

Investigating SUN1 as a INM Protein under Mechanical Stress

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ABSTRACT

Eukaryotic cells have a nuclear envelope—a membrane barrier made up of two lipid bilayer membranes—that surrounds the nucleus. In this region exists a protein complex responsible for cellular response to mechanical force called the LINC complex (Linker of Nucleoskeleton and Cytoskeleton). LINC complexes contain SUN1, SUN2 and nesprin proteins with SUN1 bind with emerin within the inner nuclear envelope (INE) whose mutation can cause X-linked Emery-Dreifuss muscular dystrophy (EDMD)

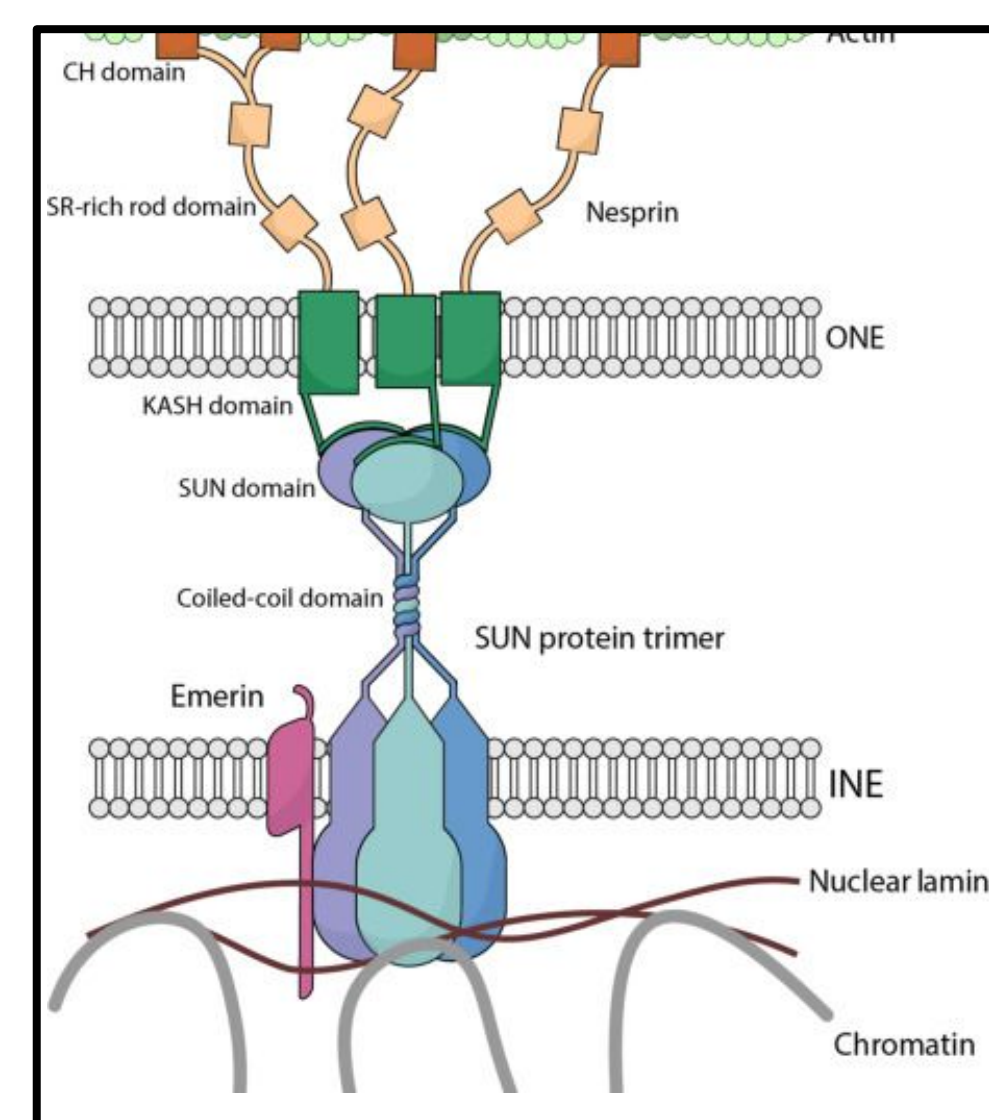
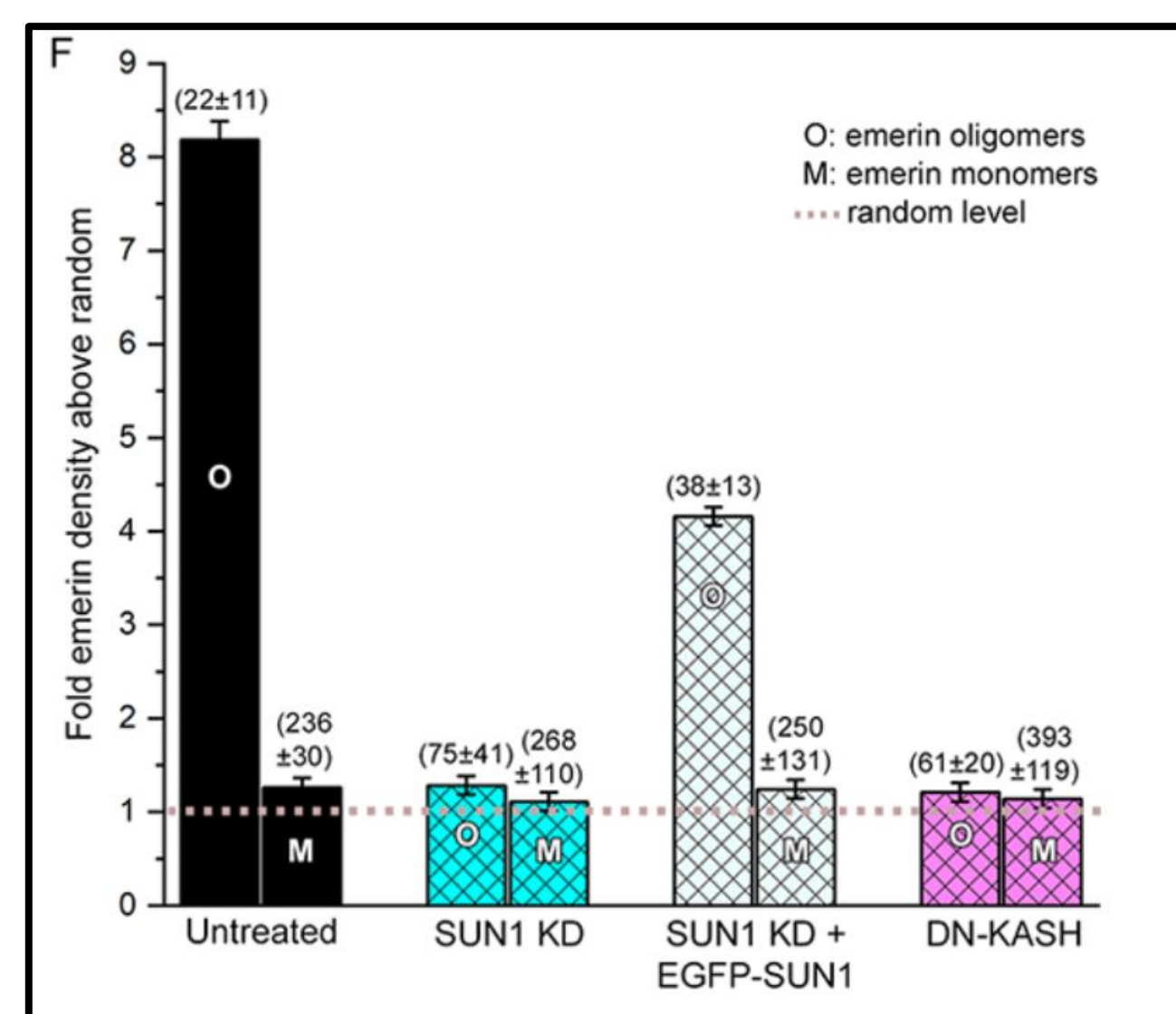
EDMD is a specific muscular dystrophy that weakens the muscle cells when mechanical stress is applied to that region, comparable to lifting weights or waving one's arm around. This is due to an emerin mutation in which force cannot be correctly taken in, resulting in the nucleus becoming rigid and even deteriorating. As the nucleus slowly destroys itself, the cell follows that behavior, resulting in the slow collapse of muscle cells in patients with EDMD.

