Declaration

I hereby declare that the work presented in this thesis titled "***Spectroscopic Understanding of Structure, Function, and Dynamics in Biomolecules: From Ensemble to Single Molecule***" submitted to the Indian Institute of Technology Jodhpur in partial fulfilment of the requirements for the award of the degree of Doctor of Philosophy, is a bonafide record of the research work carried out under the supervision of Dr. Dibyendu K. Sasmal. The contents of this thesis in full or in parts, have not been submitted to, and will not be submitted by me to, any other Institute or University in India or abroad for the award of any degree or diploma.

Pratibha Agarwala
P20CY008

Certificate

This is to certify that the thesis "***Spectroscopic Understanding of Structure, Function, and Dynamics in Biomolecules: From Ensemble to Single Molecule***" submitted by Pratibha Agarwala (P20CY008) to the Indian Institute of Technology Jodhpur for the award of the degree of Doctor of Philosophy, is a bonafide record of the research work done by her under my supervision. To the best of my knowledge, the contents of this report, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma.

Dr. Dibyendu K. Sasmal
Ph.D. Thesis Supervisor
Date:

Abstract

Biomolecules are intricate entities in which the structure, function, and dynamics are intrinsically interrelated. The function of a biomolecule not only depends on the structure but also the dynamics in real-time. Deciphering the interlink among structure, function and dynamics is critical for elucidating the molecular mechanisms that drive important biological processes at the cellular level. Single molecule fluorescence spectroscopy is ideal for understanding these dynamics because it enables observation of structural changes, molecular interactions, and conformational transitions of a single specific molecule in real-time. In order to gain a comprehensive understanding of the interactions and functions of biomolecules in biological systems, our research employs a range of spectroscopic techniques to investigate these dimensions. In the first part of my thesis, we investigated the interactions between proteins and hydrophobic drugs in presence of drug carrier. We used fluorescence spectroscopy and molecular dynamics simulations to show how diflunisal interacts with cyclodextrin complexes in the presence of protein. The observations enhance the development of more effective pharmaceutical delivery methods. We studied how curcumin interacts with bovine serum albumin (BSA) in presence of micelle carrier and found that strong hydrophobic interactions drive the binding, which may help us understand how well curcumin works in clinical trials. In this thesis, we also explore the single-molecule technique to understand molecular heterogeneity and complex dynamics, which ensemble studies typically obscure. In order to observe conformational changes in molecules in real-time with high spatial-temporal resolution at the single-molecule level, we built a prism-based total internal reflection microscope setup to perform single molecule fluorescence resonance energy transfer (smFRET) experiments. We use the smFRET technique to study the conformational heterogeneity in a DNA Holliday Junction (HJ), with a thorough statistical analysis we have found patterns of resonance, coherence, and decoherence like intermittent conformational dynamics. In the subsequent chapter we also performed smFRET experiments and molecular simulations to investigate the kinetic choreography and preferential stability of HJ conformations under ionic conditions that closely mimic the physiological environment relevant to cellular biology. Our findings predict the prevalence of three distinct conformational macrostates in HJ dynamics. Using a kinetic network model based on a multi-order time correlation function (TCF), we delineate the thermodynamic parameters that govern the heterogeneous dynamics as a function of divalent ion concentration. Stabilization of conformations due to the ionic environment and activation barriers concertedly affect the transition rates between open and closed conformations. Furthermore, we observed a significant enhancement of Mg^{2+} condensation in the central region of the HJ rather than the branch ends, leading toward a plausible conclusion that the observed differential stability of conformational states may be governed by the junction region of the HJ rather than the duplex branches.

This study provides new insights into the complex interplay between the ionic environment and HJ dynamics, offering a comprehensive understanding of HJ behavior under conditions relevant to cellular biology, and its roles in key biological process create the heterogeneous nature of life.

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List of Publications

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2. Arun K. Upadhyaya, **Pratibha Agarwala**, Chanchal Sharma, and Dibyendu K. Sasmal* "Efficient Delivery of Hydrophobic Drug in Live Cancer Cell Using Carbon Quantum Dots Synthesized by Eco-friendly Methods."
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List of Abbreviations

α	Alpha
Å	Angstrom
K	Kelvin
η	Reflective Index
π	pi
λ	Wavelength
A	Adenine
AFM	Atomic Force Microscopy
APD	Avalanche Photodiode
AOTF	acousto-optic tunable filter
β-CD	beta- Cyclodextrin
βME	2-Mercaptoethanol
bp	Base Pair(s)
BP	Bandpass Filter
BSA	Bovine Serum Albumin
C	Cytosine
CD	Circular Dichorism
CCD	Charge-coupled device
CMC	Critical Micelle Concentration
CTAB	Cetyltrimethylammonium bromide
Cy	Cyanine Dye
DLS	Dynamic Light Scattering
DM	Dichroic Mirror
DNA	Deoxyribonucleic acid
DNA HJ	DNA Holliday Junction
dsDNA	double-stranded DNA
EMCCD	Electron Multiplying Charge-coupled device
E. Coli	Escherichia coli
FRET	Fluorescence Resonance Energy Transfer
G	Guanine
HP-β-CD	2- hydroxypropyl beta- cyclodextrin
HMM	Hidden markov model
HOMO	Highly Occupied Molecular Orbital
I_A	Intensity of Donor
I_D	Intensity of Acceptor
LED	Light emitting diode
LUMO	Lowest Unoccupied Molecular Orbital
MD	Molecular dynamics
MDS	Molecular dynamic Simulation
mg	Miligram
Mg	Magnesium
µg	Microgram
ml	Mililiter
µl	Microliter
mm	milimeter
µm	Micrometer
mM	Milimolar
µM	Micromolar
MW	Molecular weight
Mili Q	
NA	Numerical Aperture
nM	Nanomolar
ns	Nano second

NMR	Nuclear Magnetic Resonance Spectroscopy
PDB	Protein data bank
PDF	Probability Distribution Function
pM	Picomolar
pTIRF/pTIRF	Prism based Total Internal Reflection Fluorescence
R_0	Förster radius or distance, giving 50% FRET efficiency
R6G	Rhodamine-6G
RNA	Ribonucleic acid
rpm	Rate per minute
T	Thymine
TCSPC	Time Correlated Single Photon Counting
TIR	Total Internal Reflection
TIRF	Total Internal Reflection Fluorescence
TIRFM	Total Internal Reflection Fluorescence Microscopy
Tris	Tris(hydroxymethyl)amino methane
Trolox	6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid
TCF	Time Corelation function
TRES	Time Resolved Emission Spectra
Trp	Truptophan
SEM	Scanning Electron Microscopy
SDS	Sodium dodecyl sulphate
smFRET	single molecule Forster Resonance Energy Transfer
ssDNA	single stranded DNA
SSE	Steady State Emission
SSI	Steady State Intensity
UV-vis	Ultra-violet Visible