Paulina Panek


# Final Project Report

Molecular dynamics simulations of wild-type ENH and engineered ENH dimers were conducted and the stability was evaluated through comparisons of trajectory RMSDs, center of mass distances between monomers, and radii of gyration. In an attempt to improve stability of the engineered dimer, a mutation was proposed and the MD data for the mutated dimer was evaluated as well.

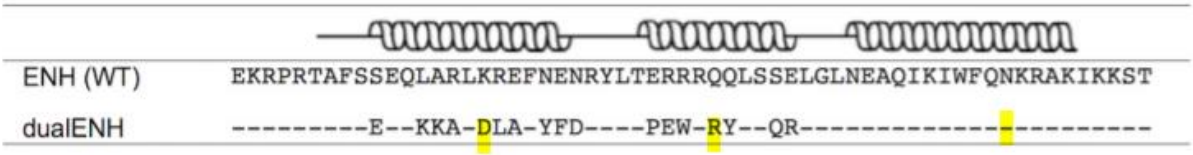



**Fig. 1.** Sequences of the wild-type and engineered dimers[1]
The three 'coils' at the top show the location of the three helices in the wild-type based on PDB structure 1ENH. Sites 17, 32, and 51 where further mutations were introduced to produce the mutated dimer are highlighted in yellow.

Since the engineered dimer showed less stability than the wild-type, a set of mutations on the engineered strands was proposed as shown in table 1. Other than the residues 17, 32, and 51, the amino acid sequence remained the same as that of the engineered dimer.

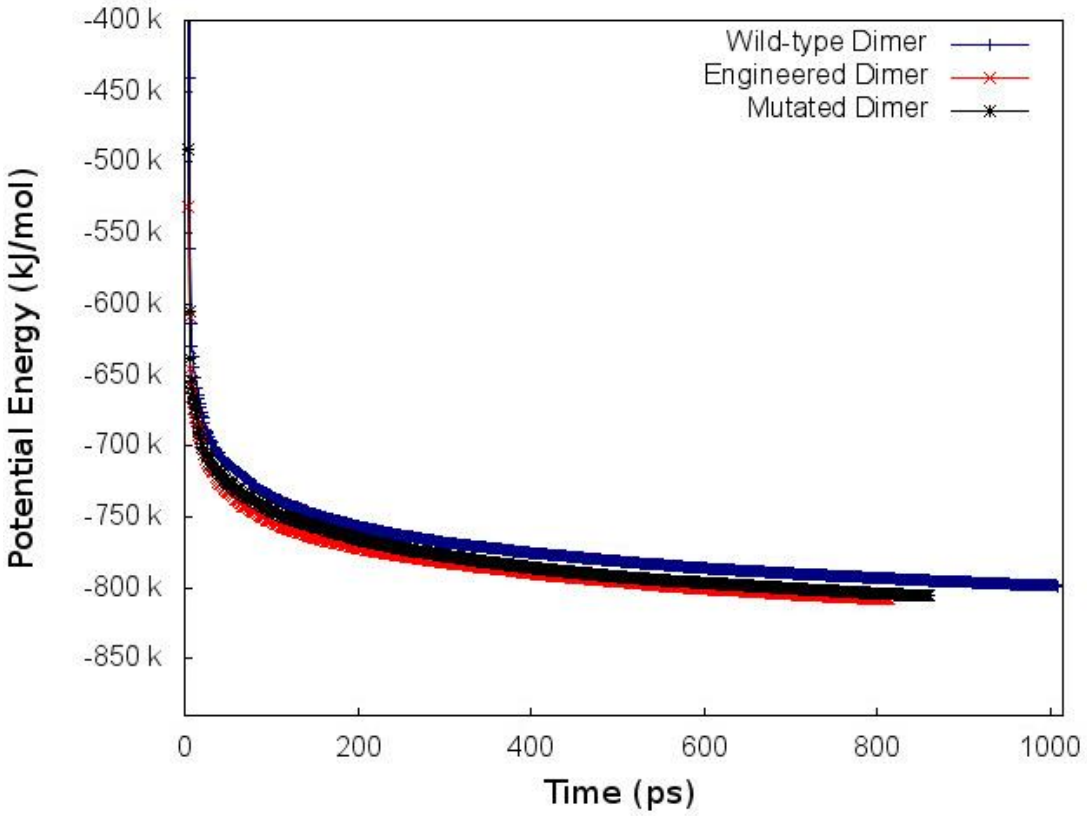
The specific sites of the mutation were chosen as follows. A residue from approximately the middle of each α-helix was chosen and was substituted with an amino acid that has a stronger propensity for helical conformation. Table 1 provides data on free energies of the amino acids in question in α-helices relative to alanine that has an arbitrary value of 0. Mutation was chosen in a way that the change in the helical penalty is not extreme, but always lower in the mutated dimer than in the engineered one. Stabilizing the α-helices should stabilize the dimer.

