

# RNA is special

what could possibly go wrong?

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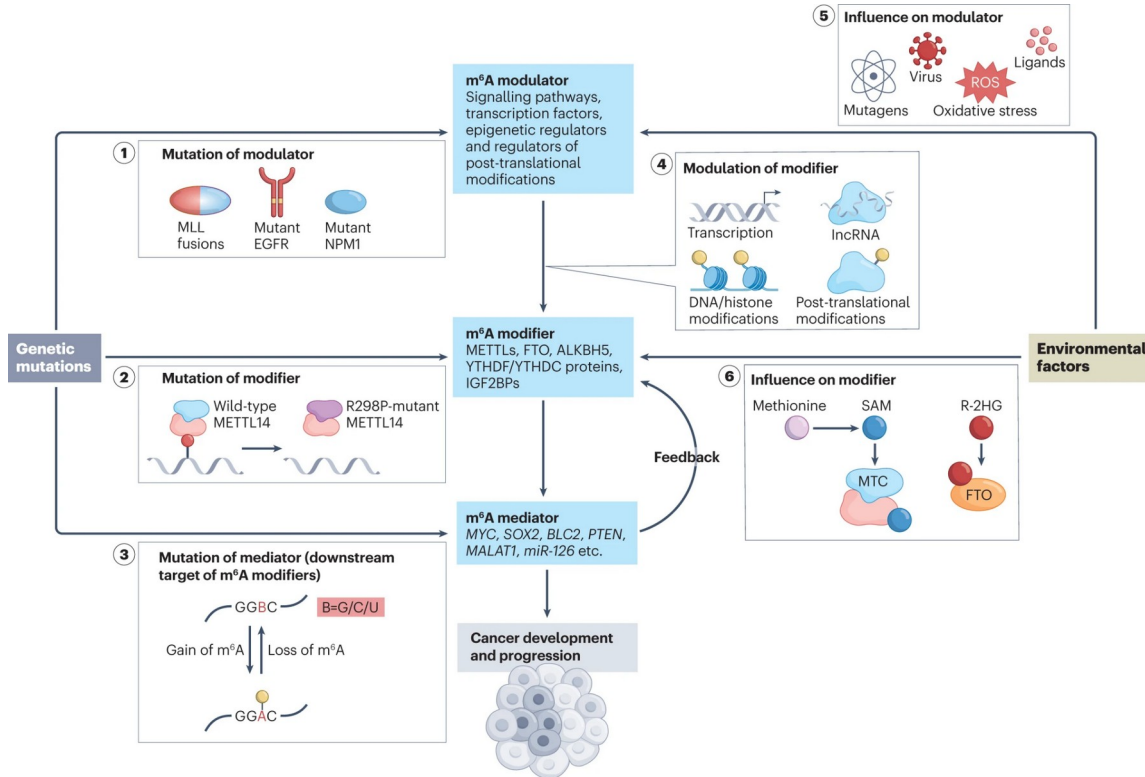


# Outline

- RNA-protein interactions
- Allostery and RNA
  - $\pi$ - $\pi$  interactions
  - The case of ions
- Integrative modelling  
(dimensionality)

# RNA-protein interaction

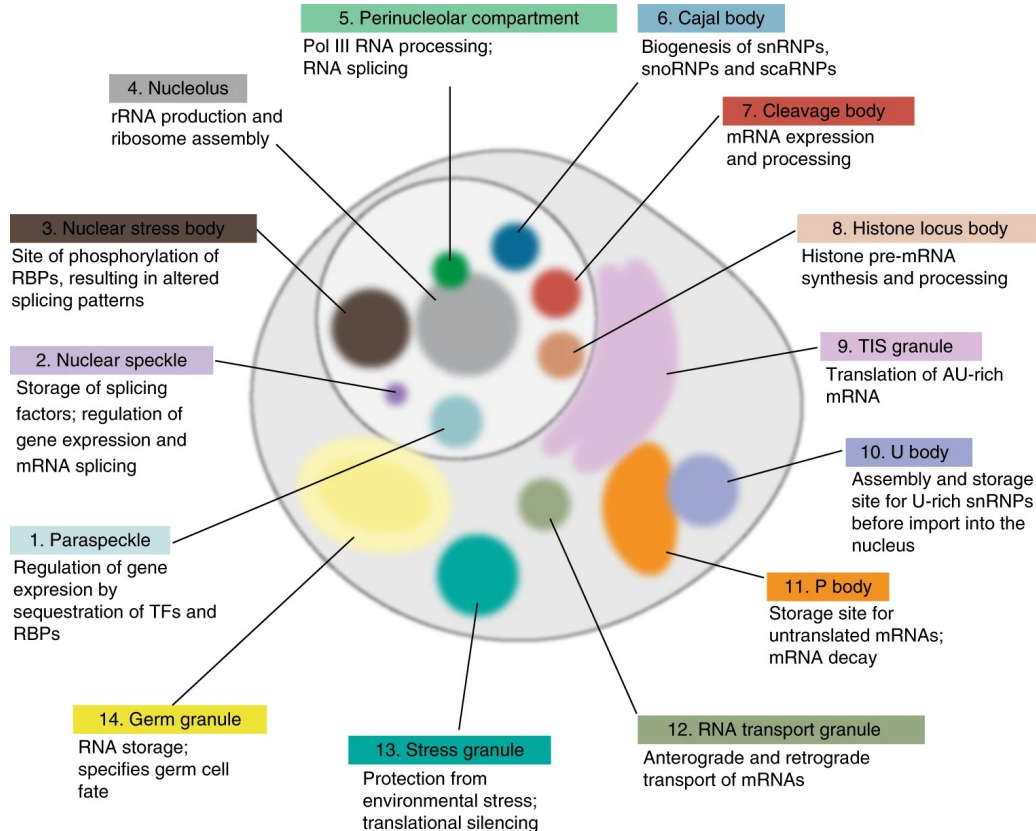
# Mechanisms underlying the dysregulation of RNA m6A modification and m6A-dependent processes in cancer.



Deng, X., Qing, Y., Horne, D., Huang, H., & Chen, J. (2023). The roles and implications of RNA m6A modification in cancer. *Nature Reviews Clinical Oncology*, 20(8), 507-526.

