

## **Controlling and interfering with pathological condensates in ALS disease-relevant cell models**

Micrometer-sized biomolecular condensates are emerging as key organizers of cellular biochemistry and are often proposed to form through liquid–liquid phase separation (LLPS). LLPS has been implicated in diverse processes, from immune signalling to synaptic organization, and is increasingly linked to neurodegenerative disease. In several proteinopathies, misfolding-prone proteins may pass through a condensate-like state before forming pathological solid aggregates, as suggested for Tau and TDP-43. However, the functional relevance of LLPS in living cells remains debated, as much evidence comes from *in vitro* reconstitution with purified components. In cells, condensates have complex, poorly defined compositions, rapid exchange dynamics, and viscoelastic properties that complicate mechanistic interpretation. Consequently, key questions remain unresolved: how aberrant phase transitions drive aggregation and spreading, how aggregation relates causally to toxicity, and whether targeting condensate states could slow disease progression.

TDP-43 is a central case study, as cytoplasmic TDP-43 aggregates are a pathological hallmark of nearly all forms of amyotrophic lateral sclerosis (ALS). However, whether these aggregates are directly toxic, and why aggregate-reduction therapies have largely failed, remains unclear. Testing these causal relationships requires tools that directly manipulate phase transitions and condensate material states in disease-relevant cells.

Here, our IPV consortium unites complementary expertise in physical chemistry/biophysics (Zoher Gueroui, CPCV) and cellular neurobiology/ALS models (Delphine Bohl, ICM) to develop and apply new strategies to control TDP-43 condensate transitions in human iPSC-derived motor neurons. We will (1) engineer motor neuron cell lines enabling controlled induction of TDP-43 phase transitions and aggregation, and (2) generate *de novo* protein binders that disrupt defined TDP-43 interactions, combining AI-driven protein design with *in cellula* characterization. Together, these tools will enable systematic testing of how biophysical states of TDP-43 aggregates relate to ALS biochemical hallmarks and neuronal viability. This interdisciplinary platform will provide mechanistic insight into ALS-relevant TDP-43 proteinopathy and establish a scalable framework for therapeutic exploration.