SELF-ASSEMBLY PATHWAYS OF INTRINSICALLY DISORDERED GLYCOMACROPEPTIDE

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Glycomacropeptide (GMP) is a C-terminal, hydrophilic casein-derived peptide composed of 64 amino acid residues and characterized by a low isoelectric point (pI < 3.8). Understanding GMP self-assembly provides a molecular model for the broader, essentially unstudied question of how mucin-type O-glycosylation governs the self-organization of intrinsically disordered glycopeptides. Indeed, GMP shares its core O-glycan architecture with the mucin family, the dominant structural and functional proteins of the intestinal mucus layer, which are difficult to manipulate experimentally.

In this study, we investigate the in vitro conditions leading to different self-assembly pathways for GMP, from liquid–liquid phase separation (LLPS) to gelation and solid aggregate formation. By varying ionic strength, ion type, molecular crowder as well as temperature and pH, we aim to identify the dominant intermolecular interactions driving condensate formation and liquid-to-solid transition. Using a combination of spectroscopy and fluorescence microscopy, we analyze both the intrinsic optical properties of GMP and their evolution during phase separation. These results provide insight into the behavior of glycosylated, intrinsically disordered peptides under charge-screened and crowded conditions.