Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente - Ciclo XXXVIII

Multiscale approach to control Biomolecular condensates for wide-range therapeutics (BIODENS)

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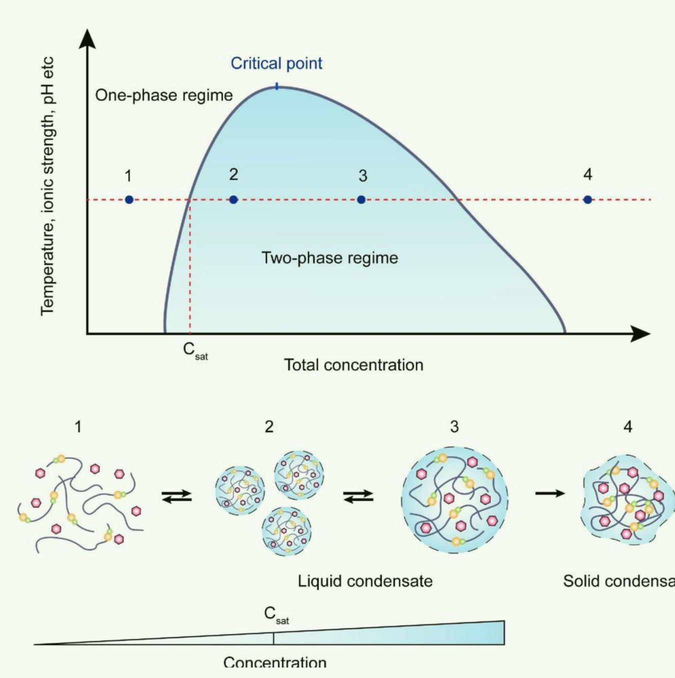
Background

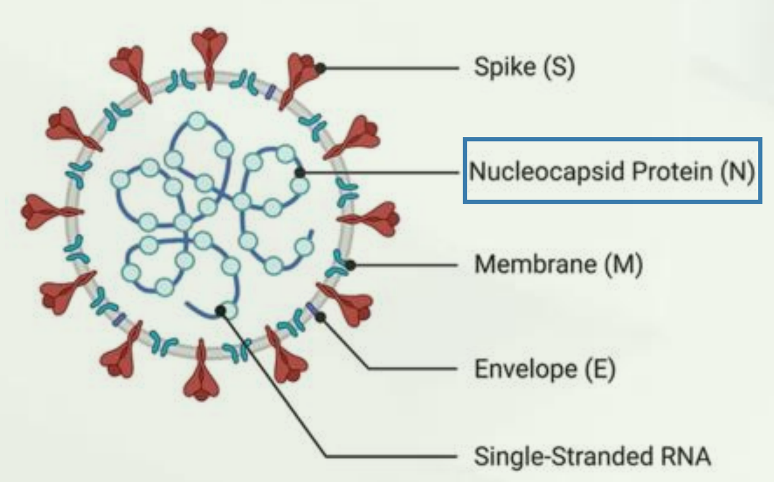
Recently, it has been discovered that certain proteins can undergo liquid–liquid phase separation (LLPS) inside the cell, driving the formation of diverse membraneless organelles in the form of biological condensates [1]. These biomolecular condensates are involved in multiple cellular processes, including gene control, ribosome function, and regulation of signal transduction. In recent years, studies on LLPS have increased remarkably, and the relationship between aberrant condensates and complex human diseases, including neurodegeneration and cancer, has been demonstrated [2, 3]. Furthermore, the formation of condensates is naturally involved in the replication and genome packaging processes of beta coronaviruses, and it is a potential target for developing antiviral drugs [4]. Therefore, it is becoming increasingly clear that understanding the biophysical principles underlying the formation of biomolecular condensates is vital for investigating the physiology and pathophysiology of a wide range of biological processes.

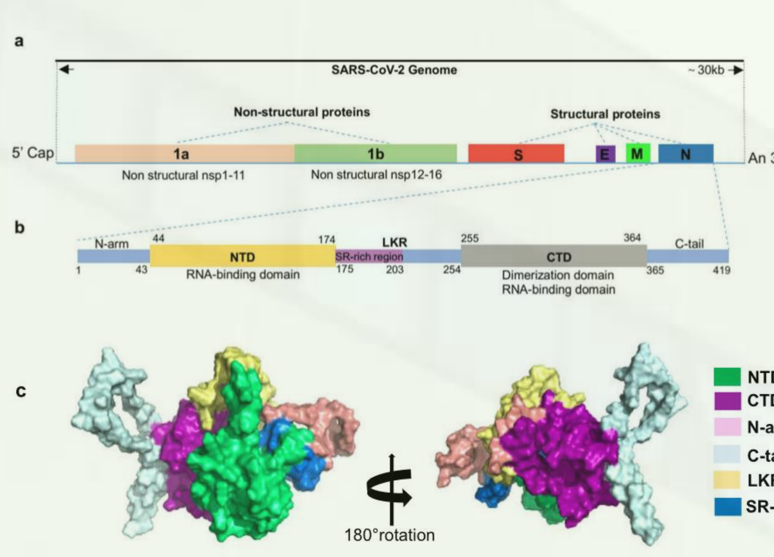
For this purpose, we focused our attention on the nucleocapsid (N) protein of the SARS-CoV-2 virus. The N-protein has gained immense interest in current and future public health as it is the protein structurally associated with the RNA of the coronavirus responsible for the COVID-19 pandemic. Our goal is to provide the understanding, down to the nanoscale, of the LLPS properties of the nucleocapsid protein of the SARS-CoV-2 virus and the way that small molecules modulate relevant condensates.



