

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

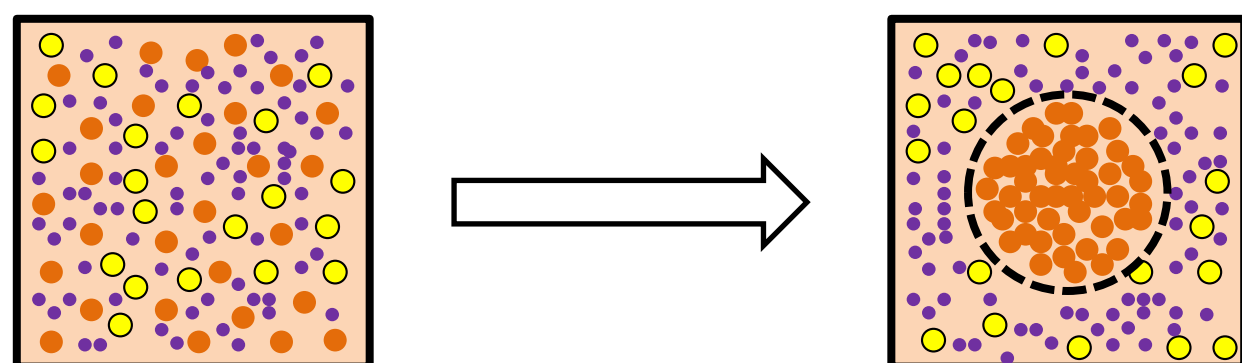
Greta Bianchi<sup>1</sup>, Sonia Longhi<sup>2</sup> and Stefania Brocca<sup>1</sup>

<sup>1</sup>Department of Biotechnology and Biosciences, University of Milano-Bicocca, Piazza della Scienza 2, 20126 Milano, Italy; <sup>2</sup>CNRS and Aix-Marseille University, Laboratoire Architecture et Fonction des Macromolécules Biologiques (AFMB), UMR 7257, Marseille, France

Email: [g.bianchi31@campus.unimib.it](mailto:g.bianchi31@campus.unimib.it)

## Liquid-liquid phase separation

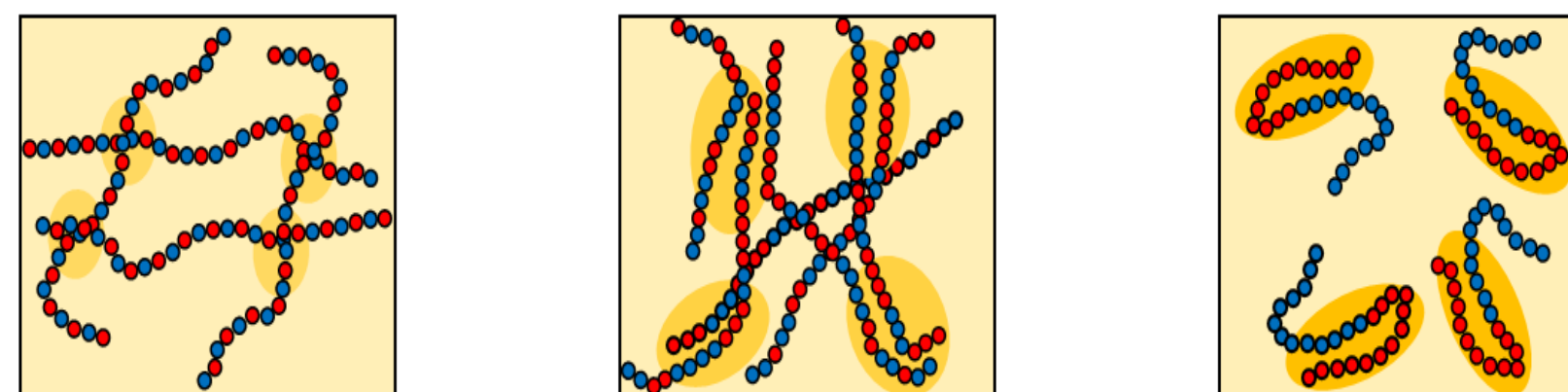
Liquid-liquid phase separation (LLPS) underlies the formation of non-membrane bound compartments<sup>1</sup>



