Self-assembly pathways of intrinsically disordered glycomacropeptide

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INTRODUCTION

Glycomacropeptide (GMP)

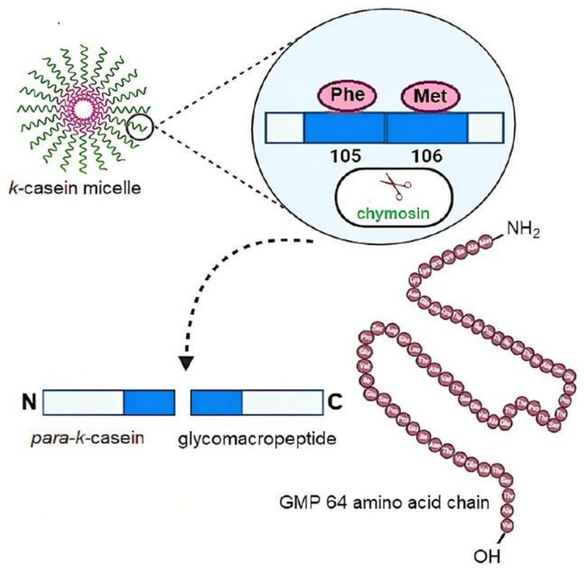
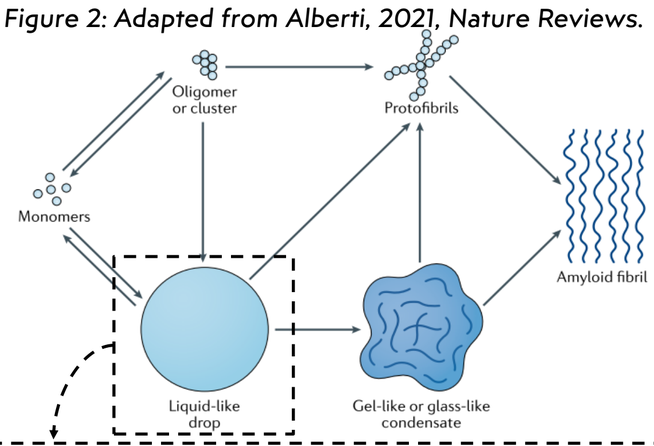


Figure 1: Chymosin action in the production of bovine glycomacropeptide (GMP). Adapted from Ebrahimi, 2024, Compr Rev Food Sci Food Saf.

Glycomacropeptide (GMP) is an **intrinsically disordered** casein-derived peptide composed of 64 amino acid residues and characterized by a low isoelectric point (pI < 3.8).
It is rich in polar amino acids and presents up to 4 carbohydrate chains.

Self-assembly pathways

Figure 2: Adapted from Alberti, 2021, Nature Reviews.



Why self-assembly?