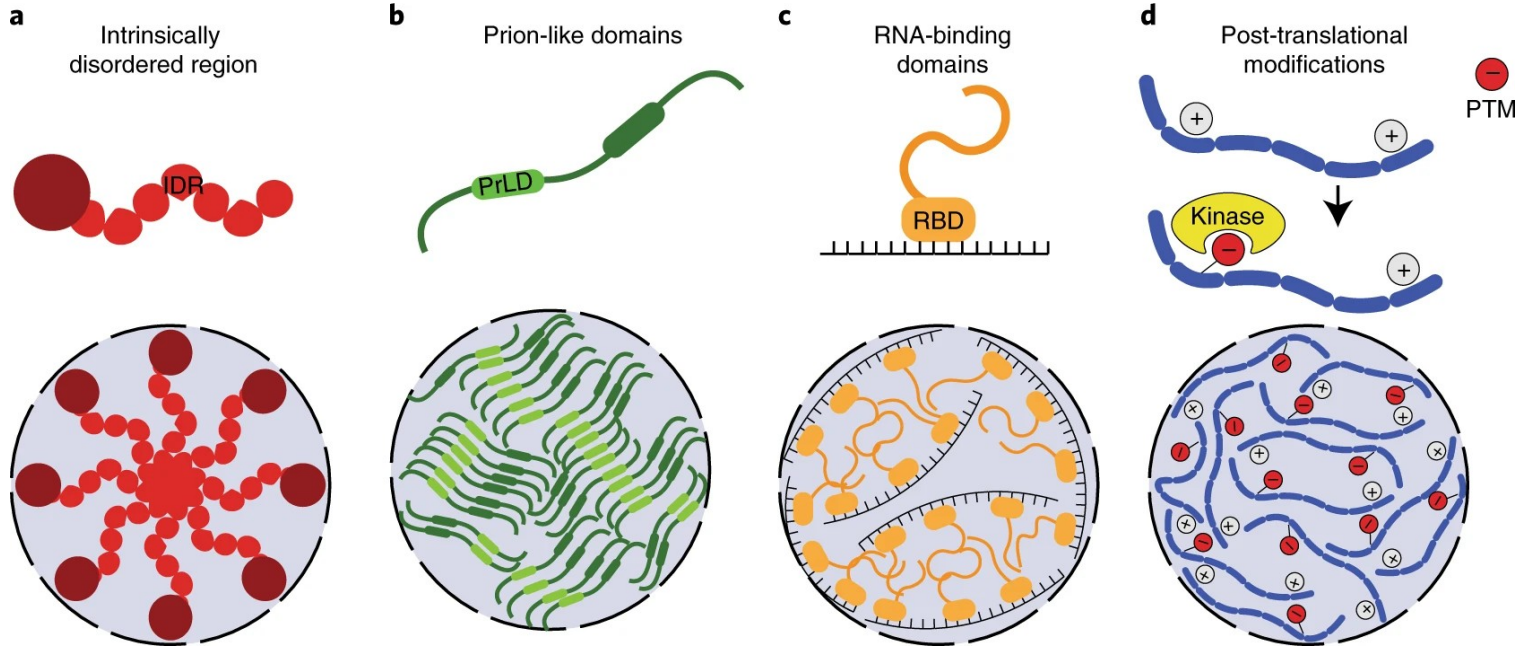
# RNA-protein interaction... and phase separation

# RNA/protein interactions and phase separation



Wiedner, H. J., & Giudice, J. (2021). It's not just a phase: function and characteristics of RNA-binding proteins in phase separation. *Nature structural & molecular biology*, 28(6), 465-473.

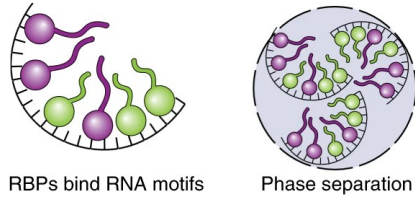
# RNA/protein interactions and phase separation



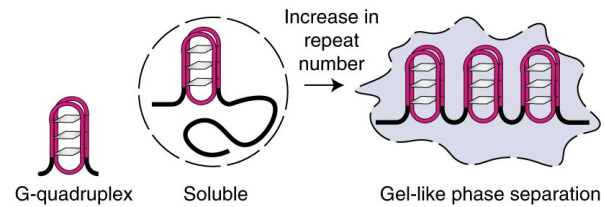
Wiedner, H. J., & Giudice, J. (2021). It's not just a phase: function and characteristics of RNA-binding proteins in phase separation. *Nature structural & molecular biology*, 28(6), 465-473.

# RNA/protein interactions and phase separation

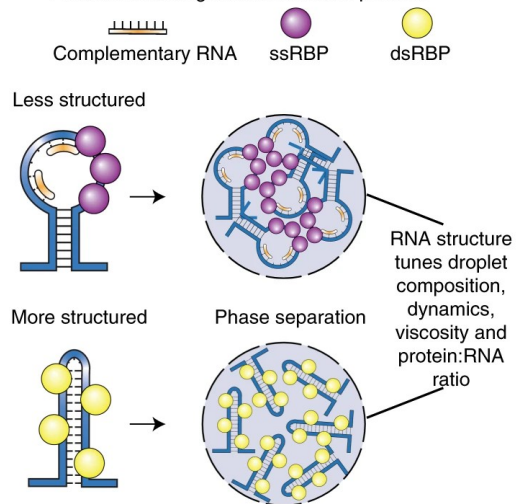
**a** RNA acts as a scaffold for RBP interactions



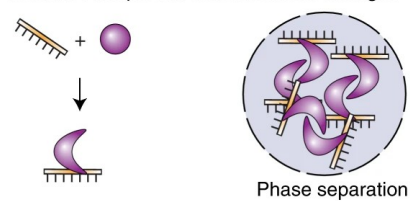
**c** Intramolecular RNA interactions promote gelation



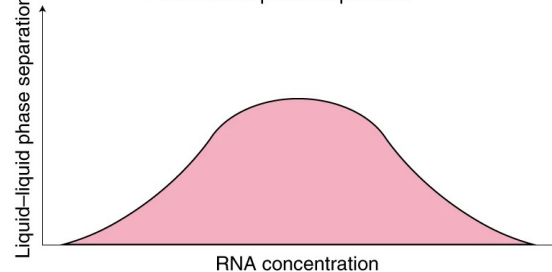
**b** RNA structure regulates interaction partners