Emerin's mutation often leaves the protein of interest, SUN1, unbound to itself, resulting in EDMD. In this project, we have prepared two distinct mutations of the protein SUN1 to replicate a similar outcome to if it were to experience mechanical stress without that stress being put forward through biochemical engineering processes.

OBJECTIVE

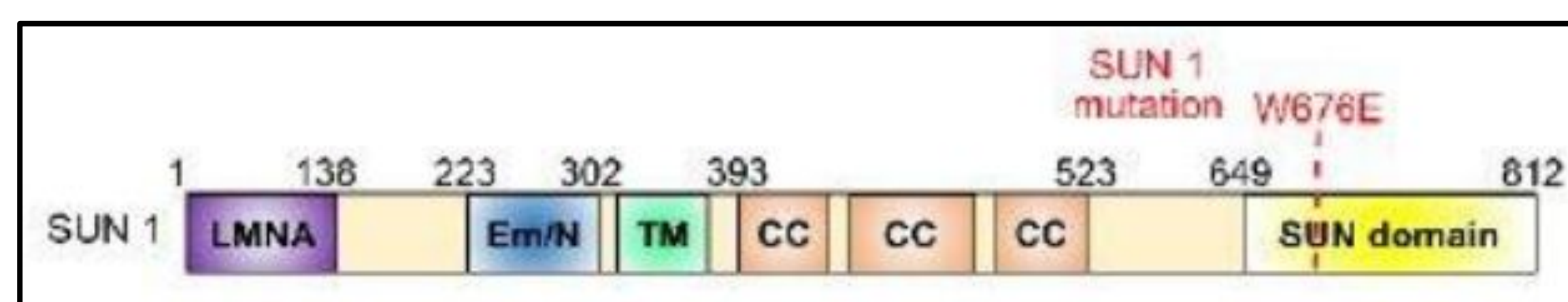
- The objective of this project is to explore how SUN1, a binding partner of emerin, may be affected under conditions of mechanical stress without mechanical stress through mutations within the coiled-coil domain.
- This was done through the preparation of these cells through mutagenesis, digestion, transformation, inoculation, miniprep, transfection, staining, imaging, and finally analyzing.