- Drug delivery
- Studying biological colloid stability
- Model system

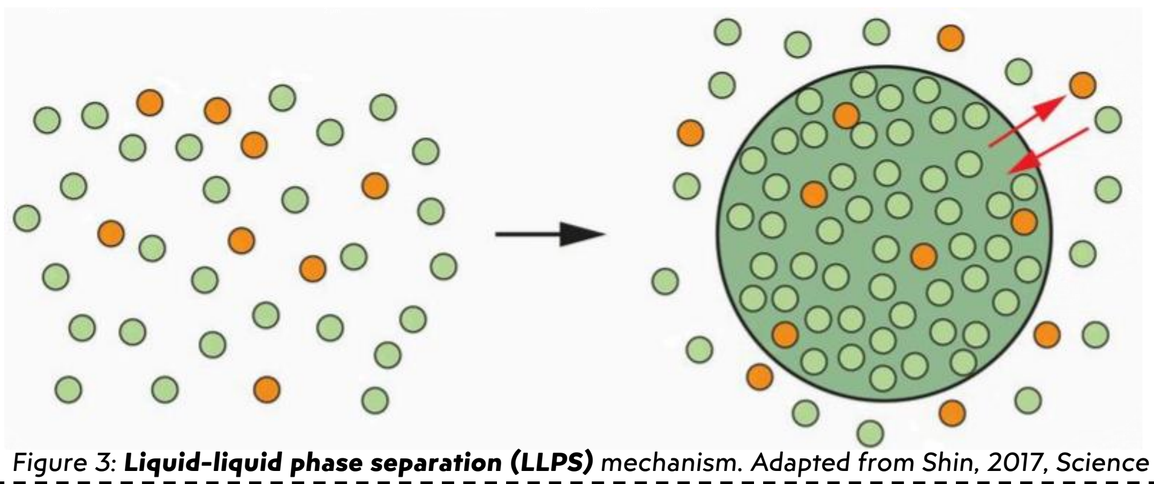
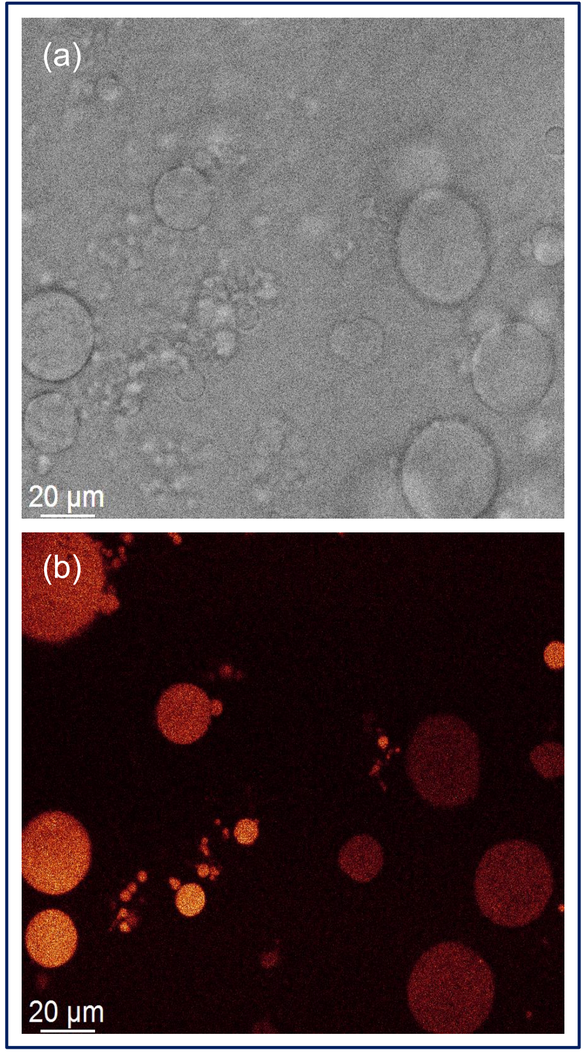


*Figure 3: **Liquid-liquid phase separation (LLPS)** mechanism. Adapted from Shin, 2017, Science*

RESULTS

Setting conditions for phase separation

Lower GMP Concentration (37.5 mg/mL)