**d** RNA induces protein conformational changes

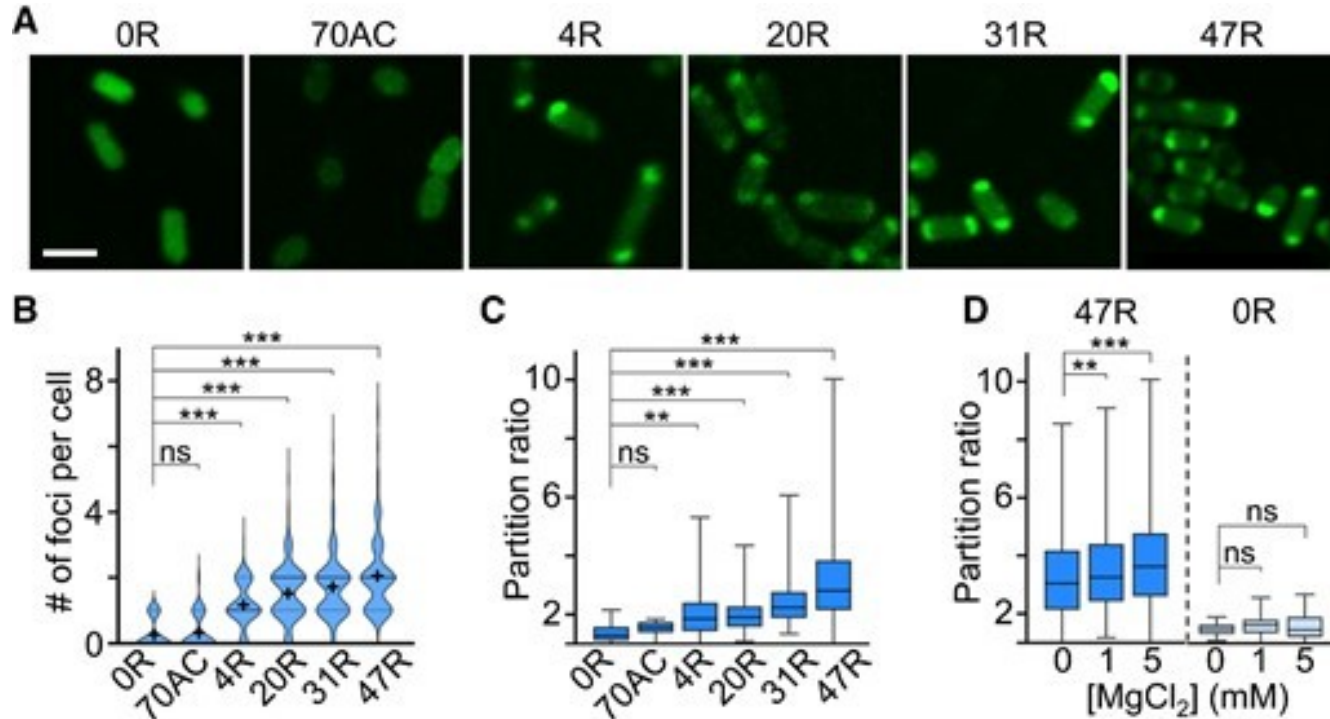


**e** RNA buffers phase separation



Wiedner, H. J., & Giudice, J. (2021). It's not just a phase: function and characteristics of RNA-binding proteins in phase separation. *Nature structural & molecular biology*, 28(6), 465-473.

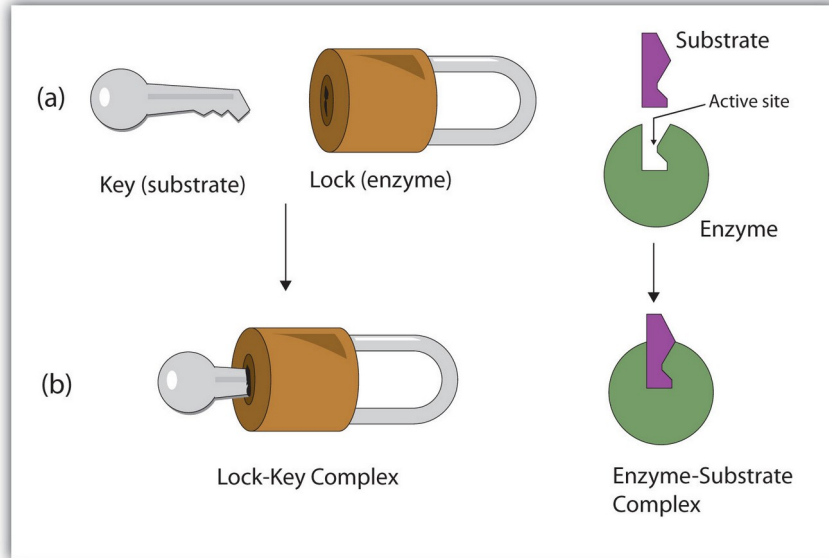
# RNA condensate formation in living bacterial cells



Zhaolin Xue, Kewei Ren, Rigumula Wu, Zhining Sun, Ru Zheng, Qian Tian, Ahsan Ausaf Ali, Lan Mi, Mingxu You, Targeted RNA condensation in living cells via genetically encodable triplet repeat tags, *Nucleic Acids Research*, 51 (16), 2023, 8337–8347

Allostery

# Lock and key (“Schlüssel-Schloss-Prinzip”)



“Um ein Bild zu gebrauchen, will ich sagen, dass Enzym und Glucosid wie Schloss und Schlüssel zu einander passen müssen, um eine chemische Wirkung aufeinander ausüben zu können.”

Fischer, Emil. "Einfluss der Configuration auf die Wirkung der Enzyme." *Berichte der deutschen chemischen Gesellschaft* 27.3 (1894): 2985-2993.

Cramer, F. (1995). Biochemical correctness: Emil Fischer's lock and key hypothesis, a hundred years after—an essay. *Pharmaceutica Acta Helvetica*, 69(4), 193-203.

