Who am I?

My name is Lorenzo Rovigatti and my email is lorenzo.rovigatti@uniroma1.it

How did I get here?

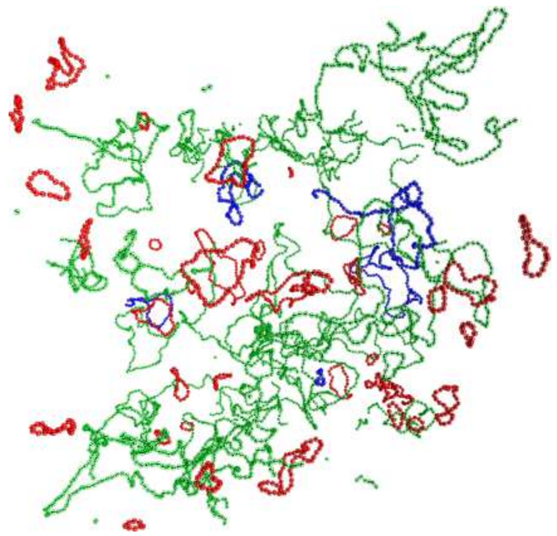
- 2009 - Laurea in Fisica here (with FS)
- 2012 - PhD in Materials Science here (with FS)
- 2013-2014 - Post-doc here (with FS)
- 2014-2016 - Post-doc at the University of Vienna
- 2016-2017 - Post-doc at the University of Oxford
- 2017-2018 - Researcher (RTDA) at CNR
- 2018-now - Tenure-track researcher (RTDB) here

What are my research interests?

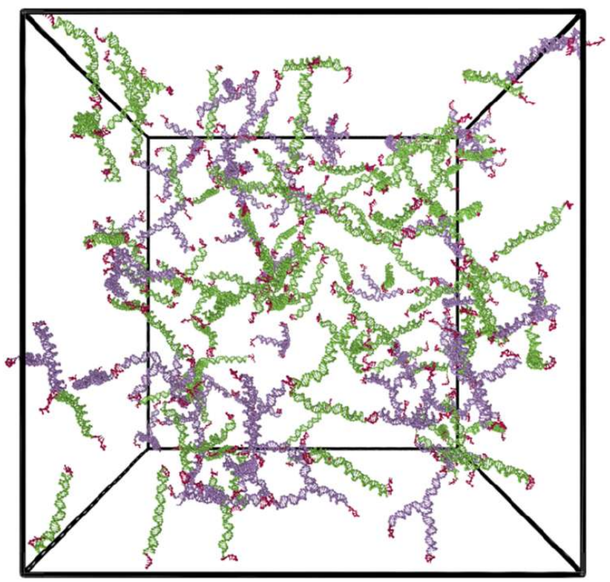
- Soft matter (including some biophysics)
- Self-assembly (colloids, proteins, nucleic acids)
- DNA nanotechnology
- Polymeric assemblies (star polymers, microgels)

Some pictures

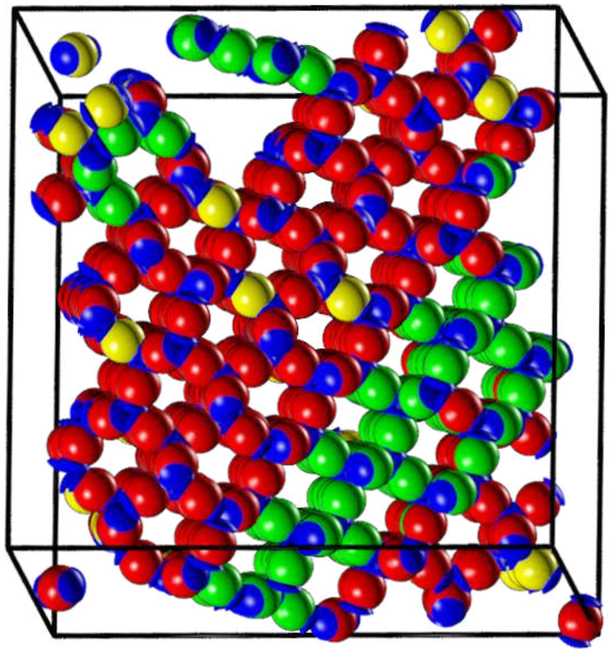
Self-assembly vs. phase separation



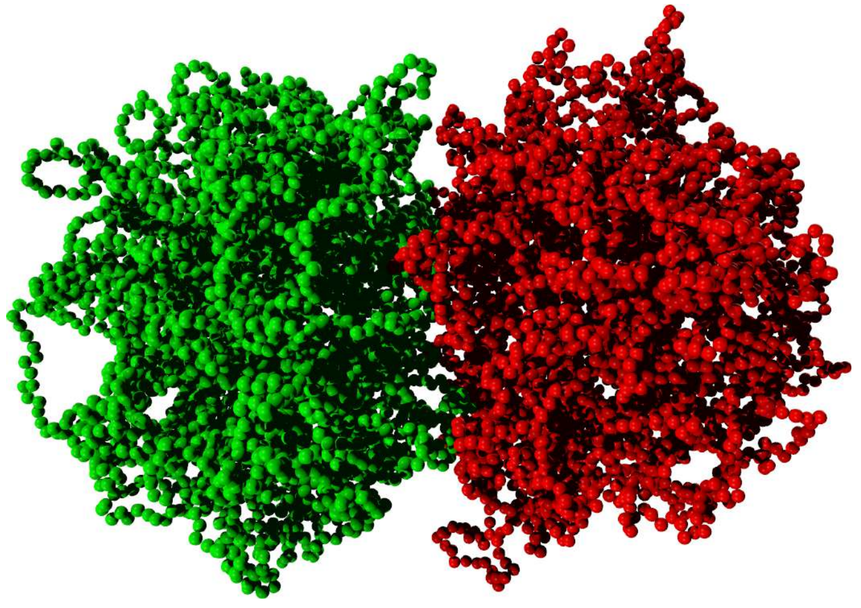
All-DNA materials



Patchy particles



Polymeric objects (microgels)



This is not a scientific talk at a conference, it is a lesson

Please do interrupt me to ask questions

SELF-ASSEMBLY AND PHASE SEPARATION IN THE CELL

—

OR

All the arguments you studied in a single place![†]

[†]"all" may be an exaggeration, but you get the point

Lorenzo Rovigatti

Physics Department, Sapienza University of Rome

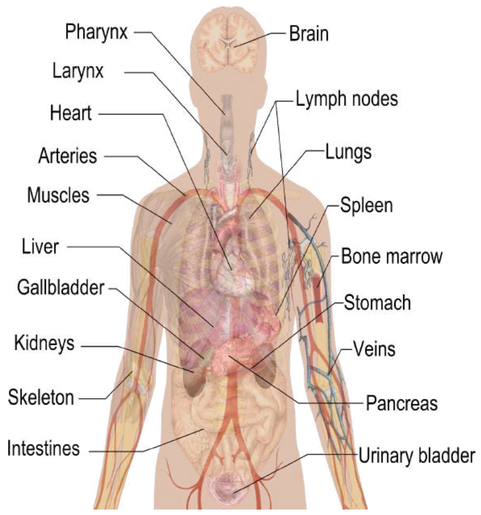
Invited lecture for the Soft and Biological Matter course, January 14th 2021

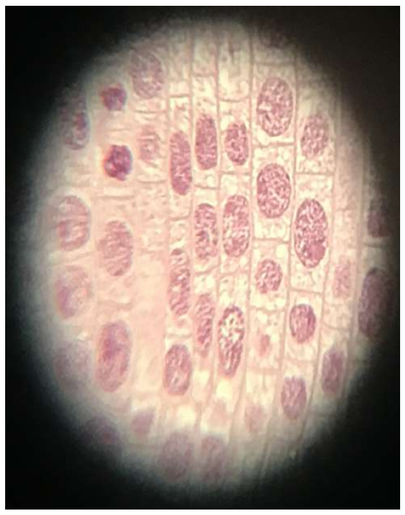
A physicist take on biology†

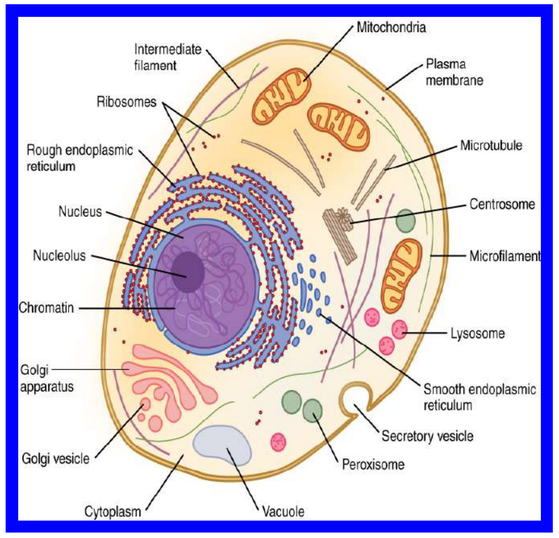
It's a matter of organisation all the way down

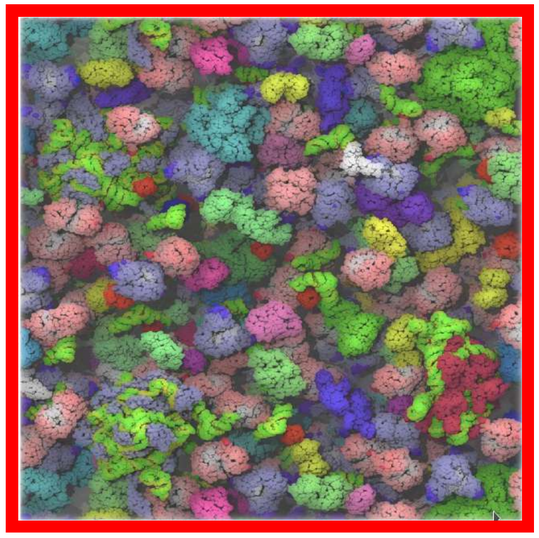
Larger → Smaller

1 m	$\approx 10\,\mu$m	$\approx 1\,\mu$m	≈ 10 nm





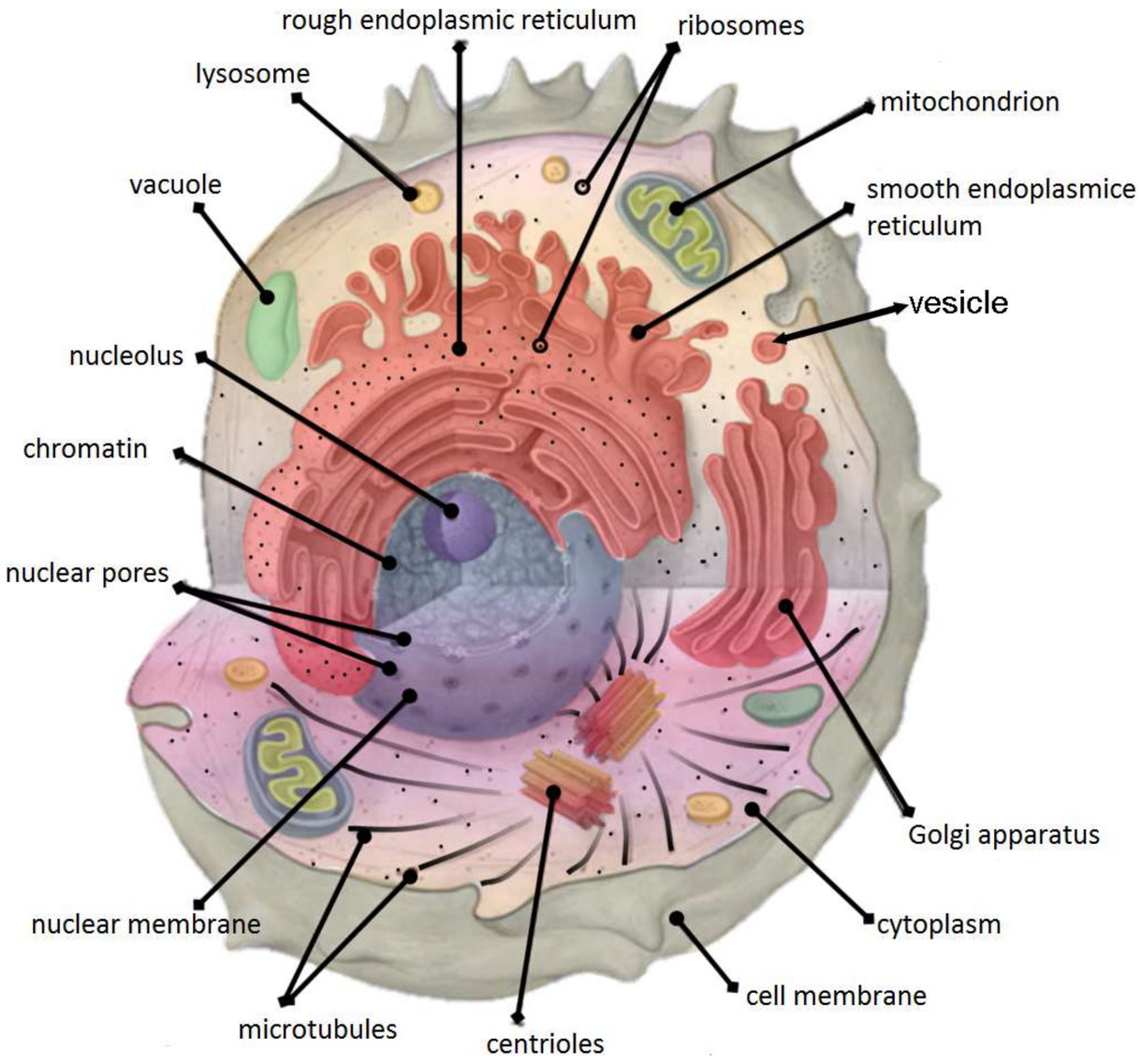




McGuffee and Elcock, *PLoS Comput. Biol.* 2010

†I apologise for this gross oversemplification

Organelles



- Perform specialised tasks
- Well-separated from the rest of the cell
- Membrane-bound
- Maintain their "identity" over time
- Communicate through molecular signals

