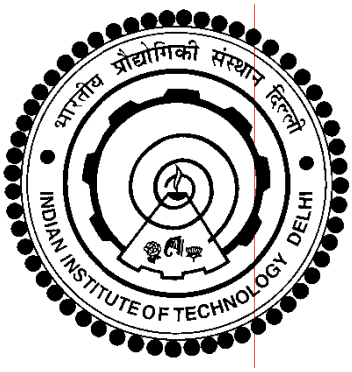
PROTEINS IN CROWDING AND CONFINEMENT

ASHIMA MALIK



DEPARTMENT OF CHEMISTRY

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PROTEINS IN CROWDING AND CONFINEMENT

by

ASHIMA MALIK

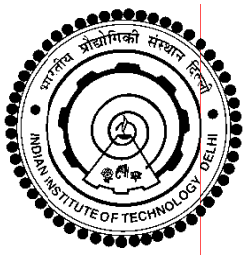
Department of Chemistry

Submitted

in fulfilment of the requirements of the degree of

Doctor of Philosophy

to the



Indian Institute of Technology Delhi

January 2016

DEDICATED
TO
MY GRANDFATHER

Late Ch. Mukhtiar Singh Malik

CERTIFICATE

This is to certify that the thesis titled **"Proteins in Crowding and Confinement"** being submitted by **Ms. Ashima Malik** to the **Indian Institute of Technology Delhi**, for the award of degree of **Doctor of Philosophy** is a record of bonafide research work carried out by her. **Ms. Ashima Malik** has worked under my guidance and supervision and has fulfilled the requirements for the submission of this thesis, which to my knowledge has reached the requisite standard.

The results contained in this thesis are original and have not been submitted, in part or full, to any other University or Institute for the award of any other degree or diploma.

Dr. Pramit K. Chowdhury
Associate Professor of Chemistry
Department of Chemistry
Indian Institute of Technology Delhi,
Hauz Khas, New Delhi- 110016

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Ashima Malik

ABSTRACT

The thesis entitled "***Proteins in Crowding and Confinement*"** focuses on the understanding of the effect of macromolecular crowding and confinement on the folding and unfolding of proteins and their differential effects on the respective conformational ensembles. The thesis features detailed investigation of the unfolding of two model proteins by thermal and/ or chemical denaturation means in presence of different macromolecular crowders utilizing various spectroscopic techniques.

The thesis has been divided into seven chapters.

Chapter I titled ''**Introduction**'' provides brief introduction to the phenomena of macromolecular crowding and confinement and their influence on the protein folding and unfolding. It also describes the objectives of the studies carried out in the thesis and the strategy devised to achieve the same. An effort has been made to present an overview of crowding and confinement studies that have been studied during the past years.

Chapter II titled "**Materials and Methods"** describes chemical procurement, purification and storage along with techniques used during the investigation. Specifically, steady-state and time-resolved fluorescence, UV-VIS spectroscopy, Circular Dichroism (CD) and Dynamic light scattering (DLS) measurements were used to carry out the requisite characterization. The techniques of fluorescence (steady-state and time-resolved) and CD spectroscopy have been employed to study the conformational changes of the proteins in confinement.

C**hapter III** titled "**Myoglobin unfolding in Crowding and Confinement"** includes a detailed investigation of the unfolding of the heme protein myoglobin (Mb) in presence of macromolecular crowders (Ficoll 70, Dextran 70 and Dextran 40) and in a confined (AOT reverse micelles in Isooctane (IO)) environment. Our results reveal that confinement effect can be quite destabilizing in nature for Mb with the extent of distortion depending on various factors such as water localized in the microemulsion water pools having different properties than bulk water and/or the interaction of Mb with the charged interfacial wall of AOT reverse micelle apart from the effects that are purely confinement related . Effects of the crowding agents on myoglobin show a deviation from the general notion that synthetic macromolecular crowding agents are always stabilizing in nature. Ficoll 70 was observed to be particularly destabilizing in its influence on Mb unfolding which indicates that effect of macromolecular crowders can be quite protein specific.

Chapter IV titled "**Interaction of ANS with Human Serum Albumin under Confinement: Important insights and relevance**" focuses on the conformational disposition of the multidomain protein HSA (585 amino acids) within the confinement of water pools of AOT reverse micelles and its interaction with a commonly used fluorescence probe 1-anilinonapthelenesulfonate (ANS) inside the water pools. Our study reveals the presence of little or no interaction between HSA and ANS inside the AOT water pools which is expected based on the observed distortion of the protein secondary structure on reverse micelle entrapment, the latter resulting in disruption of the binding pockets available to ANS. These observations are in sharp contrast to what has been alluded to earlier wherein such probes have been hypothesized to bind to the well-defined binding cavities (e.g. subdomain IIA) that this serum albumin possesses.

Chapter V titled "**Dramatic Effects of Macromolecular Crowders on Drug Binding to Serum Albumins: Consequences and Physiological Relevance**" lays out a detailed investigation of drug binding to the serum proteins, HSA and BSA in presence of a series of macromolecular crowding agents, Dextran 6, Dextran 40, Dextran 70 and Ficoll 70, using the fluorescence probe Coumarin-153 as a representative drug molecule. Since crowding agents can modulate protein structures (both shape and size) in the native and denatured states, these would then also be expected to affect the ligand binding properties of such proteins. Collectively our results provide important insights into the mechanism by which the cellular interior can modulate the binding aspects of proteins, starting from the number of pockets available (hence increasing the transfer yield per protein) to changes in the binding affinity, the latter being very important for release of bound species.

Chapter VI titled "**Urea induced unfolding of Human Serum Albumin in presence of Macromolecular Crowders using Prodan as an extrinsic probe''** provides a detailed investigation of the unfolding behavior of the protein as a function of urea in presence of different macromolecular crowders by probing with the help of Prodan. Our data provide an in-depth analysis of how domain II of HSA gets perturbed in presence of the synthetic crowding agents by using intrinsic (Trp) and extrinsic (Prodan) probes, the latter being non-covalently bound. We conclude that the crowding agents provide significant modulation of the HSA structure, the latter depending on the type of excluded volume that the crowders exude.

Chapter VII titled "**Conclusions and Future Prospects**" presents the conclusions drawn from the overall investigation, starting from the conformational dispositions of proteins in crowded and confined environments to how the native state ligand binding properties can get

affected by macromolecular crowders. This thesis opens the door to a host of future research possibilities that will contribute significantly to the fundamental understanding of crowding and confinement.

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