**Tab. 1.** Free energy per residue at mutation sites[2]

| | Residue 17 | Helical Penalty (kJ/mol) | Residue 32 | Helical Penalty (kJ/mol) | Residue 51 | Helical Penalty (kJ/mol) |
|---|---|---|---|---|---|---|
| Wild-type | K | 1.09 | Q | 1.63 | N | 2.72 |
| Engineered | D | 2.89 | R | 0.88 | | |
| Mutated | S | 2.09 | A | 0.00 | Q | 1.63 |

Before starting the MD simulations, the energy of the system had to be minimized as shown in figure 2:

**Fig. 2.** Energy minimizations




Then the systems were equilibrated for NVT (303.15K) and NPT. Evidence for proper equilibration is shown on figures 3-5 that plot temperature, pressure, and density as a function of equilibration time.

**Fig. 3.** Temperature equilibrations

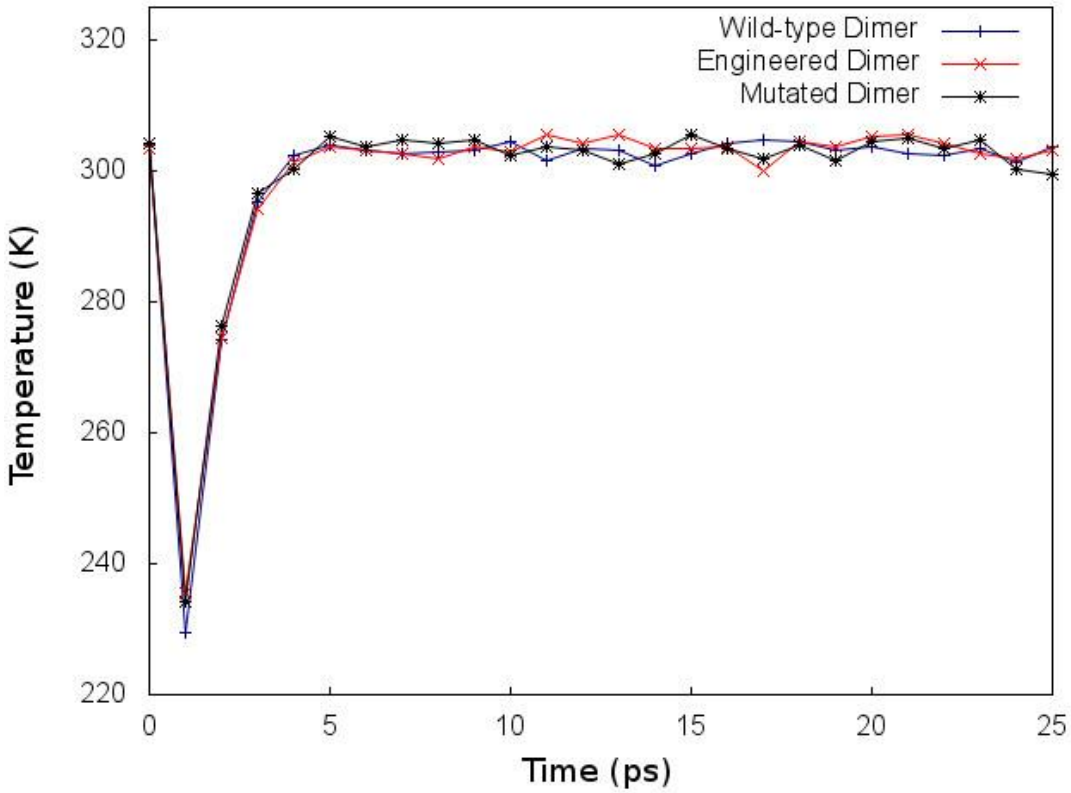



**Fig. 4.** Pressure equilibrations

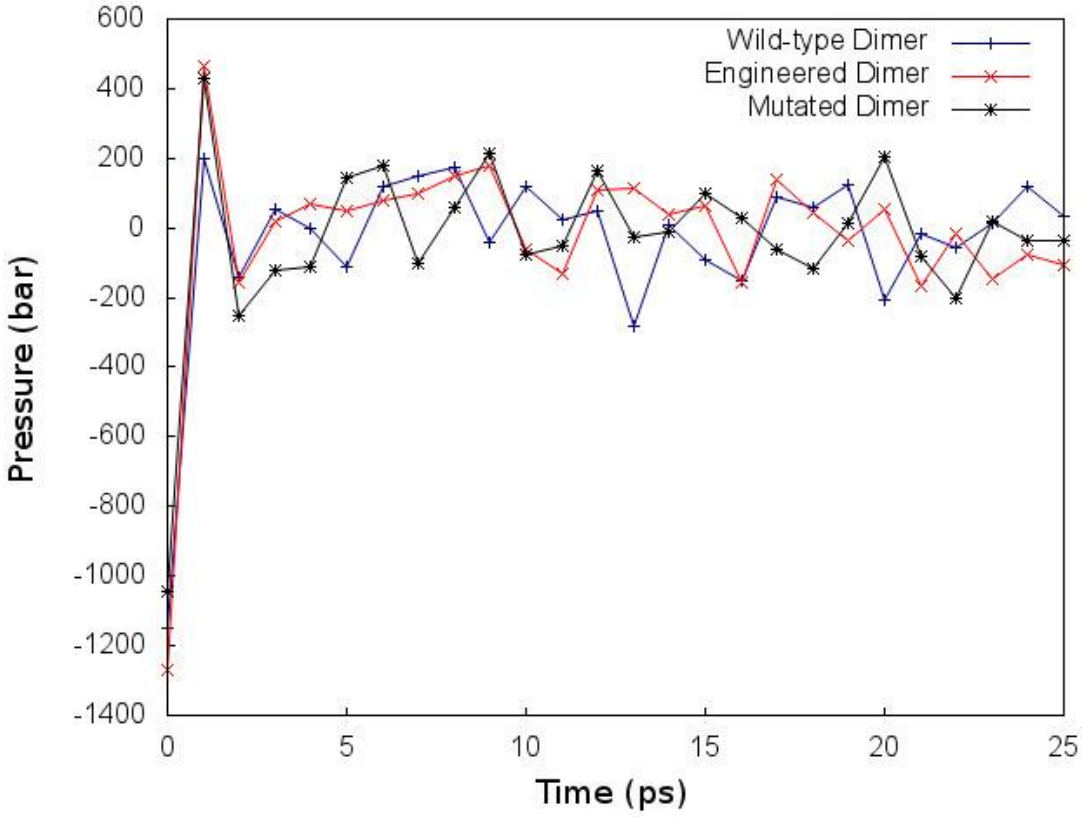
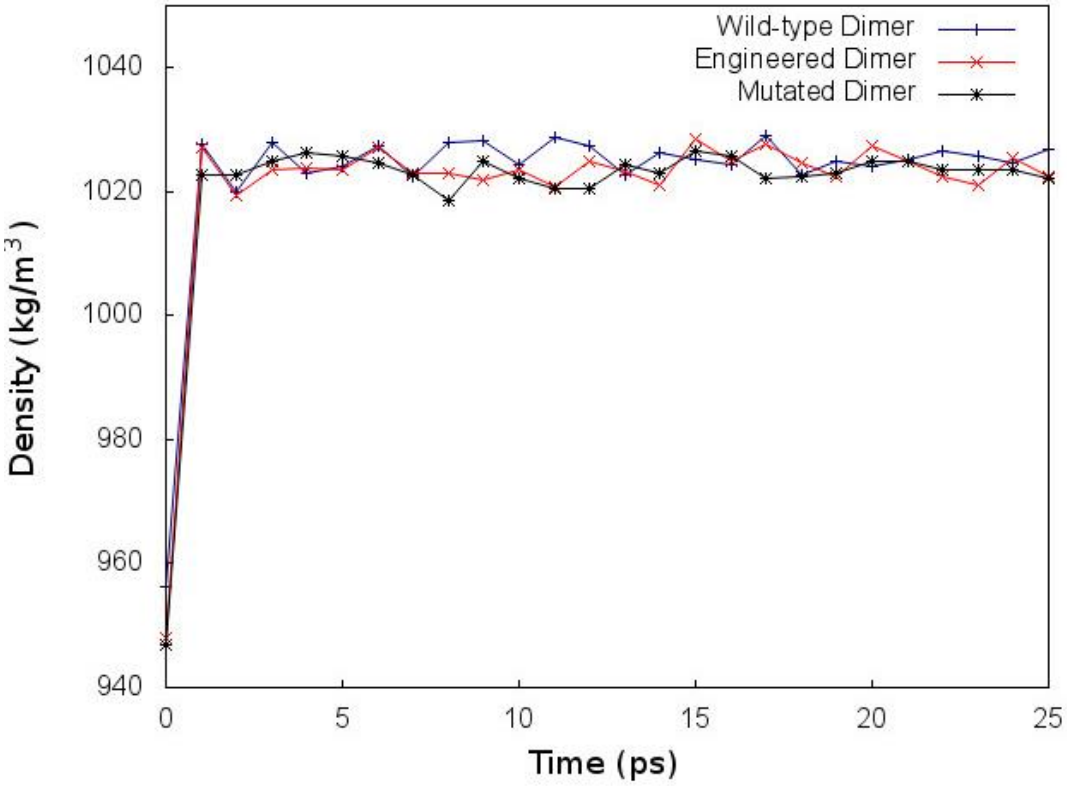


