**Advances in single molecule microscopy: protein characterization, force analysis and fluorescence localization**

Recent advances in single molecule techniques have allowed scientists to address biological questions which cannot be resolved by traditional ensemble measurements. In this dissertation, I integrate single molecule and bulk measurements to establish a direct link between copper exposure and neurotoxicity in prion disease. Furthermore, I develop a new analysis method to improve the accuracy of kinetic parameter estimation in single molecule Atomic Force Microscope (AFM) experiments. Finally, I develop a new fluorescence localization microscopy to identify the axial position of a single fluorescent object with sub-nanometer accuracy.

Prion diseases are characterized by the misfolding and oligomerization of prion protein (PrP). Copper exposure has been linked to prion pathogenesis; however, the molecular mechanism is still unknown. In the first part of this dissertation, I use single molecule fluorescence assay, dynamic force spectroscopy (DFS) with AFM, and real-time quaking-induced conversion (RT-QuIC) a to resolve, for the first time, the mechanistic basis by which Cu2+ ions induce a structural change in PrP, further promote oligomerization, template amyloid formation and neurotoxicity.

In the second part of this dissertation, I established a more accurate analysis method for single molecule DFS experiments. DFS is a widely used technique to characterize the dissociation kinetic between individual biomolecules. In AFM-DFS, receptor-ligand complexes are ruptured at different force rates by varying the speed at which the AFM-tip and substrate are pulled away from each other. The rupture events are grouped according to their pulling speeds and the mean force and loading rate of each group is calculated. This data is subsequently fit to the established models to extract the kinetic parameters such as the intrinsic off-rate (*koff*) and the width of the potential energy barrier (*xβ*). However, due to the large uncertainty in determining the mean forces and loading rates, errors in the estimated *koff* and *xβ* can be substantial. Here, I demonstrate that these errors can be dramatically reduced by sorting rupture events into groups using cluster analysis. Monte Carlo simulations show that cluster analysis is very effective at improving the accuracy of parameter estimation, especially when the number of unbinding events are limited and not well separated into distinct groups.

Finally, I describe a new technique, standing wave axial nanometry (SWAN), to image the axial location of a single nanoscale fluorescent object with sub-nanometer accuracy and 3.7 nm precision. A standing wave, generated by positioning an AFM tip over a focused laser beam, is used to excite fluorescence; axial position is determined from the phase of the emission intensity. I use SWAN to measure the orientation of single DNA molecules of different lengths, grafted on surfaces with different functionalities.