Current Stage

The Nucleocapsid Protein

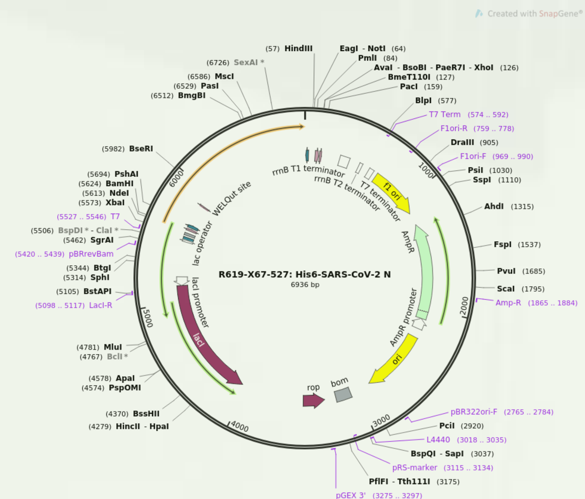


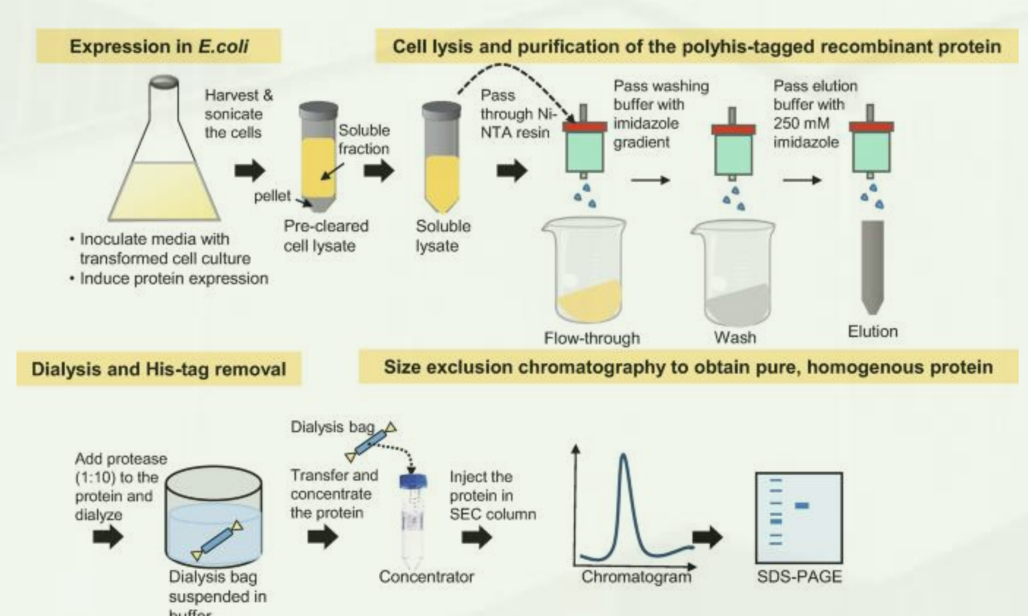


419-amino acid-long
MW: 46 KDa

Protein purification

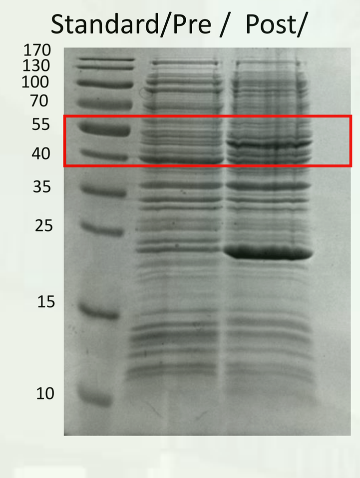
General Process



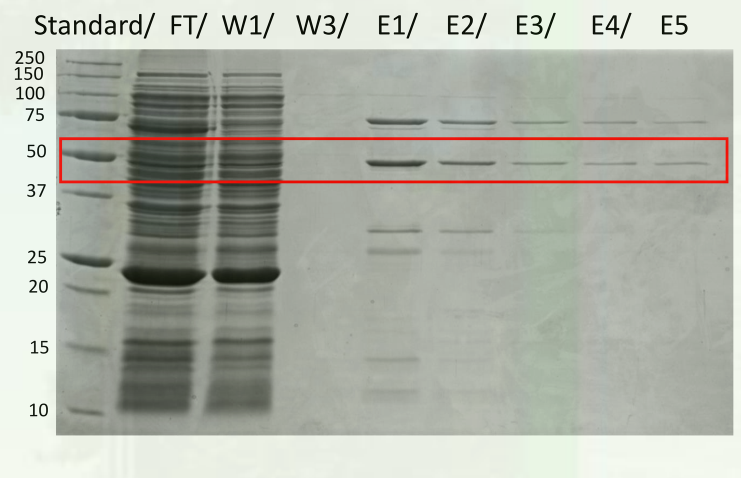


Results

Pre-Post Induction

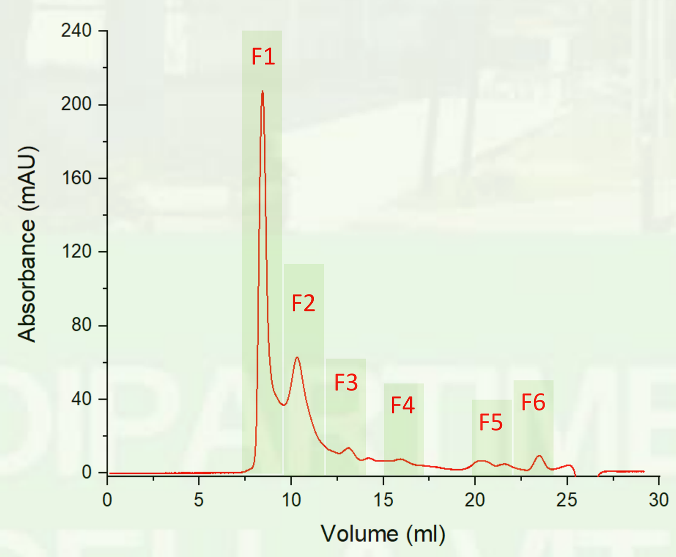


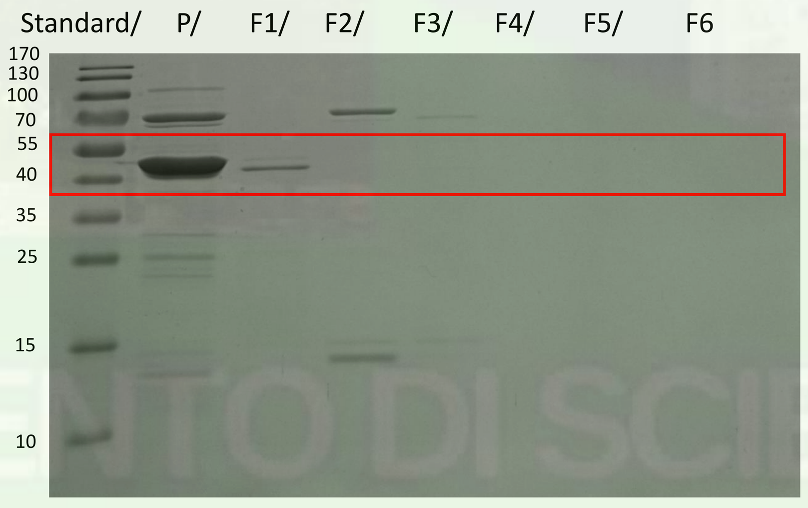