BACKGROUND INFORMATION



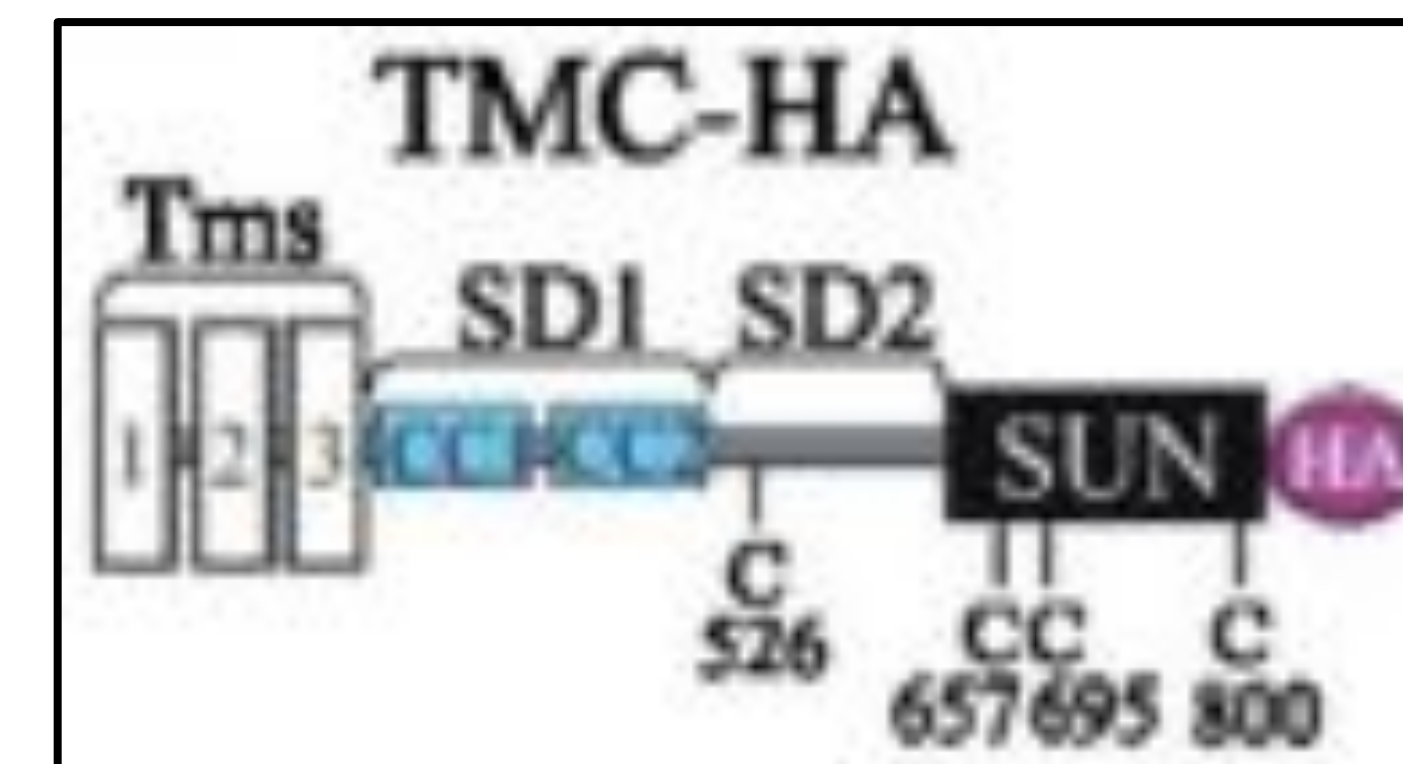
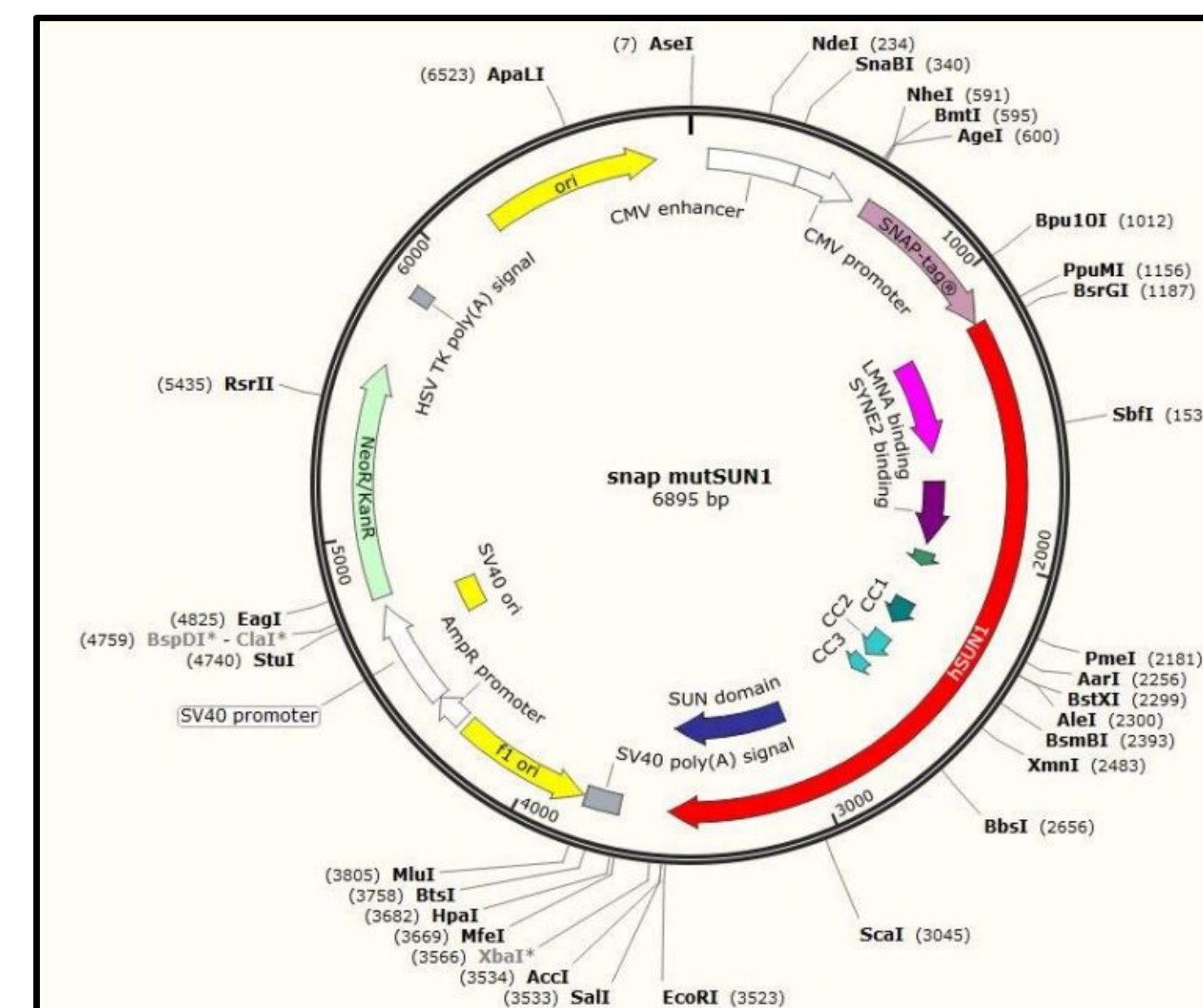
SUN1 KD Effect on Emerin Density

