Experimental report

01/02/2024

Proposal: 8-03-1078 **Council:** 4/2023

Title: Structure of peptide-RNA condensates under passive and active conditions

Research area: Soft condensed matter

This proposal is a new proposal

Main proposer: Guillaume TRESSET

Experimental team: Guillaume TRESSET
Sandrine MARIOT
Kalouna KRA
Tamizhmalar SUNDARARAJAN

Local contacts: Lionel PORCAR
Anne MARTEL

Samples: Peptide
Protein kinase A (PKA)
polyU

Instrument	Requested days	Allocated days	From	To
D22	3	2	22/09/2023	23/09/2023
			23/09/2023	24/09/2023

Abstract:

Living cells are organized into distinct sub-compartments to facilitate spatiotemporal regulation of biological reactions. While this organization is traditionally thought to rely on membrane-delimited organelles, such as the nucleus and mitochondria, it also involves membraneless assemblies of proteins and nucleic acids that are kept together only by weak, dynamical interactions between their components. In the last years, groundbreaking studies suggested that these cellular assemblies have liquid-like physical properties and are formed via liquid-liquid phase separation (LLPS) processes. The objective of this project is to explore the structure of peptide-RNA condensates under varying conditions, namely at various charge ratios, temperatures and D2O contents in order to probe either peptides or RNA. We aim first at elucidating the correlation and possibly clustering peaks within condensates at the nanometered scale as well as the condensate structure at the large scale. Next, we will test an original fluidic setup that will dissociate the condensates through enzymatic reactions maintained in a steady state, thus mimicking the active milieu of the cytoplasm.

Report on experiment 8-03-1078

Structure of peptide-RNA condensates under passive and active conditions

Guillaume TRESSET, Tamizhmalar SUNDARARAJAN, Sandrine MARIOT

Université Paris-Saclay, CNRS, Laboratoire de Physique des Solides, Orsay

Context and motivation

Living cells are organized into distinct sub-compartments to facilitate spatiotemporal regulation of biological reactions. This organization relies not only on organelles delimited by membranes, such as the nucleus and mitochondria but also on membrane-less organelles, which are formed by liquid-liquid phase separation (LLPS) of proteins and nucleic acids.

In this project, we investigate the structural dynamics of peptide-RNA condensates under active and passive conditions, which are a perfect model system for membrane-less organelles and are shown to exhibit liquid-like properties. The experiment at ILL aimed to probe either peptides or RNA at various conditions, such as charge ratios, temperatures, and D_2O contents, using the contrast variation method. Next, we tested a home-built fluidic setup that allows us to study the dynamics and dissolution of the condensates through enzymatic reactions such as dephosphorylation in a controlled manner.

Materials and methods

The condensates were formed by mixing a cationic peptide (RRASLRRASL) and polyU RNA (~1,000 nucleotides) in a physiological buffer containing 50 mM HEPES, pH 7.4, 4 mM $MgCl_2$, 2.25 mM ATP, 1.6 mM dithiothreitol [1]. Experiments were carried out with a polyU concentration of 0.5 $g.L^{-1}$ and a peptide concentration ranging from 0.4 to 4.0 $g.L^{-1}$ at 37°C. For static measurements, the samples were placed in 1-mm-thick quartz cuvettes.

Since we focus on studying a wide range of length scales, the sample-to-detector distance was set at 17.6 m, which enabled us to collect patterns for q values ranging from 3×10^{-3} $Å^{-1}$ to 0.6 $Å^{-1}$ and hence, to explore the large-scale structures of the condensates.

Contrast match points

In this experiment, we used the contrast variation method to probe either one component in the condensates by matching the scattering length density of the other component to that of the solvent. The individual contrast match points for the peptide and polyU in pure samples were measured by varying the D_2O/H_2O ratio. The theoretical contrast match points of the peptide and polyU were 45% and 63% D_2O, respectively.

For polyU (1 $g.L^{-1}$), the samples were prepared with 58, 60, 63, 66, and 68% D_2O, and for peptide (5 $g.L^{-1}$), the samples were prepared with 40, 42, 45, 47, and 50% D_2O. We used the high flux delivered at ILL to collect data with a good signal-to-noise ratio in spite of the low peptide concentration. The obtained contrast match points of the peptide and polyU were 45% and 61% D_2O, respectively, which are in good agreement with the theoretical values.

We attempted to do SANS measurements on condensates by varying the peptide concentration at the contrast match points of the peptide and polyU. However, we encountered low signal and high background noise issues, preventing us from obtaining reliable results. The signal was

comparatively better with 100% D_2O, so for the next part of the experiment with the fluidic cell, we decided to use 100% D_2O to study the growth evolution of condensates.

Enzymatic activity with a fluidic cell

To investigate the formation of condensates under active conditions, a fluidic device (Fig. 1) was mounted on the beamline. The focus was to study the dephosphorylation of double-phosphorylated peptide (RRApSLRRApSL) by LPP (Lambda Protein Phosphatase), a Ser/Thr protein phosphatase that requires Mn^{2+} for its activity. LPP dephosphorylates the serine residues of the peptide, thus driving coacervation [1].

