# Liquid-Liquid Phase Transitions in RNA-Peptide Mixtures: A Balance of Competing Entropies


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## Motivation

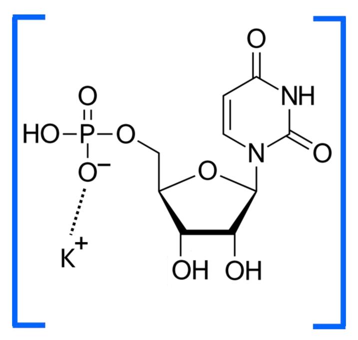
Membraneless organelles (MLOs) are biological liquid-liquid phase separation (LLPS) highly exploited both in the cell nucleus and cytosol in order to maintain homeostasis[1-2]. Nonetheless, the physical mechanisms driving their formation are not fully understood. In this study, we investigate the formation of MLOs in the proximity of the LLPS boundary using a simplified molecular system composed of independent unstructured RNA chains, to which we progressively add cationic peptides. We quantify the molecular aggregation states on both sides of the phase boundary, and show that our observations match the predictions obtained by balancing the competing entropy losses involved in the compression of the RNA chains and in the non-uniform peptide distribution.

## 1. Model system

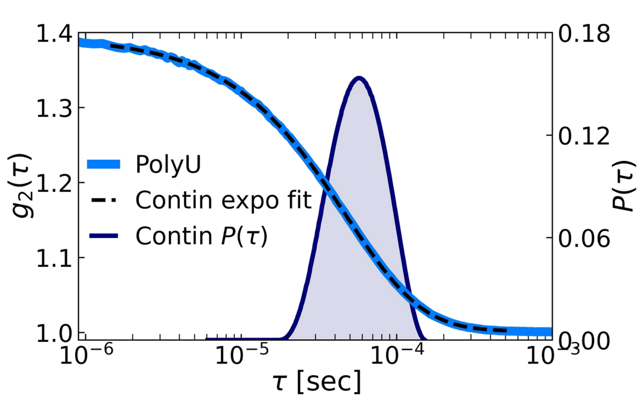
We consider mixtures of oppositely charged **model polymers**

**Polyanion**

**PolyU**




- **Homopolymeric** RNA (~1000 nt)
- $Q_{RNA}$ **~1000e⁻** net charge
- **Unstructured** → Random coil
- No self pairing interactions, negligible base stacking
- **Ideal chain** at high salt with persistence length $\xi_p \approx 8$ Å [3]



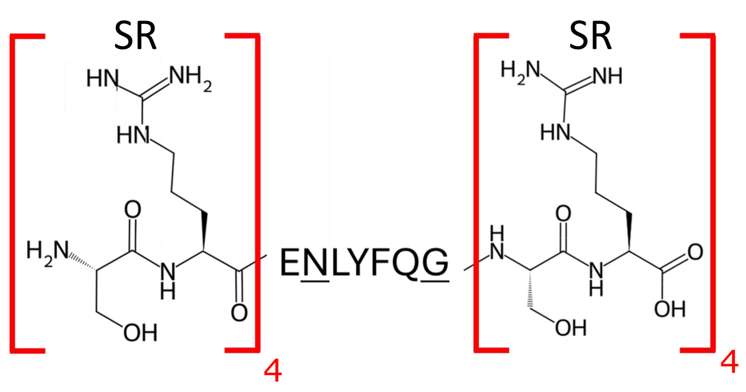

Contin polydispersity analysis

$\langle R_h \rangle = 6 \pm 1.5$ nm

↓

$\langle R_g \rangle = 9 \pm 3$ nm [4]

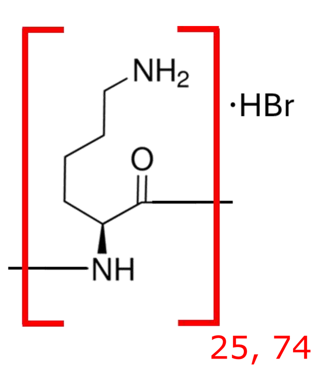
**Polycations**




**meCP**

- $Q_P$**= 7e⁺** net charge
- 23 a.a. sequence
- Inspired from **RNA binding domains** [5]
- **Sticker-and-spacer** architecture [6]
- **Methylated** on N and G backbone nitrogen