# Allostery - Biological problem

## (a) *The kinetics of allosteric systems*

In Fig. 3, results for the fractional saturation of haemoglobin by oxygen at different partial pressures (Lyster, unpublished work) have been fitted to equation (2). While the fit is satisfactory, we feel that strict quantitative agreement is neither sufficient

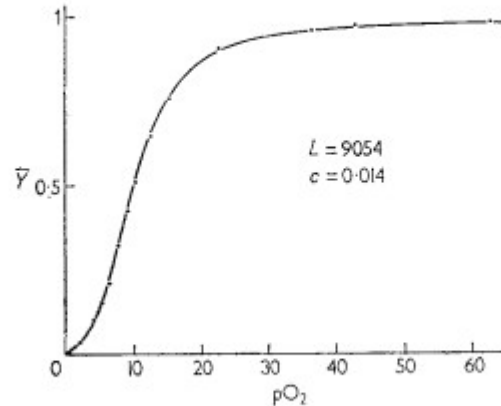


FIG. 3. Saturation of haemoglobin with oxygen. Results (points) obtained by R. W. J. Lyster (unpublished work) with horse haemoglobin (4.6%) in 0.6 M-phosphate buffer (pH 7) at 19°C. Solid line drawn to equation (2) using the values of the constants  $L$  and  $c$  given on the graph.

Monod, J., Wyman, J., & Changeux, J. P. (1965). On the nature of allosteric transitions: a plausible model. *J Mol Biol*, 12(1), 88-118.

# Allostery - The MWC Model

94

J. MONOD, J. WYMAN AND J. P. CHANGEUX

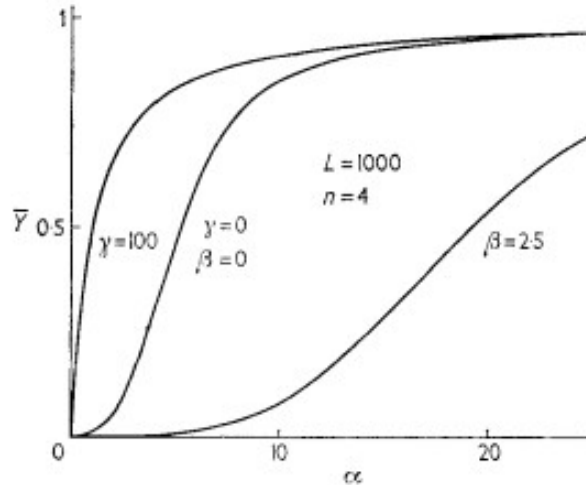


FIG. 2. Theoretical curves showing the heterotropic effects of an allosteric activator ( $\gamma$ ) or inhibitor ( $\beta$ ) upon the shape of the saturation function for substrate ( $\alpha$ ) according to equation (3).

Monod, J., Wyman, J., & Changeux, J. P. (1965). On the nature of allosteric transitions: a plausible model. *J Mol Biol*, 12(1), 88-118.

# Allostery - The KNF Model

VOL. 5, NO. 1, JANUARY 1966

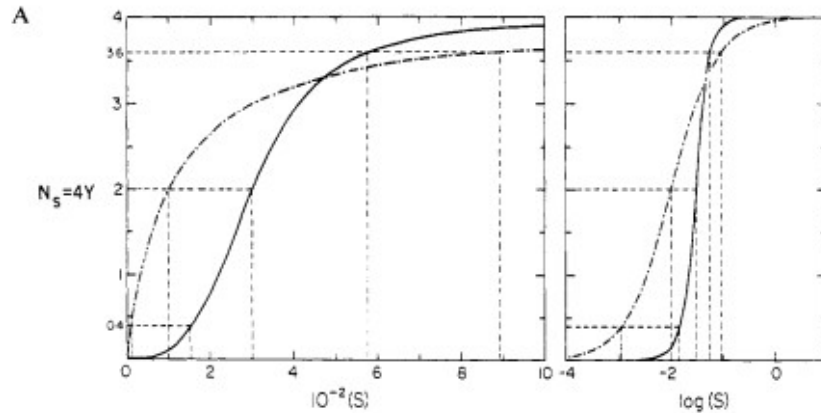
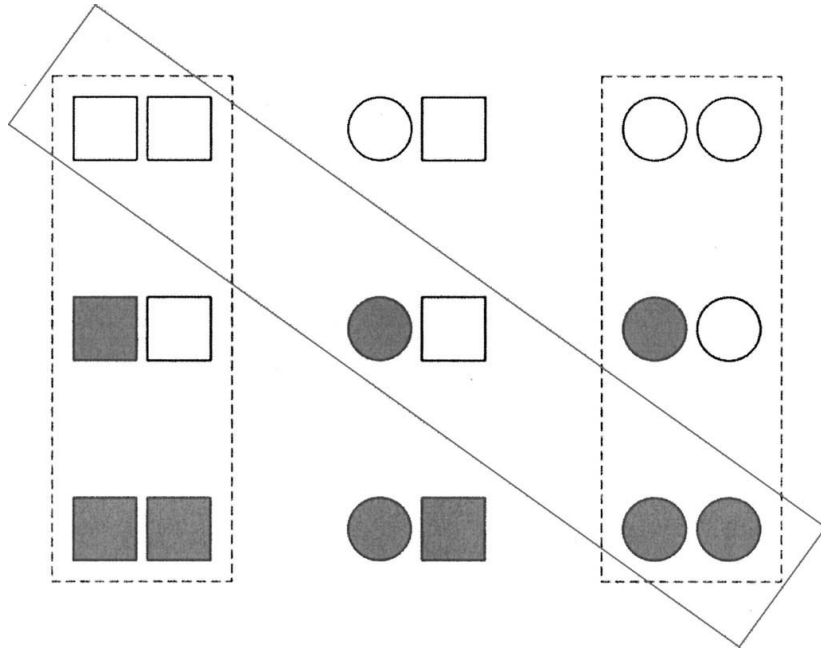


FIGURE 1: Comparison of a sigmoid saturation curve (—) with a Michaelis-Menten saturation curve (---). (A) Plotting  $N_s$  vs.  $(S)$  and (B) on a plot of  $N_s$  vs.  $\log(S)$ . On both plots, the levels of saturation ( $N_s = 0.4, 2.0$ , and  $3.6$ , corresponding to 10, 50, and 90% saturation, respectively) used to define the characteristic parameters ( $S_{0.1}$ ) and  $R_s$ , and the concentrations corresponding to each, are indicated by dashed lines.

