Single-molecule investigation of pathological protein condensates by combining microfluidics with fluorescence microscopy

Lambert-Paul Jorissen [1,2,3] , Jef Hooyberghs [4], Jelle Hendrix [3] and Ronald Thoelen [1,2]

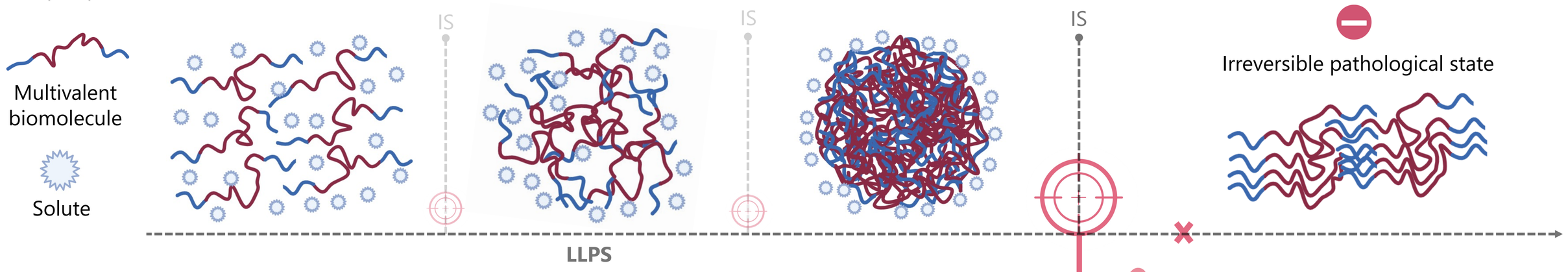
1. UHasselt, Biomedical Device Engineering Group, Institute for Materials Research (IMO), Agoralaan, 3590 Diepenbeek, Belgium
2. Division IMOMEC, IMEC vzw, Wetenschapspark 1, 3590 Diepenbeek, Belgium
3. UHasselt, Dynamic Bioimaging Group, Biomedical Research Institute, Agoralaan, 3590 Diepenbeek, Belgium
4. UHasselt, Theory Lab, Faculty of Sciences, Agoralaan, 3590 Diepenbeek, Belgium

BIOMEDICAL DEVICE ENGINEERING
IMO-IMOMEC
UHASSELT imec

HENDRIX LAB
DYNAMIC BIOIMAGING
UHASSELT
www.uhasselt.be/dbi @prof_jelle

BACKGROUND: phase separation

- Biological **liquid-liquid phase separation (LLPS)** is the spontaneous demixing of multivalent biomolecules, such as intrinsically disordered proteins (IDPs), into distinct liquid phases within a solution.



- Crucial for **compartmentalizing** specific biomolecules in subcellular environments
- Regulates **cellular processes**