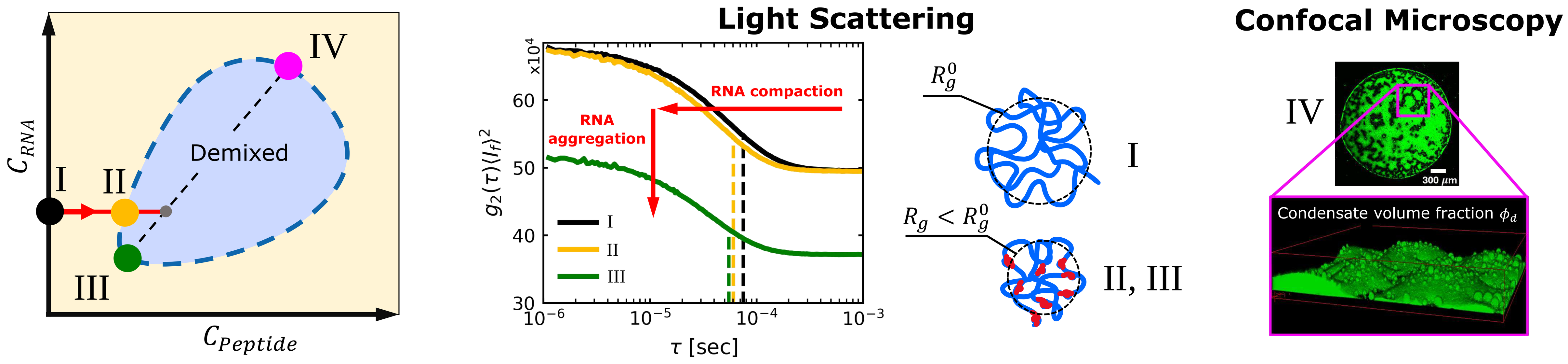
**Poly-L-Lysine (PLL)**




- **Homopolymeric** peptide
- Two sizes, 25 and 74 a.a.
- **Uniformly positively charged**
- $Q_P$**= 25e⁺, 74e⁺** net charge

