Chapter 1

Overview

1.1 Motivation of the Work

Detailed reports on the structure and function of biological macromolecules in condensed phases and subcellular diverse microenvironments are developed independently when novel cutting-edge technologies emerge. Nonetheless, comprehending the interplay among the structures, functions, and dynamics of biomolecules remains unclear. This research is driven by function, and dynamics of biomolecules, which are vital for fundamental biological processes and therapeutic applications. Biomolecules are intricate entities whose structure, function, and dynamics are intrinsically interconnected. The functionality of a biomolecule is contingent not only upon its structure but also on its real-time dynamics. Understanding these links is crucial for clarifying the molecular pathways that underlie significant biological events. Spectroscopic techniques, particularly those applicable to single molecules, are optimal for elucidating these dynamics as they enable observation of structural changes, interactions, and molecular transitions across time. To achieve a thorough understanding of biomolecule interactions and functions in biological systems, our research utilizes several spectroscopic techniques to explore these aspects. This thesis identifies critical deficiencies in our comprehension of biomolecular dynamics, laying the groundwork for prospective treatment strategies targeting genetic illnesses and improving drug efficacy.

1.2 Objective

In the initial part of the thesis, we discussed the interactions between proteins and hydrophobic drugs in the presence of a drug carrier. We employed fluorescence spectroscopy and molecular dynamics simulations to demonstrate the interaction of diflunisal with cyclodextrin complexes in the presence of protein. The above-mentioned observations improve the advancement of more efficient medication delivery systems. We studied the interaction between curcumin and bovine serum albumin (BSA) in the presence of a micelle carrier and found that significant hydrophobic interactions facilitate the binding, which may enhance our understanding of curcumin's efficacy in clinical trials. Next, we investigate the single-molecule approach to elucidate the conformational dynamics that are often obscured in ensemble studies. To study conformational changes in molecules in real time using high spatial-temporal resolution at the single-molecule level, we built a p-TIRF microscope to conduct smFRET investigations. With the smFRET experiment, we studied the conformational dynamics in DNA HJ, and by rigorous statistical analysis, we have identified patterns of resonance, coherence, and decoherence resembling intermittent conformational dynamics. In the following chapter, we evaluated the kinetic choreography and favored stability of HJ conformations in ionic conditions by smFRET and molecular dynamic simulation that closely resemble the physiological environment relevant to cellular life. Stabilization of conformations due to the ionic environment and activation barriers concertedly affect transition rates between open and closed conformations. Moreover, we noted a substantial increase in Mg^{2+} condensation in the central region of the Holliday junction compared to the branch ends, suggesting that the differential stability of conformational states may be influenced more by the junction region of the Holliday junction than by the duplex branches. This study elucidates the intricate relationship between the ionic environment and HJ dynamics, enhancing our understanding of their behavior in cell biology.

1.3 Summary of the Work Done

1.3.1 Molecular Mechanism of Interaction of Curcumin with BSA, Surfactants and Live E. Coli Cell Membrane Revealed by Fluorescence Spectroscopy and Confocal Microscopy[1]

Clinical trials on the therapeutic effect of curcumin have proven to be highly effective against many diseases including cancer and Alzheimer's. However, the molecular mechanism of interaction of curcumin with protein and live cell membrane is poorly understood. Here, we report the mechanism of interaction of curcumin with bovine serum albumin (BSA) and live *E. coli* cell membrane in the presence of organized assemblies of sodium dodecyl sulfate (SDS) and cetrimonium bromide (CTAB) by fluorescence spectroscopy, laser-scanning confocal microscopy, and molecular docking study. Enhanced binding constant, blue-shifted emission spectra, and imaging of heterogeneous FRET on live bacteria cell membrane strongly indicate the complex formation of curcumin with strong hydrophobic interaction, which is further validated by docking results. Finally, our results may shed light on the efficient strategy of applications of curcumin as a natural therapeutic lead in clinical trials against many life-threatening diseases.

