Summary

Subcellular components like the cytoskeleton, chromatin, and membranes play crucial roles in many cellular functions and have acquired specific structures during the evolutionary process tailored for specific tasks. Understanding the structure-function relationship of subcellular components is fundamental to understanding how these structures work and have been optimized by nature. In particular, inside the cell, these structures interact dynamically, respond to external mechanical stimuli, and exert mechanical forces. Therefore, this structure-function relationship is essential, as cells sense and adapt to mechanical stimuli, which influence in this way processes like growth, differentiation, and tissue repair. The scientific field investigating how cells sense and respond to physical stimuli is known as mechanobiology and is gaining a growing interest in the research community. This field emerges from biomechanics and exploits results and information coming from it. At the single-cell level, understanding the mechanical behavior of the cytoskeleton as well as the cell nucleus is crucial to characterize how mechanical stimuli are sensed by cells. In this context, computational molecular modeling can assist the study of subcellular structures with a resolution that can reach a single atom. Different *in silico* approaches can be used to create models of subcellular structures starting from experiments and study them in terms of mechanical behavior and conformational changes, suggesting insights into their structure-function relationship. However, since subcellular structures can be characterized by different mechanical properties, different methodologies need to be employed. On one side, microtubules (MTs) are stiff structures with persistence lengths that can reach several millimeters. These filaments are usually named *short*, since the usual length is shorter than their persistence length, as opposed to *long* filaments like chromatin, which usually are found in highly convoluted shapes inside the cell. In this dissertation, different approaches are presented to characterize the dynamics, conformation, and mechanics of stiff filaments, i.e., the MTs, and flexible filaments, i.e., chromatin, ultimately analyzing the relationship between the mechanics, function, and structure of those components.

In the case of MTs, the presented research aimed at characterizing the so-called structural communication of tubulin assembly, i.e., the way in which

vibrational stimuli propagate within the MT structure. The importance of structural communication resides in the highlight of key subunits, regions, and residues involved in the stability of the assembly and the propagation of stimuli. The structural communication of a standard MT has been compared to a taxol-stabilized MT but also to MTs with different architectures. At the same time, the mechanics and deformability of the MT have been studied through elastic network modeling (ENM). The analysis allowed the characterization of how the MT architecture is stabilized, the mechanical effect of stabilizing agents, as well as the relationship between architecture, mechanics, and specific functions.

As for chromatin, the described studies aimed at showing how molecular modeling can help in gaining insight into experimental evidence, but also how the existing ENM standard can be improved to model large-scale conformational changes involving nucleic acids. One study here presented provided atomistic insights into the effect of epigenetic modifications on histone dynamics, with possible implications in chromatin folding. The considered epigenetic modifications are associated with the calcification of arterial valves and variations in cell nucleus mechanics. Then, since the current ENM implementation still provides limited performance in modeling nucleic acid vibrations, an optimized essential dynamics-based implementation of ENMs, namely the edENM, has been developed. Moreover, a novel approach to parametrize protein-nucleic acid complexes was proposed. The edENM has been also integrated into an advanced simulation platform that can model large-scale non-harmonic conformational transitions in protein, extending is applicability domain to the nucleic acid field.

In summary, the studies presented in this dissertation illustrate how molecular modeling can be used to provide mechanistic insights into the structure-function relationship of subcellular components. In this Ph.D. thesis, the insights have been obtained by exploiting existing methodologies, supporting experimental results, and tailoring standard approaches to better perform in a specific applicability domain. Overall, these approaches were useful for gaining insights into the biomechanics of subcellular structures, paving the way for innovations in life and material sciences, with a specific focus on mechanobiology.