Conclusion

The thesis focused on the development and application of fluorescence-based spectroscopic and microscopic techniques to detect molecular interactions essential for understanding the organization and functioning of biological organisms. Single-molecule fluorescence techniques integrate great spatial and temporal resolution to investigate biochemical and cellular processes. By observing oscillations in fluorescence intensity over time, we can infer structural mechanisms, conformational dynamics and reaction kinetics, thus establishing a connection between structure and function. Ensemble approaches facilitate the examination of equilibrium distributions, whereas single-molecule techniques elucidate the stochastic characteristics of molecular dynamics. We also integrate the experimental techniques with the molecular dynamic simulations for the cross-validation, as demonstrated in several projects. This work enhances the comprehension of protein-drug interactions, micelle-mediated drug delivery systems, and DNA conformational dynamics, specifically addressing the issues of drug solubility, delivery efficacy, and the structural transformations of biomolecules.

The first project (fourth chapter) of this study illustrates the efficacy of protein and micelle systems as effective carriers for poorly water-soluble substance curcumin. Through the utilization of fluorescence spectroscopy and confocal imaging, we clarified the interaction of curcumin with protein and micelle systems, demonstrating a blue shift in emission maxima and increased binding constants. This was mainly attributable to interaction of tryptophan residues into the hydrophobic core of the micelles. Our research demonstrates that curcumin integrates into the hydrophobic cores of micelles and proteins, rendering the combined system a viable candidate for drug delivery applications. These insights may facilitate the advancement of the curcumin as a natural medicinal drug, addressing existing issues associated with solubility and physiological delivery.

In the subsequent chapter, we analyzed the interactions among the anti-inflammatory agent diflunisal, cyclodextrins (β-CD and HP-β-CD), and the protein lysozyme via fluorescence spectroscopy. The research emphasized the non-cooperative characteristics of drug-cyclodextrin interactions and demonstrated the impact of cyclodextrin cavity size and hydrophobicity on binding affinities and quantum yields. Additionally, FRET and solvation dynamics investigations elucidated the close proximity of diflunisal to the ligand-binding domain of lysozyme and the nuanced alterations in the polar environment surrounding the protein during drug engagement. The findings are supported by molecular dynamics simulations, providing a new perspective on the interaction between drug-carrier complexes and proteins, which is essential for the advancement of more effective drug delivery systems.

In the following projects we first build a pTIRF setup by using a confocal microscope to perform smFRET experiment. We investigate how DNA HJ in the presence of Mg^{2+} demonstrated intricate oscillatory patterns, encompassing resonance, coherence, and decoherence in conformational changes. Through the formulation of a mathematical model to elucidate these patterns, we identified a memory effect in HJ dynamics, characterized by the periodic recurrence of specific conformations. Utilizing two-dimensional regional correlation analysis, we discerned stochastic frequency variations and semi-periodic motion characteristic of biological systems, essential for accurately extracting information from smFRET data. The research yielded insights into the energy landscape of the HJ, identifying three conformational states and determining the activation energies for transitions among these states.

In the last chapter of the thesis, we have integrated experimental and computational studies and identified three thermodynamically stable macrostates of DNA HJ, with transition speeds influenced by the concentration of divalent ions like Mg^{2+}. We demonstrated that the open conformation of the HJ predominates under physiological settings, highlighting the essential role of ions in maintaining these structures. These findings enhance our comprehension of HJ dynamics and indicate wider implications for genome maintenance and genetic recombination, underscoring possible pathways for therapeutic intervention in genetic diseases and for potential therapeutics. Previous reports predominantly highlighted conformational transitions between isoI and isoII forms or concentrated on force-field-dependent stabilization of stacked states;

however, our study identifies a significant open state under physiological Mg^{2+} concentrations and uncovers distinct ion localization patterns, particularly at the junction center. This observation addresses a significant knowledge gap by correlating local ion distribution with macrostate stability. These findings underscore the significance of ionic microenvironments in determining the free-energy landscape of DNA junctions and provide opportunities for additional investigation into how specific ion types or sequence variants affect DNA structure-function interactions in recombination and repair mechanisms.