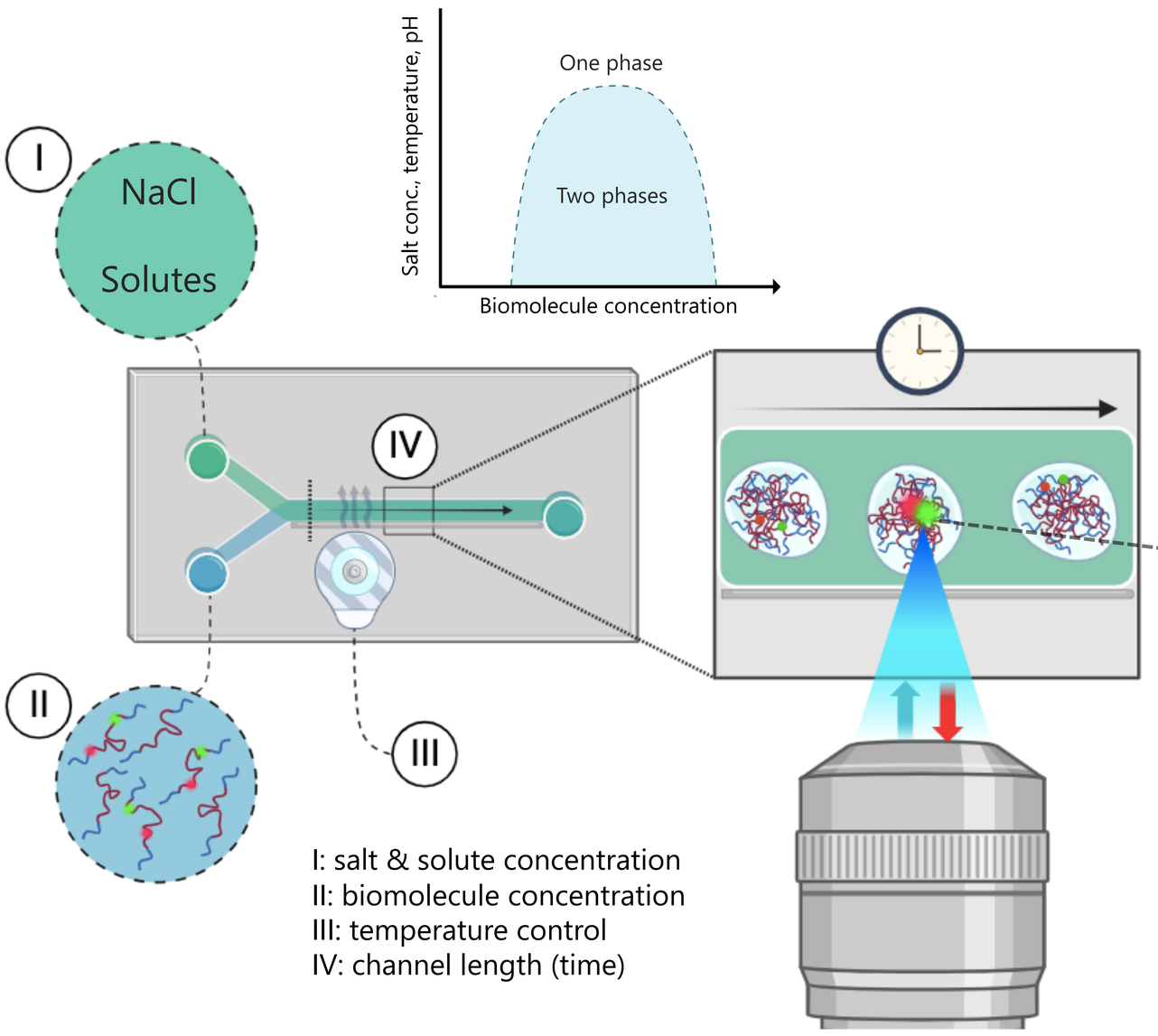
- **Dysregulated** condensate formation (pathological states) has been linked to the development of **neurodegenerative disorders and cancers**

Intermediate states (IS) and local **microenvironmental changes** in the pathological condensate formation pathway may act as potential **therapeutic targets**

Incomplete knowledge of local microenvironmental changes and intermediate conformational states responsible for pathological condensation hinders the development of targeted drugs → **Objective**: elucidate the microenvironmental changes and intermediate conformational states responsible for pathological condensation on a **molecular level**