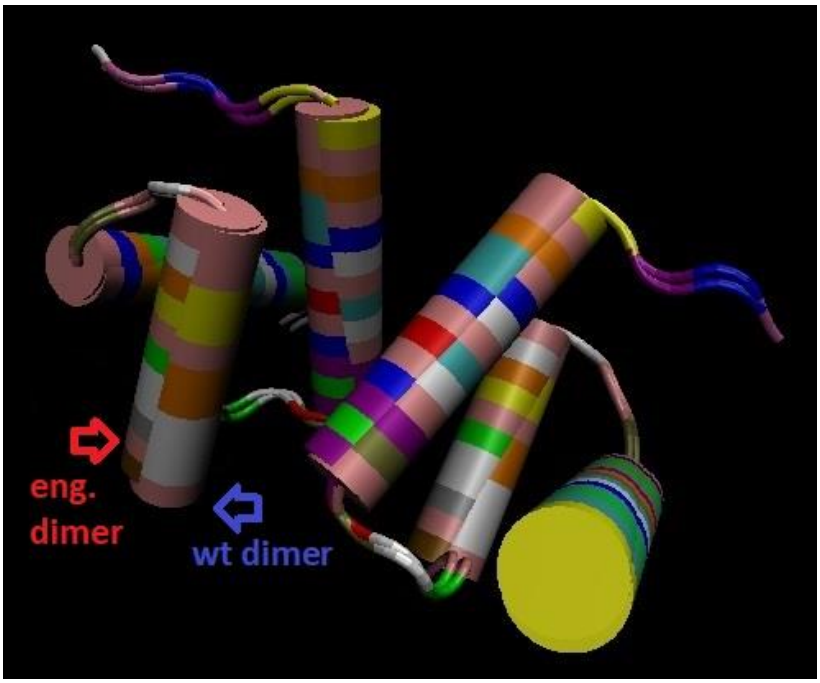
**Fig. 5.** Density equilibrations



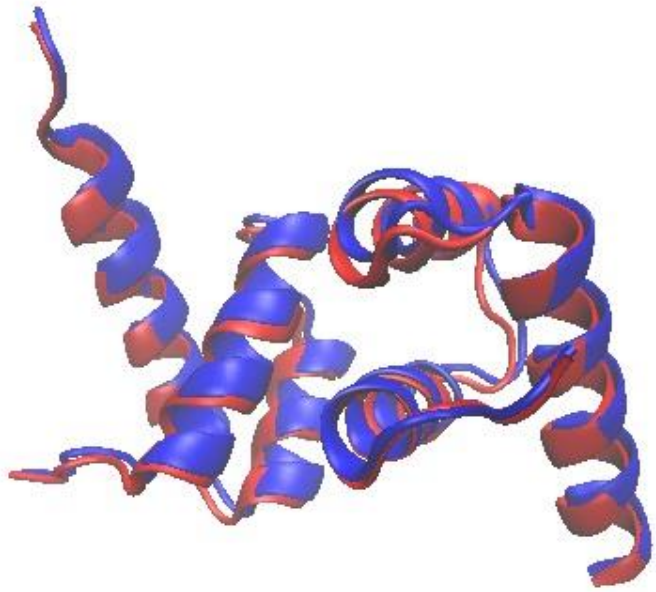

The equilibrated structures of wild-type and engineered dimer were overlaid (figure 6) using Multiseq extension of VMD software and RMSD was calculated. Similar comparisons were performed for the mutated dimer (figure 7).

**Fig. 6.** Wild-type vs engineered dimers: visual representations

a) Cartoon representation colored according to amino acid identity shows sites of mutations. The two outermost α-helices where the amino acid sequence is not well-visible have the exact same sequence.