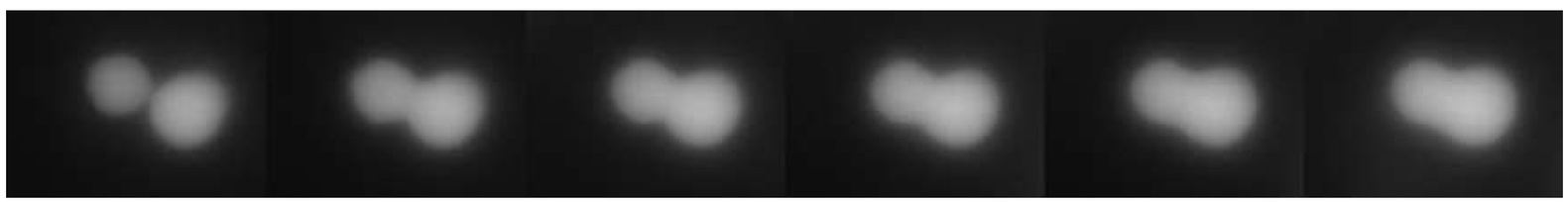
More organelles?

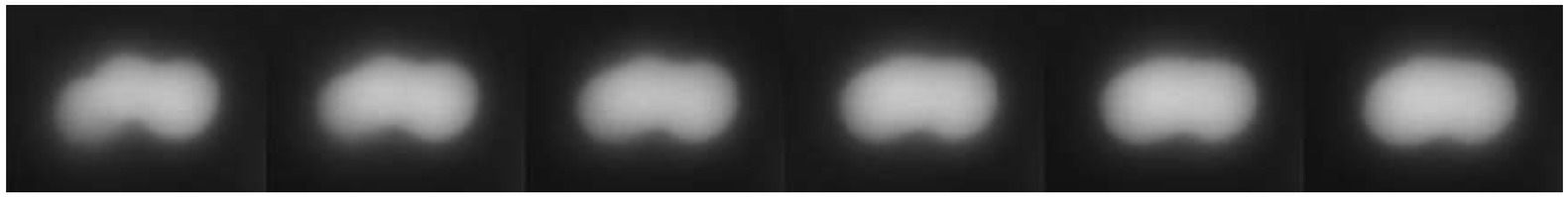
REPORT

Germline P Granules Are Liquid Droplets That Localize by Controlled Dissolution/Condensation

Science 26 Jun 2009:
Vol. 324, Issue 5935, pp. 1729-1732
DOI: 10.1126/science.1172046

Clifford P. Brangwynne[1,2,3], Christian R. Eckmann[1], David S. Courson[3], Agata Rybarska[1], Carsten Hoege[1], Jöbin Gharakhani[2]...





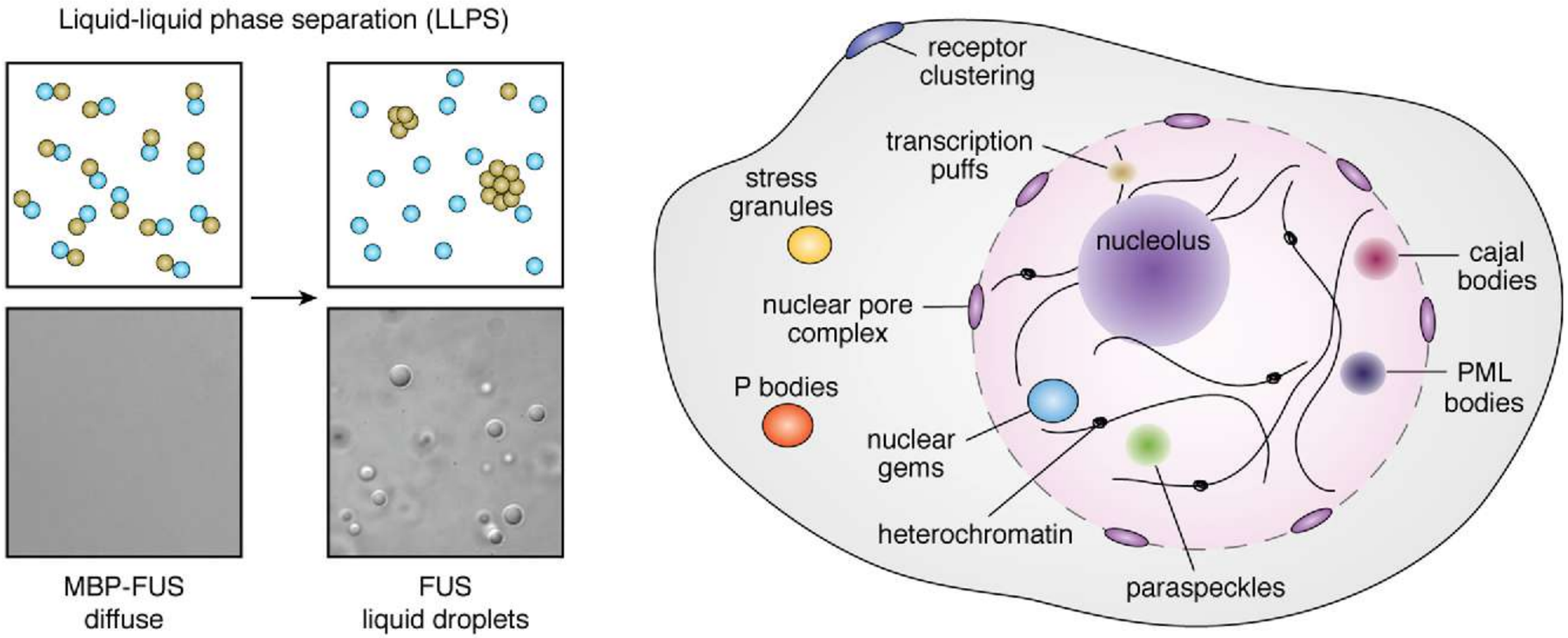
Droplets $\rightarrow$ phase separation

What kind of phase separation?

A (gas-like) highly-diluted phase coexist with a (liquid-like) dense phase

Protein liquid-liquid phase separation (LLPS) = colloidal gas-liquid phase separation

Membraneless organelles



E. Gomes and J. Shorter, *J. Mol. Bio.* 2018

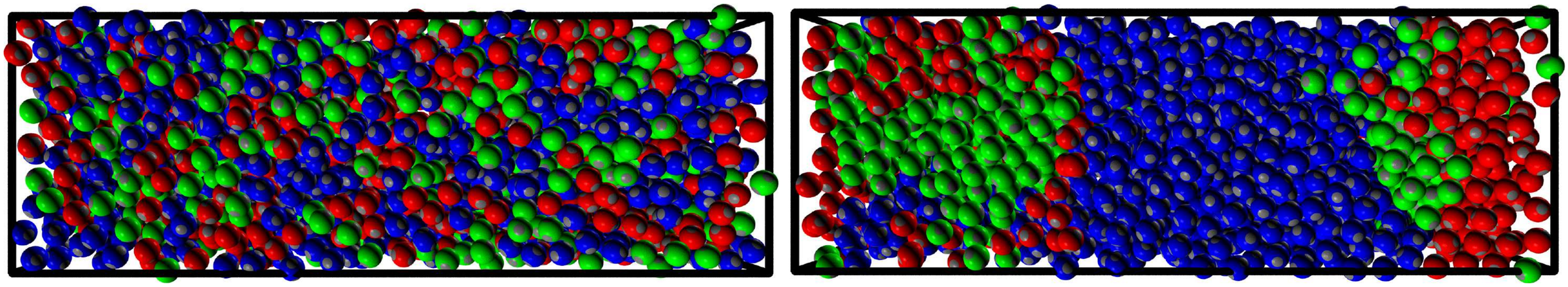
- Also known as biomolecular condensates
- Liquid-like (they can and do flow, cfr. Brangwynne *et al*)
- Made of multivalent (intrinsically-disordered) proteins and/or RNA
- Act as reservoirs of biomolecules or as microreactors
- The mechanisms behind their formation are linked to the pathogenesis of several diseases (*e.g.* Alzheimer's, ALS)

On-the-fly compartmentalisation

- Phase separation relies on a subtle interplay between entropy and enthalpy
- Slightly changing some conditions can suppress/enhance phase separation
- Condensates can quickly adapt to environmental changes!

A visual example

- We take a multi-component mixture $\rightarrow$ we need to set many different interactions (red-blue, red-green, green-blue, *etc.*)
- For this particular choice the resulting system is homogeneous
- We change **a single** interaction $\rightarrow$ phase separation



LLPS in proteins

- Abnormal LLPS is connected to many diseases
  - Alzheimer's, Huntington's disease, ALS, frontotemporal dementia
- LLPS seems to be involved in transcriptional control
  - It helps controlling when, how and in what quantity proteins are synthesised
- Some types of condensates act as scaffolds that concentrate other molecules
  - LLPS is used in the cell to generate "micro-reactors"
- Mechanical properties affect the morphology and localisation of the condensates
  - Condensates are influenced by how stiff/flexible is the cell's local environment
- Relative concentrations are connected to the overall biocondensate stability
  - the thermodynamics can become **really** complicated

Some motivating problems

- The interior of the cell contains thousands of different macromolecules
- Evolutionary pressure optimises mutual interactions in the proteome
- *In vivo* and *in vitro* experiments don't always match (Jain and Vale, *Nature* 2017)

More motivation?

Letter to the Editor | Published: 08 September 2020

Liquid–liquid phase separation by SARS-CoV-2 nucleocapsid protein and RNA

Hui Chen, Yang Cui, Xuling Han, Wei Hu, Min Sun, Yong Zhang, Pei-Hui Wang, Guangtao Song, Wei Chen ✉ & Jizhong Lou ✉

Cell Research **30**, 1143–1145(2020) | Cite this article

Article | Open Access | Published: 27 November 2020

Nucleocapsid protein of SARS-CoV-2 phase separates into RNA-rich polymerase-containing condensates

Adriana Savastano, Alain Ibáñez de Opakua, Marija Rankovic & Markus Zweckstetter ✉

Nature Communications **11**, Article number: 6041 (2020) | Cite this article

Article | 4 December 2020 | OPEN ACCESS | SOURCE DATA | TRANSPARENT PROCESS

SARS-CoV-2 nucleocapsid protein phase-separates with RNA and with human hnRNPs

Theodora Myrto Perdikari, Anastasia C Murthy, Veronica H Ryan, Scott Watters, Mandar T Naik, Nicolas L Fawzi ✉

Author Information

EMBO J (2020) 39: e106478 | https://doi.org/10.15252/embj.2020106478

Where is the physics?

EVERYWHERE, AT EVERY LEVEL

- Everything is hydrated $\rightarrow$ thermodynamics of water
- Proteins and nucleic acids are polymers $\rightarrow$ polymer physics (elasticity, *etc.*)
- Proteins and nucleic acids bind reversibly $\rightarrow$ self-assembly, gels
- Cells are **very** crowded $\rightarrow$ entropic effects (depletion, nematic transition, *etc.*)
- The final boss: living organisms are, by definition, out of equilibrium

We need simple[†] systems and models we can play with to disentangle all these effects!

