Distribution of charged residues affects the liquid-liquid phase separation of proteins

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Liquid-liquid phase separation (LLPS) is a phenomenon of solute and solvent demixing that occurs in supersaturated solutions, generating a two-phase regime – a "dense phase" and a "diluted phase" with respect to solute concentration[1]. In living cells, LLPS involves the condensation of proteins and nucleic acids, giving rise to so-called "membraneless organelles" (MLOs), which are dynamic and functional compartments retaining liquid-like properties and playing key roles in the cellular homeostasis. Proteins that undergo LLPS often exhibit intrinsically disordered regions (IDRs) held as interaction hubs for protein-protein interactions. Electrostatic interactions have been proved to be involved in LLPS. In turn, they are strongly influenced by both charge density and distribution in the primary structure of proteins[2]. A positive correlation between charge clustering and phase separation propensity has been recently discussed in a computational[3] and a few experimental works[4,5].

In this project, a highly charged IDR was isolated from human topoisomerase I. This IDR includes a 100-residue region (NDR) with regularly alternating cationic and anionic residues and an NLS region with nuclear localisation signals. The distribution of charged residues within the primary structure of NDR was changed to improve charge clustering in a series of synthetic proteins. These were recombinantly produced and tested to experimentally prove their disordered nature and their propensity to undergo LLPS *in vitro*.

Confocal microscopy imaging and turbidity assays suggest that charge clustering strongly increases the propensity for phase separation of our model IDR. Overall, data collected so far confirm the existence of a relationship between charge patterning and LLPS.

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