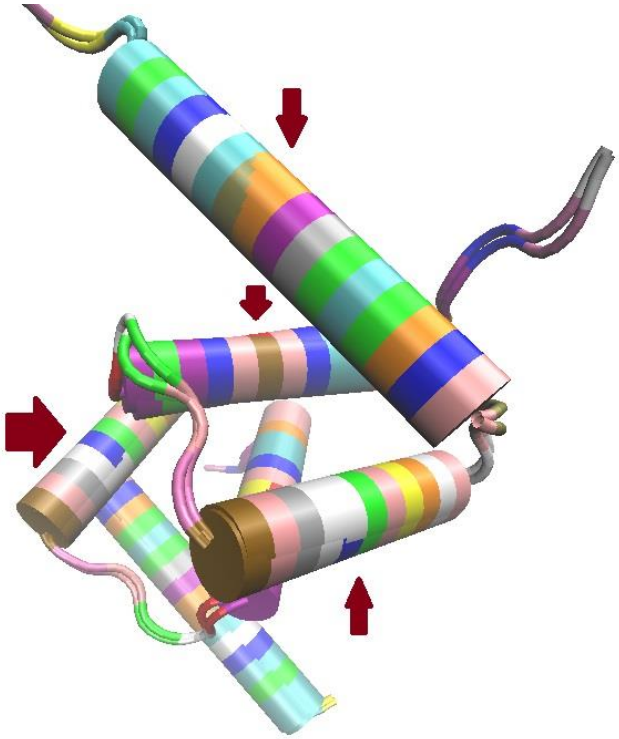


b) Different representation of the same two dimers. Navy protein is the wild-type while red is the engineered one. The two structures are very similar.

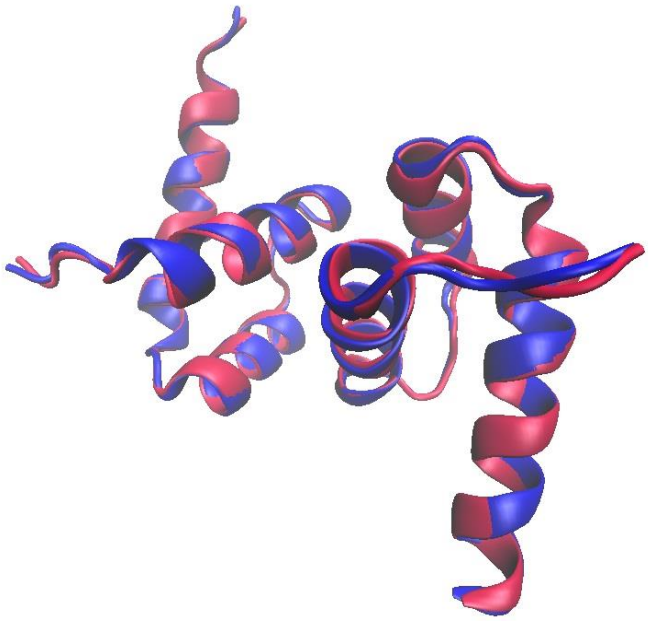


The RMSD for these two equilibrated structures was calculated using both RMSD and Multiseq modules of the VMD software. Multiseq that uses STAMP structural assignment showed RMSD of 0.5108 while the RMSD Tool calculated it to be 0.4741.

**Fig. 7.** Mutated dimer vs others: visual representations



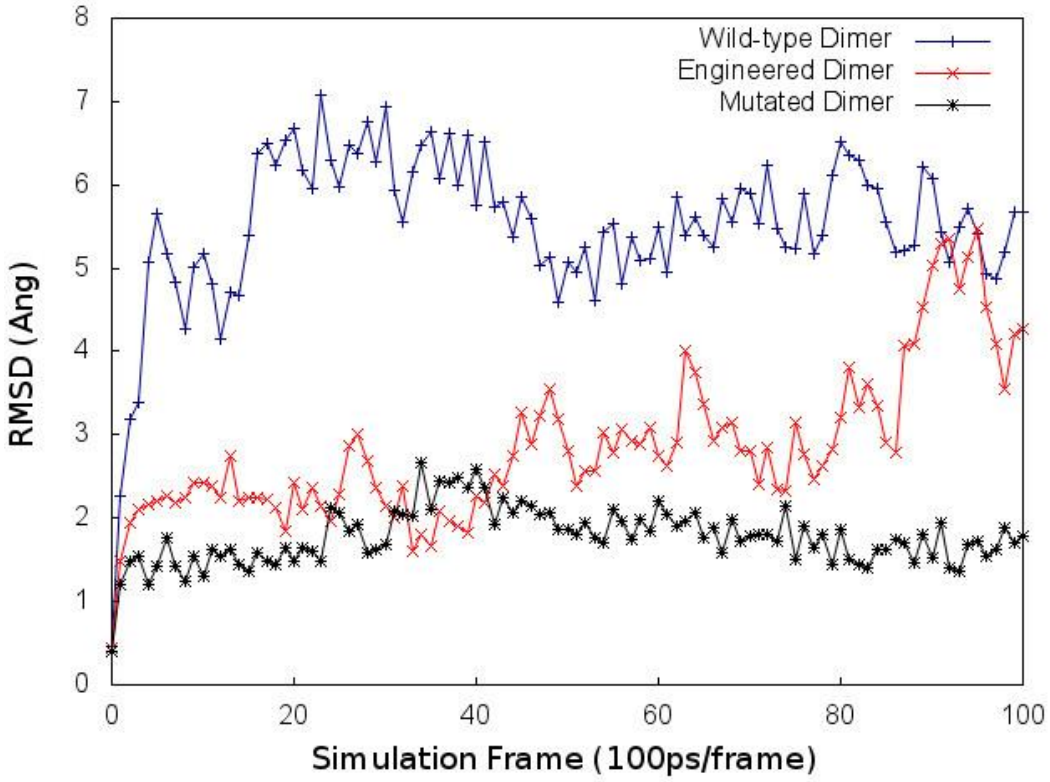
a) Cartoon representation colored according to amino acid identity shows sites of mutations. Arrows point at residues 17, 32, and 51 where the mutation is visible.