[†] in a reductionist sense

Our idea†

Convince a living organism to stochastically express artificial proteins designed to phase separate

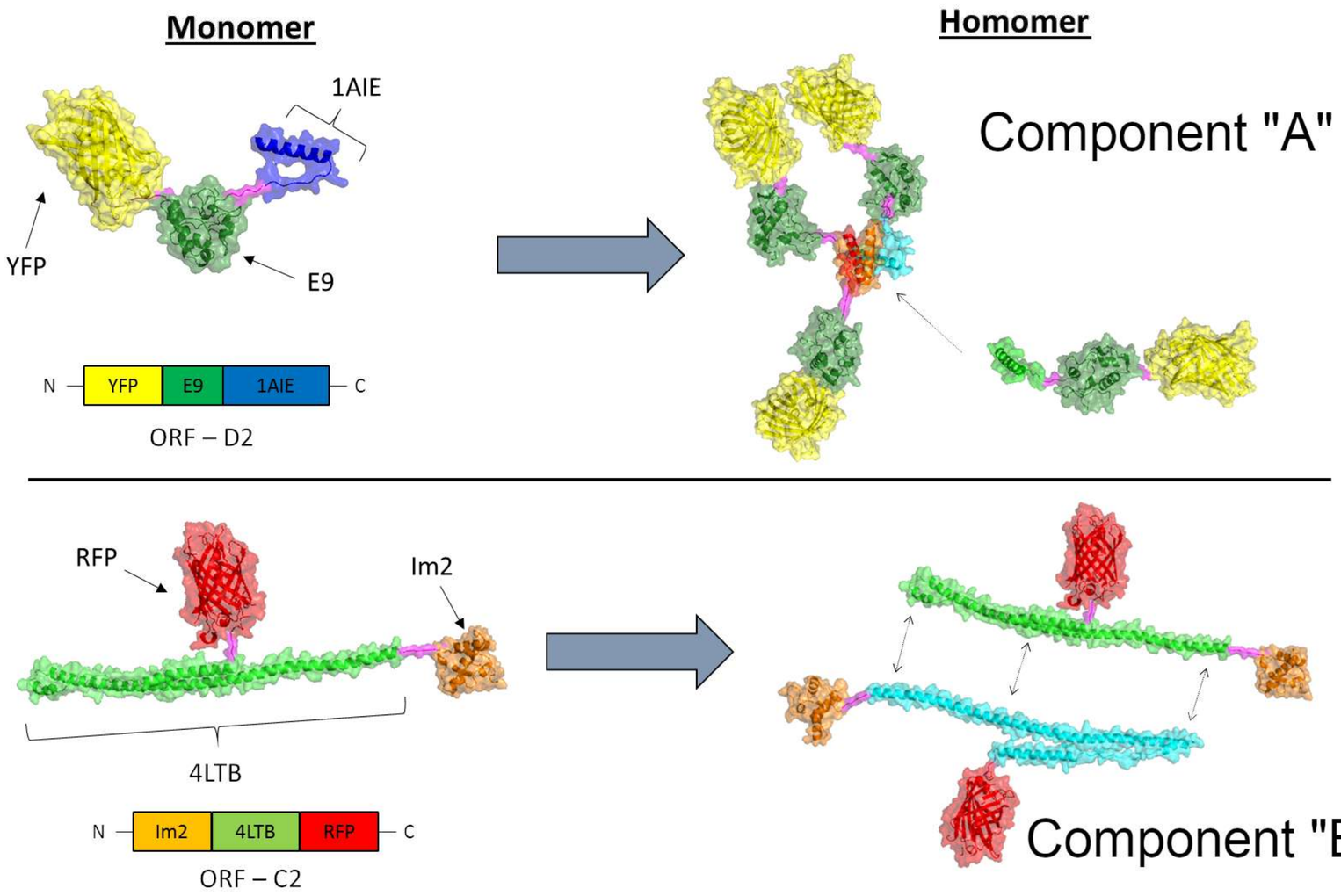
The artificial proteins (expressed in a yeast strain through plasmids) should

- be extraneous to the cell
- bond through a lock-and-key (L&K) mechanism
- have a tunable bonding strength (*i.e.* a tunable affinity)
- be multivalent (*e.g.* can bind to multiple partners)

The goal: build a synthetic toolkit to study LLPS *in vivo*

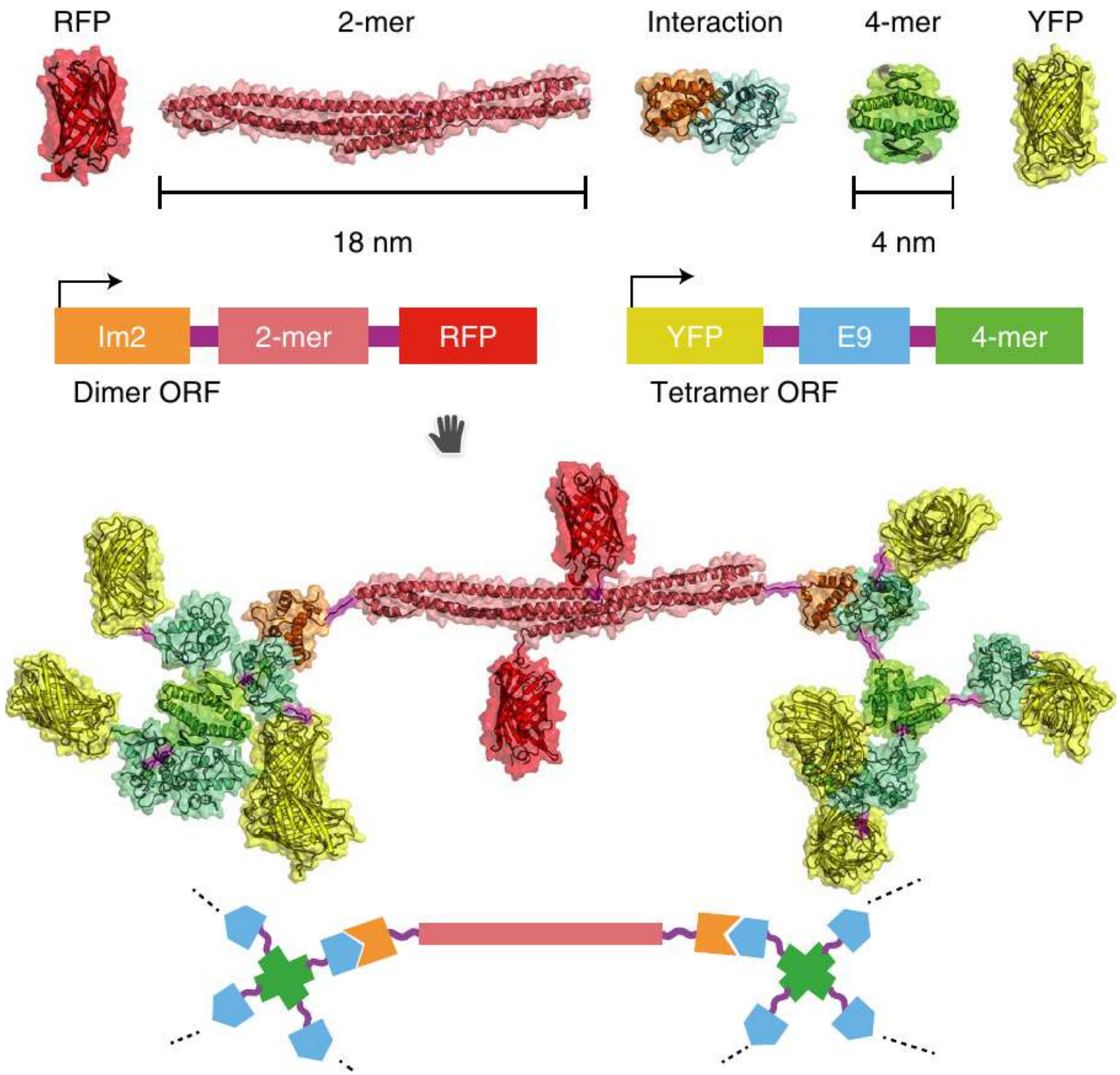
† Emmanuel Levy's ingenious idea, to be really honest

The building blocks

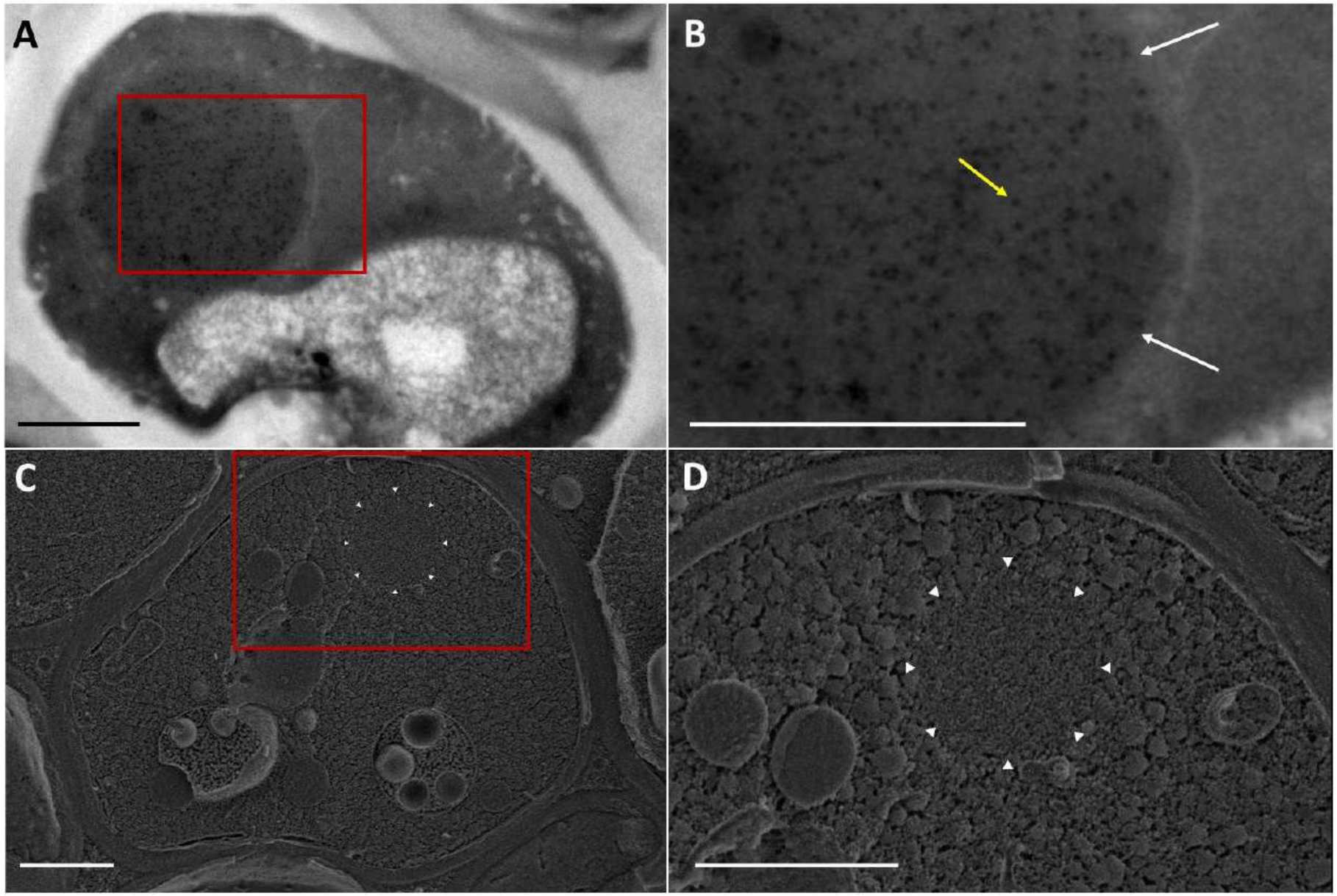


- Two components (A and B) that can form up to 4 and 2 bonds, respectively
- Their size is chosen so that multiple bonding is unlikely (if not impossible)
- The L&K attraction strength can be tuned with point mutations

A closer look



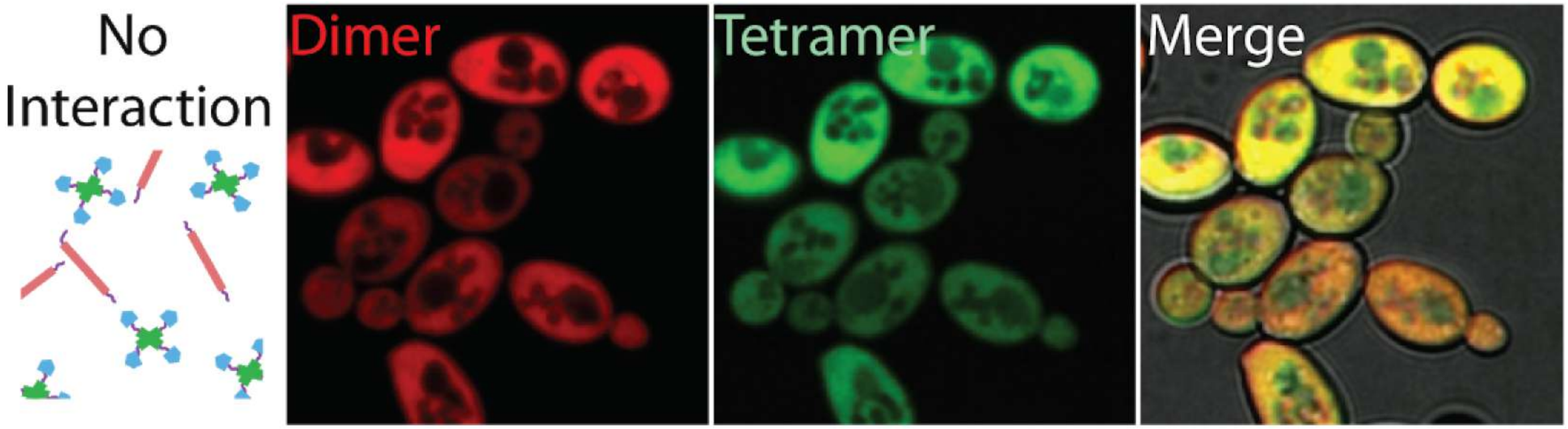
Do they really form condensates?



