Proteins are central to life. More than 30% of proteins in any organism are transported from the site of synthesis into or through cell membranes to properly localize and function. The general secretory or Sec system is the major route of export for proteins from the cytosol of Escherichia coli and all eubacteria. The Sec system consists of the heterotrimeric translocon SecYEG along with cytosolic factors SecA (the ATPase of the system) and chaperone SecB. Many significant questions remain unanswered regarding SecYEG mediated secretion, including how many SecYEG and SecA copies are required during translocation. Recently, atomic force microscopy (AFM) has emerged as an important complementary tool in biophysics and is well suited for studying membrane proteins in near-native conditions. My thesis work represents the first characterization of Sec system using this technique.

We directly visualized the changes in the structure of SecYEG as a function of time. The dynamics observed were significant in magnitude and were attributed to the cytoplasmic loops spanning transmembrane helices 6-7 and 8-9 of SecY. In addition, we identified a distribution between monomers and dimers of SecYEG as well as a smaller population of higher order oligomers. We have also imaged SecA engaged on SecYEG and related the structural states observed to the activity of the translocase. Lastly we imaged the active Sec system in the presence of two different precursors. The measured heights of Sec system protrusions with proOmpA were generally in the lower range (~10 to 32 Å) indicating the release of SecA during translocation. In contrast, the major height distribution was around ~40-60 Å for pGBP indicating SecA remains bound during translocation. Overall, this work represents the novel view of the topographical details of the Sec system in near native conditions.