Modeling of liquid-liquid phase separation in biological systems

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SUMMARY

Biological cells organize their processes in two different types of organelles: the membrane-bound and the membraneless organelles. While the membrane-bound organelles separate their interiors from the surrounding cytoplasm or nucleoplasm through a lipid membrane, the membraneless organelles, also known as biomolecular condensates, make use of the liquid-liquid phase separation phenomenon for the same purpose. Biomolecular condensates play a significant role in many cellular processes and their formation, dissolution and localization is modulated either by modifying the environmental conditions or by enzymes.

Molecular dynamics (MD) and in particular coarse-grained MD simulations (CGMD) can provide detailed insights on the conditions that drive or disrupt condensation, on the properties of the condensates and on the dynamics of their components, while being not too computationally demanding. The Martini model is a widely applied, explicit solvent CG force field that can be used to study condensate systems. The advantage of the Martini model lies in its ability to represent fine chemical details of the condensates' constituents. While other CG force fields use a one CG bead per aminoacid model offering important insights on the physicochemical driving forces of the condensate systems, the Martini force field maps 2-4 heavy atoms to one CG bead offering a near atomic resolution of the condensates' constituents.

In **chapter 2**, we show that the Martini model is able to accurately represent the salt dependent condensation of systems composed of poly-lysine and poly-glutamate peptides and the partitioning of RNA molecules inside these condensates. Furthermore, we investigate the dynamics of the water molecules, the polypeptides and ions from which it is apparent that all these molecules diffuse freely in the aforementioned complex systems. We further investigate the diffusion of water molecules both in the bulk phase (the dilute phase of the condensates) and in the coacervate phase (the dense biomolecule rich phase of the condensates) and show that the diffusion coefficient of the water in the bulk phase is approximately one order of magnitude larger than in the coacervate phase. The results in this chapter demonstrate that the Martini model can capture accurately the experimental trends of pLys/pGlu condensate systems.

In **chapter 3**, we make use of the latest version of the Martini force field to investigate the formation and dissolution of condensate systems consisting of polyuridylic acid (polyU) molecule and an arginine-rich peptide (RRASLRRASL). This is done by firstly modulating the environmental conditions such as the ionic strength and the positive to negative charge ratio and then by phosphorylating the arginine-rich peptides. Our simulations show that the Martini model can capture qualitatively the formation and the dissolution of such condensates by modulating such environmental conditions. However in contrast to experimental findings, the phosphorylation of the peptides does not affect noticeably the condensation, which may be attributed to a shortcoming of the model and

warrants further optimization.

In **chapter 4**, we study the salt and pH dependent condensation of systems consisting of polyuridylic acid (polyU) and two polyamines — spermine and spermidine. While we were able to capture qualitatively both the salt dependent condensation and the pH-dependent dissolution of these condensates, our results demonstrate that the currently used model of the polyU molecule is too sticky and further optimization is required. Moreover, we investigate the effect of these condensates on the structural integrity of subcellular compartments such as a DOPC membrane and a DOPC/POPC vesicle. By attaching cholesterol anchors to the polyU molecules, we show the partial bending of the membrane, in line with the experimental findings. On the other hand, the condensates when they are encapsulated in a DOPC/POPC vesicle do not noticeably change its shape during the simulation.

This thesis contributes to the understanding of several condensate systems, of their properties and the parameters that affect them, while also demonstrating some of the shortcomings of the Martini model.

SAMENVATTING

Biologische cellen organiseren hun processen in twee verschillende soorten organellen: de membraanomsloten en de membraanloze organellen. Membraanomsloten organellen gebruiken een lipidemembraan om hun interne componenten te scheiden van het omringende cytoplasma of nucleoplasma. Membraanloze organellen, ook wel bekend als biomoleculaire condensaten, gebruiken hiervoor daarentegen het fenomeen vloeistof-vloeistof fasescheiding. Biomoleculaire condensaten spelen een belangrijke rol in veel cellulaire processen. Hun vorming, ontbinding en lokalisatie wordt beïnvloed door enzymen of veranderingen in de omgevingscondities.

