# Chapter 1

## Introduction to self-assembled structures.

Bio-molecules self-assemble into functional structures that are dynamic. Such self-assembly is driven primarily by electrostatic interactions, hydrophobic/hydrophilic interactions and hydrogen bonds. The energies required to destabilize these structures are greater than the thermal energy and hence are stable under physiological conditions. Some examples of self-assembled structures in biology are membranes bilayers, chromatin organization, assembly of virus capsid and microtubule networks. The reorganization of these structures *invivo* render them dynamic as well as functional. The continual reorganization of self-assembled structures is achieved by various ATP dependent molecular motors.

Emerging technology has enabled us to measure "pico-Newton" forces and "nano-meter" distances, providing tools to understand the mechanics and dynamics of biological self-assembled structures. A variety of measurement techniques, like the optical trap, magnetic trap, the micro-cantilevers have made possible measurement of forces in a broad range from ~sub-pico-Newton to 1000`s of pico-Newtons. For example, optical trap has been applied to study the step size and forces applied by molecular motors [1, 2, 3]. In addition, these methods have been applied to understand cell mechanics, DNA and chromatin elasticity, protein unfolding, rheology of soft systems, to name a few. Some of these methods that can be adapted to study *invivo* offer tremendous advantages in the study of live systems. In this thesis we have used an optical trap to apply localized forces on self-assembled structures specifically on membrane bilayers and chromatin organization. We have developed an "intensity modulated optical trap" to study the dynamics of these structures.

Biological membrane bilayers consist of amphiphilic molecules that have hydrophobic and hydrophilic regions. At room temperature they exhibit fluid phase with diffusion constant ~$10^{-11}$-$10^{-12}$$m^2$/s, enabling rapid diffusion of lipids and other molecules in the membrane. The extraordinary number of shapes that the membranes can assume is a result of their fluidic structure and small bending energies [4]. Bio-membranes undergo various shape changes and active fission/fusion events required for intra-cellular transport [5, 6]. Cargo vesicles and membrane tubules from the membrane organelles like endoplasmic reticulum, plasma membrane and the golgi are involved in transport of proteins in the cell [7]. Molecular motors, such as Kinesin, participate in the formation of

extensive membrane tubule networks that are dynamic in accord with cellular requirements [8]. The microtubule based motors can apply localized pico-Newton forces to pull out and extend the membrane tubules. The step sizes of the motors and the forces that they apply have been measured. Myosin can apply a force of about 3-4pN, with a step size of ~11nm [1] and Kinesin has been shown to be stalled at a force of ~5.4pN [2]. In Chapter 2 we describe an interesting regime of membrane flow into the tubule beyond a threshold force in multi-lamellar vesicles using an optical tweezer. Nanotubules from multilamellar vesicles show a frequency dependent response enhancement at the threshold force of membrane flow.

In Chapter 3 we describe our work on chromatin organization, achieved by the self-assembly of the eukaryotic genomic DNA with the highly basic histone proteins, which is driven by electrostatic interactions [9]. The DNA based molecular motors, for example, the remodeling enzymes like SWI/SNF, ISWI etc, helicase, RNA polymerases and DNA polymerases, cause alterations in the chromatin and DNA structures. The chromatin organization is highly regulated through the cell cycle and small stretches of the genome are opened up when required to enable access to proteins. This is achieved by a variety of "chromatin remodeling enzymes" and "histone modifying enzymes" [10, 11]. Such chromatin remodeling is highly localized to the specific stretch and is controlled by an elaborate regulatory mechanism. For example, during transcription initiation, modifications of the specific histone residues by acetylation, phosphorylation, etc. mark the DNA regions to be transcribed [12]. Such modifications are recognized by remodeling and other transcription related enzymes to disrupt the chromatin organization specifically and access the DNA [13]. The ATP dependent chromatin remodeling enzymes can apply localized forces to achieve chromatin remodeling in specific stretches of the genome [14]. The modification and remodeling enzymes thus alter the chromatin organization locally and hence its fluidity and accessibility.

In the first part of Chapter 3 we have explored a mechanistic basis for chromatin fluidity controlled by its state of compaction, using the intensity modulated optical trap. Here we discuss our experiments on measurement of local fluidity of chromatin fibers isolated from HeLa cells. We have used sensitive phase detection measurements to probe the local fluidity as a function of chromatin decompaction either mechanically or by

chemical means. The chromatin organization into the 30nm fiber and higher order structure is primarily driven by histone tail-tail interactions [15]. Tension applied on the chromatin fiber resulted in the disruption of the tail-tail interactions leading to an intermediate increase in the fluidity of the chromatin. Chemical digestion of the tails using trypsin also resulted in similar increase in fluidity.

In the second part of Chapter 3 we have studied the role of the chromatin in defining the nuclear architecture and mechanical stability. Although the architecture of the nucleus and its cell cycle dependent reorganization has been a subject of study for long, it is far from clear what determines such order in the nucleus. The elements of the nuclear architecture are the double nuclear membrane, the lamin scaffold which is an intermediate filament protein network under the nuclear membrane and the chromatin [16]. The outer nuclear membrane and inner nuclear membrane integral proteins interact across the nuclear lumen and mechanically couple the cytoskeleton with the nucleoskeleton. The chromatin is organized in an interphase nucleus with repeated attachments to the nuclear envelope. Hence the lamin network is connected to the membrane as well as the chromatin, rendering the nucleus a complex self-assembled structure of membranes, protein network and chromatin.

The higher order condensation of the chromatin, driven by histone tail-tail interactions, enables the packing of chromatin into the nucleus of diameter ~20μm [15]. Disruption of the tail-tail interactions led to the entropic swelling of the chromatin and hence the nucleus, as described in Chapter 3 of this thesis. Chromatin decondensation induced pressure resulted in expansion of the lamin network and the nuclear membrane prior to the rupture of the nuclear envelope. Our results suggest that the chromatin anchorage controls the lamin network and the two components together provide an architectural scaffold for the nucleus.

# Publications

The work discussed in this thesis resulted in publications as listed below.

1. Soni G. V.*, Feroz Meeran Hameed*, Roopa T.* and Shivashankar G. V. Development of an optical tweezer combined with micromanipulation for DNA and protein nanobioscience. *Current Science.* **83**, 1464 (2002).

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2. Roopa T. and Shivashankar G. V. Nanomechanics of membrane tubulation and DNA assembly. *Applied Physics Letters.* **82**, 1631 (2003).

3. Roopa T., Kumar N., Bhattacharya S. and Shivashankar G. V. Dynamics of membrane nanotubulation and DNA self-assembly. *Biophysical Journal.* **87**, 974 (2004).

4. Roopa T. and Shivashankar G. V. Direct measurement of local chromatin fluidity using optical trap modulation force spectroscopy. (Under review)

5. Roopa T., Basu A. and Shivashankar G. V. Higher order chromatin assembly is a structural element of the cell nucleus. (Under submission)

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