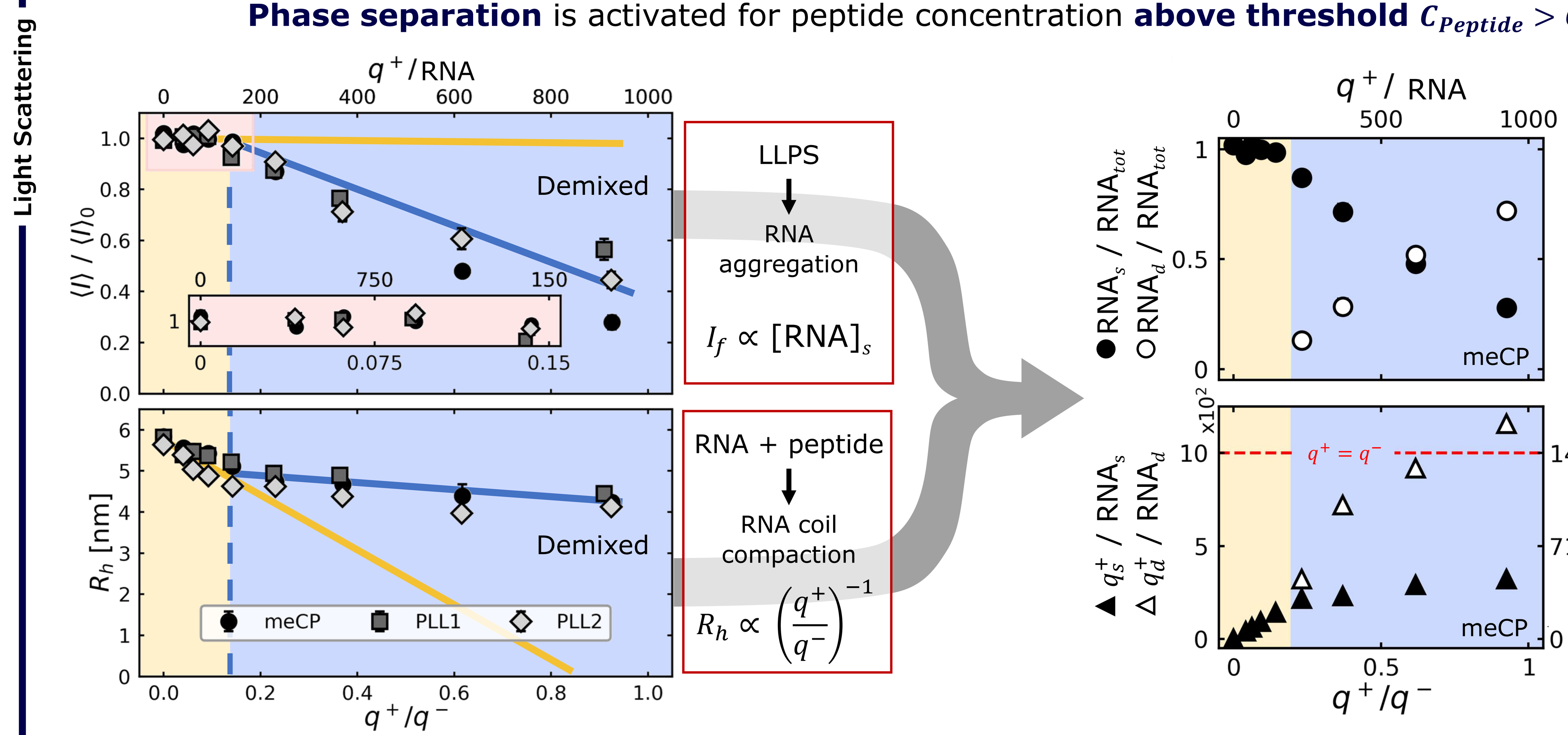
## 2. Experimental outline

We explored the **phase behaviour of PolyU** chains in **increasing peptide concentration** $C_{Peptide}$




## 3. Threshold behaviour

**Phase separation** is activated for peptide concentration **above threshold** $C_{Peptide} > C^T_{Peptide}$

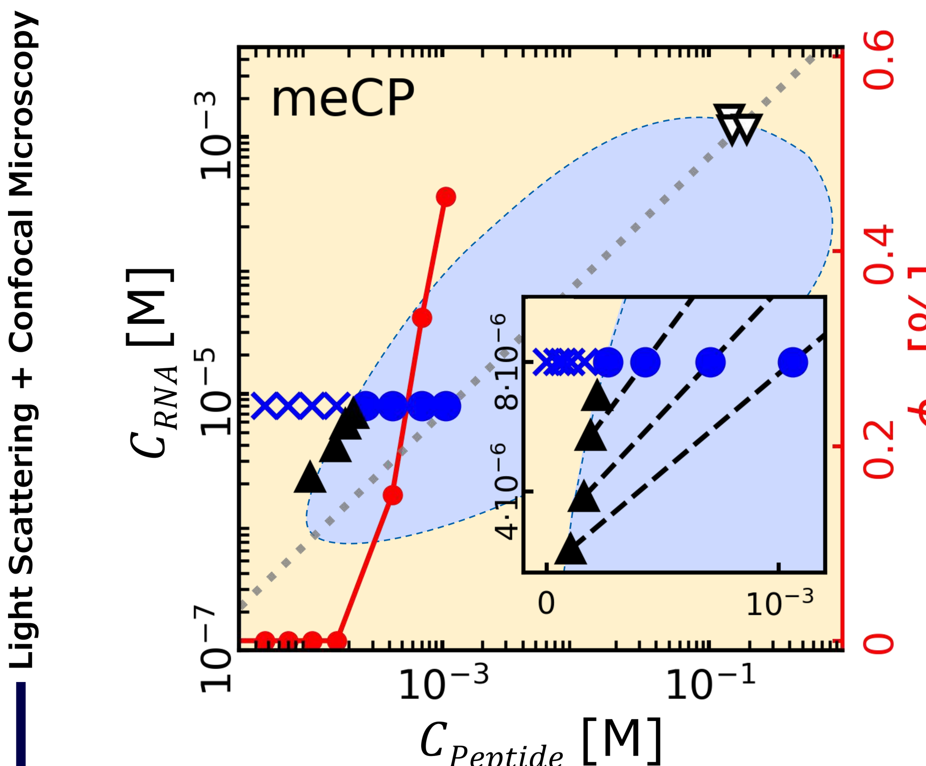



**Relative RNA abundance in the two phases**

**Peptide/RNA molecular ratio**

Through **light scattering** we quantitatively measure **molecular quantities** and **stochiometric ratios** in both phases