LINC Complex Diagram



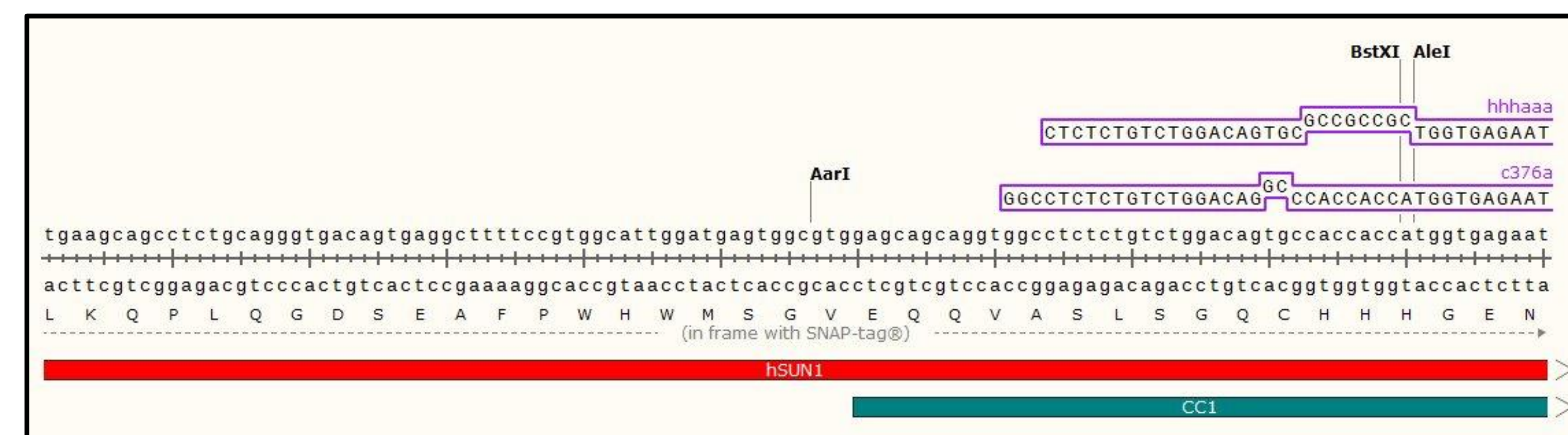
Schematic Sequence of SUN1 Protein

METHODS

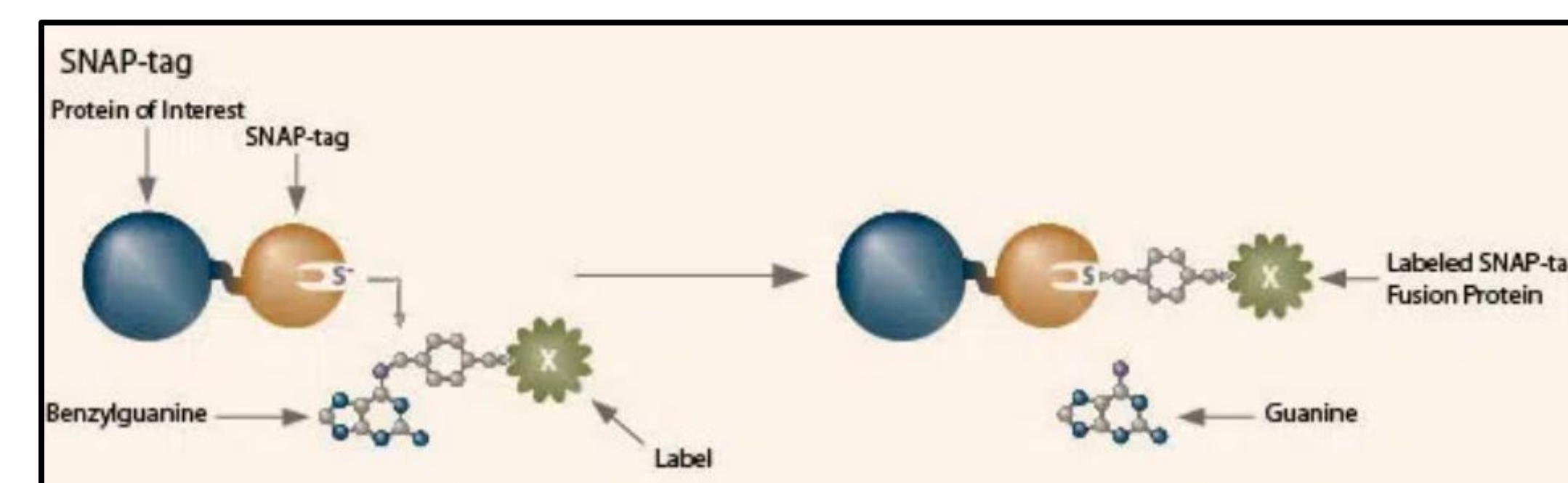


Location of Mutations

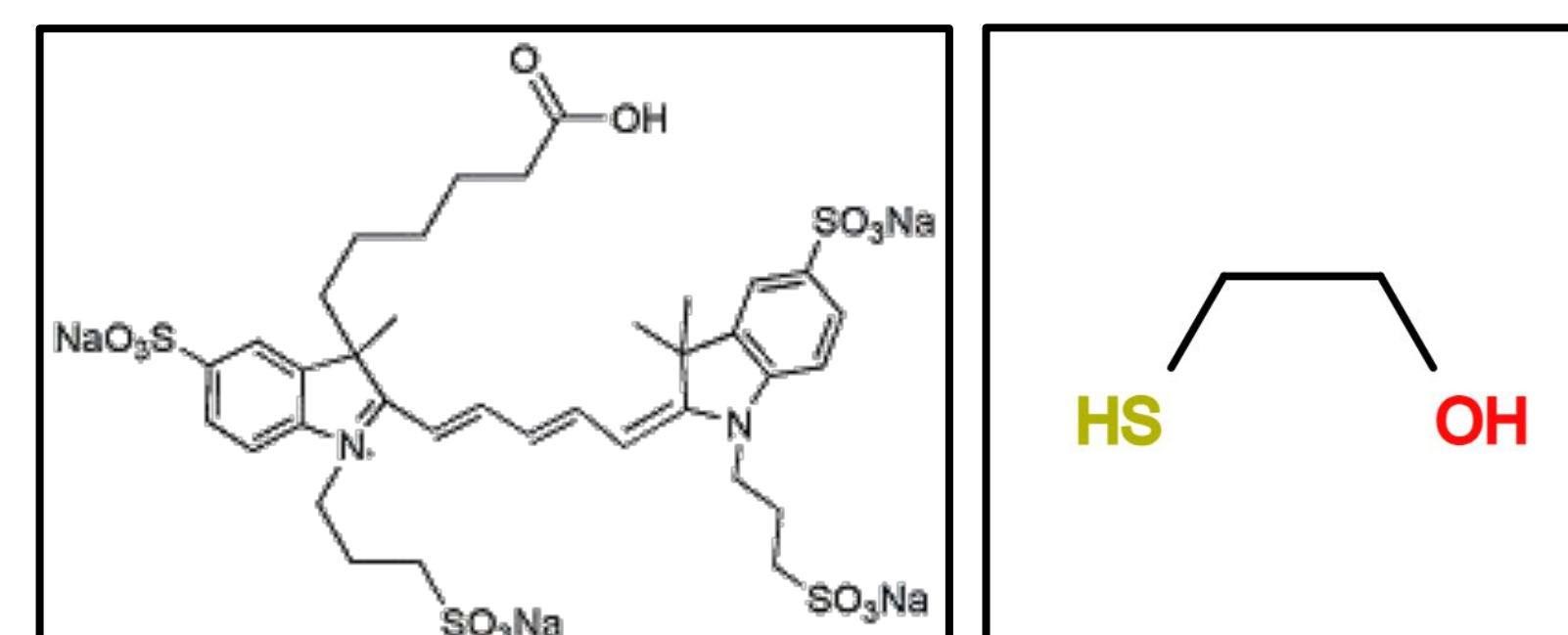