b) Wild-type dimer (navy) superimposed on the mutated dimer (pink). The structural similarity between is still of high degree. The RMSD between this two structures according to Multiseq extension was 0.5061, so slightly lower than for the engineered dimer.

After a 10 ns MD simulation, first the trajectory RMSDs were compared as shown in figure 8. Wild-type dimer changed the most compared to its equilibrated form, but its RMSD leveled off around 5.5Å, which means once a stable form was reached, the molecule remained stable.

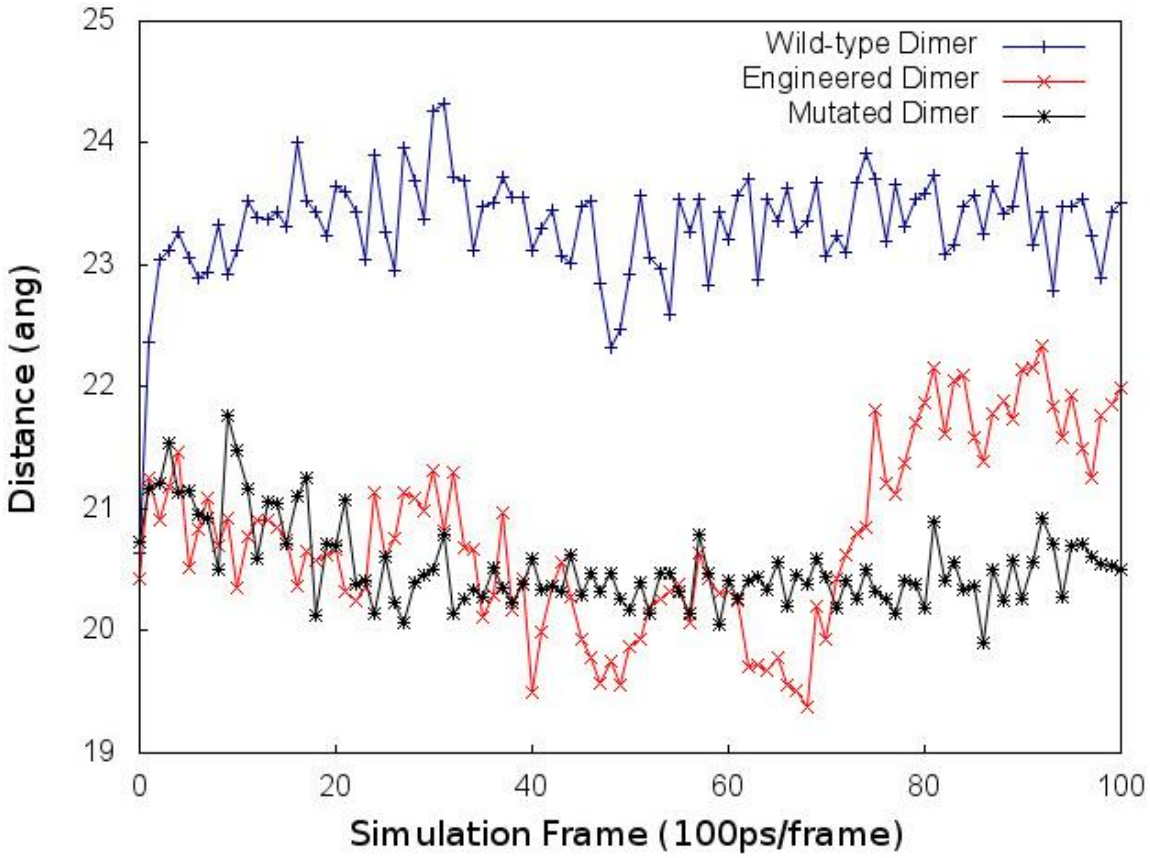
**Fig. 8.** RMSD during the simulation



The RMSD for the engineered dimer was lower at first but kept increasing which means the molecule kept changing its conformations and it is unclear if it would stabilize if the simulation time was extended. As anticipated, the RMSD for the mutated dimer had very little fluctuations, even less than the wild-type, and remained around 2 Å. As expected, improving the propensity for α-helices of middle amino acids in the α-helix domains, improved the overall stability of the dimer.