1.3.2 Unraveling the Interaction of Diflunisal with Cyclodextrin and Lysozyme by Fluorescence Spectroscopy[2]

Understanding the interaction between drug:carrier complex and protein is essential for the development of a new drug delivery system. However, the majority of reports are based on an understanding of interactions between drug and protein. Here, we present our findings on the interaction of the anti-inflammatory drug diflunisal with a drug carrier cyclodextrin (CD) and the protein lysozyme, utilizing steady-state and time-resolved fluorescence spectroscopy. Our findings reveal a different pattern of molecular interaction between the inclusion complex of beta-cyclodextrin (β-CD) or hydroxypropyl-beta-cyclodextrin (HP-β-CD) (as the host) and diflunisal (as the guest) in the presence of protein lysozyme. The quantum yield for the 1:2 guest:host complex is twice that of the 1:1 guest:host complex, indicating a more stable hydrophobic micro-environment created in the 1:2 complex. Consequently, the non-radiative decay pathway is significantly reduced. The interaction is characterized by ultrafast solvation dynamics and time-resolved fluorescence resonance energy transfer (FRET). The solvation dynamics of lysozyme become 10% faster under the condition of binding with drug, indicating a negligible change in the polar environment after binding. In addition, the fluorescence lifetime of diflunisal (acceptor) is increased by 50% in the presence of lysozyme (donor), which indicates that the drug molecule is bound to binding pocket on the surface of protein, and the average distance between active tryptophan in hydrophobic region and diflunisal is calculated to be approximately 50 Å. Excitation and emission matrix spectroscopy reveals that tryptophan emission increases 3-5 times in the presence of both diflunisal and CD. This indicates that tryptophan of lysozyme may be present in more hydrophobic environment in the presence of both diflunisal and CD. Our observations on interaction of diflunisal with β-CD and lysozyme are well supported by molecular dynamics simulation. This study may have impact on the development of better drug-delivery system in the future. It also reveals fundamental molecular mechanism of interaction of drug-carrier complex with protein.

1.3.3 Probing Single-Molecule Intermittent Coherence Conformation Dynamics and Multistate Transition Kinetic of DNA Holliday Junction by smFRET[3,4]

The relationship between conformational dynamics and multistate transition kinetics has been the fundamental focus for understanding the DNA Holliday Junction (HJ) intermediated

homologous genetic recombination process. Although crystal structure and Markovian dynamics of HJ are known, the nature of dynamics related to transition frequency to thermodynamically stable multistate and intermediate states remain unexplored. Using single-molecule fluorescence resonance energy transfer (smFRET) and statistical analysis, we have identified intermittent conformational dynamics with three different patterns: Resonance, Coherence, and Decoherence. Furthermore, we found that the intermittently coherence conformational dynamics of DNA Holliday Junction goes through multistep transition kinetics along the transition coordinates, presenting an extreme pattern of conformational dynamics. There is also a heterogeneity in the coherence frequency due to heterogeneity in the molecular dynamics. Our results on conformational dynamics of DNA HJ also support the multistate transition model. We applied multipoint time-correlation function (TCF) analysis to smFRET efficiency time-trajectories resulting from Cy3/Cy5 labeled DNA HJ to determine and compare the kinetics of various possible multistate conformational pathways. This TCF analysis show that intermediate states between the energy states of two extreme conformations likely play a critical role in loop-gated conformational change mechanisms in HJ-mediated genetic recombination processes.

1.3.4 Differential Mg^{2+} Deposition on DNA Holliday Junctions Dictates the Rate and Stability of Conformational Exchange[3,5]