Koshland Jr, D. E., Nemethy, G., & Filmer, D. (1966).

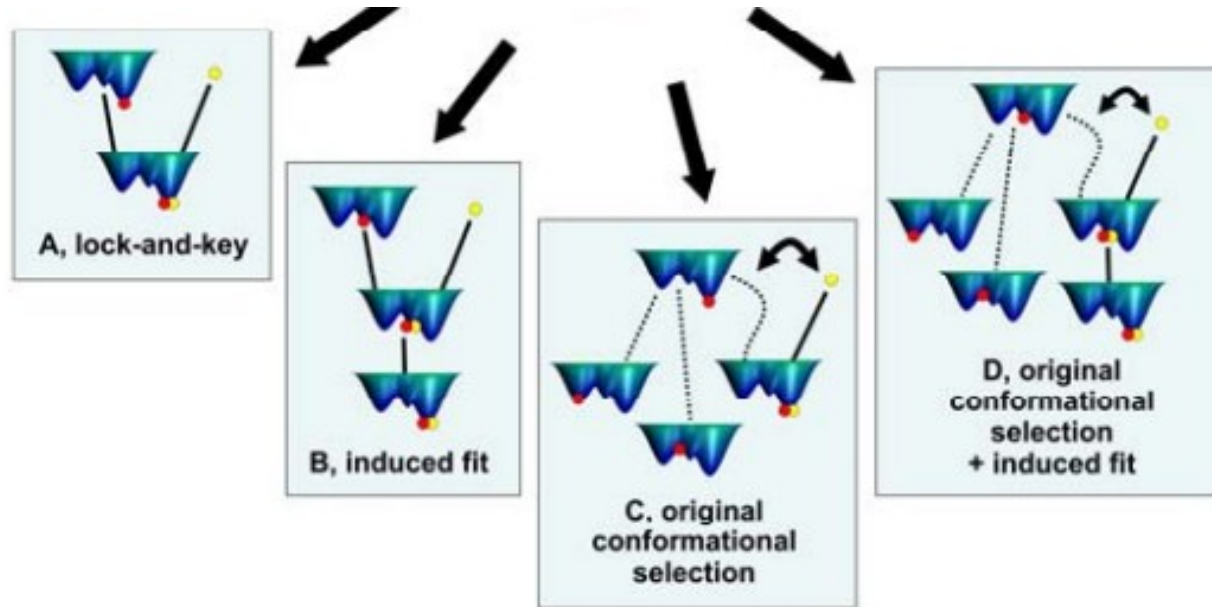
Comparison of experimental binding data and theoretical models in proteins containing subunits. *Biochemistry*, 5(1), 365-385.

# Allostery - Comparing MWC and KNF



Cui, Q., & Karplus, M. (2008). Allostery and cooperativity revisited. *Protein science*, 17(8), 1295-1307.

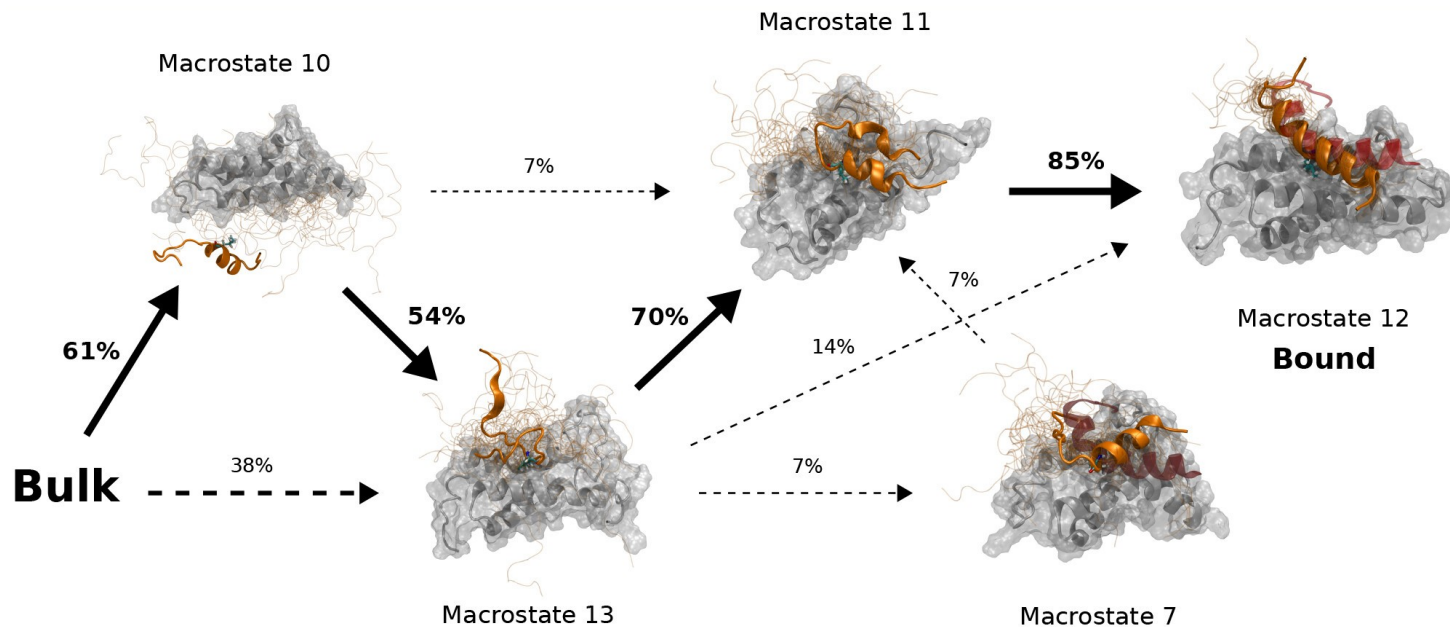
# Allostery - Modern view



Csermely, P., Palotai, R., & Nussinov, R. (2010).

