DECIPHERING THE FUNCTIONALITY OF THE UNSTRUCTURED N-TERMINAL DOMAIN OF THE BACTERIAL INITIATION FACTOR 2

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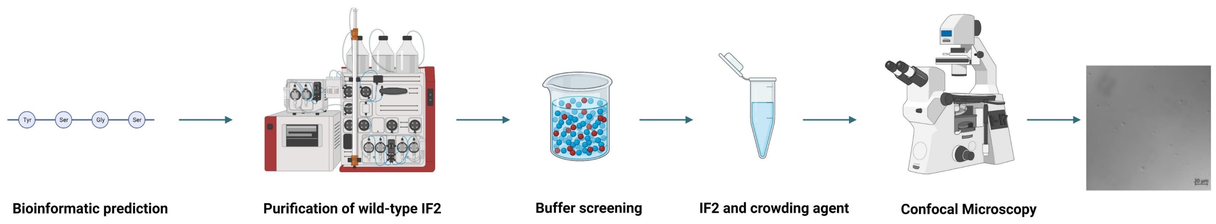
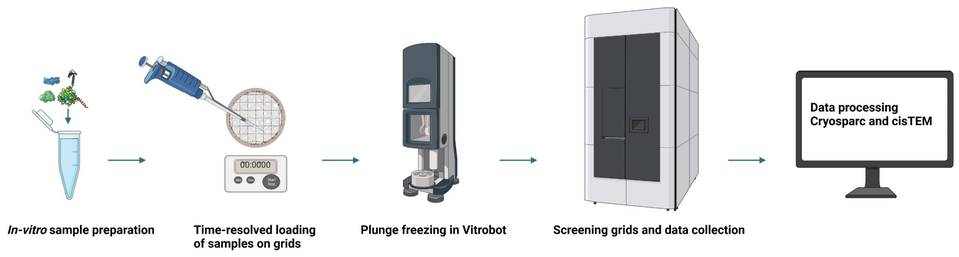
Introduction

This study focuses on examining the disordered N-terminus (intrinsically disordered region; IDR[1]) of bacterial initiaton factor 2 (IF-2) and its impact on Liquid-Liquid Phase Separation (LLPS) formation[2,3]. Additionally, we employ time-resolved cryo-electron microscopy (cryo-EM) to capture the intermediate states of the translation initiation process to determine the role of the N-terminus of IF-2. Our goal is to enhance our understanding of how the disordered N-terminus of IF-2 functions in ribosomal subunit joining[4] and contributes to the compartmentalization of the translation machinery within bacterial cells.



Flowchart for the LLPS experiments



Flowchart for the time-resolved cryo-EM experiments

LLPS studies

For the LLPS studies we performed different salt and protein concentration gradients to pinpoint the IF-2 LLPS formation. We started to observe clear condensate formation at 10-20 µM IF-2 concentration in a low salt buffer (10mM NaCl) with 5% Dextran (Fig. 1A,B). The condensate formation was also visible in a medium salt buffer (25mM NaCl) (Fig. 1C). The condensates dissolve in the presence of 5% 1,6-Hexanediol (Fig. 1D). The fluorescence microscopy of the labelled protein (IF-2/Alexa Flour 633) confirms the formation of condensates *in vitro* (Fig. 2).

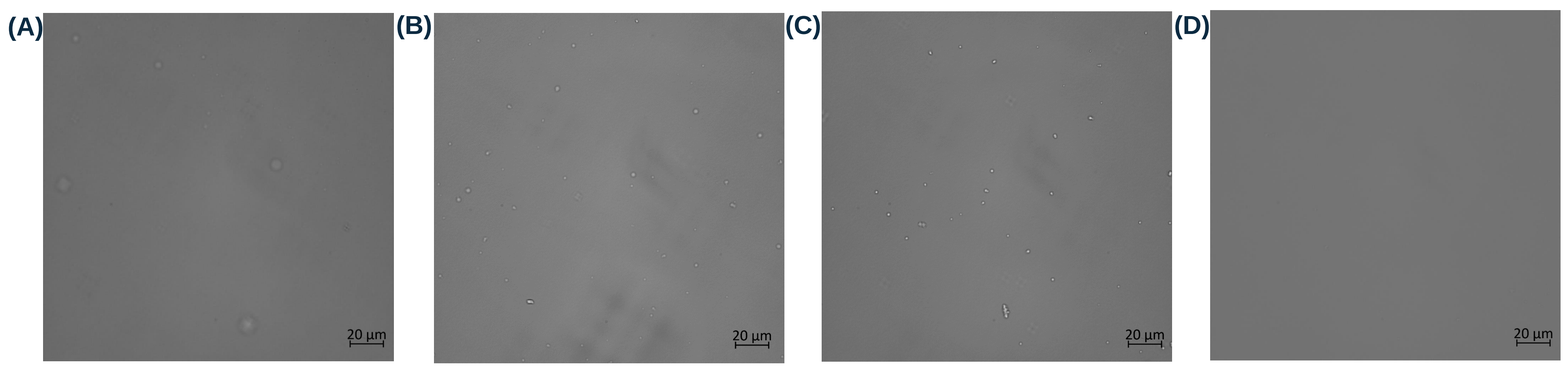


Figure 1. Condensate formation of IF-2. (A) Condensate formation of IF-2 (10 µM) in low salt buffer (NaCl 10mM) with 5% Dextran, (B) IF-2 (20 µM) in low salt buffer (NaCl 10 mM) with 5% Dextran and (C) IF-2 (15 µM) in medium salt buffer (NaCl 25 mM) with 5% Dextran. (D) Dissolved condensates in the presence of 5% 1,6 Hexanediol.

