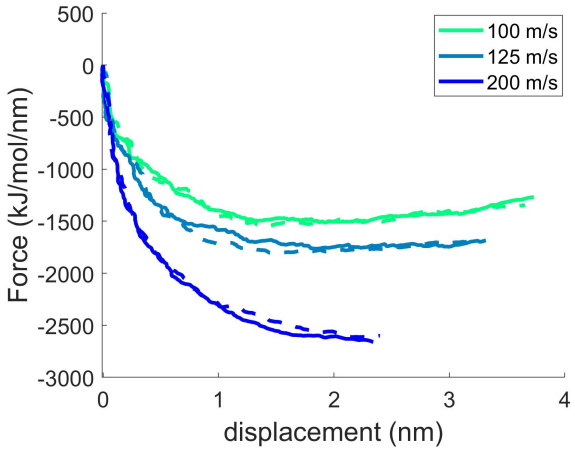

Figure 3: Force applied to the pull group by the harmonic biasing potential plotted against the displacement of the pull group as it moves towards the reference group; several pulling speeds simulated, indicated by line color. Solid lines depict measurements taken on the modified structure (cysteines added); dashed lines depict measurements taken on the original structure (no cysteines). No significant difference between these structures can be seen.