Single-Molecule Dynamics: Fluorescent Tracking and Analysis Techniques

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Preparation

We prepared fluorescent protein samples including AF594 Streptavidin, Neutravidin, Calmodulin (CaM) with avi-tagged wild-type nitric oxide synthase (nNOS), and a 10X fluorescence buffer. These samples were prepared to enable fluorescence imaging and analysis of protein-protein interactions.

Collection

We collected fluorescence data using a Nikon Eclipse Ti2 scanning confocal inverted microscope. Glass cover slips and silicone adhesive wells were used to contain our protein samples for imaging. We performed single-molecule fluorescence correlation spectroscopy (FCS) measurements over time to record photon counts and fluorescence lifetimes. By recording photon counts and lifetimes, we gained information on molecular motions of nNOS, interaction kinetics, and more. These time-resolved single-molecule measurements provided key insights into our protein systems.

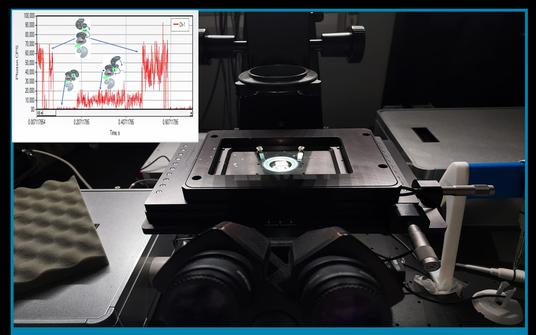
Analysis

This project aims to extract a kinetic model from sets of trajectories obtained from individual molecules. We are interested in both the timescale and sequencing for transitions observed in our signals. This information will be available from a properly derived transition plot, two examples of which are shown on the bottom right for example sequences of states. The publicly available program mashFRET can derive these plots, and the corresponding mechanisms, from a set of trajectories. So, in this project, we are seeking to properly format our data so that it can be analyzed through mashFRET.

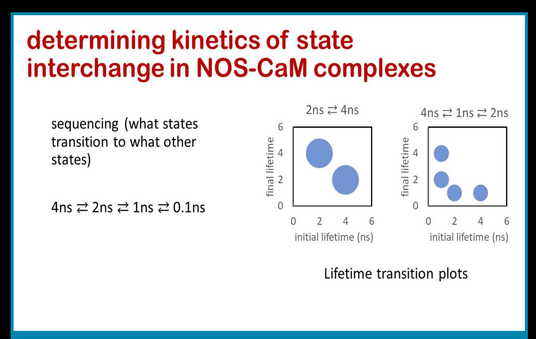
Results

Although this work is ongoing, we have made substantial progress in learning to analyze fluorescence data from single molecules. We have become proficient in using methods like mashFRET to interpret quenching changes. We have optimized our MATLAB code and created documentation to guide others. We have significantly advanced our abilities to collect and interpret fluorescence trajectories, revealing details of CaM-NOS interactions that were previously hidden. While more work remains, establishing these foundational techniques and tools will enable future discoveries about how calcium sensor proteins dynamically regulate enzyme activity.

Step 1: Prepare Samples

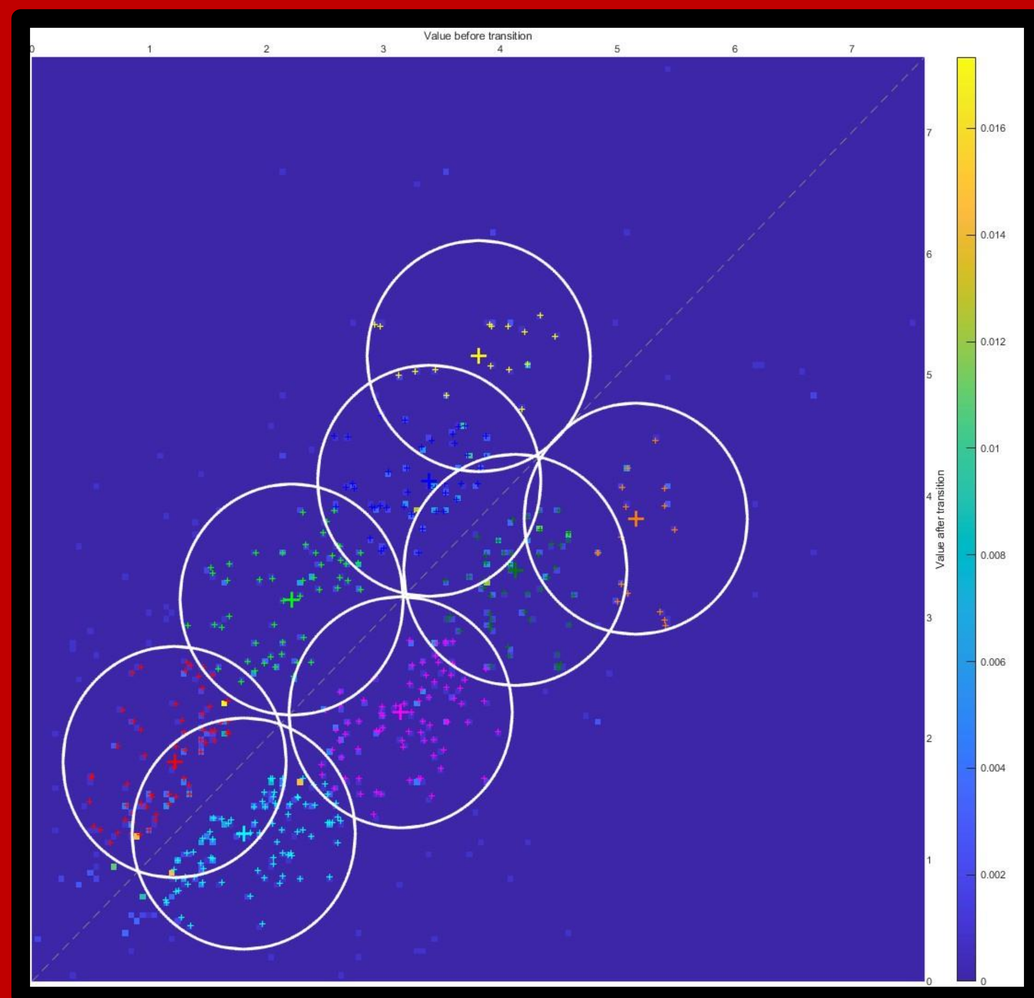


Step 2: Record Data



Step 3: Transition Analysis

This project successfully collected and interpreted trajectory data for various samples using fluorescence spectroscopy, offering insights into the behaviors and interactions of the studied molecules, while also optimizing existing data analysis code. The normalization of the trajectories should produce results similar to those of Figure 3, but processing this data remains an ongoing effort.



References

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Supplementary Information

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