Charge patterning and phase separation propensity in IDPs: is there a possible interplay?

Greta Bianchi[1], Lorenzo dell'Orto, Sonia Longhi and Stefania Brocca[1]
E-mail: g.bianchi31@campus.unimib.it;
[1]*Department of Biotechnology and Biosciences, University of Milano-Bicocca, Piazza della Scienza 2, 20126 Milano, Italy;*
[2]*CNRS and Aix-Marseille University, Laboratoire Architecture et Fonction des Macromolécules Biologiques (AFMB), UMR 7257, Marseille, France*

Keywords: protein conformation, protein coacervation, distribution of electrostatic charges

Liquid-liquid phase separation (LLPS) underlies the formation of non-membrane bound compartments, employed to concentrate proteins and nucleic acids and further control cellular biochemistry in space and time [1, 2]. Intrinsically disordered proteins (IDPs) and regions (IDRs) have been recently suggested as triggers for LLPS, due to their peculiar composition and conformational dynamism, both favouring intermolecular interactions.
Electrostatic forces were proved to have a crucial role in IDR-mediated liquid demixing. Both net charge and charge patterning – *i.e.* the linear distribution of charged residues within the primary structure – seem to determine IDR propensity to phase separate, as assessed by computational[3] and experimental evidences[4,5]. Nevertheless, a unitary and comprehensive understanding of such a correlation is still missing.
In this respect, the connection between charge patterning and LLPS will be investigated in a human IDR, the N-terminus of topoisomerase I, which induces the relaxation of supercoiled DNA in the nucleolus. Keeping the nuclear localisation sequence unchanged, charge scrambling has been performed on the 100-residue N-terminal domain, in order to obtain synthetic constructs differing in charge distribution. Their LLPS propensity will be assessed *in vitro*, through turbidity assays and FRAP (Fluorescence Recovery After Photobleaching) measurements, and possibly *in vivo* as well.

References

1. Banani, S. F, Lee, H. O., Hyman A. A. and Rosen M. K. (2017). *Nature reviews Molecular cell biology*, **18**, 285-298.
2. Boeynaems, S., Alberti, S., Fawzi, N. L., Mittag, T., Polymenidou, M., Rousseau, F., Schymkowitz, J., Shorter, J., Wolozin, B.; Van Den Bosch, L. (2018). *Trends in cell biology*, **28**, 420-435.
3. Lin Y.H. and Chan H. S. (2017). *Biophysical journal*, **112**, 2043-2046.
4. Nott, T.J., Petsalaki, E., Farber, P., Jervis, D., Fussner, E., Plochowietz, A., Craggs, T. D., Bazett-Jones, D.P., Pawson, T. and Forman-Kay, J. D. (2015). *Molecular cell*, **57**, 936-947.
5. Schuster, B. S., Dignon, G. L., Tang, W. S., Kelley, F. M., Ranganath, A. K., Jahnke, C. N., Simpkins A. G., Regy R. M., Hammer D. A., Good M. C and Mittal, J. (2020). *Proceedings of the National Academy of Sciences*, **117(21)**, 11421-11431.