Liquid-liquid phase separation of a bacterial translation factor

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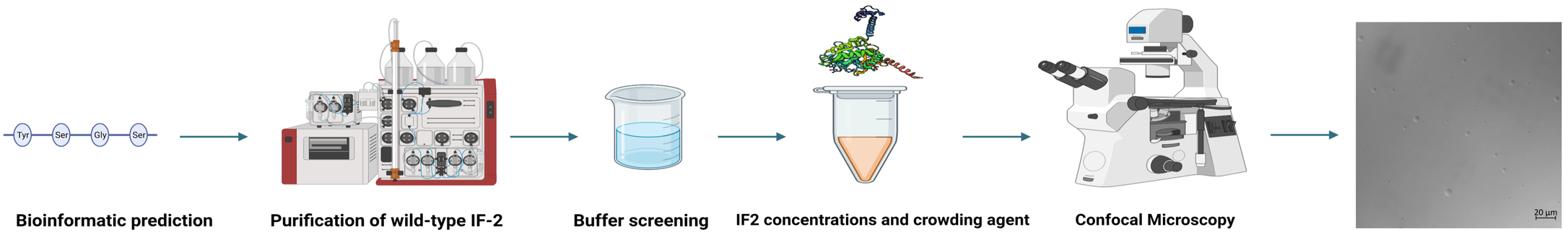
INTRODUCTION

Compartmentalization is a hallmark of living cells that allow them to perform complex tasks by dynamically coordinating matter and energy fluxes in space and time. This compartmentalization of membraneless organelles in bacteria is driven by Liquid-Liquid Phase Separation (LLPS)[1]. These condensates are comprised of proteins that are generally rich in intrinsically disordered regions (IDRs) and nucleic acids[2]. In bacteria, translation iinitiation factor 2 (IF-2) is a GTPase that binds the initiator tRNA and catalyzes the ribosomal subunit joining to form the 70S ribosome[3]. Large portion of IF-2 is intrinsically disordered and might form LLPS to localize the translation in specific foci in bacteria. This study investigates IF-2 and its formation of LLPS to provide deeper insight into compartmentalized translation machinery in bacteria.



Flow diagram of experiments

BIOINFORMATIC ANALYSIS AND LLPS SETUP

We analyzed various bacterial strains using bioinformatic tools and found that IF-2 has a high percentage of IDRs across various strains (Fig. 1).

For LLPS analyses, the purified protein was subject to buffer (range of salt concentrations) and protein concentration screening assays. The analyses also included microscopic examination of the reactions after different time intervals. Dextran was used as a crowding agent to promote the condensate formation.

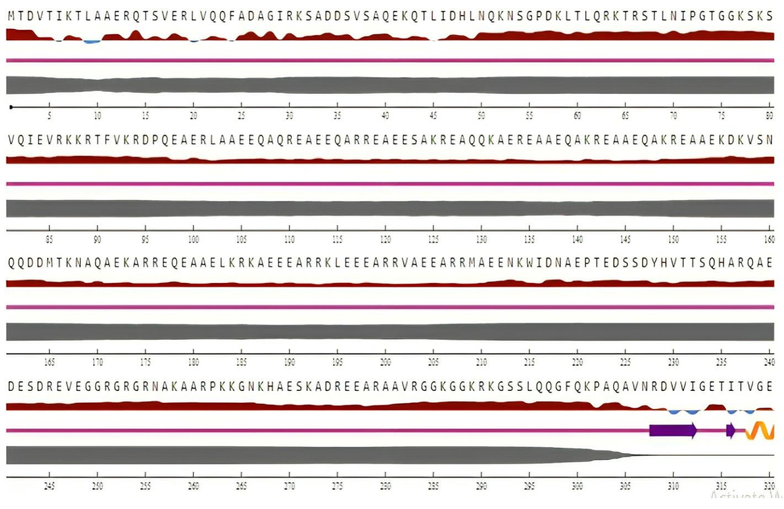


Figure 1. Bioinformatic prediction[4] shows that the first 305 residues of IF-2 at the N-terminus are disordered (IDR region).