Ni-NTA Purification



- *FT: Flow-Through*
- *W1: First Washing step*
- *W3: Third washing step*
- *E1, E2, E3, E4, and E5: Eluates 1, 2, 3, 4, and 5 respectively*

Size Exclusion Chromatography



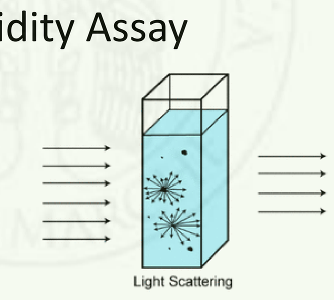


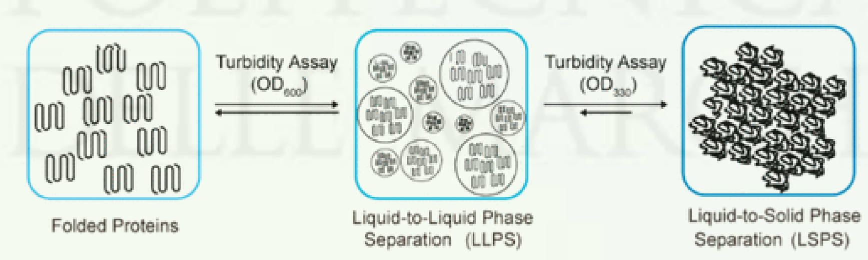
- *P: Protein before purification*
- *F1, F2, F3, F4, F5, and F6: Fractions 1, 2, 3, 4, 5, and 6 of the purification respectively*

