Effects of Tubulin Tail Post-Translational Modifications on Microtubule-Severing Protein's Dynamics

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Background

- Microtubules (MTs) are long filamentous molecules used in the cell for structure, support, and transport[1]. They're made up of smaller proteins called tubulin, which have long tail regions called carboxy terminal tails (CTTs)[2]
- MTs are constantly being polymerized and depolymerized, a trait called "dynamic instability" (Figure 1).[2] Many proteins are responsible for this, including some known as the microtubule-severing enzymes.[3]
- Spastin and katanin are two microtubule-severing enzymes. They are both are both monomeric proteins that, when bound with adenosine triphosphate (ATP) assemble into hexameric ring states around the carboxy-terminal tails (CTTs) on tubulin.[1,4] Once assembled, hydrolysis of ATP causes a change from the ring state to a spiral state, generating a mechanical force on the CTT that depolymerizes the tubulin lattice and thus breaks the MT (Figure 2).[1,4]

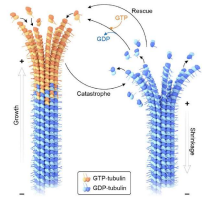

Figure 1: The polymerization and depolymerization of a microtubule composed of many protofilaments. Tubulin is shown in pairs, with two head regions and two tail regions.[2] These tail regions are the CTTs referred to in the text.

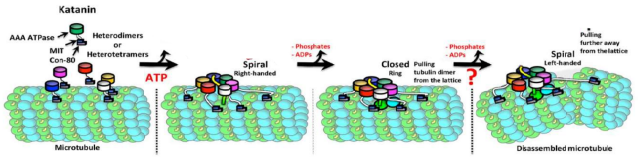

Figure 2: A cartoon diagram showing the assembly of katanin into a hexamer ring on a microtubule around a CTT and the subsequent conversion to the spiral state that breaks the MT[1]. A similar diagram can be drawn for spastin.

- The cell regulates microtubule-severing proteins via chemical modifications on the CTTs. How these modifications affect the activity of proteins is an open question.[2]
- In this project, we use a type of computer simulation called an all-atomistic molecular dynamics simulation to analyze the dynamics of spastin and katanin associated around the wild-type CTT found on β-tubulin along with other modified CTTs.

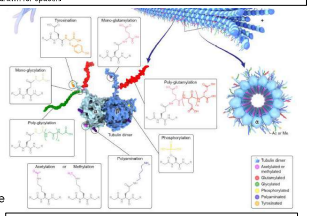

Figure 3: A collection of common chemical modifications found on tubulin dimers, along with their locations[2].

Methods

- Five trajectories for spastin hexamers assembled around a β-wild type CTT were simulated in GROMACS[5], with visualizations in PyMOL[6] and VMD[7]. Spastin was docked with the CTT using GRAMM-X[8]
- Simulation results were analyzed with several machine learning techniques.
- RMSD and DCCM were used to verify that the trajectories had converged.
- RMSF was used to identify regions of the protein that underwent significant variation from their time-averaged positions.
- Principal component analysis (PCA) was performed on all trajectories. PCA is a dimensionality reduction tool that diagonalizes the covariance matrix for the atomic positions, finding derived features with the greatest variance. (Figure 3)
- All techniques were performed on a concatenation of all five trajectories

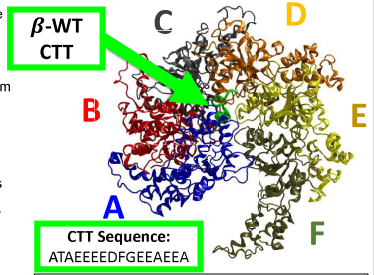

Figure 4: The initial structure of spiral-state spastin used in simulation. Monomers are labelled and CTT is indicated (light green). The primary sequence of the CTT is also given in one-letter code form

Results

Trajectory Convergence

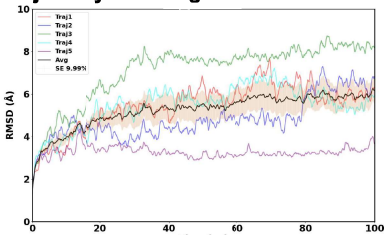

Figure 5: RMSD plotted over time for all five trajectories, alongside the average with standard error bars.

