Modeling of liquid-liquid phase separation in biological systems

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OUTLOOK

One of the current scientific challenges is to understand how an eukaryotic cell organizes its organelles and performs functions essential to the organism that it is part of. Building a synthetic cell, specifically building first its molecular building blocks and then assembling them, has been the aim of the Building a Synthetic Cell (BaSyC) project, which this thesis' work is part of. Cells consist of membrane-bound organelles and membraneless organelles, the latter ones are formed through the LLPS phenomenon. The process of complex coacervation, a type of LLPS, was studied in this thesis in different biological systems as models for intracellular compartments. This was done to gain physical insight on the molecular mechanisms and the parameters that drive the formation and dissolution of such organelles as well as the dynamics and the physicochemical properties of their components. While the research performed in this thesis provides insights on several condensate systems, it also indicates possible shortcomings of the Martini model. These shortcomings and possible future directions for the simulation of condensates with the Martini model are discussed next.

Resolving the shortcomings of the model. In chapters 3 and 4, we showed the shortcomings of the Martini model, which resulted in the inaccurate representation of the formation and the dissolution of the condensates. Specifically, in chapter 3 our simulations showed that interactions between the polyU molecules and the arginine-rich peptides are too strong and the phosphorylation of the arginine-rich peptide does not dissolve the condensate. Moreover, in chapter 4 our simulations showed that interactions between polyU are too strong, indicating a shortcoming of the current polyU model. Work is currently done to fix these shortcomings by further refining the protein-solvent interactions as well as the base-base interactions of nucleic acids. In particular, by increasing the strength of the protein-solvent interactions and decreasing the base-base interactions of the polyU molecule these problems will probably be corrected. Improving these interactions paves the way to study a vast number of proteins, especially intrinsically disordered proteins and nucleic acids that undergo phase separation and compose many membraneless organelles. In addition, such refinements enable the study of post-translational modifications such as the phosphorylation and the methylation, processes

that cells use to regulate the formation/dissolution of these organelles.

Furthermore, a growing body of the literature suggests that membraneless organelles possess a highly crowded environment, which is also indicated from our results in chapters 2 and 3. Specifically these organelles are able to selectively admit in their interiors proteins, nucleic acids, water molecules, ions and organic molecules. Although parameters such as the sequence, the charge and the length of the biomolecules seem to play a role in this selective admission, the exact parameters are still unclear. Thus, extensive research is still needed to understand and determine the parameters that are responsible for the selective compartmentalization of biomolecules in the membraneless organelles.

In addition, such a crowded environment provides a favourable medium for various enzymatic reactions. Work is currently done to provide the possibility to model chemical reactions with the Martini model. This will improve our understanding on such reactions as well as their significance in many cellular processes. In particular, modelling and reproducing the phosphorylation/dephosphorylation processes will be of significant importance for the BaSyC project, since cells employ these processes to regulate the formation/dissolution of the membraneless organelles. Such reactions are described thoroughly in the experiments of Aumiller et al, [18] and could be simulated in the future with the Martini model, where proteins such as kinase and phosphatase act on the arginine-rich peptides by adding and removing phosphate groups to and from the peptides in the presence of adenosine triphosphate (ATP).

Predicting the proteins that participate in membraneless organelles. Although in this thesis we studied biomolecules that have been shown through experimental work to form condensates, it is of great interest to also predict which biomolecules and especially which proteins are able to form condensates in living cells. This can be achieved by determining the specific sequences/regions of the proteins that drive phase separation. Building a database with these regions will be beneficial when selecting the molecular building blocks of a synthetic cell, which is the goal of the BaSyC project.

Moreover, such a database will be beneficial in understanding which proteins and under which conditions they tend to aggregate and solidify leading to many neurodegenerative diseases and cell aging. This can provide useful insights on comprehending the molecular basis of such diseases but also predict disease-associated mutations and possible ways to prevent them (e.g. alternating the conditions that cause them or enhancing post-translational modifications).