Future Perspectives

1 *Microscale analysis*

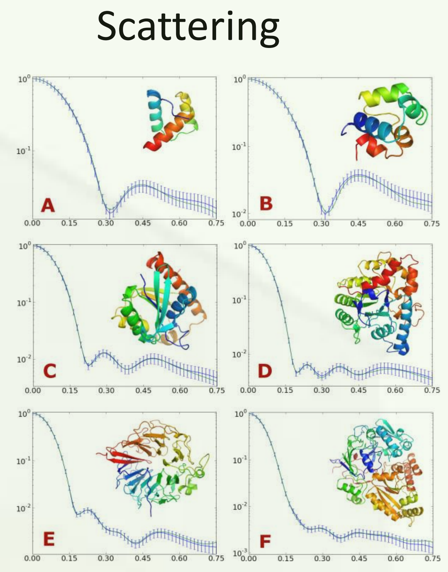
Turbidity Assay





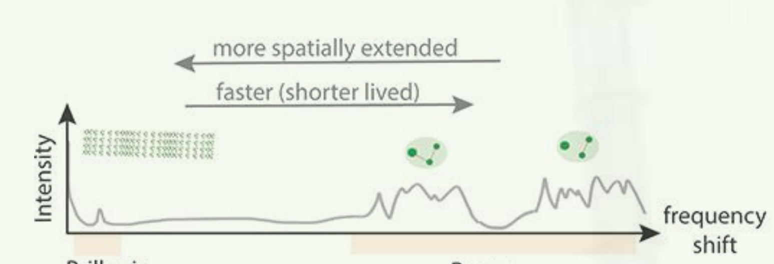
2 *Micro-nanoscale analysis*

Small-angle X-ray and Neutron Scattering



Study the overall shape and structural transitions of biological macromolecules in solution

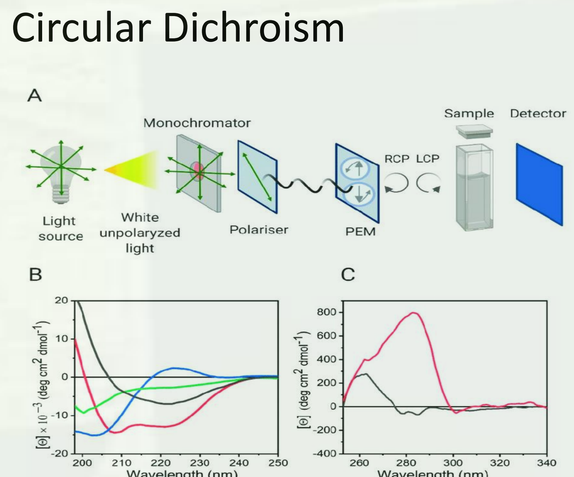
Correlative Brillouin-Raman Microscopy



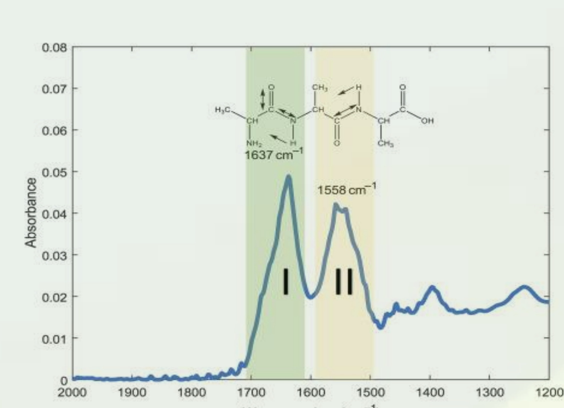
Brillouin spectroscopy measures the elastic behavior of a sample using an interferometer. Raman spectroscopy determines the chemical composition and molecular structure

3 *Nanoscale analysis*

Circular Dichroism



Micro FTIR



Protein structure characterization

References

1. Sun, Y., et al., Liquid–liquid phase separation of proteins and peptides derived from biological materials: Discovery, protein engineering, and emerging applications. MRS Bulletin, 2020. 45(12): p. 1039-1047.
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4. Lu, S., et al., The SARS-CoV-2 nucleocapsid phosphoprotein forms mutually exclusive condensates with RNA and the membrane-associated M protein. Nat Commun, 2021. 12(1): p. 502.