Samples were prepared by mixing RRApSLRRApSL (~1,000 µM) and polyU (~0.5 $g.L^{-1}$) at different concentrations in the fluidic device, followed by adding LPP (400 to 800 $U.mL^{-1}$) and Mn^{2+} (1 mM). The initial clear solution with double phosphorylated peptide and polyU gradually became turbid after adding LPP, indicating the formation of condensates. We maintained a steady state by continuously supplying fresh ATP (2.25 mM) and removing the produced ADP using ultrafiltration membranes with a molecular weight cutoff of 1 kDa.

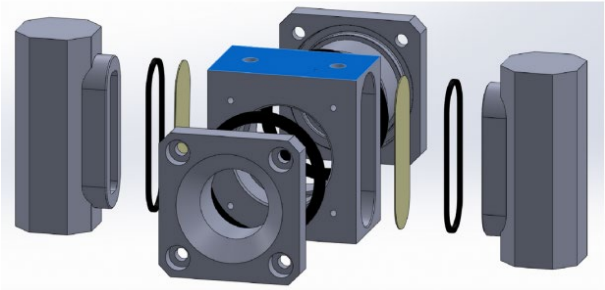

Figure 1 : 3D exploded view of the fluidic device which was used to maintain active conditions in condensates. It consisted of a central cell containing the condensates and two lateral reservoirs with circulating buffer solution.

To study the temporal evolution of the condensates, the average intensity over the *q*-range of interest (0.01 to 0.06 $Å^{-1}$) was calculated for each kinetics using GRASP software. The average intensity linearly increased by 25% (Figure 2) within 113 minutes (8,000 s) for the sample containing 0.5 $g.L^{-1}$ polyU, 1,000 µM peptide, and 400 $U.mL^{-1}$ LPP denoting an increase in the number and/or size of the condensates over time.

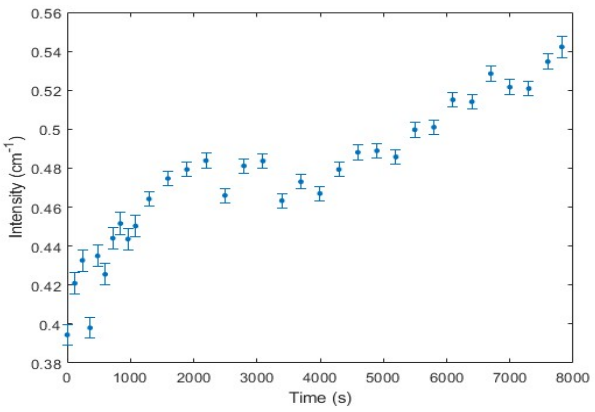

Figure 2: Average intensity versus time for the sample containing 0.5 $g.L^{-1}$ polyU, 1,000 µM peptide, and 400 $U.mL^{-1}$ LPP in 100% D_2O. The graph shows an increase in intensity over time.

The next few kinetics were performed by doubling the sample concentration. From Figure 3, we can see that the average intensity was higher from the outset and remained relatively stable at around 1.0 cm^{-1} for the first 113 minutes (8,000 s). This could be attributed to the higher concentration of the sample, which may have increased the rate of condensation, resulting in an early equilibrium. However, after this period, a noticeable decline was observed, with the intensity dropping by 20% to about 0.8 cm^{-1} within the next 83 minutes (6,000 s), signifying the dissociation of condensates occurring over time.

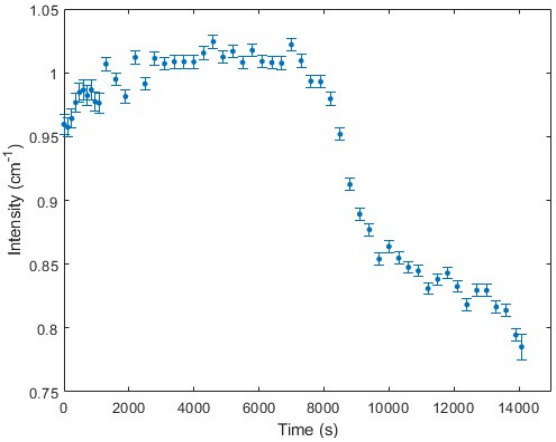

Figure 3: Average intensity versus time inferred from SANS curves for sample containing 1 g.L^{-1} polyU, 2,000 µM peptide, and 800 U.mL^{-1} LPP in 100% D_2O.The charge ratio was estimated to be 2.45. The graph shows a rapid increase in intensity followed by a gradual decrease over time.

Conclusion

By performing SANS experiments with a fluidic cell, we successfully studied the formation of condensates under active conditions. Our preliminary findings demonstrate that the enzymatic activity of LPP induces dephosphorylation of phosphorylated peptide resulting in coacervation in the presence of polyU RNA. The sample concentration influences the kinetics of this process, leading to either a rapid or slow condensate formation. The contrast match points obtained from static experiments were found to be too close to apply the contrast variation method. As a consequence, deuterated polyU will be used in future experiments in such a way that the scattering intensity arising from polyU will be matched by 100% D_2O. Future experiments will also be focused on studying the dissolution of condensates upon the action of Protein Kinase A (PKA) and the formation of condensates under nonequilibrium steady-state conditions.

[1] W.M. Aumiller, C.D. Keating, Nature Chem. 8 (2016) 129-137