## 4. Phase behaviour

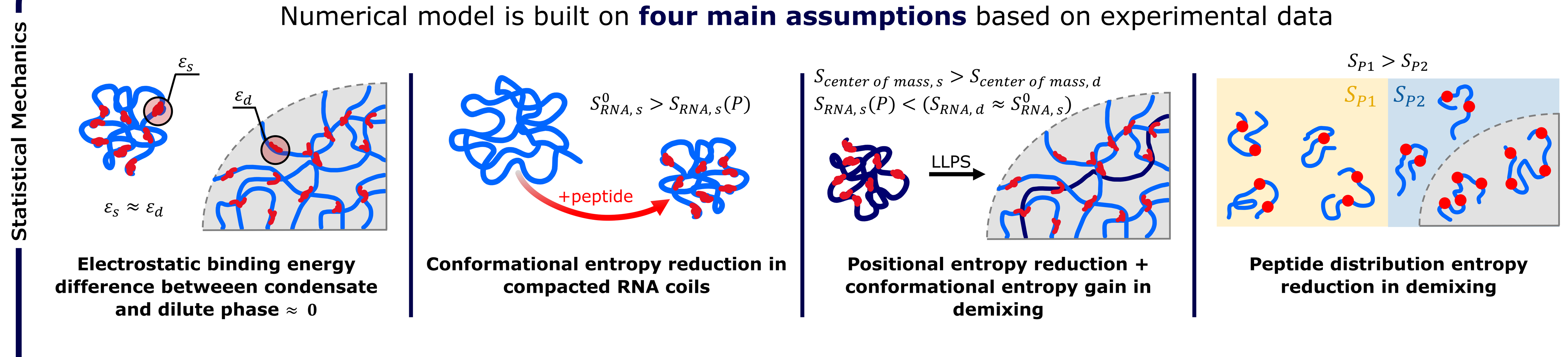



Integrating LS data with **confocal measurements** of **dense phase volumes fraction** $\phi_d$

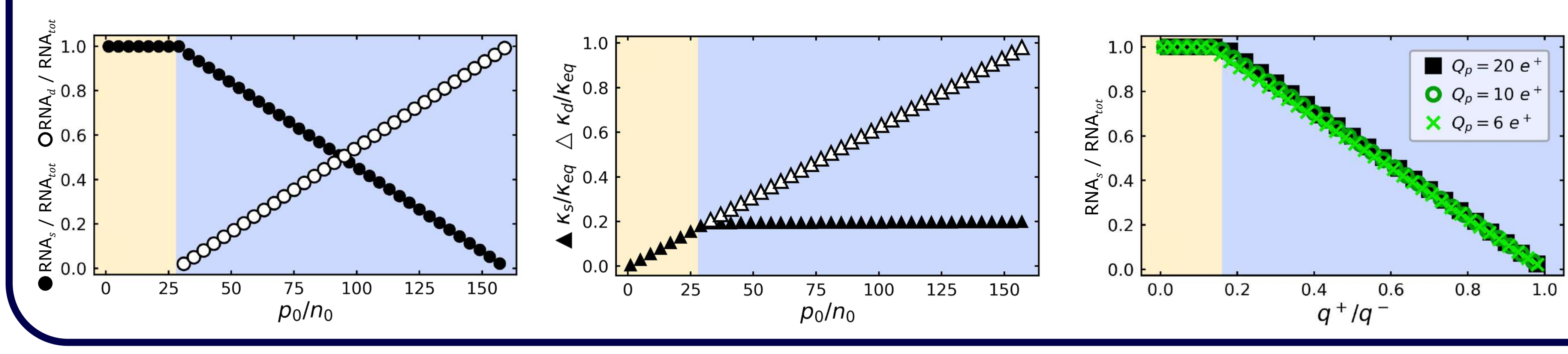
allowed to define an **experimental phase diagram**

## 5. Entropic interplay

Numerical model is built on **four main assumptions** based on experimental data




The model **quantitatively predicts** the **threshold** and the **molecular partitioning**



Threshold position is solely determined by **charge ratio**

**Complex coacervate onset is regulated by local charge ratio and driven by an entropic balance involving polyelectrolytes interactions, conformations and distribution**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