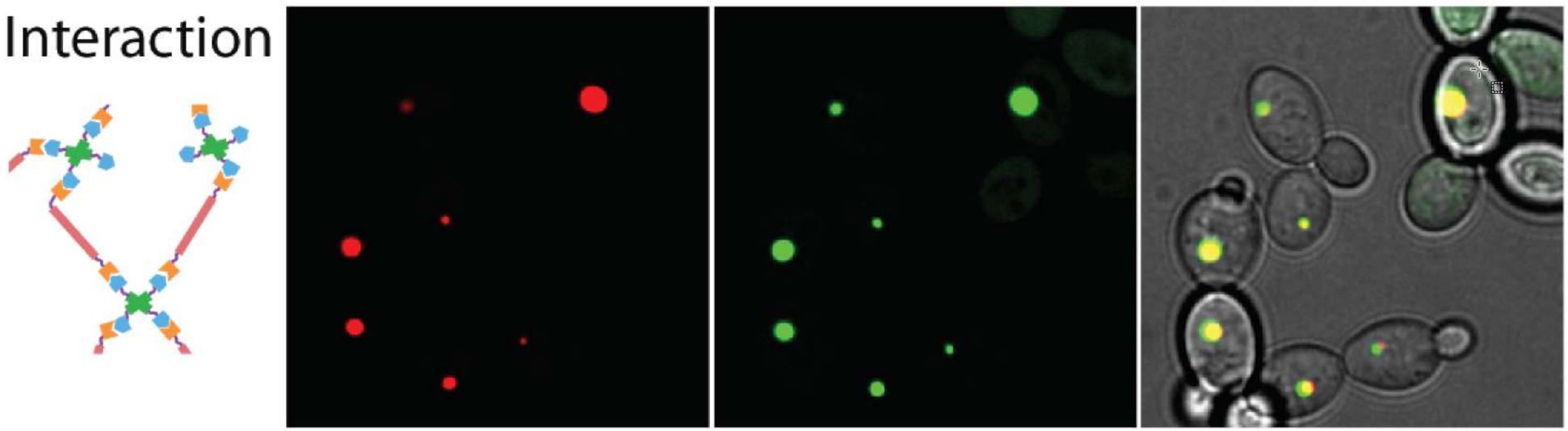
Transmission & scanning electron micoscropy images

A preliminary check

Control sample: no phase separation if the L&K attraction is disabled

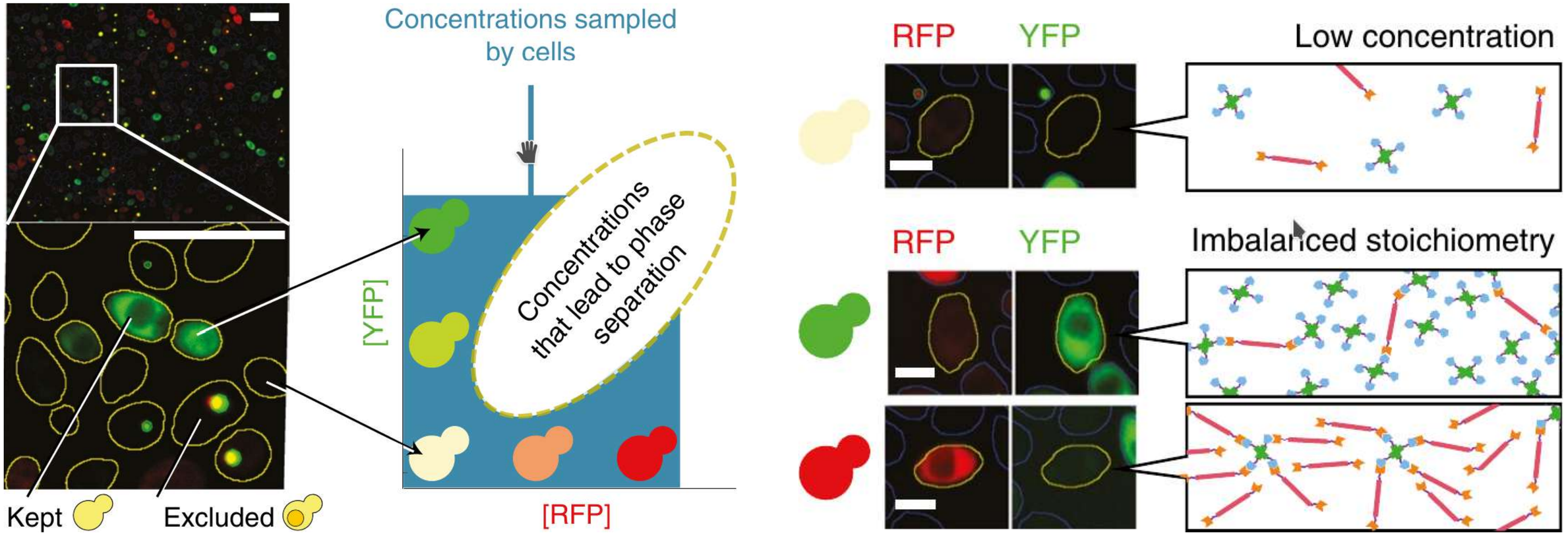


L&K enabled: the components co-localise and phase separate!



Quantifying the phase behaviour

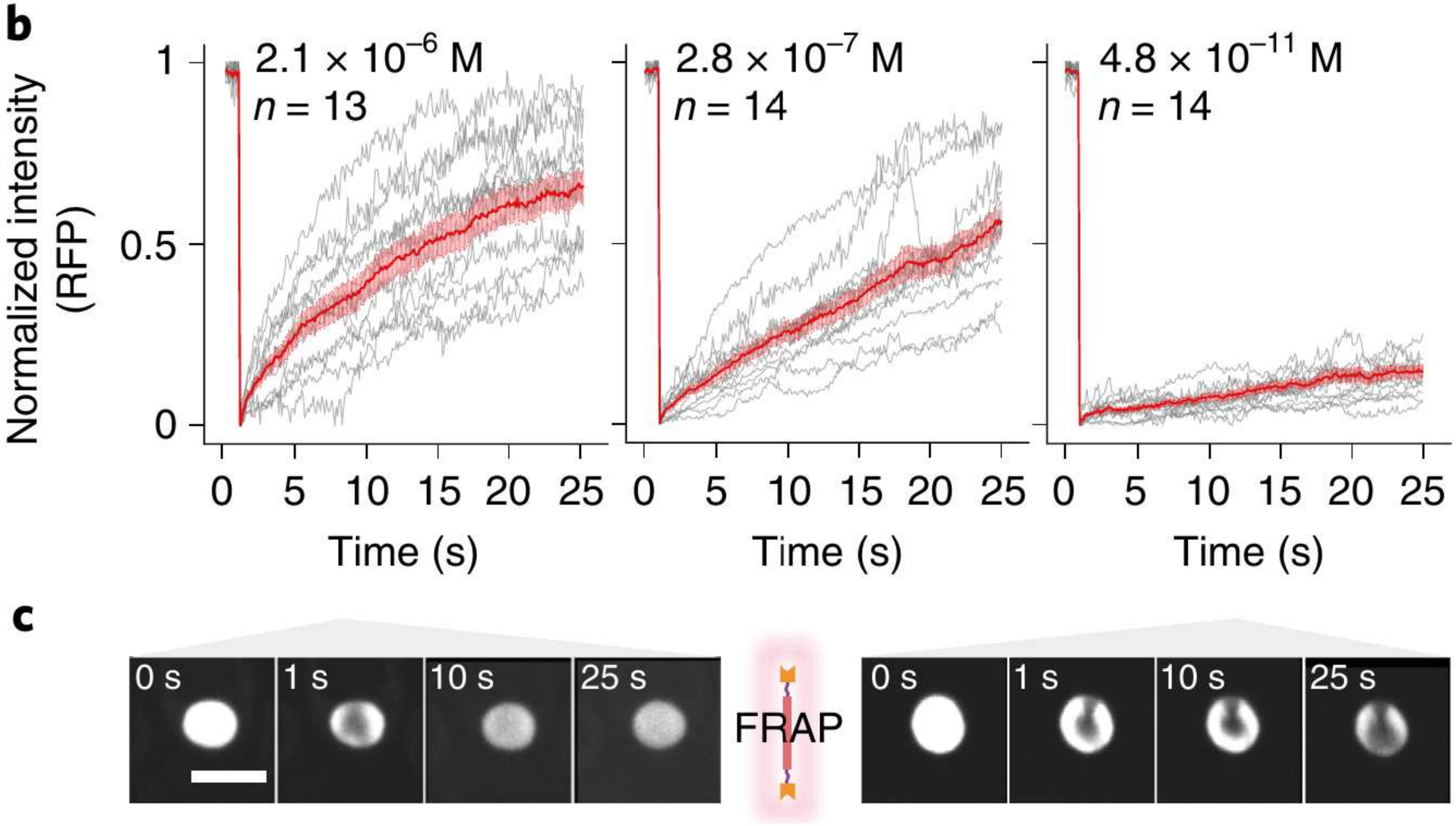
We draw a *negative* of the low-ρ part of the phase diagram!



NB: the high-concentration part of the phase diagram cannot be accessed

Investigating the dynamics

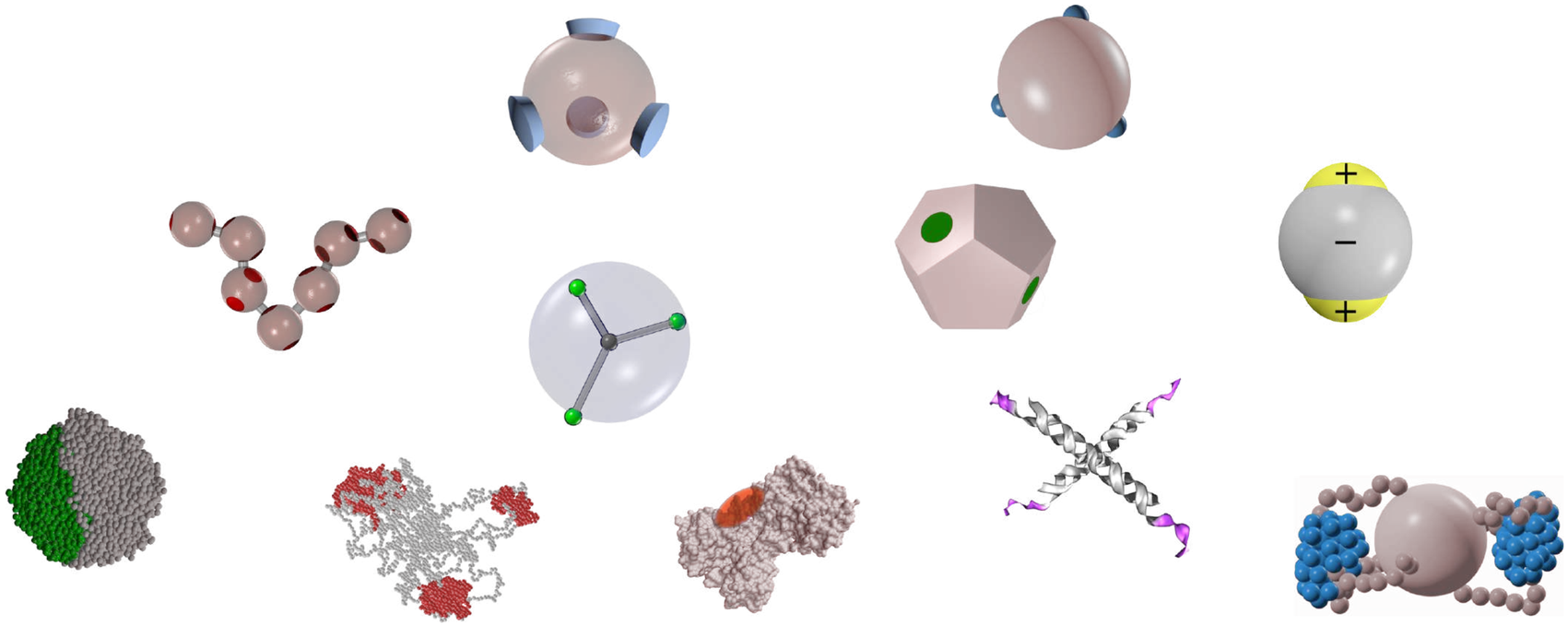
FRAP: fluorescence recovery after photobleaching