PRELIMINARY RESULTS

IF-2 undergoes LLPS formation *in vitro* in specific buffer and protein concentrations. For IF-2, we started to observe clear condensate formation at 10µM IF-2 concentration in a low salt buffer (10mM NaCl) with 5% Dextran (Fig. 2A). The condensate formation was also visible at 20µM IF-2 in low salt buffer (Fig. 2B) and at 15 µM IF-2 in a medium salt buffer (25mM NaCl) (Fig. 2C). The condensates dissolve in the presence of 5% 1,6-Hexanediol (Fig. 2D). The fluorescence microscopy of the labeled protein (IF-2/Alexa Flour 633) confirms the formation of condensates *in vitro* (Fig. 3).

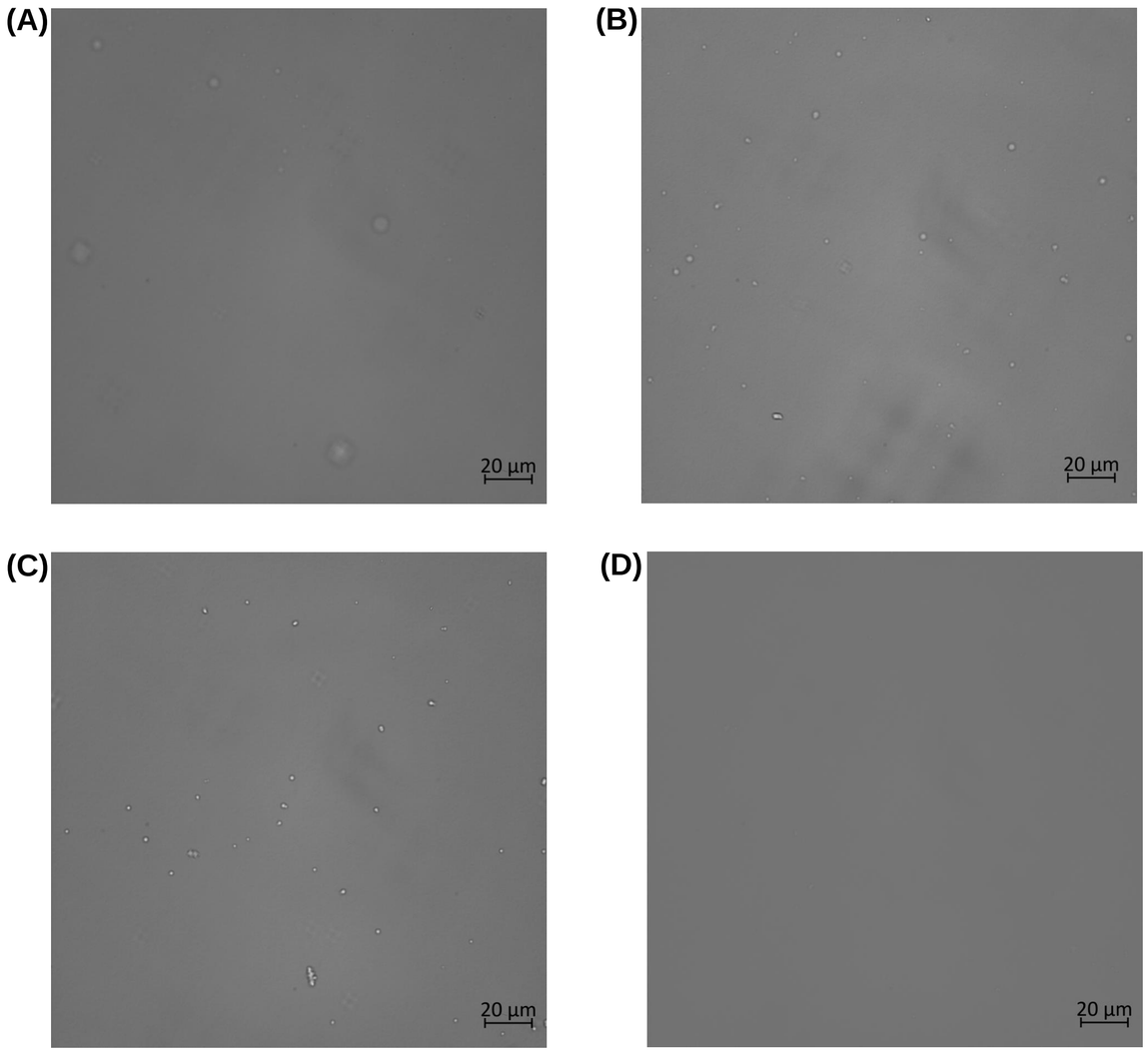


Figure 2. Condensate formation of IF-2. (A) Condensate formation of IF-2 (10 µM) in low salt buffer (NaCl 10mM) with 5% Dextran, (B) IF-2 (20 µM) in low salt buffer (NaCl 10 mM) with 5% Dextran and (C) IF-2 (15 µM) in medium salt buffer (NaCl 25 mM) with 5% Dextran. (D) Dissolved condensates in the presence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

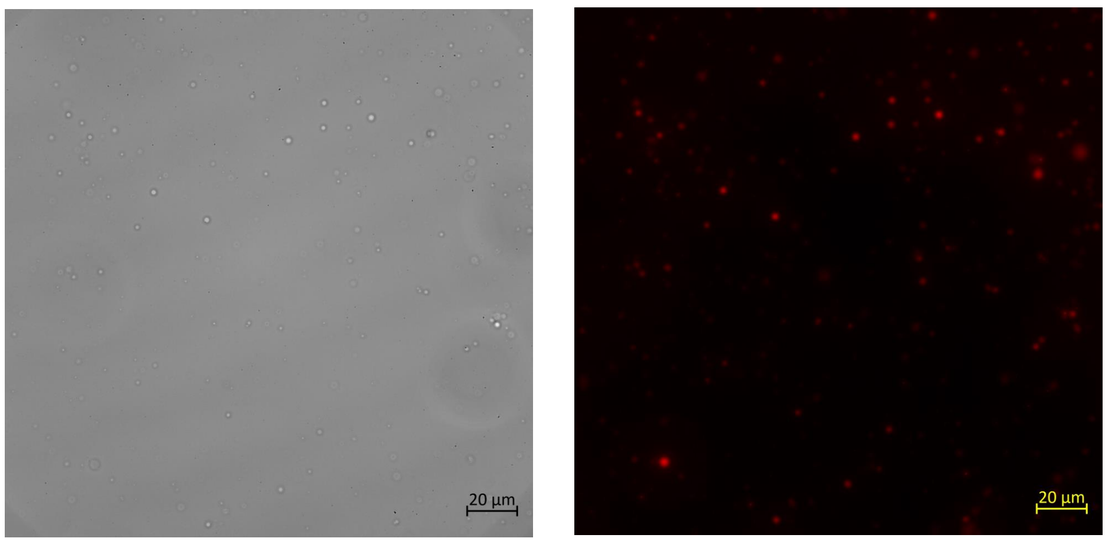


Figure 3. Condensate formation by mixing labeled and unlabeled (1:5 ratio) IF-2 in medium salt buffer (NaCl 25 mM) with 10% Dextran. Bright field and fluorescent images are shown.

FUTURE PERSPECTIVES

We plan to use different isoforms of IF-2 with deletions in the N-terminus, to analyze the formation of LLPS condensates. Our 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.

Acknowledgement

This study was supported by the project National Institute of virology and bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) - Funded by the European Union - Next Generation EU.

References

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