Intrinsically disordered regions (IDRs) trigger LLPS

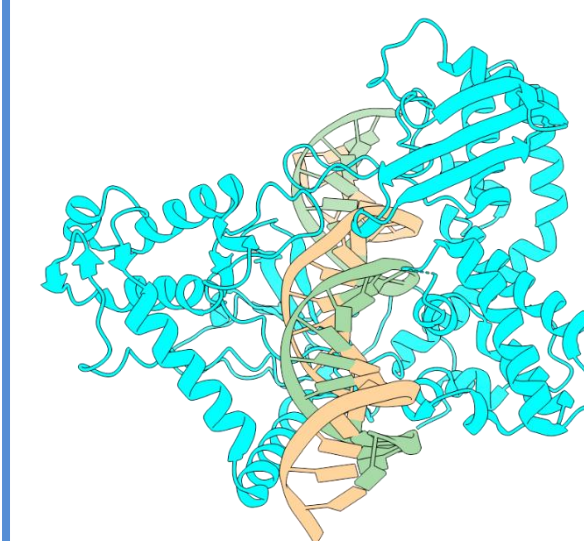
## Electrostatic interactions

Electrostatic forces were proved to have a crucial role in IDR-mediated demixing<sup>2,3,4</sup>



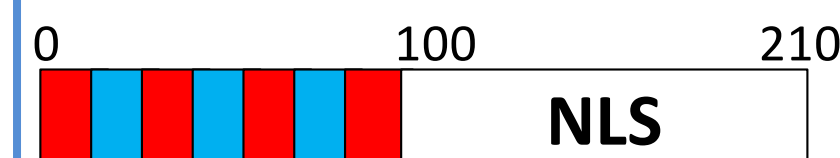
Short charged clusters should strengthen intermolecular interactions and favour LLPS

## Aims and experimental design



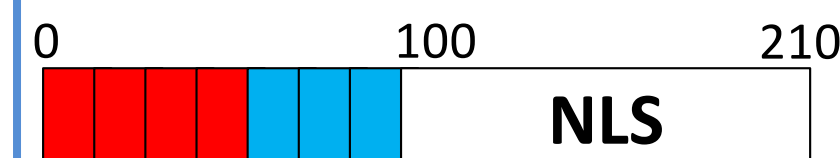
Human topoisomerase I (*hTOP1*) is a nucleolar enzyme involved in the reduction of DNA torsional stress

wt *hTOP1*



*hTOP1* contains a charge-rich IDR, with evenly distributed charged residues.