SNAP-mutSUN1 Template



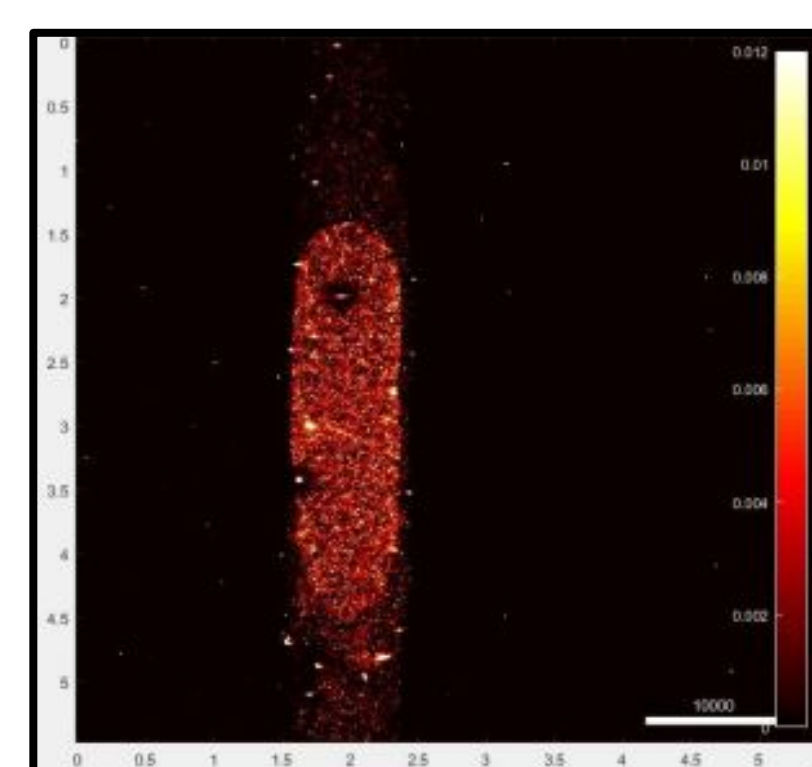
SUN1 Primers (C376A & 3HA)



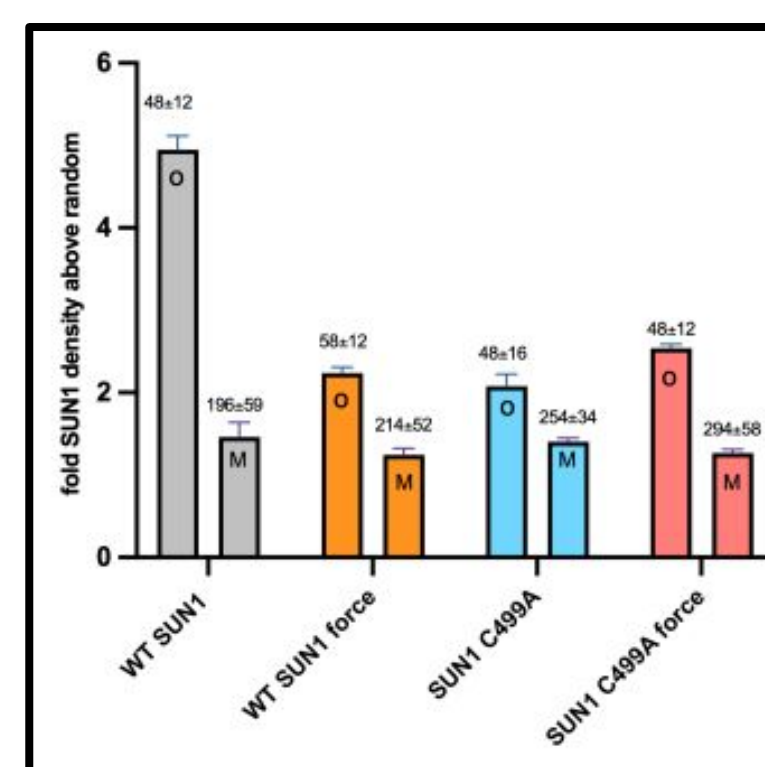
SNAP-tag Function in Recognizing Alexa-647 Dye