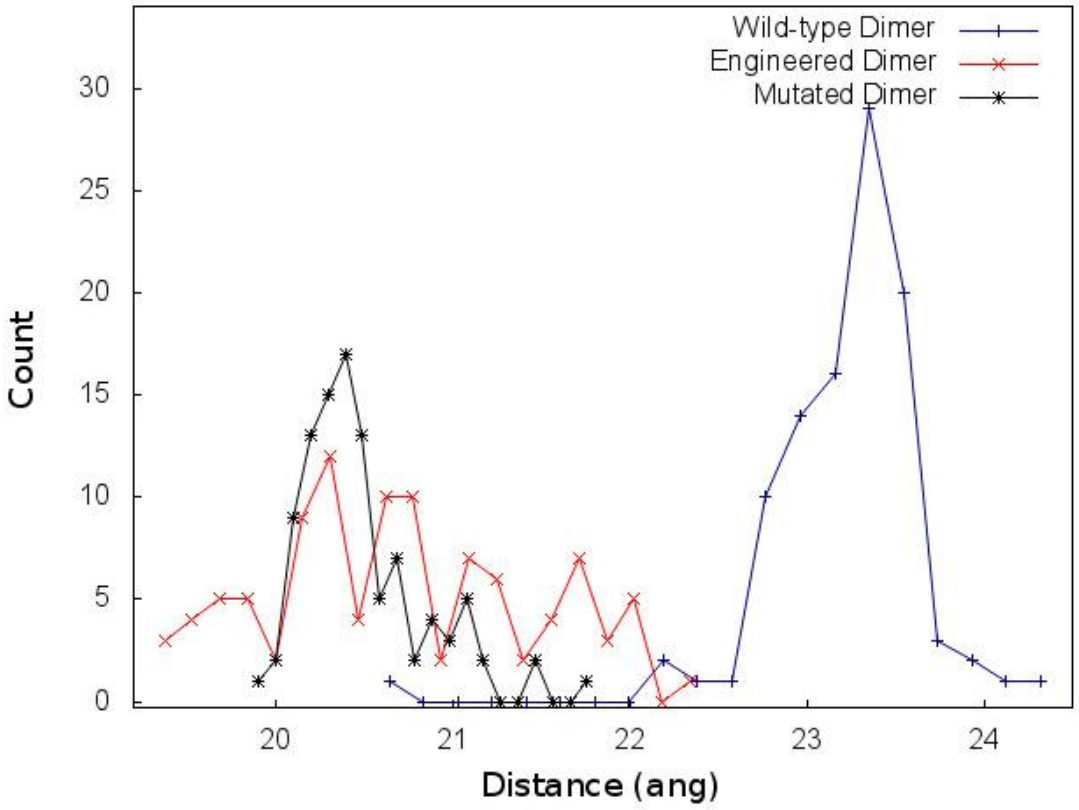
Center of mass distance between monomers over the trajectory is another measure of the stability of the dimers. Figure 9 shows how it changed during the simulation and figure 10 is a histogram of these distances. Figure 9 reveals that the distance is quite stable for the wild-type protein, but for the engineered dimer it kept increasing further implying low stability. Again, it is unclear if the distance would keep increasing if the simulation was continued. The mutated dimer again behaved as expected, had little fluctuations over the simulation time, and leveled off around 20.5 Å. It is worth noting the wild-type dimer stabilized at a higher value around 23.5 Å.