A simple (but not too simple) model

The main ingredients

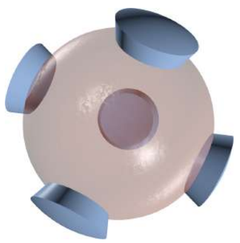
- No water $\rightarrow$ implicit solvent, effective interactions
- No (or few) internal degrees of freedom $\rightarrow$ spheres or quasi-rigid bodies
- Multivalency (a.k.a. limited valence in the colloidal field)
- A lock-and-key (L&K) mechanism (a.k.a. bond specificity)

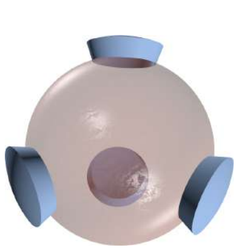


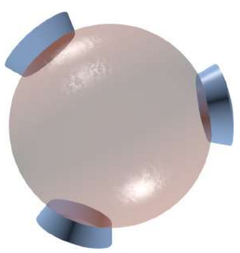
Bianchi *et al., Phys. Chem. Chem. Phys.* 2017

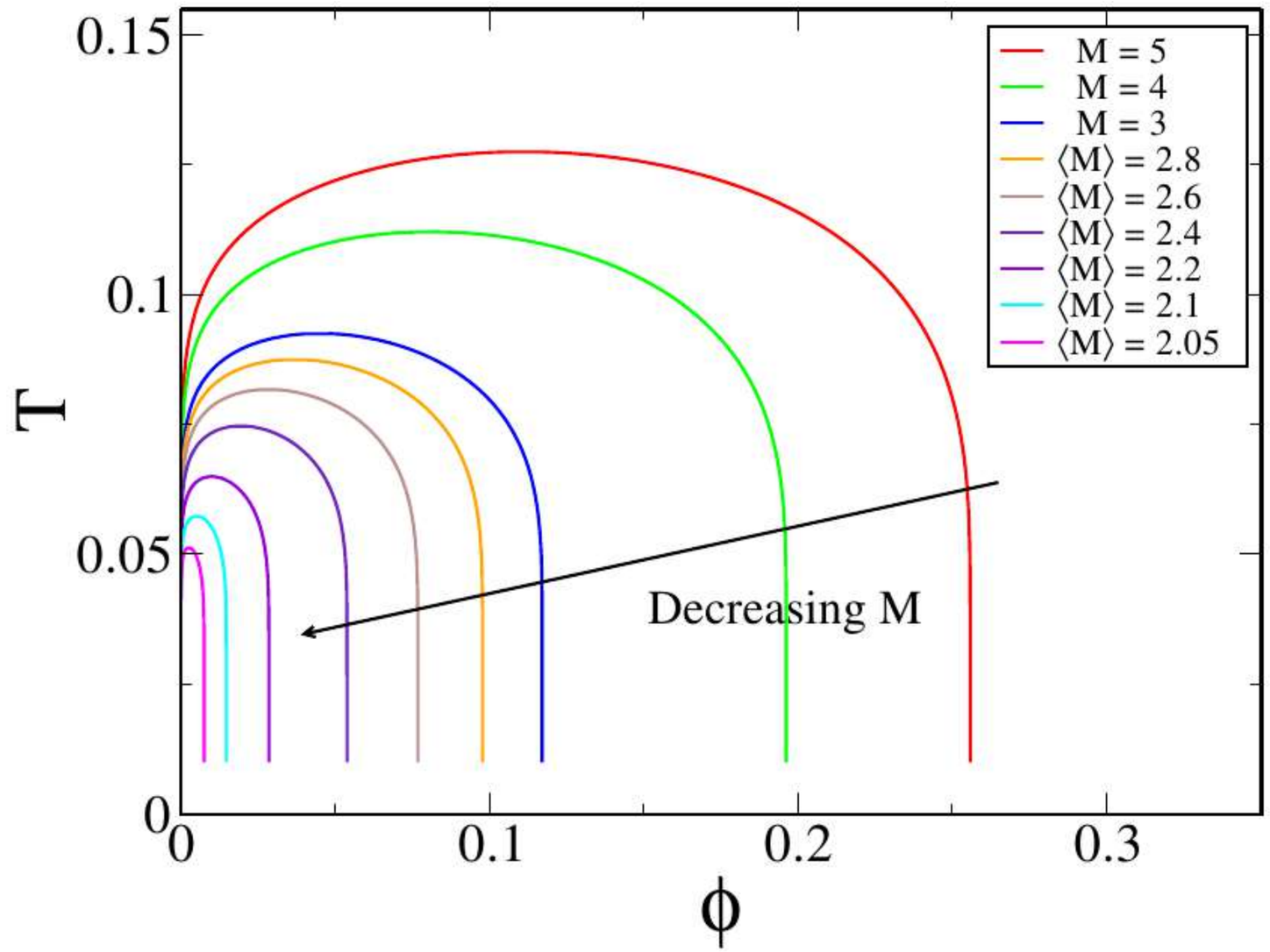
Simple models $\rightarrow$ complex behaviour

Limited valence





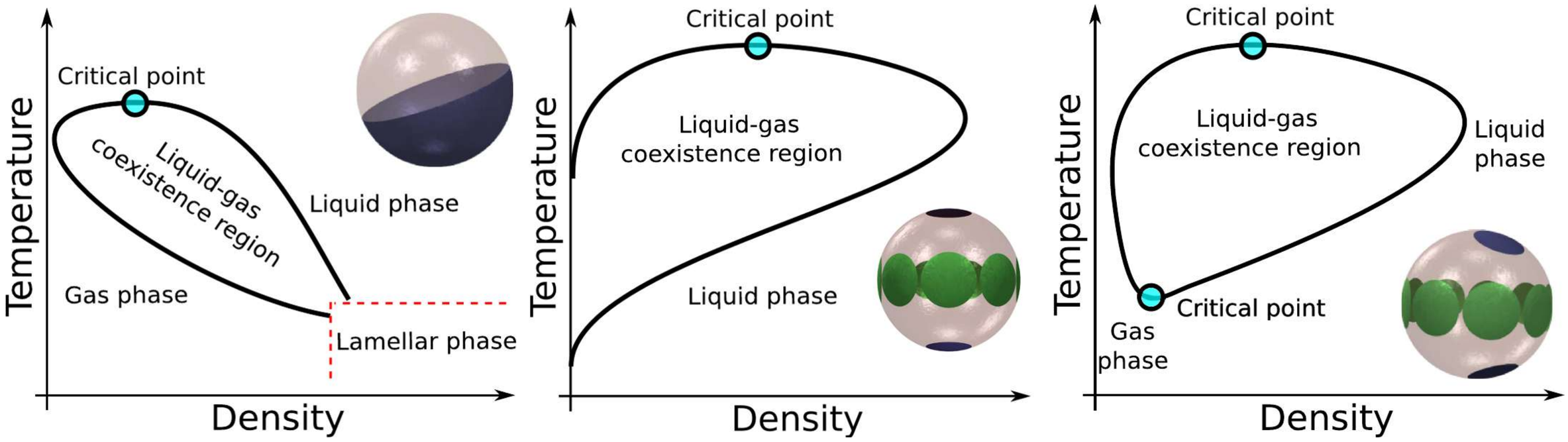




E. Bianchi, J. Largo, P. Tartaglia, E. Zaccarelli and F. Sciortino, *Phys. Rev. Lett.* (2006)

Simple models $\rightarrow$ complex behaviour

Self-assembly *vs.* phase separation



F. Sciortino, A. Giacometti and G. Pastore, *Phys. Rev. Lett.* (2009)

J. Russo, J. M. Tavares, P. I. C. Teixeira, M. M. Telo da Gama and F. Sciortino, *Phys. Rev. Lett.* (2011)

L. Rovigatti, J. M. Tavares and F. Sciortino, *Phys. Rev. Lett.* (2013)

The theory

What you know: the F per particle of a self-assembling ideal gas of valence M

$$\beta f \equiv \frac{\beta F}{N} = \beta f_{\mathrm{id}} + M \ln(1 - p_b) + \frac{M p_b}{2}$$

It can be generalised to the case where the reference system is not an ideal gas

$$\beta f = \frac{\beta F_{\mathrm{ref}}}{N} + \frac{\beta F_{\mathrm{bond}}}{N} = \beta f_{\mathrm{ref}} + \beta f_{\mathrm{bond}}$$

A few definitions when we deal with L&K binary mixtures

- x is the composition (fraction of A particles, for instance)
- M_A and M_B is the number of sites of species A and B
- X_A and X_B is the probability that a A or B site is unbonded
- B_2^{AA}, B_2^{AB}, B_2^{BB} are the second virial coefficients between the species

The theory

The two terms of the free energy now become

$$\beta f_{\text{ref}} = \beta f_{\text{id}} + x\ln(x) + (1-x)\ln(1-x) + \beta f_{\text{ex}}$$

$$\beta f_{\text{bond}} = x\left(M_A \ln(X_A) - \frac{M_A X_A}{2} + \frac{M_A}{2}\right) + (1-x)\left(M_B \ln(X_B) - \frac{M_B X_B}{2} + \frac{M_B}{2}\right)$$

where βf_{ex} is the *excess* free energy (*e.g.* the hard-sphere excess free energy).

In the case of soft systems (ρ is the overall density)

$$\beta f_{\text{ex}} \approx \rho\left(x^2 B_2^{AA} + 2x(1-x)B_2^{AB} + (1-x)^2 B_2^{BB}\right)$$

Free energy $\longrightarrow$ whole phase diagram

Connecting theory and simulations

The second virial coefficients can be estimated as

$$B_{ij} = -\frac{1}{2}\int_0^\infty 4\pi r^2 \left(e^{-\beta V_{ij}(r)} - 1\right) dr$$

where $V_{ij}(r)$ is the reference interaction potential between species i and j

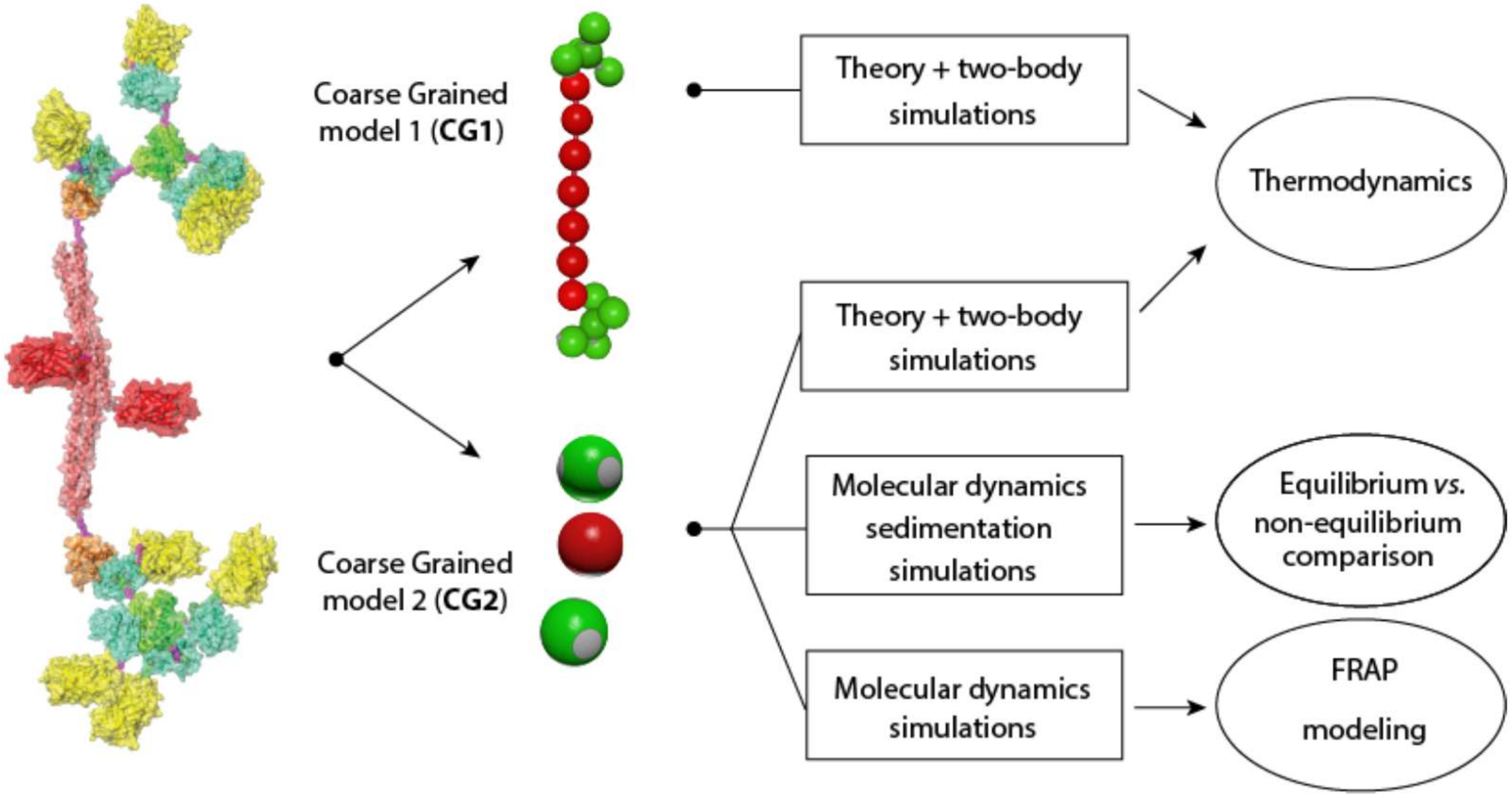
X_A and X_B are connected to the bonding free energy Δ