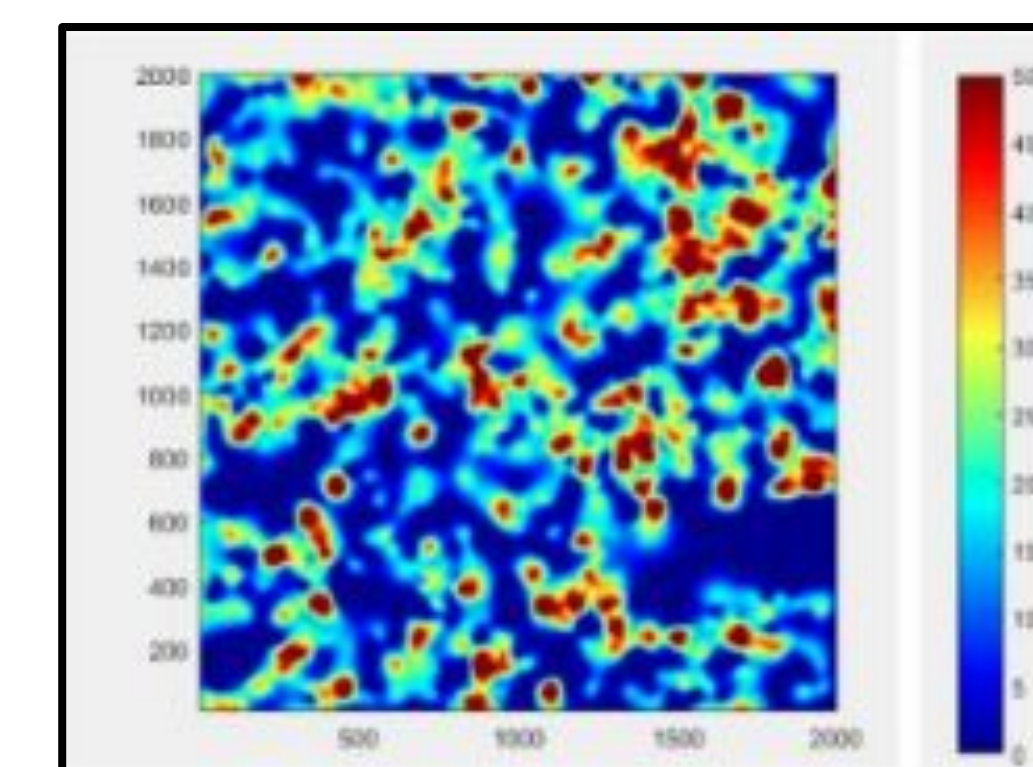
PRELIMINARY RESULTS



Micropatterning of Nucleus

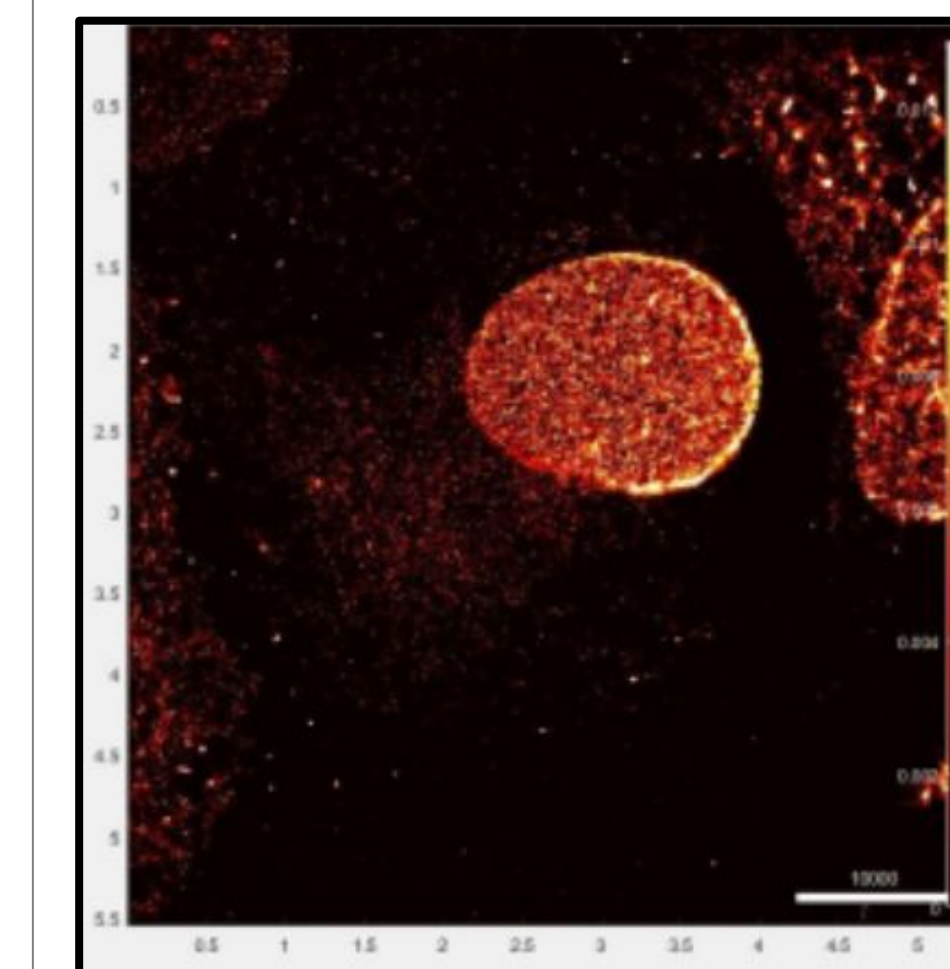


C499A Mutation Force Simulation