**Fig. 9.** Center of mass distance between monomers during simulation



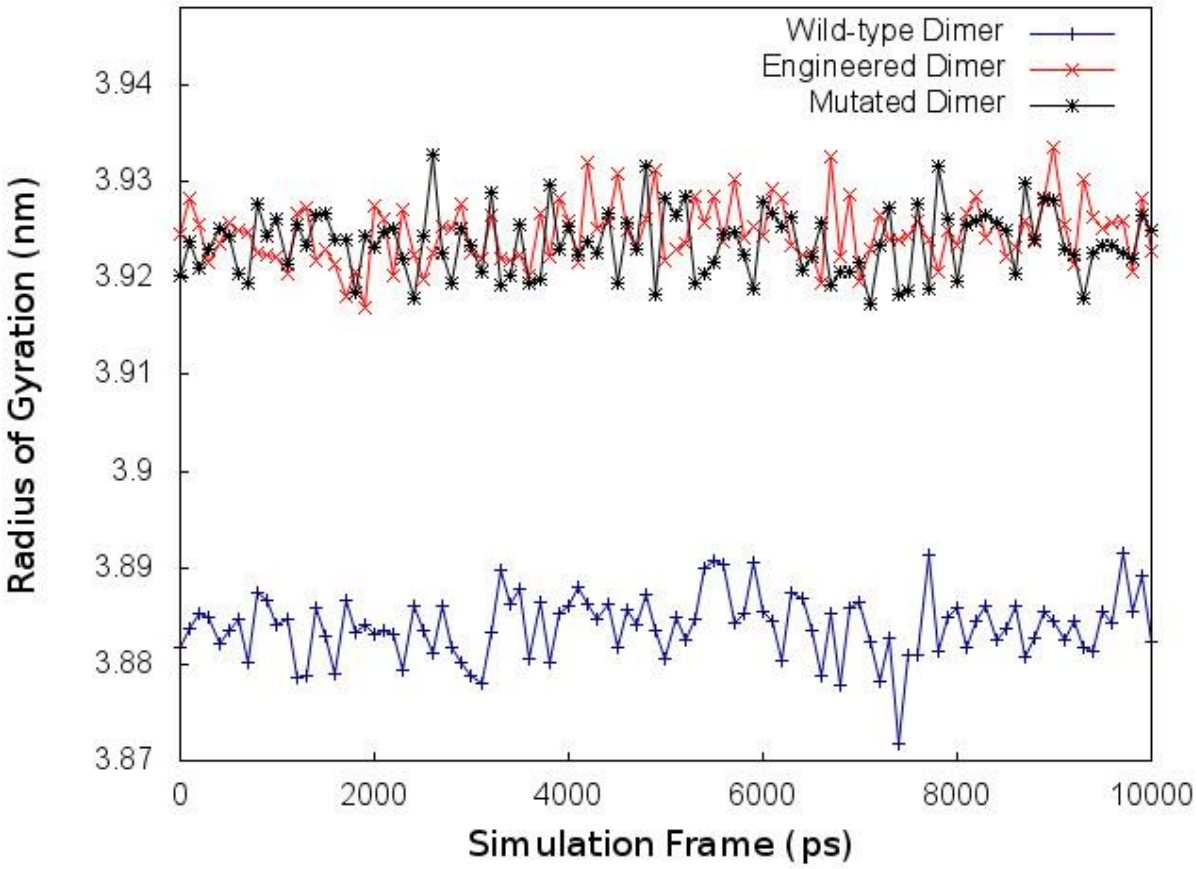
The histogram of the distances only confirms the conclusions. The wild-type has a clear single peak, but the engineered dimer has several of almost equal heights suggesting the distance kept changing rather than find an optimum and stay there. The histogram for the mulated dimer shows its behavior is in between the other two dimers. It does have a clear peak, but a few smaller ones at higher distances are also present.

**Fig. 9.** Histograms of center of mass distance between monomers




Lastly, radius of gyration was evaluated (figure 11). It appears that the results for the engineered and mutated dimers are very similar. The variation over time for the wild-type is also comparable, however this one stabilized at a lower value than the other two. Possibly the wild-type protein is more compact to begin with than the other two.

**Fig. 11.** Radius of gyration




Overall, as expected the stability of the mutated dimer improved as compared to the engineered one, but was still not as good as that of the wild-type. A few additional considerations should be taken into account. First, it is unclear from the simulation data if the parameters for the engineered dimer would eventually stabilize. For instance, it is possible that the center of mass distance between monomers needs 6 ns to stabilize and remains relatively constant afterwards (and resembles the wild-type value more than the proposed mutated dimer). To test whether it is true, a longer simulation time would be needed. These dimers were investigated by Mou et. al. for a specific reason, that is binding to DNA, and even though my proposed mutated structure appears to be more stable, it is unknown whether its DNA binding property is affected. Choosing more α-helical amino acids did not take into consideration acidic vs basic character of the residues or any other characteristic that might affect interactions with DNA. It should be also noted that helical penalty values cited in table 1 are only approximations and are affected by neighboring residues as well. A more throughout study would be needed to conclude if such approach is a sound way of designing proteins.

# References

1. Mou, Y.; Jiun-Yann, Y.; Wannier, T. M.; Guo, C. L.; Mayo, S. L. Computational design of co-assembling protein-DNA nanowires. *Nature*, **2015**, 525, 230-233.
2. Pace, C. N.; Scholtz, J. M. A Helix Propensity Scale Based on Experimental Studies of Peptides and Proteins. *Biophys. J.*, **1998**, 75, 422-427.