Induced fit, conformational selection and independent dynamic segments: an extended view of binding events. *Nature Precedings*, 1-1.

Herrera-Nieto, P., Pérez, A., & De Fabritiis, G. (2023). Binding-and-folding recognition of an intrinsically disordered protein using online learning molecular dynamics. *Journal of Chemical Theory and Computation*.



Ion, RNA and Allostery modeling

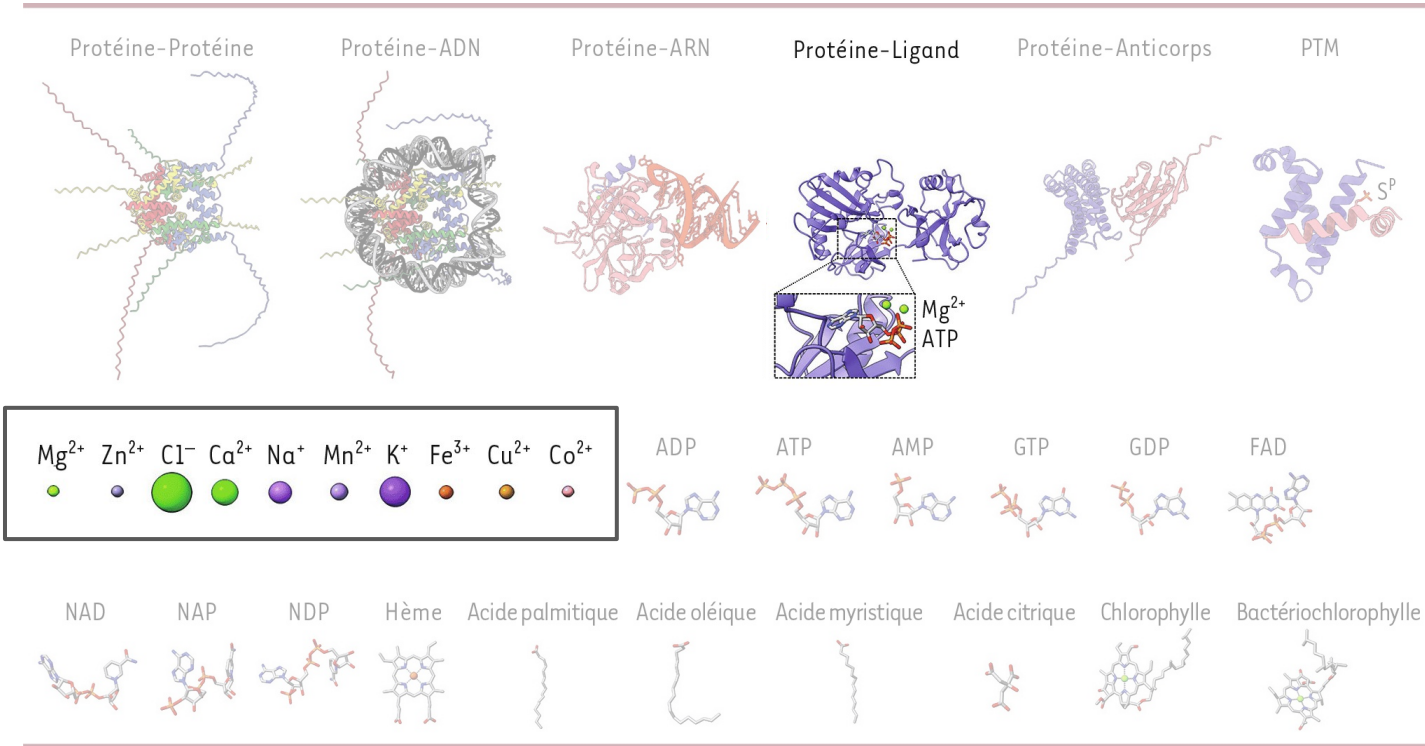
# Understanding nucleic acid-ion interactions

- Nucleic acids are highly charged.
- RNA folding [and RNA-protein interactions] involve bringing charges in close proximity
- This require counterions.with an enormous electrostatic energy barrier.

Lipfert J, Doniach S, Das R, Herschlag D. Understanding nucleic acid-ion interactions. *Annu Rev Biochem.* 2014;83:813-41. doi: 10.1146/annurev-biochem-060409-092720.

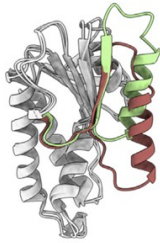


# Modelling ion-associated conformational changes with AF

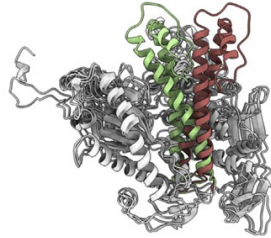


Taly, A., & Verger, A. (2024). Prediction of complex biomolecular structures by AlphaFold 3. *Medecine sciences: M/S*, 40(10), 725-727.

Adenosylcobinamide kinase apo  
(green), holo (red)



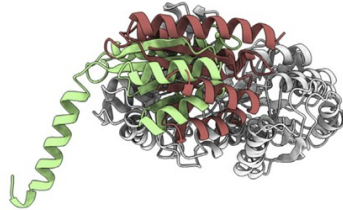
Pol alpha DNA polymerase apo  
(green), holo (red)



Glutamine-binding protein apo  
(green), holo (red)



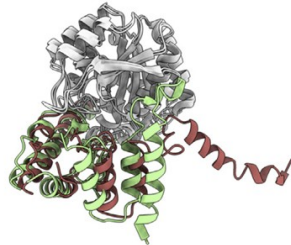
Leucine-binding protein apo (green), holo (red)



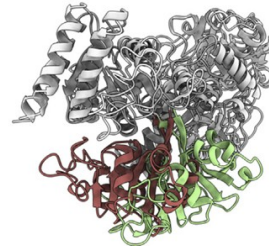
DNA polymerase I active (green), open (red)



Aspartate aminotransferase open (green), closed (red)

