FLORY-HUGGINS LIQUID PHASE SEPARATION DETERMINATION

We wanted to first characterise the RNA organelles under different repeat lengths to ensure liquid phase separation. This was done through applying the Flory Huggins solution theory, which is a mathematical model of the thermodynamics involved in polymer-solvent solutions (Brangwynne et al. 2015), in our case- water and RNA (1).

$$\Delta G_m = RT[n_1 \ln \phi_1 + n_2 \ln \phi_2 + n_1 \phi_2 \chi_{12}]$$

	Notation	Parameter
Solvent properties	n1	Concentration of water
	Phi 1	Volume fraction of water/RNA strands
Polymer	n2	Concentration of RNA strands
	Phi2	Volume fraction of RNA strand / water.
Interaction	Chi12	The interaction parameter
Conditions	R	Gas constant
	T	Absolute temperature

(1)

Since the RNA volume and concentration scan be assumed to be negligible compared to the overall volume of a cell, n1>> n2 and Φ1>> Φ2

Implementing this into our equation gives us (2):

$$\Delta G_m = RT[n_1 \ln \phi_1 + n_1 \chi_{12}] \quad (2)$$

If we assume that the RNA levels remain at all times too small to be considered, then the molar concentration and volume fraction of the solvent become constant, and the only parameter that is important in the variation of the free energy is the chi12- interaction parameter. This parameter is defined as (3):

The χ12 parameter is defined as (3):

$$\chi_{12} = \frac{z}{kT}(\omega_{12} - \frac{1}{2}\omega_{11} - \frac{1}{2}\omega_{22}) \quad (3)$$

Notation	Parameter	Value
ω11	Solvent-solvent interaction	-2 kJ/mol^{-1}
ω12	Solvent-polymer interaction	Depending on polymer
ω22	Polymer-polymer interaction	Depending on polymer length
K	Boltzmann Constant	1.38065*10^-23
T	Absolute temperature; 310 K	310 K
Z	Iteracting possibilites	3 (3D model)

We make the simplifying assumption that the cellular environment is composed entirely of water as other elements are at much lower concentrations and have a negligible effect. Therefore, the solvent-solvent interaction of water (ω11) was determined as Gibbs free energy of the hydrogen binding of one water molecule to another, which is -2 kJ/mol^{-1} (Chaplin, 2010).

To characterise the solvent - monomer interactions of the RNA strands with water, we used structural modelling software to calculate the free energy of the unboud structure, and calculated it for each individual repeat. We tested eight different RNA repeat legnths ranging between 5 and 70.

To do this, the cofold structure was calculated for each of the length with 10 strands, and the monomer-monomer interaction free energy was averaged between all the differernt cofold predictions and used as the ω22.

No of strands	5xCAG	15x CAG	20x CAG	24xCAG	40xCAG	45xCAG	50xCAG	70xCAG
10	-74.6	-213.5	-279.9	-321.6	-545.4	-611.7	-678.1	-943.4
9	-65.7	-191.3	-251.1	-288.4	-490.0	-549.7	-609.4	-848.2
8	-58.7	-169.2	-222.3	-256.3	-434.6	-487.7	-540.7	-753.0
7	-49.7	-147.1	-193.6	-223.0	-379.3	-425.7	-472.1	-88.4
6	-42.7	-125.1	-164.9	-190.9	-324.0	-363.7	-403.5	-657.8
5	-33.7	-103.0	-136.2	-157.6	-268.7	-301.8	-335.0	-562.6
4	-26.7	-81.2	-107.6	-125.5	-213.5	-240.0	-266.5	-182.9
3	-18.8	-59.2	-79.0	-92.3	-158.4	-178.2	-198.1	-467.5
2	-13.0	-39.0	-52.1	-62.4	-104.2	-117.3	-130.4	-372.5
1	-1.8	-15.8	-22.8	-27.8	-49.0	-55.4	-62.1	-277.5
Mean:	-38.5	-114.5	-150.9	-174.6	-296.7	-333.1	-369.6	-515.4

ω12 was calculated by estimating the monomer interaction of an unfolded RNA strand:

Strand	ω12
5xCAG	-0.4980
15xCAG	-1.0500
20xCAG	-1.1375
24xCAG	-1.1663
40xCAG	-1.2240
45xCAG	-1.2311
50xCAG	-1.2420
70xCAG	-1.3086

(1) Brangwynne, C.P., Tompa, P. and Pappu, R.V., 2015. Polymer physics of intracellular phase transitions. Nature Physics, 11(11), pp.899-904.

(2) Chaplin, M.F., 2010. Water's hydrogen bond strength. *Water and Life: The unique properties of H2O*, pp.69-86