$$\Delta = 4\pi \int_0^\infty g_{\mathrm{ref}}(r) \langle f_{12}(r)_{\omega_1\omega_2} \rangle r^2 dr$$

- $g_{\mathrm{ref}}(r)$ is the reference hard-sphere fluid pair correlation function
- $\langle f_{12}(r)_{\omega_1\omega_2} \rangle$ is the angle-averaged Mayer function at distance r

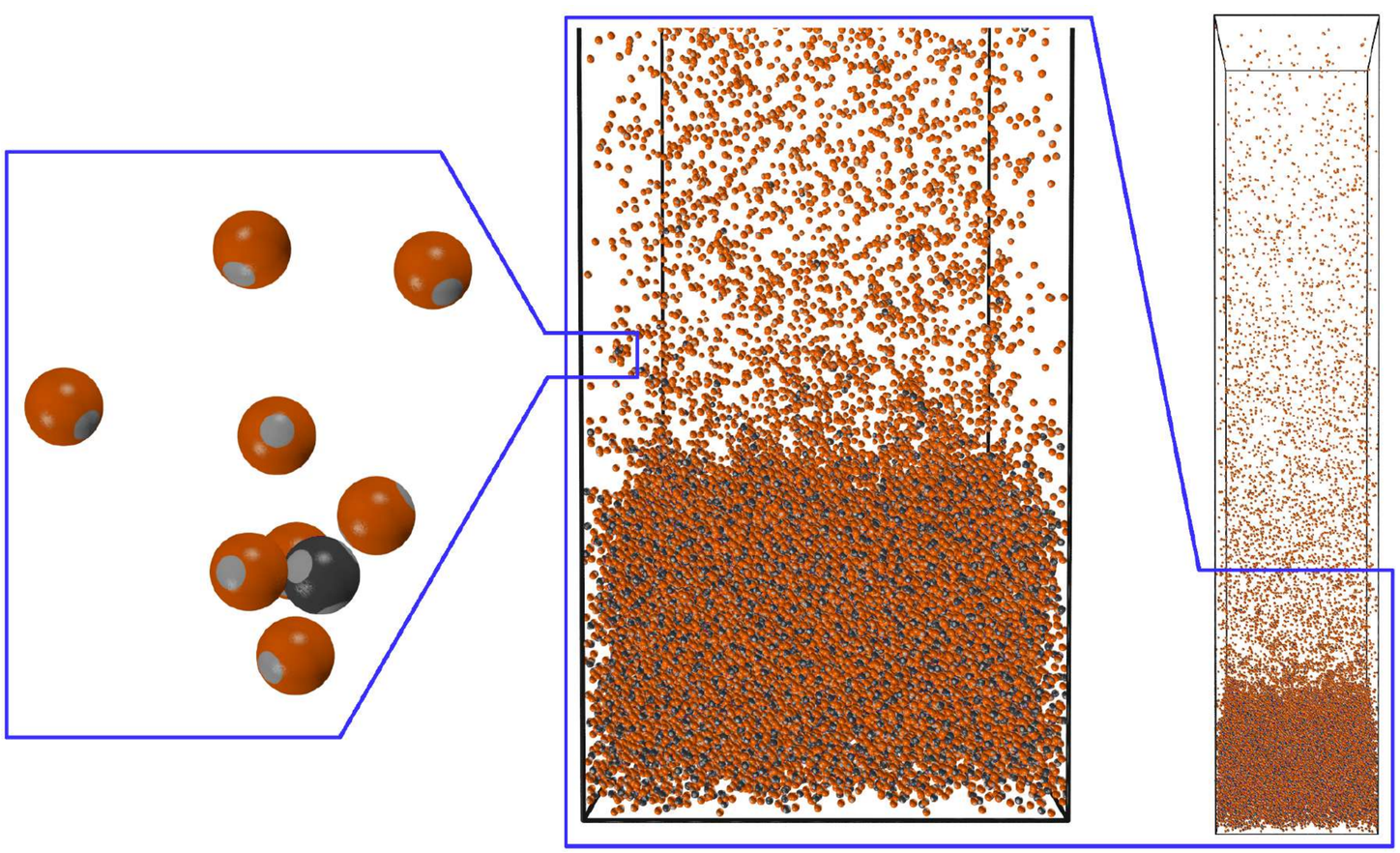
These quantities can be computed with two-body simulations

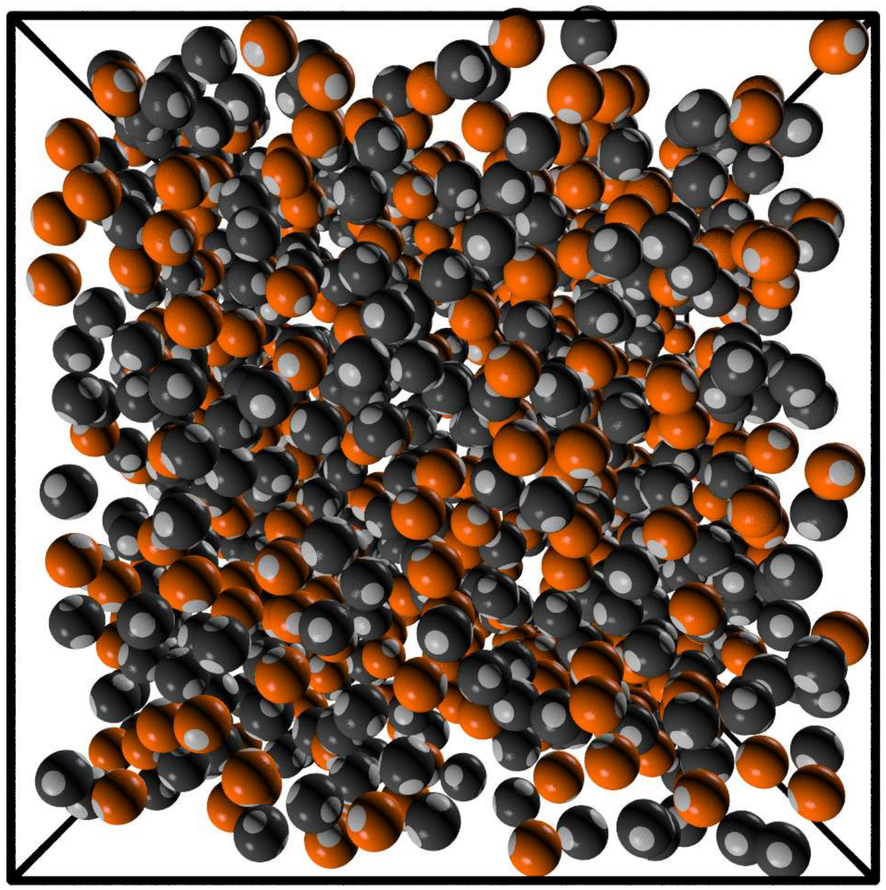
The coarse-graining strategy



Adding simulations

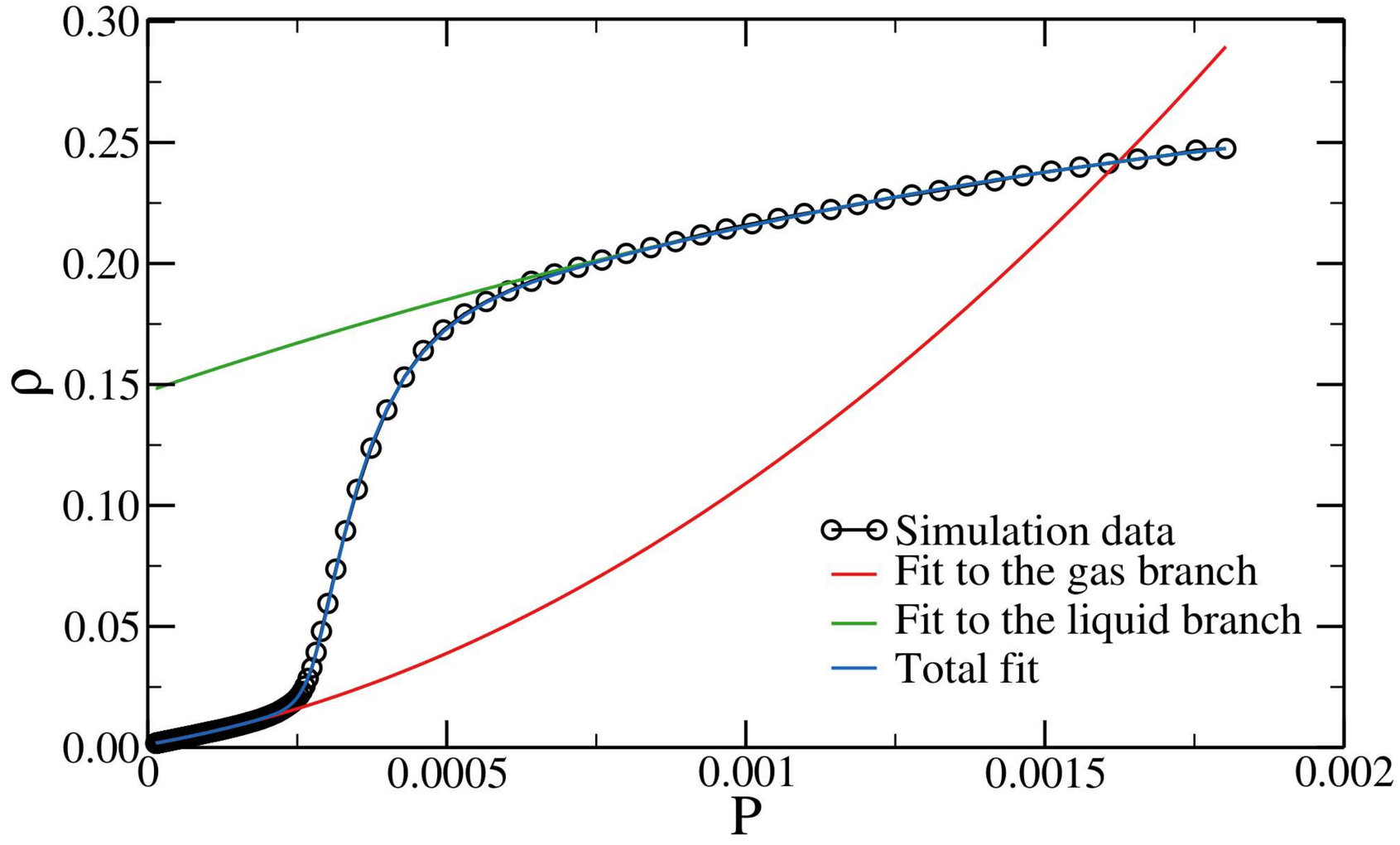
- Binary mixture of divalent and tetravalent particles, varying ρ, x, T ($\propto$ affinity^{-1})
- Sedimentation simulations for the phase diagrams in and out of equilibrium
- Constant-volume simulations to compare to dynamic data
- Explore how changing the model affects the thermodynamics