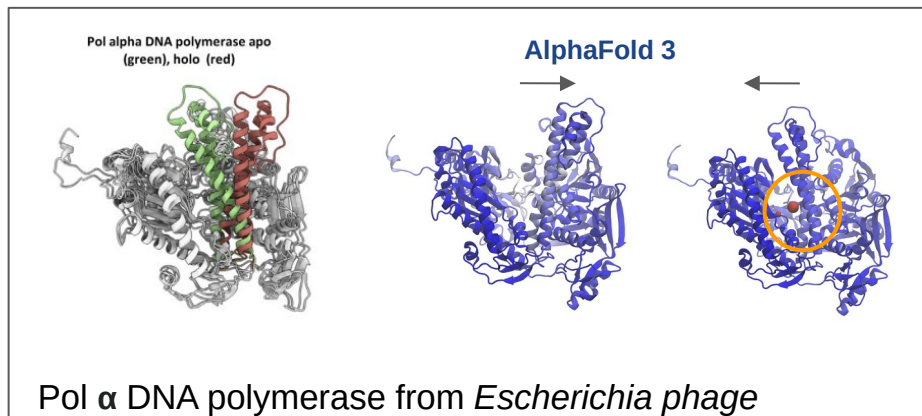
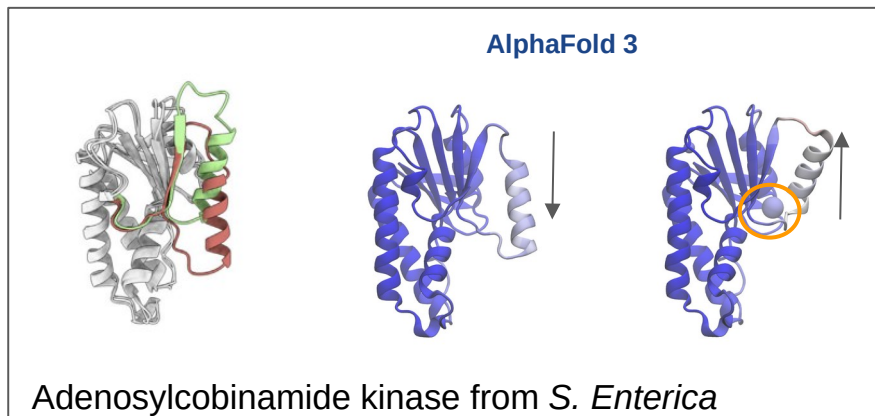
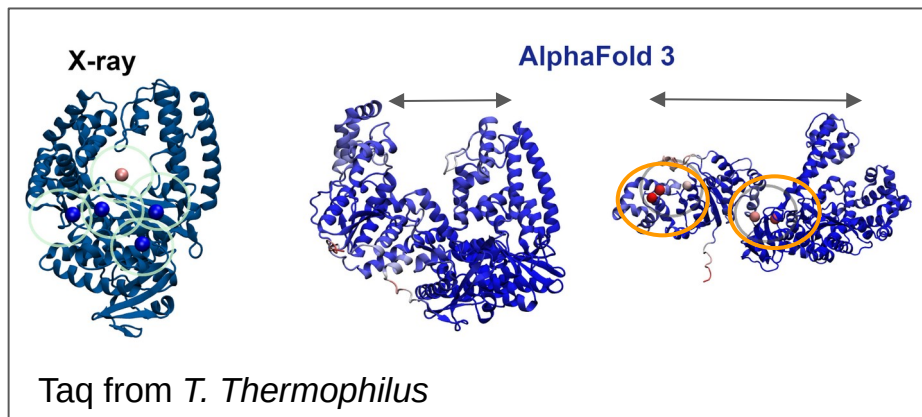
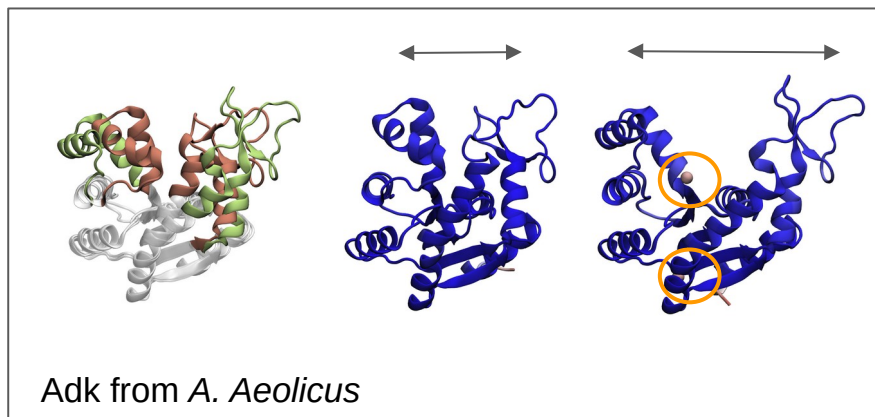


Lactoferrin apo (green), holo (red)



Sala et al.: Modeling of protein conformational changes with Rosetta guided by limited experimental data (2022)

# Extremophiles



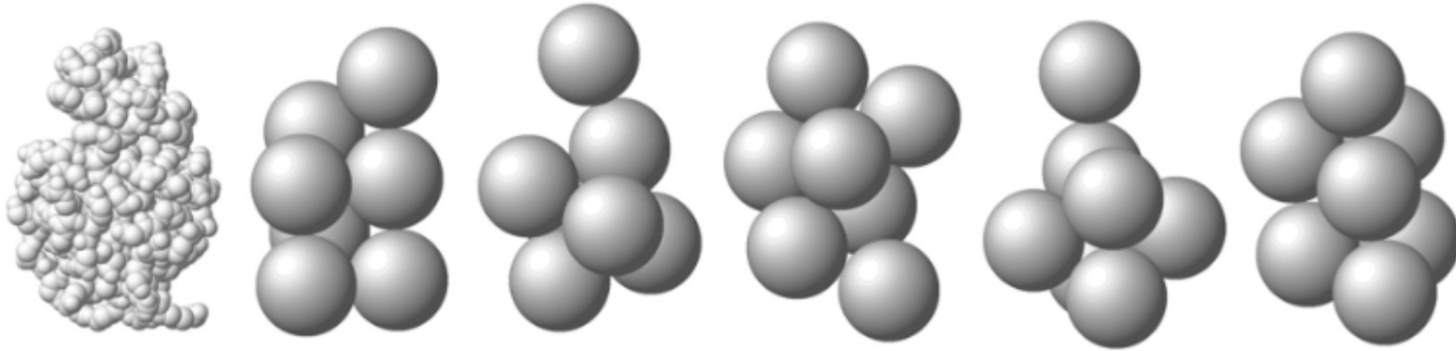
# AF has issues with modelling ion-associated conformational changes