DNA Holliday Junctions (HJ) are characterized by dynamic nature and transitioning among multiple conformations on the timescale ranging from sub-millisecond to second. While the influence of ions on HJ dynamics has been extensively studied, precise quantification of thermodynamic feasibility of transitions and detailed kinetic cooperativity remains unexplored. Understanding heterogeneity in providing stochastic gene recombination is extremely difficult to characterize using ensemble averaged experimental techniques because of its lack of ability to differentiate dynamics and function in a high spatiotemporal resolution. Here, we have developed a new technique that combines smFRET experiments and molecular simulation to investigate the kinetic choreography and preferential stability of HJ conformations under ionic condition that closely mimics physiological environment relevant to cellular biology. Our findings predict the prevalence of three distinct conformational macrostates in HJ dynamics. At low ion concentration, HJ transitions rapidly among three thermodynamically stable conformational macrostates. However, under physiological ionic environment, the open conformation becomes predominant. Using a kinetic network model based on multi-order TCF, we delineate the thermodynamic parameters that govern the heterogeneous dynamics as a function of divalent ion concentration. Stabilization of conformations due to ionic environment and activation barriers concertedly affect transition rates between open and closed conformations. Furthermore, we observed significant enhancement of Mg^{2+} condensation in the central region of HJ than branch ends, lead toward a plausible conclusion that differential stability of conformational states may be governed by junction region of HJ than duplex branches. This study provides a new insight into the complex interplay between ionic environment and HJ dynamics, offering a comprehensive understanding of their behavior under conditions relevant to cellular biology, and roles in key biological process for creating heterogeneous nature of life.

1.4 Plan of the Thesis

The plan of the thesis is as follows:

Chapter 1: An overview of the thesis's scope and motivation is presented in this chapter. Covered in this chapter is a brief description of the work completed.

Chapter 2: This chapter summarizes of the fundamental concepts of proteins, drugs, organized assembly and DNA Holliday Junction.

Chapter 3: This chapter briefly describes the instrumentation and data analysis and a detailed method to build a pTIRF setup inexpensively using an existing confocal microscope.

Chapter 4: This chapter deals with the mechanism of interaction of curcumin with bovine serum albumin (BSA) and the live *E. coli* cell membrane in the presence of organized assemblies of sodium dodecyl sulfate (SDS) and cetrimonium bromide (CTAB) by fluorescence spectroscopy, laser-scanning confocal microscopy, and computation.

Chapter 5: This chapter discusses our work on the interaction of the anti-inflammatory drug diflunisal with a drug carrier cyclodextrin (CD) and the protein lysozyme, utilizing steady-state and time-resolved fluorescence spectroscopy.

Chapter 6: In this chapter, we have discussed our work on probing single-molecule intermittent coherence conformation dynamics and multistate transition kinetics of DNA Holliday junction by smFRET.

Chapter 7: This chapter is focused on investigating the kinetic choreography and preferential stability of HJ conformations under an ionic condition that closely mimics the physiological environment relevant to cellular biology.

References:

1. Molecular Mechanism of Interaction of Curcumin with BSA, Surfactants and Live *E. Coli* Cell Membrane Revealed by Fluorescence Spectroscopy and Confocal Microscopy Pratibha Agarwala, Turban Bera, and Dr. Dibyendu K. Sasmal* ChemPhysChem 2022, 23, e202200265

2. Unraveling the Interaction of Diflunisal with Cyclodextrin and Lysozyme by Fluorescence Spectroscopy. Pratibha Agarwala, Arabinda Ghosh, Priyanka Hazarika, Debopam Acharjee, Shirsendu Ghosh, Debasish Rout, Dibyendu K. Sasmal,* J. Phys. Chem. B 2023, 127, 45, 9710–9723.

3. Economical Method to Construct a Prism-based TIRF Setup on an Existing Confocal Microscope to Perform smFRET Experiments. Pratibha Agarwala, and Dibyendu K. Sasmal*

4. Probing Single-Molecule Intermittent Coherence Conformation Dynamics and Multistate Transition Kinetics of DNA Holliday Junction by smFRET. Pratibha Agarwala, Saikat Sadhukhan, Soumen Mandal, and Dibyendu K. Sasmal*

5. Differential Mg^{2+} Deposition on DNA Holliday Junctions Dictates the Rate and Stability of Conformational Exchange. Pratibha Agarwala, Arumay Pal, Milan Kumar Hazra*, and Dibyendu K. Sasmal*