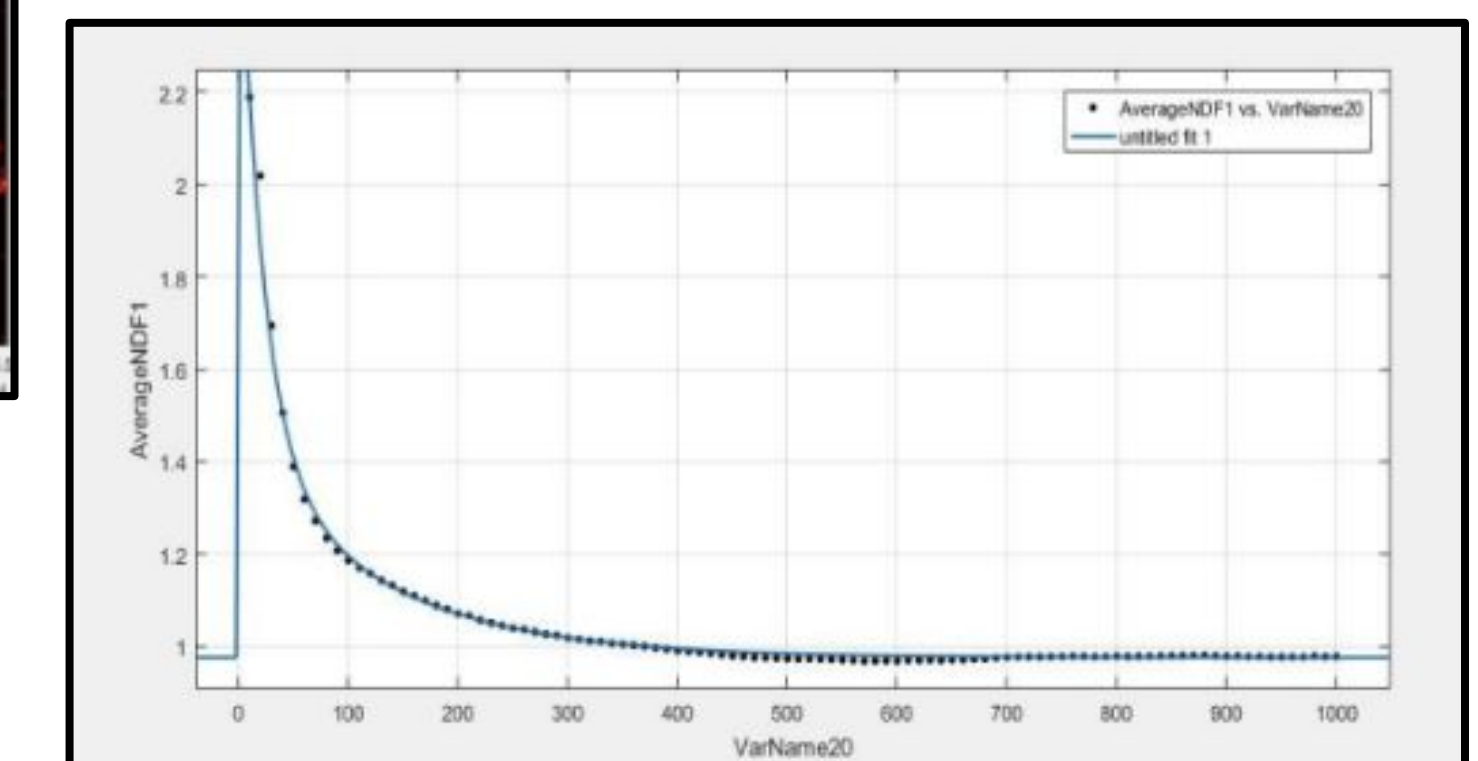


SUN1 Local Clusters

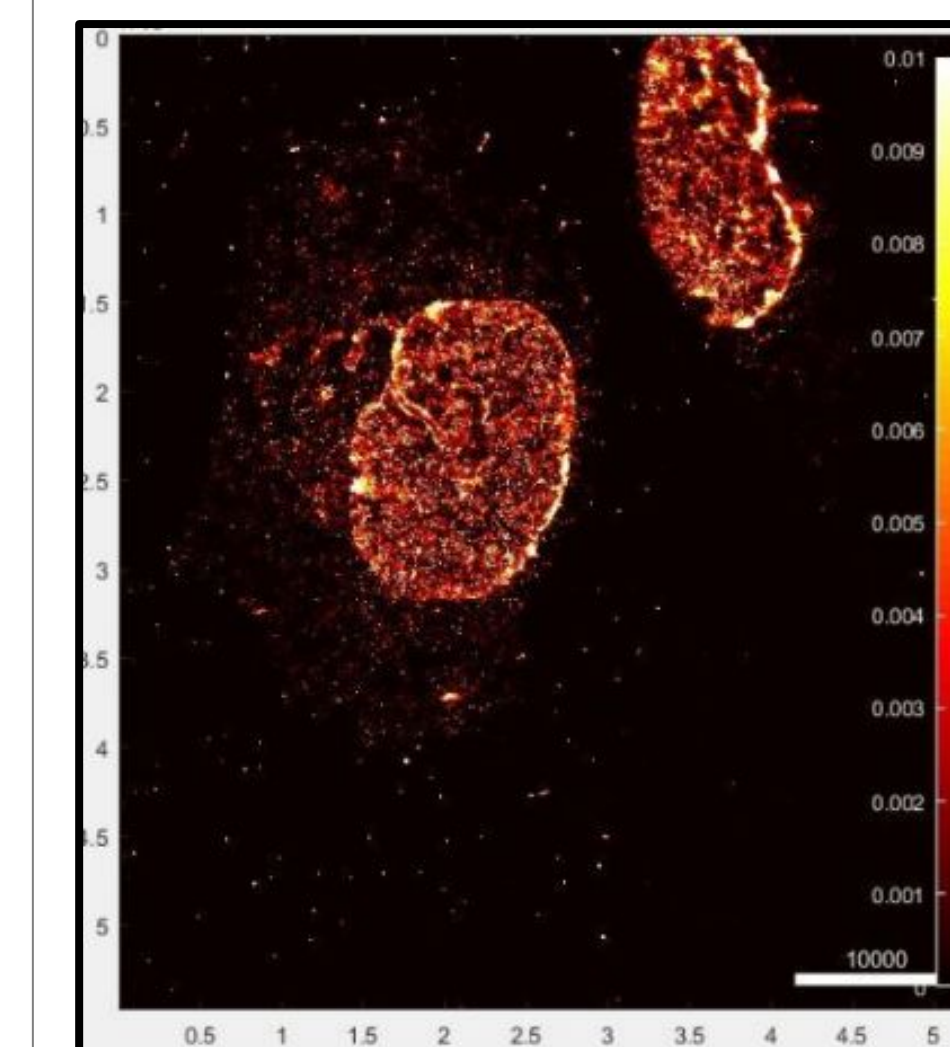
SNAP-mutSUN1 C376A Storm Imaging Results