Modified *hTOP1*

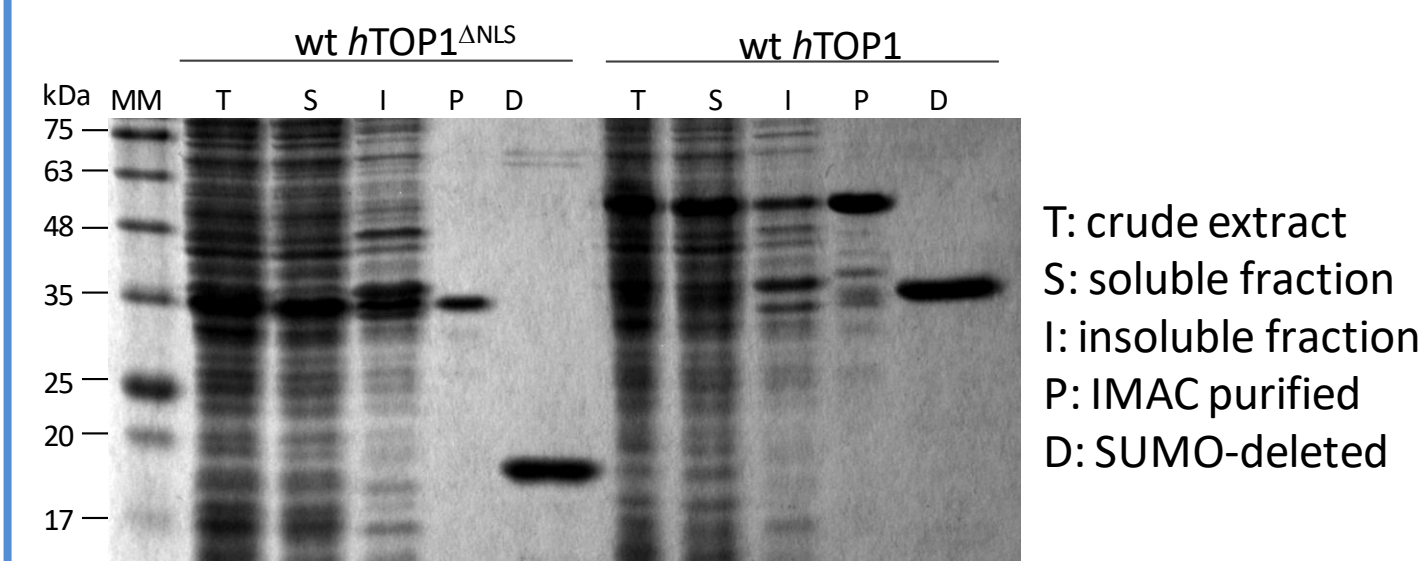


A *hTOP1* variant was designed, with clustered charged residues

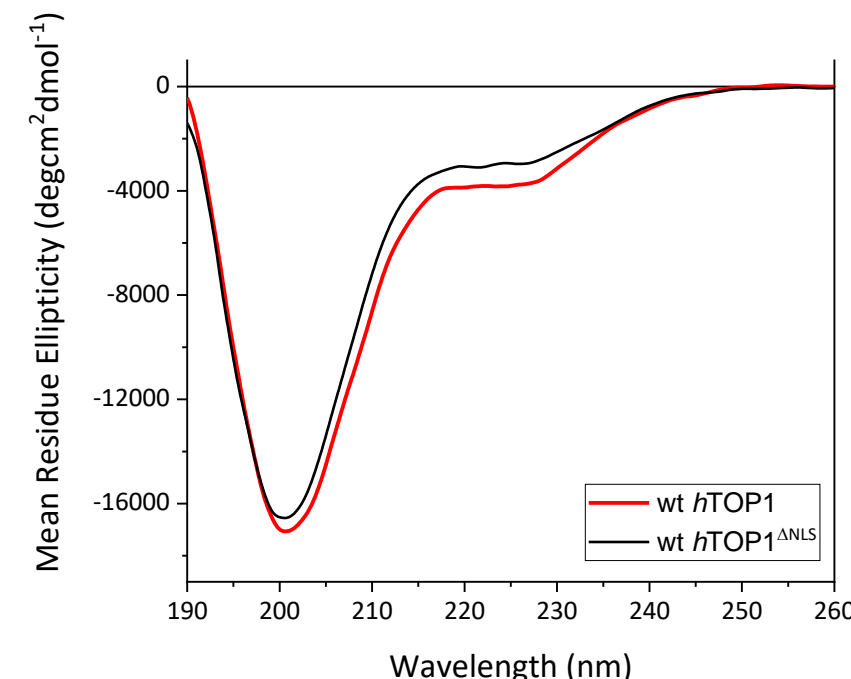
- Are *hTOP1* and its isolated IDR able to drive LLPS?
- How does charge patterning affect LLPS propensity?

## Preliminary results

### SDS-PAGE



### Far-UV circular dichroism

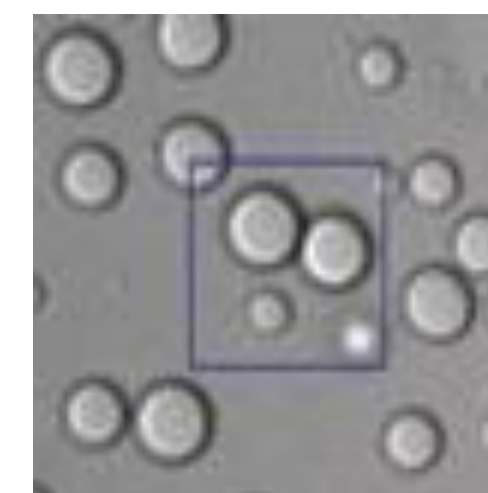


They both show the typical traits of structural disorder

wt *hTOP1* and *hTOP1*<sup>ΔNLS</sup> were produced as recombinant, SUMO-fused proteins and purified at homogeneity

## Future work

Assessment of LLPS propensity for wt and modified IDRs (*i.e.* through turbidity and FRAP assays).



## References

- <sup>1</sup>Banani et al. (2017). *Nature reviews Molecular cell biology*, **18**, 285-298.
- <sup>2</sup>Nott et al. (2015). *Molecular cell*, **57**, 936-947.
- <sup>3</sup>Schuster et al. (2020). *Proceedings of the National Academy*, **117**(21), 11421-11431.
- <sup>4</sup> Bianchi et al., (2020). *International Journal of Molecular Sciences*, **21**(17), 6208.