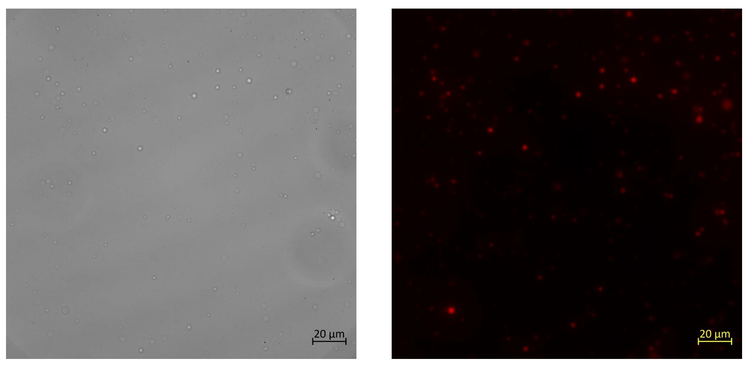


Figure 2. Condensate formation by mixing labeled and unlabeled (1:5 ratio) IF-2 in medium salt buffer (NaCl 25 mM) with 10% Dextran. Bright field and fluorescent images are shown.

Time-resolved cryo-EM studies

The aim has been to recreate the bacterial initation pathway starting with the 30S initiation complex (30S IC) and the 70S initiaton complex (70S IC). With the exception of initiation factor 3 (IF3), all major components of the translation initiation complex were assembled *in vitro*.

2D classification analysis identified a 30S IC class exhibiting density corresponding to a certain part of N-terminus of IF2 (Fig. 3A) which is effectively anchored to the 30S subunit. Furthermore, the classification revealed a class representing the 70S IC with partial density indicative of the N-terminus of IF2 (Fig. 3B).

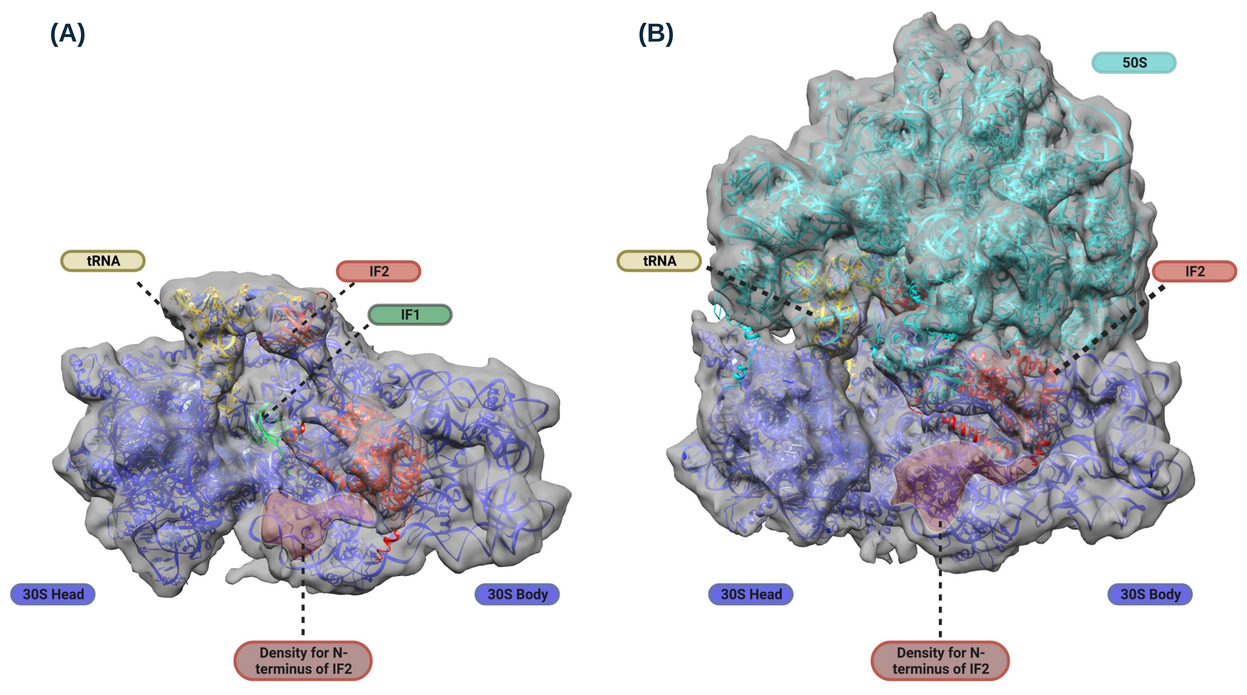


Figure 3. (A) Structure of 30S IC. Map (grey) shown for the 30S IC with partial density for the N-terminus of IF2. (B) Structure of 70S IC. Map (grey) shown for the 70S IC with partial density for the N-terminus of IF2. The color code for each of the component in 30S IC and 70S IC is highlighted in both panels.

Future Perspectives

For future LLPS experiments, the plan is to use different isoforms of IF-2, to analyze the formation of LLPS condensates. The LLPS analyses will be extended to *in vivo* studies, to decipher the relevance of IF-2 condensates on the translation process inside the bacterial cell.

For the time resolved cryo-EM studies we plan to use GTP to capture near native intermediate states in the initiation pathway with an emphasis to resolve density for the N-terminus of IF-2.

References

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