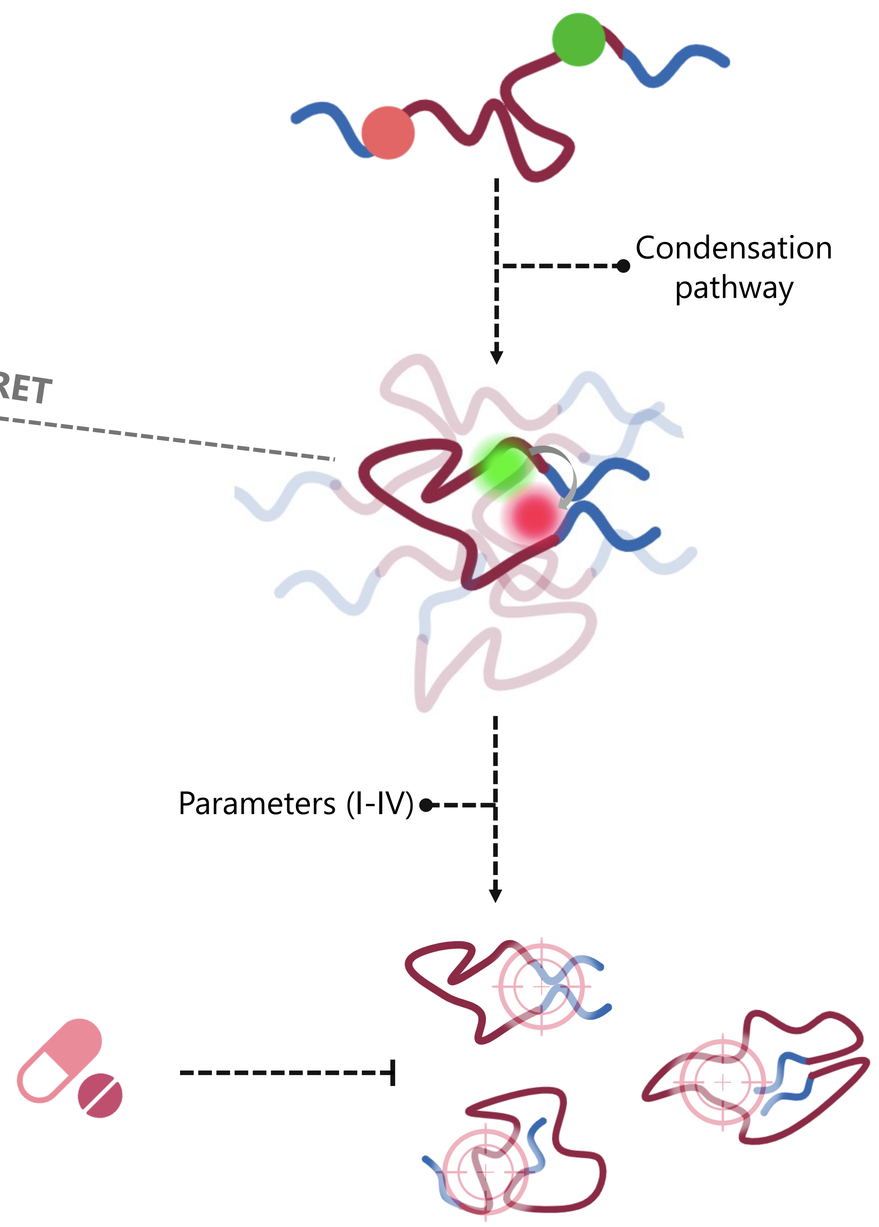
METHOD: study single molecules

The microfluidic device enables precise control of phase-separation parameters and allows a one-by-one droplet flow



Single-molecule Förster resonance energy transfer (smFRET)

- Fluorescently labeled biomolecules transfer energy upon close proximity (<10 nm)
- Measuring the energy transfer probes conformational changes of the labeled molecule and local microenvironmental changes within the droplet



Microenvironmental and **intermediate conformational changes** in the pathological condensate formation pathway

NOVELTY OF EXPERIMENTAL SET-UP:

- **Droplet formation:** a one-by-one droplet flow system with trace amounts of fluorescently labeled biomolecules enables smFRET measurements and the observation of early pathological condensate formation events.
- **Parameter flexibility**: the experimental setup offers versatile adjustment of crucial parameters, providing the capability to investigate their effect on droplet formation and molecular conformational changes of phase-seperating biomolecules.

Images generated with Biorender (https://app.biorender.com/). References: Borgia, A., Borgia, M., Bugge, K. et al. Extreme disorder in an ultrahigh-affinity protein complex. Nature 555, 61–66 (2018), Galvanetto, N., Ivanović, M.T., Chowdhury, A. et al. Extreme dynamics in a biomolecular condensate. Nature 619, 876–883 (2023), Jitao Wen, Liu Hong, Georg Krainer, Qiong-Qiong Yao, Tuomas P. J. Knowles, Si Wu, and Sarah Perrett Journal of the American Chemical Society 2021 143 (33), 13056-13064, Mitrea, D.M., Mittasch, M., Gomes, B.F. et al. Modulating biomolecular condensates: a novel approach to drug discovery. Nat Rev Drug Discov 21, 841–862 (2022)