Self-Assembling Nanodiscs Technology Exploration with Single-Molecule Biophysics Experimentation using Site-Specific Attachment Atomic Force Microscopy

Stephanie Talder

The following is an excerpt from a longer piece. For full text, please visit https://scholar.colorado.edu/concern/undergraduate_honors_theses/xg94hq786

Abstract

The relationship between membrane proteins and functional cells is not yet fully understood, in large part due to the lack of knowledge about the structure and dynamics of membrane proteins. Because of the recent advancement of biotechnology, the visualization of membrane protein dynamics and energetics has progressed significantly, in large part due to nanodisc technology. Nanodiscs allow for the formation of a native environment for membrane proteins, which is essential to learning more about their structure. Atomic force microscopy (AFM) allows for the precise imaging of membrane proteins as well as the utilization of single-molecule force spectroscopy (SMFS). When completing single-molecule experimentation, it is crucial that the covalent attachment of the probe is completed, because it allows for hundreds of force-extension traces from a single molecule to be completed. Another essential aspect of site-specific attachment is passivation is necessary for unwanted interactions between the AFM cantilever tip and a single probe molecule. The focus of my senior thesis is to work with the optimization of nanodisc technology formation embedded with the membrane protein bacteriorhodopsin (bR). The bR was inserted into nanodiscs in both wild-type and c-terminal cysteine transformed to allow for site-specific labeling. The formation of nanodiscs with c-terminal cysteine bR was then labeled with DBCO-Maleimide tagging to allow for covalent connections when utilizing AFM SMFS. Altogether, this work shows a methodology for the optimization of nanodisc formation containing c-terminal cysteine bR membrane protein and warrants further investigation utilizing AFM imaging and SMFS with varying conditions of site-specific spectroscopy to target the development of protein-membrane dynamics.