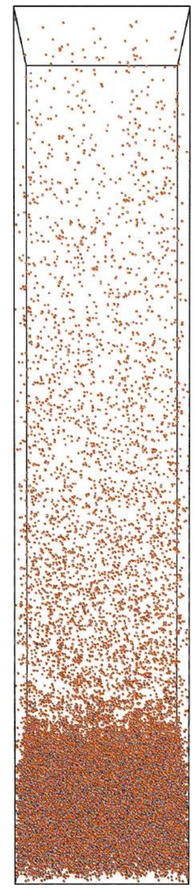




Sedimentation

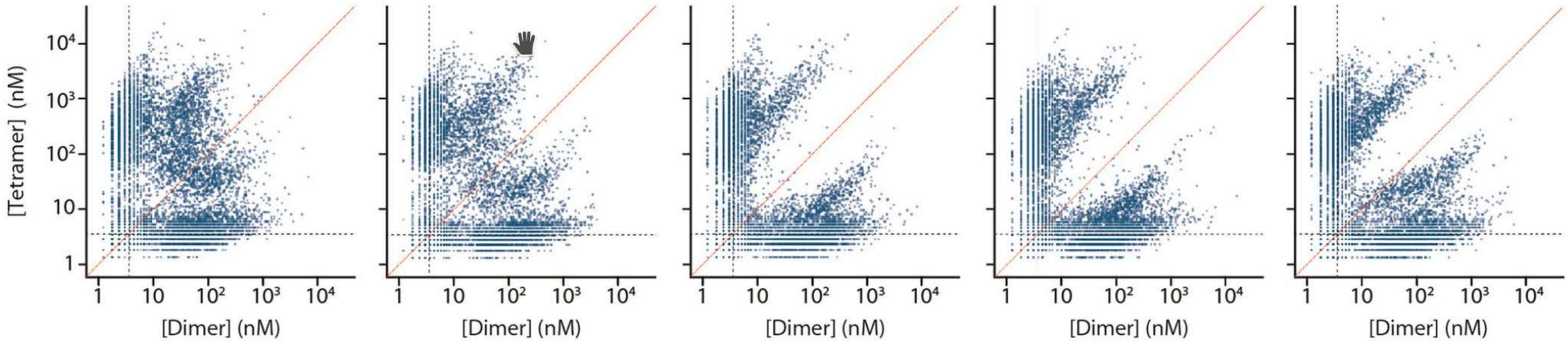
We fit the equation of state to extract the coexisting ρ_2 and ρ_4





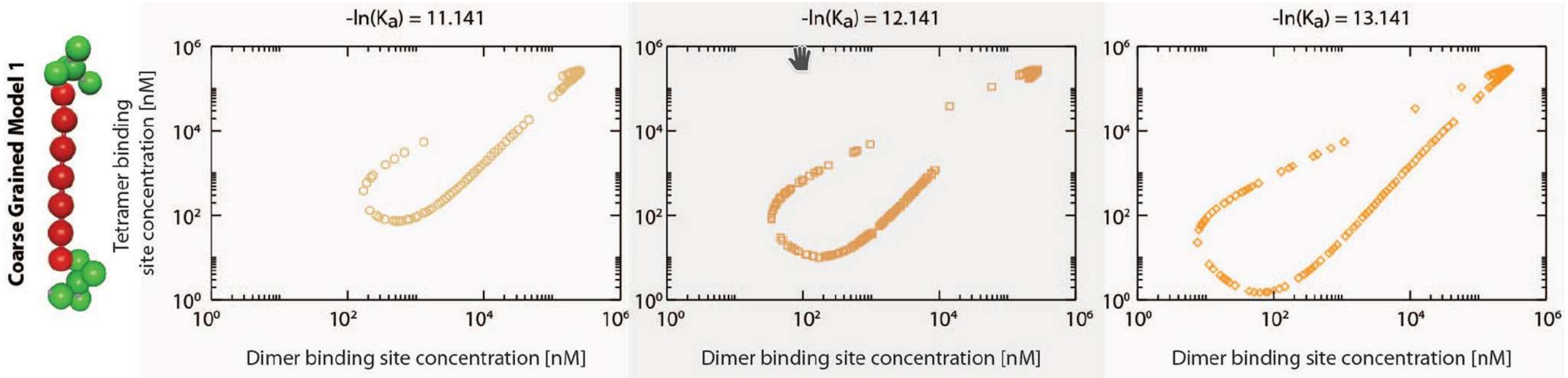
Experimental phase diagrams

Affinity (interaction strength)



- The coexistence region is ≈ symmetric with respect to stoichiometry conditions
- It enlarges as affinity (*i.e.* bond strength) increases
- Something happens at high affinities: out-of-equilibrium effects?

Theoretical phase diagrams

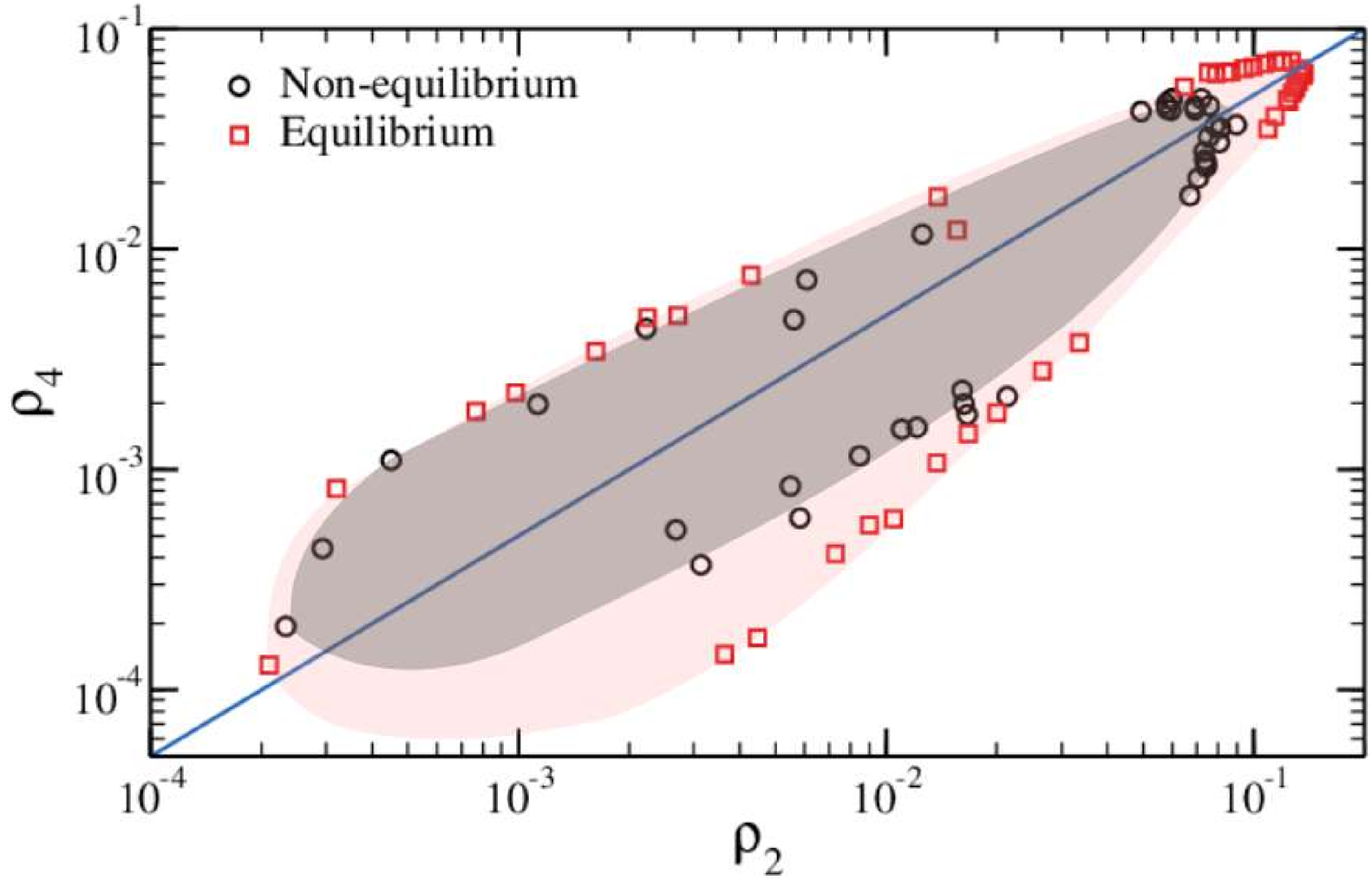


- The coexistence region is symmetric with respect to stoichiometry conditions
- It enlarges as affinity (*i.e.* bond strength) increases

We reproduce all the equilibrium qualitative trends

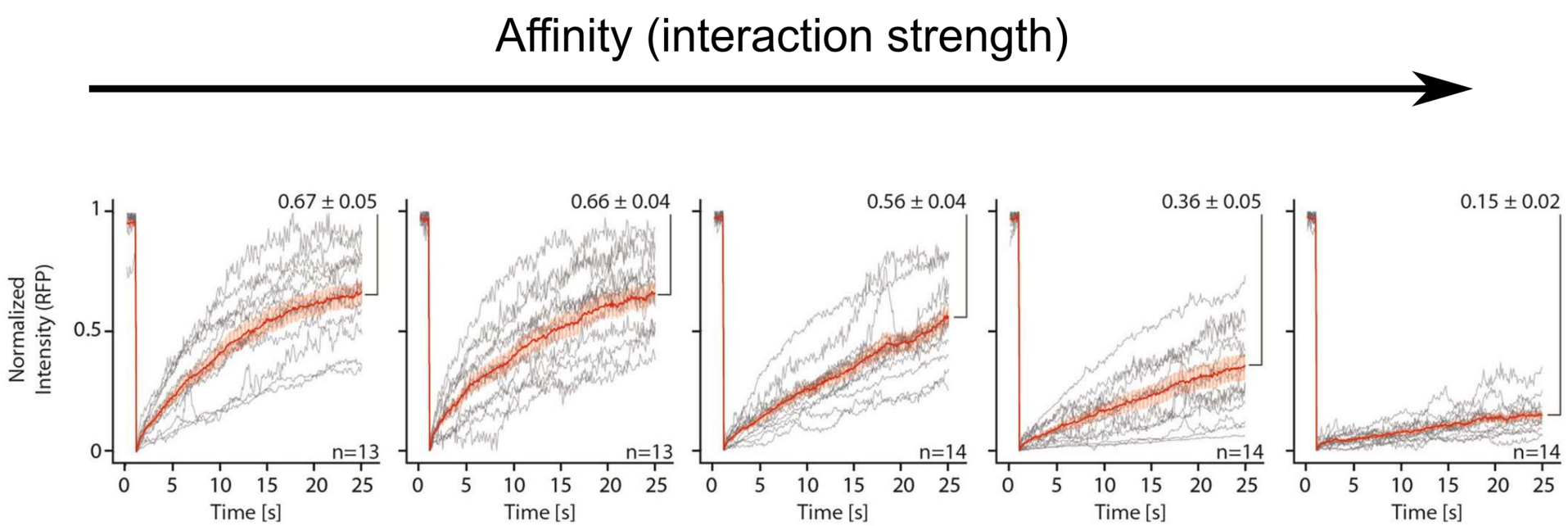
Numerical phase diagrams

Numerical phase diagrams in and out of equilibrium

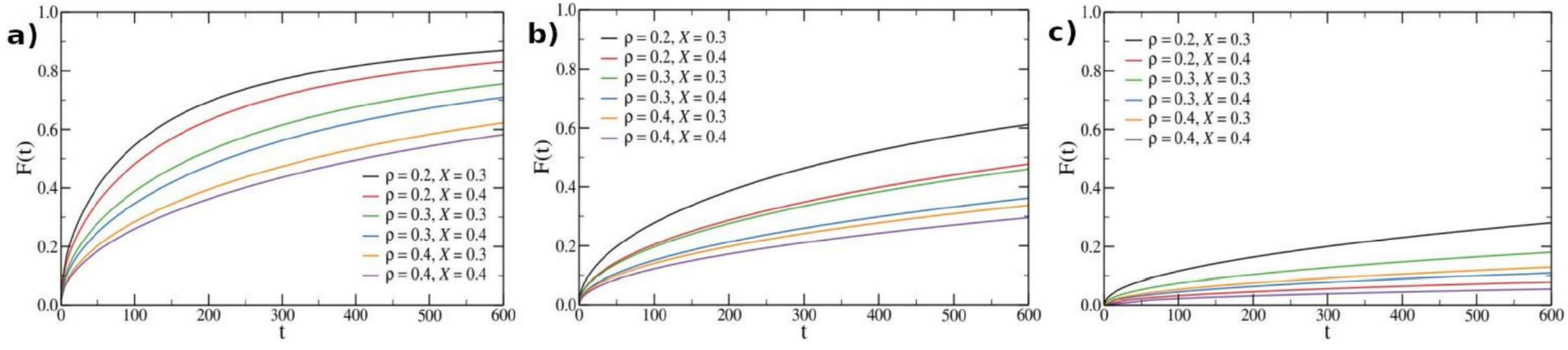


We see the same qualitative shift measured in experiments

Out-of-equilibrium effects!



