Researchers are studying phase separation behavior between an RNA molecule (polyA$_{100}$, a negatively charged polymer) and a transcription factor (TF) rich in R-K motifs (positively charged, intrinsically disordered). At low concentrations, both are uniformly mixed in solution, but above certain thresholds they form condensed droplets (liquid-liquid phase separation).

The following observations are made experimentally:

Condition	Salt (NaCl, mM)	Temperature (°C)	Droplet formation
A	50	25	Yes
B	150	25	No
C	50	37	Yes
D	50	4	No

Isothermal Titration Calorimetry

ITC experiments show that binding between RNA and TF is enthalpically unfavorable ($\Delta H>0$) but entropically favorable ($\Delta S>0$).

*NaCl dissolves into counterions Na^+ and Cl^- in water.
Reminder: $\Delta G=\Delta H-T\Delta S$

: Flory-Huggins model

$$\Delta\overline{S_{mix}}=-k_B\left(\frac{\phi}{N_A}\ln[\phi]+\frac{1-\phi}{N_B}\ln[1-\phi]\right)$$

Q1: Explain why phase separation occurs between RNA molecules and TFs at low salt concentration but not at high salt. (0.5 pts)

Q2: Why is the process more favorable at higher temperature but not at very low temperature? (0.2 pts)

Q3: List two sources of entropy that favor phase separation in this RNA-TF system. (0.2 pts)

+ opposes

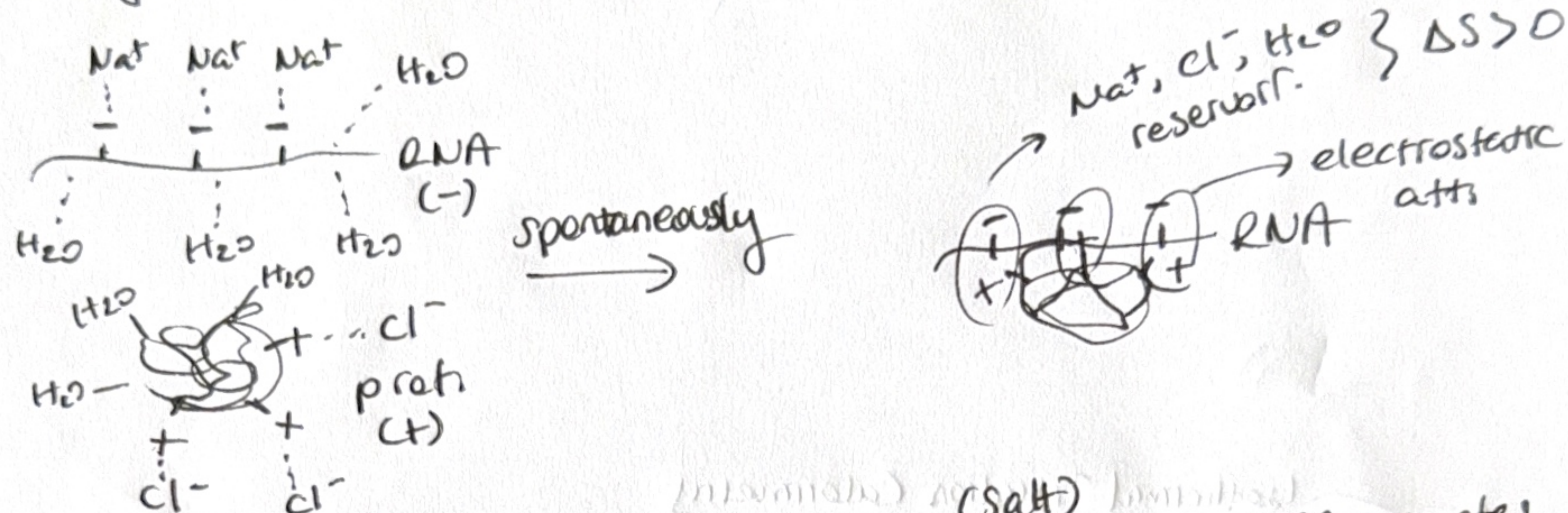
Q4: Using the given Flory-Huggins approach, describe how the polymer length (N) of RNA affects the entropy of mixing and thus its tendency to phase separate? (0.1 pts)

Q1: At low salt concentration, electrostatic attraction between negatively charged RNA molecules and positively charged TFs dominates. When RNA molecules bind to TFs, counterions (Na^+ and Cl^-) that were previously associated with each charged polymer are released into solution. This counterion release, as well as water molecule release, increases the entropy of the universe ($\Delta S > 0$), which drives phase separation. At high salt concentration, screening of electrostatic interactions occurs and fewer counterions are released to the reservoir. Entropic gain is reduced, so droplets do not form.

1 pts

(0.4 pts)

Low salt conc.:



(salt)
* Ion concentration competes with RNA−TF complex form

High salt conc.:

H_2O (Na^+) (H_2O) → prob. of being not occupied

3 Na^+, 3 Cl^-, 3 H_2O ↓

RNA $\underline{4}\ \underline{3}\ \underline{2}$ = 24 microstates

prot. $\underline{4}\ \underline{3}\ \underline{2}$ = 24 microcrestates

(Cl^-)

H_2O H_2O

(Na^+) 6 Na^+, 6 Cl^-, 3 H_2O ↑

RNA $\underline{7}\ \underline{6}\ \underline{5}$ = 210 microstates

prot. $\underline{7}\ \underline{6}\ \underline{5}$ = 210 microcrestates

(Cl^-)

(0.2 pts)

Q2: ITC data indicates the process is entropically driven ($\Delta S > 0$, $\Delta H > 0$).

Gibbs free energy eq.: $\underline{\Delta G} = \Delta H - T\Delta S$ ↑ T↑ makes ΔG more negative.

Thus higher temperature favors droplet formation. At low temperature, if the $T\Delta S$ term is not big enough, cannot overcome positive enthalpy ($\Delta H > 0$), so no phase separation occurs.

(0.2 pts)

Q3: favors: i) counterion release (Na^+, Cl^-), ii) water mol. release to the reservoir

opposes:
i) loss of translational entropy (molecules become confined/trapped in droplets)
ii) loss of conformational entropy (RNA−TF interaction)

(0.2 pts)

Q4: As RNA becomes longer (larger $\underline{N_{RNA}}$), the term $1/N_{RNA}$ decreases, which causes smaller entropy of mixing, meaning less entropic penalty to demixing. Thus longer RNAs phase separate more easily.

$\Delta S_{mix} = -k_B(\cdot\ 1/N_{RNA})$ → becomes less negative = entropy of mixing becomes smaller in magnitude.

less entropic cost to complex form.

$\uparrow S = +k_B \ln(w) \uparrow$ # w↑, S↑