Moleculaire dynamica (MD), in het bijzonder grofkorrelige MD simulaties (CGMD), kan gedetailleerde inzichten verschaffen in de factoren die de vorming van condensaten aandrijven of verstoren, in de eigenschappen van de condensaten en in de dynamica van hun componenten, zonder al te hoge computationele kosten te vergen. Het Martini model is een veel toegepast CG krachtenveld met expliciete beschrijving van het oplosmiddel dat gebruikt kan worden om condensaatsystemen. Het voordeel van het Martini model ligt in zijn vermogen om fijne chemische details van de bestanddelen van de condensaten weer te geven. Terwijl andere CG-krachtvelden één CG-kraal per aminozuurmodel gebruiken, wat belangrijke inzichten biedt in de fysisch-chemische drijvende krachten van de condensaatsystemen, brengt het Martini-krachtveld 2-4 zware atomen in kaart op één CG-kraal, wat een bijna atomaire resolutie biedt van de bestanddelen van de condensaten.

In **hoofdstuk 2** tonen we aan dat het Martini model in staat is om voor systemen, bestaande uit polylysine en polyglutamaat peptiden, de zoutafhankelijke condensatie en de interne verdeling van RNA moleculen nauwkeurig weer te geven. Verder wordt de dynamiek van watermoleculen, polypeptiden en ionen bestudeerd. Hieruit blijkt dat al deze moleculen vrij kunnen diffunderen in de onderzochte complexe systemen. Ook vergelijken we de diffusiecoëfficiënt van watermoleculen, zowel in de bulkfase (de verdunde fase van de condensaten) als in de coacervaatfase (de dichte biomoleculen rijke fase van de condensaten). We laten zien dat de diffusie van het water in de bulkfase ongeveer een orde van grootte sneller is dan in de coacervaatfase. De resultaten in dit hoofdstuk tonen aan dat het Martini model de experimentele trends van pLys/pGlu condensaatsystemen nauwkeurig kan reproduceren.

In **hoofdstuk 3** maken we gebruik van de nieuwste versie van het Martini krachtenveld om de vorming en het ontbinden van condensaatsystemen bestaande uit polyuridinezuur (polyU) en arginine rijke peptiden (RRASLRRASL) te onderzoeken. Allereerst worden omgevingscondities zoals de ionsterkte en de verhouding tussen positieve en negatieve lading gemoduleerd. Uit onze simulaties blijkt dat het Martini model de vorming en het ontbinden van deze condensaten door veranderingen in de omgevingscondities kwalitatief kan weergeven. Vervolgens wordt ook de invloed bestudeerd van het fosforyleren

van de arginine rijke peptide op de condensatie van het systeem. In tegenstelling tot de experimentele bevindingen, tonen onze simulaties hier geen significant verband aan. Dit verschil kan worden toegeschreven aan een tekortkoming van ons model. Verdere optimalisatie is nog nodig.

In **hoofdstuk 4** bestuderen we de zout- en pH-afhankelijke condensatie van systemen bestaande uit polyuridylzuur (polyU) en twee soorten polyamines — spermine en spermidine. Ondanks dat wij zowel de zoutafhankelijke condensatie als de pH-afhankelijke ontbinding van deze condensaten kwalitatief konden vastleggen, tonen onze resultaten aan dat het momenteel gebruikte model van het polyU-molecuul te plakkerig is. Dit betekend dat verdere optimalisatie vereist is. Bovendien onderzoeken we het effect van deze condensaten op de structurele integriteit van subcellulaire compartimenten. Meer specifiek hebben we dit onderzocht voor een DOPC-membraan en een DOPC/POPC-vesikel. Door cholesterol ankers aan de polyU-moleculen te bevestigen, tonen wij de gedeeltelijke buiging van het membraan aan. Dit is in overeenstemming met de experimentele bevindingen. Anderzijds veranderen de condensaten wanneer zij zijn ingekapseld in een DOPC/POPC-vesikel niet merkbaar van vorm tijdens de simulatie.

Dit proefschrift draagt bij tot een beter begrip van de eigenschappen van verschillende condensaatsystemen en de parameters waardoor ze beïnvloedt worden. Ook worden enkele tekortkomingen van het Martini model besproken.

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