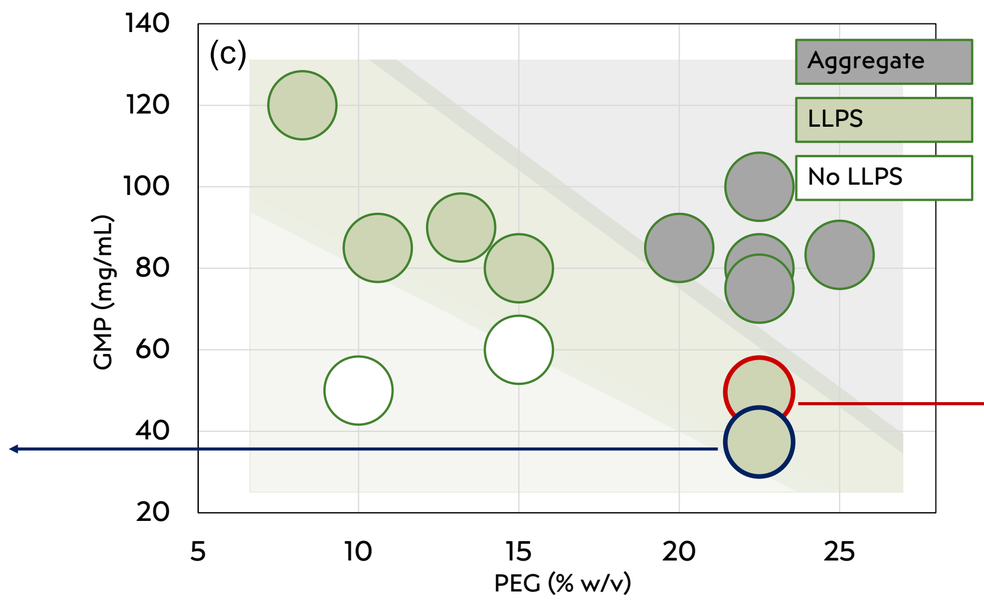
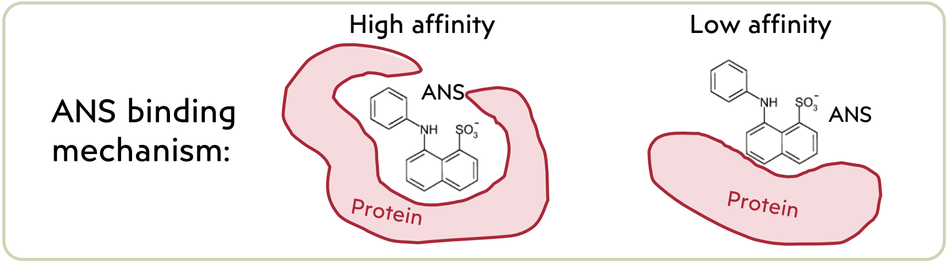
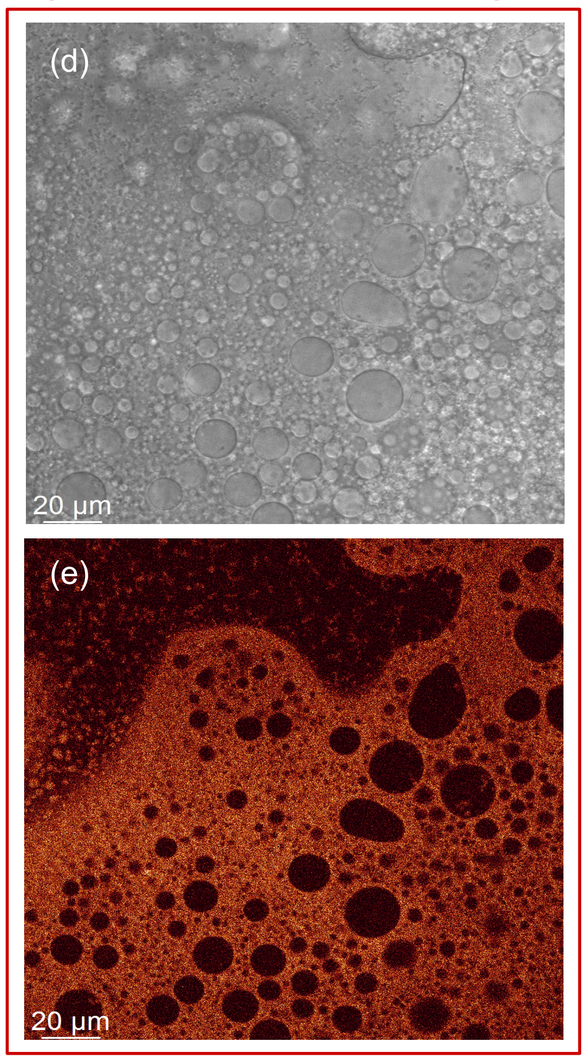


Figure 4: Transmittance (a-d) and ANS fluorescence (b-e) of GMP in the presence of PEG. (c) Phase diagram illustrating the experimental conditions for aggregation and liquid-liquid phase separation (LLPS). The blue and red markers highlight the specific condition selected for the adjacent images.



Higher GMP Concentration (50 mg/mL)

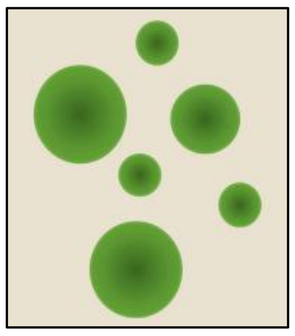


CONCLUSION AND FUTURE DIRECTIONS

Conclusions

- Conditions leading to GMP **phase separation** were identified using PEG as a crowding agent.
- Different GMP concentrations lead to droplets enriched in **different components**.
- ANS fluorescence reveals heterogeneous protein environments, supporting the coexistence of **different protein states** under phase-separation conditions.

Next steps



FRAP → Revealing internal dynamics and diffusion rates.
SpIDA → Mapping oligomerization within coacervates.
ThT-phasor FLIM → Protein conformational changes.