RMSD

- The average RMSD (in black) shows high fluctuation in the initial nanoseconds of simulation, followed by a plateau as the system approaches equilibrium (figure 4)
- The plateau is indicative of trajectory convergence
- Trajectories three and five are outliers, spending much of the 100ns outside the average's standard error (Figure 4)

DCCM

- The difference between successive DCCM matrices should approach zero as time progresses (Figure 5)
- Each of the five trajectories were concatenated when determining DCCM convergence
- The approach to zero indicates convergence

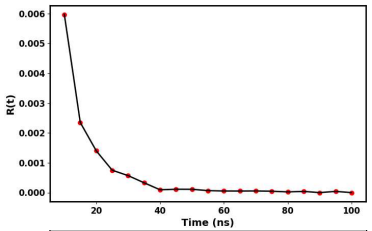

Figure 6: DCCM plotted over time, calculated at 5ns intervals

Monomer Fluctuations

- RMSF is highest in monomer F HBD region, indicating that the largest amplitude fluctuations of the protein are found in that region (Figure 7)

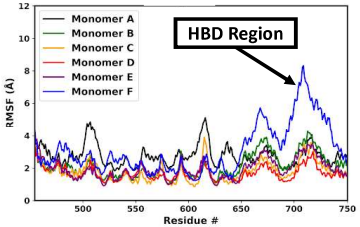

Figure 7: RMSF plotted for all six monomers. The HBD region is indicated with an arrow

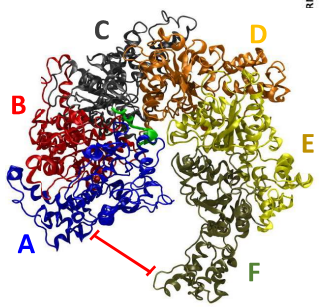

Figure 8: Spastin captured at a moment that characterizes the large fluctuation of monomer F indicated by figure 7, notice the large gap between monomers F and A in comparison to Figure 4

- The high fluctuations for F may be due to its position as the terminal monomer, giving it greater accessibility to water
- The other monomers have comparatively lower fluctuations
- The fluctuations of F result in a widening of the gap between it and monomer A, increasingly wider than in the starting spiral conformation in Figure 4 (Figure 7)

Principal Component Analysis

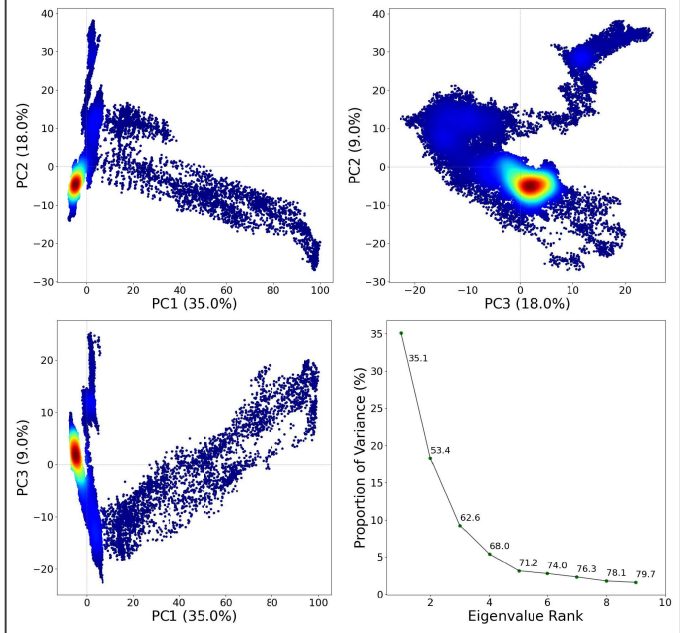

Figure 9: (First Row, Left to Right): Scoring plot for PC1 vs. PC2 and PC2 vs. PC3; (Second Row, Left to Right) Scoring plot for PC1 vs. PC3, Scree Plot showing cumulative variance explained by successive PCs. On the scoring plots, density of points is indicated by heat.

- The first three PCs explain 62.6% of the variance (Figure 9, lower right)
- Across each of the scoring plots, we notice one maximum of configuration density
- This maximum likely corresponds to a local minimum in the protein's free energy landscape
- If one assumes that the largest motions are the most important motions, the PCs would give the most important details of the protein's dynamics

Discussion

- Molecular dynamics simulations were performed for spastin in its hexameric spiral state assembled around a wild type β-tubulin CTT
- All five trajectories simulated were shown to converge by DCCM and RMSD
- HBD region of monomer F exhibited the greatest fluctuations
- Principal component analysis indicated clustering in state space

Acknowledgements

National Science Foundation
WHERE DISCOVERIES BEGIN

References

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