Chakravarty, D., Schafer, J.W., Chen, E.A. *et al.* AlphaFold predictions of fold-switched conformations are driven by structure memorization. *Nat Commun* 15, 7296 (2024). [10.1038/s41467-024-51801-z](https://doi.org/10.1038/s41467-024-51801-z)

Škrinjar, P., Eberhardt, J., Durairaj, J., & Schwede, T. (2025). Have protein-ligand co-folding methods moved beyond memorisation?. *bioRxiv* 2025.02.03.636309; [10.1101/2025.02.03.636309](https://doi.org/10.1101/2025.02.03.636309)

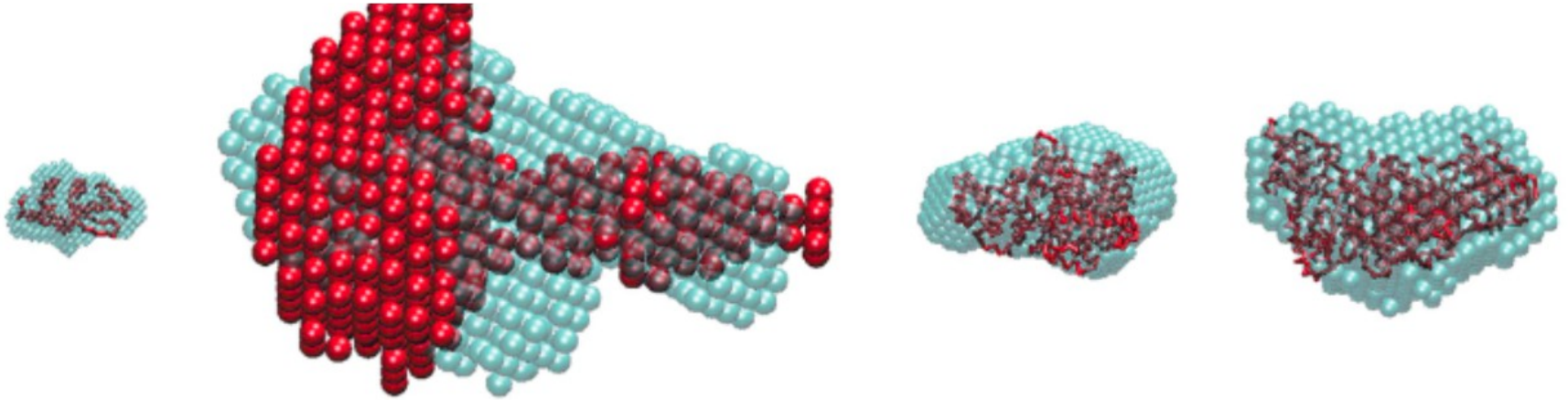
# Dimensionality and the ambiguity of fitted data

# Dimensionality



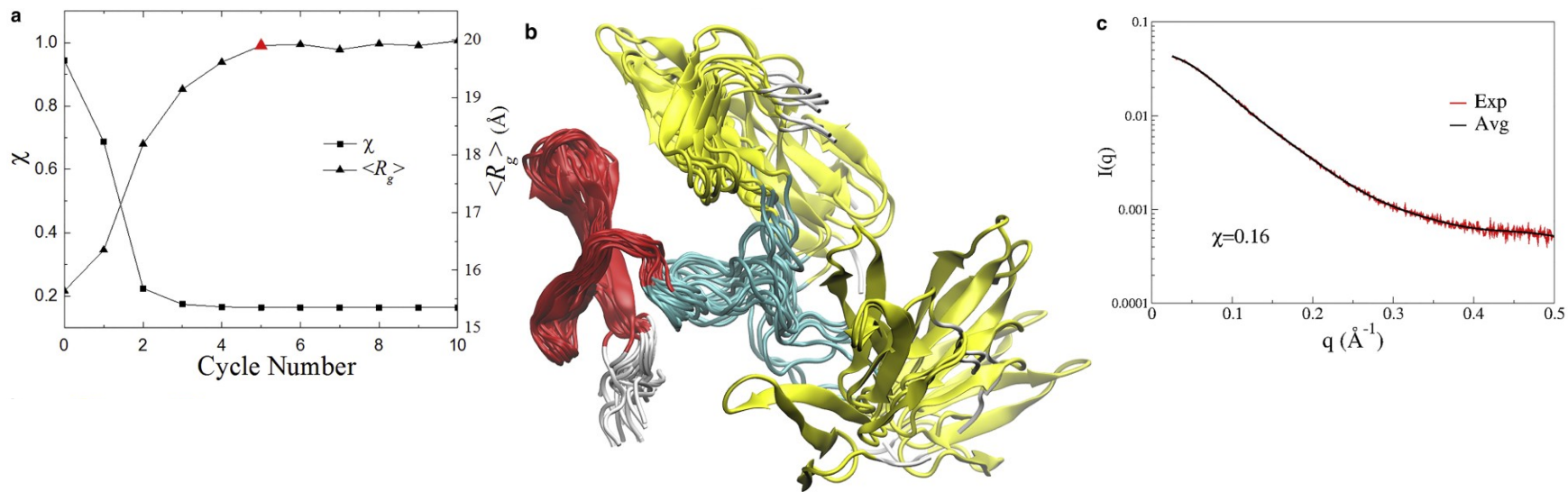
Petoukhov, M. V., & Svergun, D. I. (2015). Ambiguity assessment of small-angle scattering curves from monodisperse systems. *Biological Crystallography*, 71(5), 1051-1058.

# Dimensionality (and fit)



Petoukhov, M. V., & Svergun, D. I. (2015). Ambiguity assessment of small-angle scattering curves from monodisperse systems. *Biological Crystallography*, 71(5), 1051-1058.

# Fitting with conformation ensembles



Cheng P, Peng J, Zhang Z. SAXS-Oriented Ensemble Refinement of Flexible Biomolecules. *Biophys J.* 2017 Apr 11;112(7):1295-1301.