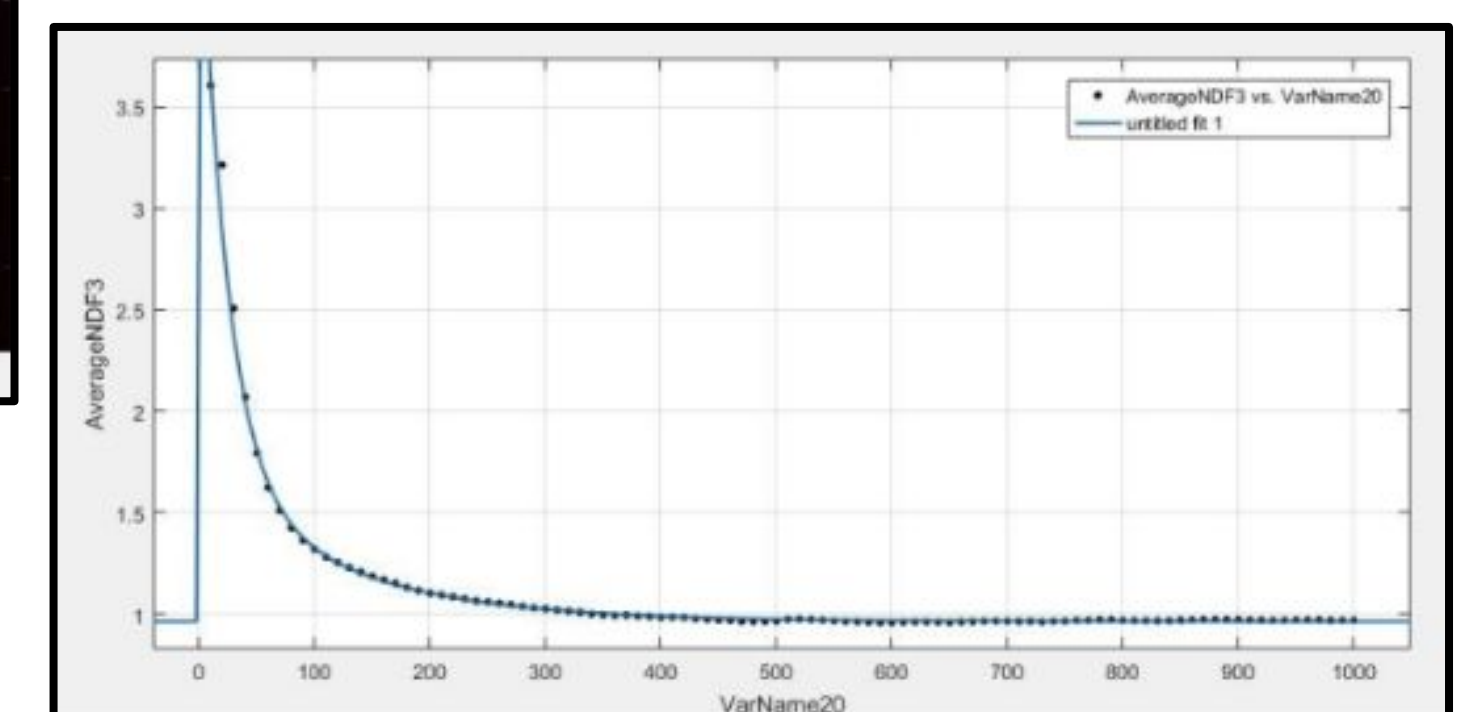
condition	Monomer density	Monomer size	Oligomer density	Oligomer size
SUN1 WT	1.46X above random	98nm	4.95X above random	24nm
SUN1 C499A	1.41X above random	127nm	2.09X above random	24nm
SUN1 C376A	1.47X above random	127nm	2.29X above random	22nm



SNAP-mutSUN1 3HA Storm Imaging Results



condition	Monomer density	Monomer size	Oligomer density	Oligomer size
SUN1 WT	1.46X above random	98nm	4.95X above random	24nm
SUN1 C499A	1.41X above random	127nm	2.09X above random	24nm
SUN1 C376A	1.47X above random	127nm	2.29X above random	22nm
SUN1 3HA	1.75X above random	121 nm	4.17X above random	23nm



SUMMARY AND CONCLUSION

- The location in which SUN1 is able to form oligomers surrounded by trimers can be found on the inner nuclear envelope (INE).
- Ways that SUN1 can respond to force application can be through decreasing oligomer density, increasing trimer domain size, and increasing diffusion speed.
- SUN C499A and C376A, a mutation located near the coiled-coil domain of the SUN1 complex, can mimic force response out of a non-mutated SUN1 protein without actual force application.
- SUN1 3HA following the C376A did not have an effect on SUN1 oligomerization but affect nuclear morphology which requires further research.

ACKNOWLEDGEMENTS

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CONTACT US

bridge.usc.edu/bugs