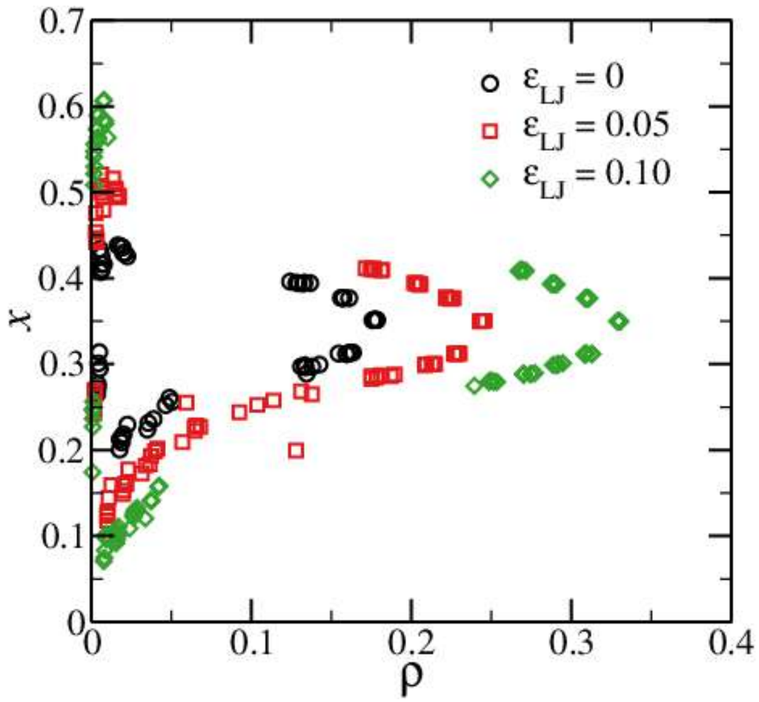
- FRAP data on single cells
- Large spread between the curves, especially at low affinity
- At large affinities there is ≈ no recovery after 25 seconds

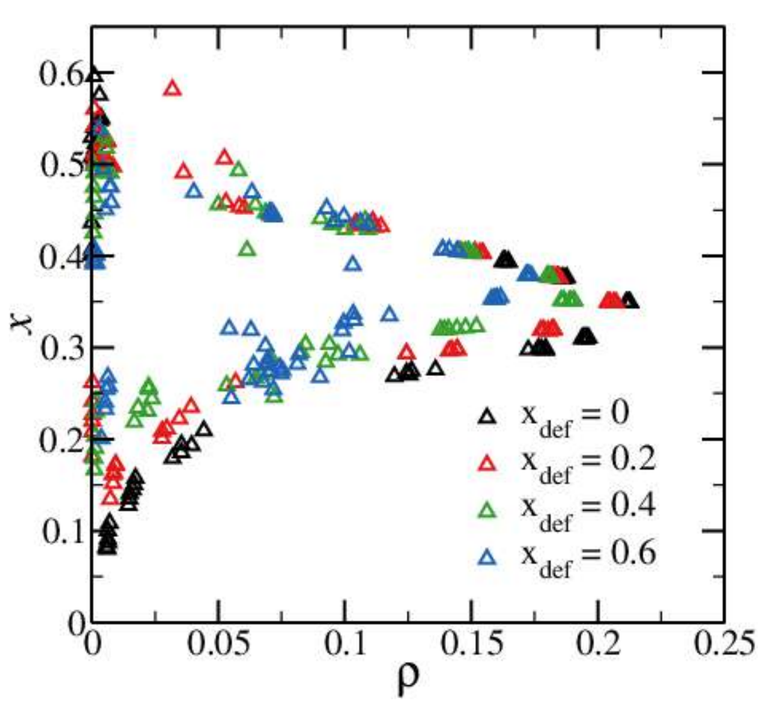


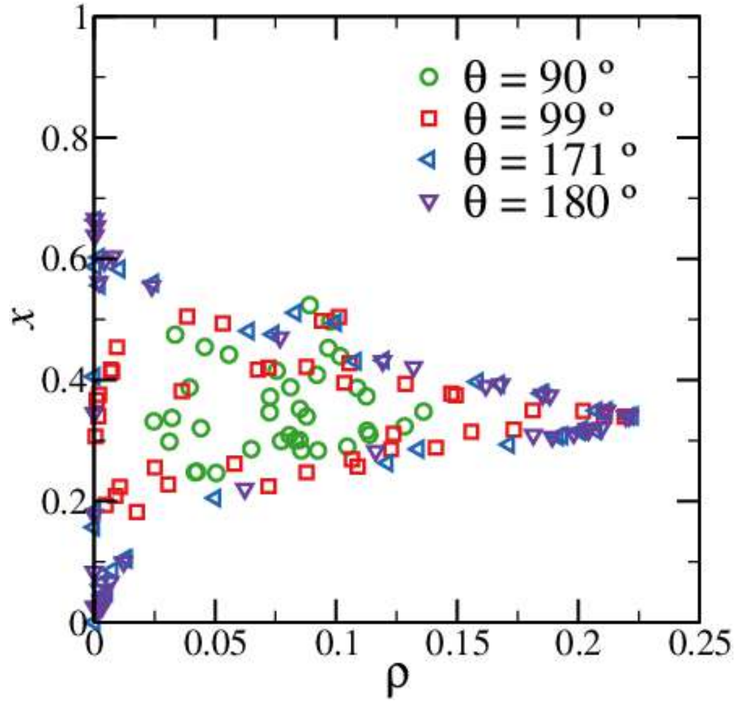
Model extensions

We can build on the model to assess the role

- of non-specific attractions
- of defects (example: some of tetramers can form only 3 bonds)
- of the flexibility/geometrical arrangements of the patches







Conclusions

- We can engineer synthetic "playground" biomolecular condensates
- The phase behaviour of the system can be directly measured
- Notwithstanding the complexity of the cell environment, experiments agree with coarse-grained simulations/theory, in and out of equilibrium
- The system can be used to test hypothesis and develop new methods (see *e.g.* Mc Laughin *et al, Mol. Biol. Cell* (2020))
- It's a starting point: we can add more ingredients!

Further questions?

- Ask me now!
- Contact me: lorenzo.rovigatti@uniroma1.it
- Read the paper: M. Heidenreich *et al.*, Nat. Chem. Biol. **16**, 939 (2020)

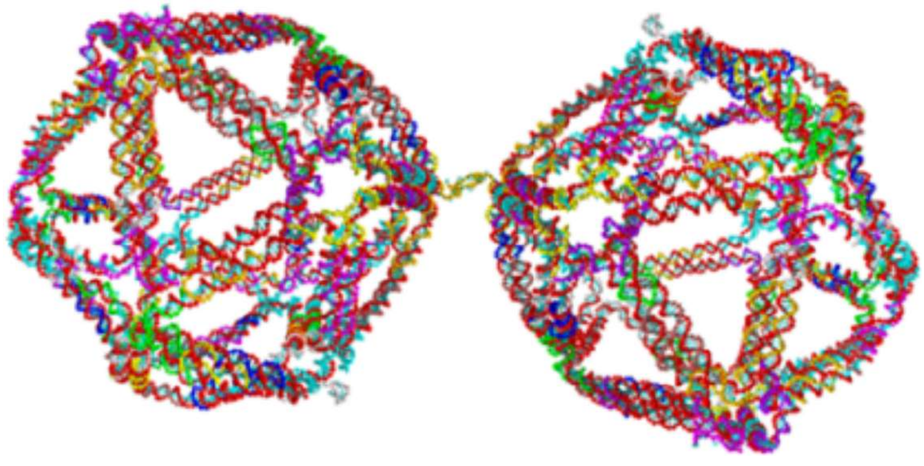
Some lines of research†

The role of polymer elasticity in the formation of condensates

- The presence of an elastic network alters the phase behaviour of liquids
- How does this relate to the formation/dissolution of molecular condensates?
- Use simulations to test (and improve!) theories and compare to experiments

Interactions between DNA nanostructures

- DNA can be used to build nano-structures, machines and devices
- We know how to predict and control the hybridisation between small strands
- What about large all-DNA nanostructures (DNA origami)?



†"Advertising"

Acknowledgements

Work done in collaboration with:

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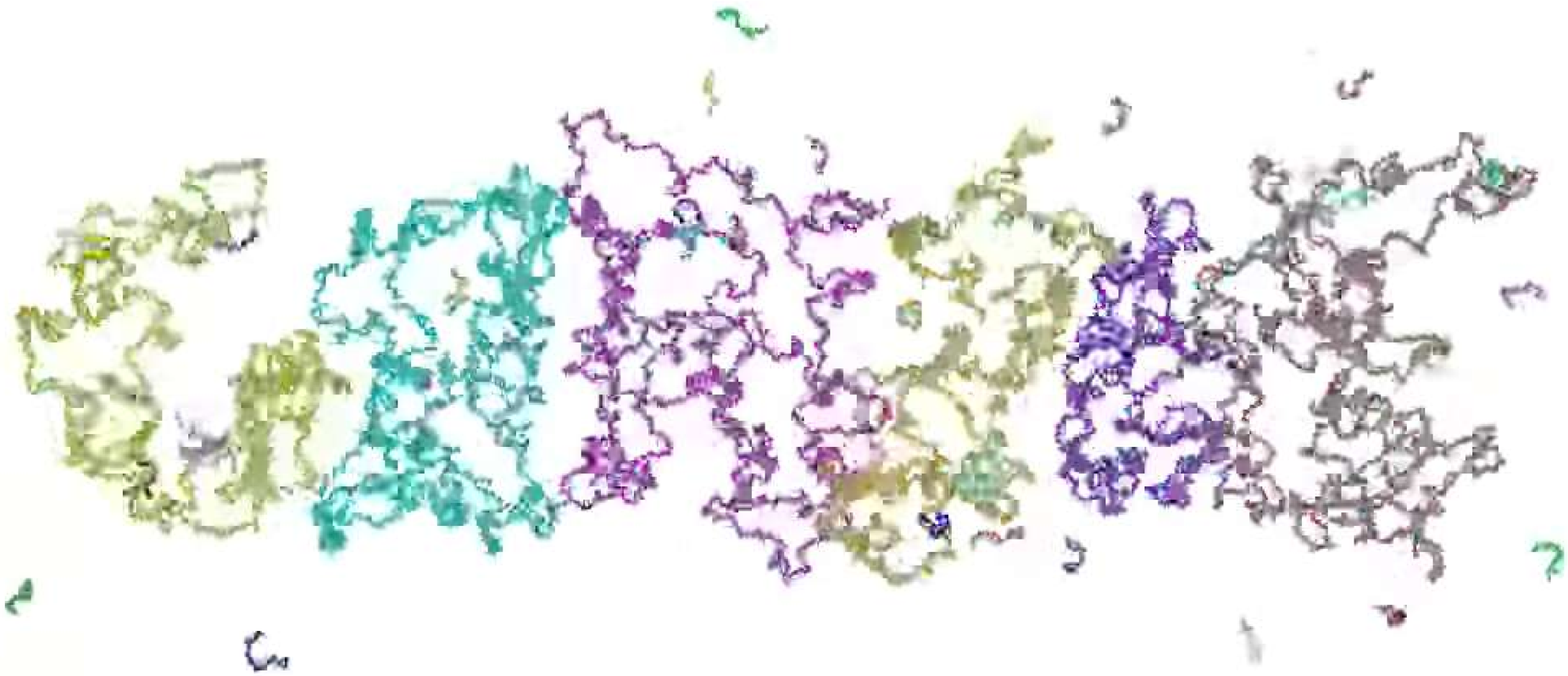
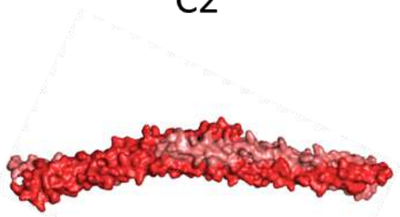
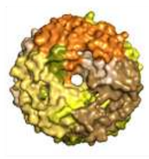
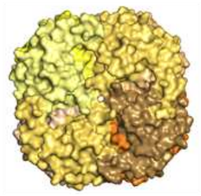
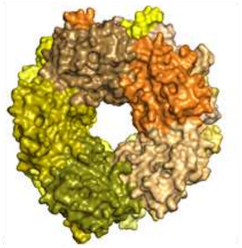
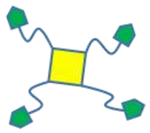
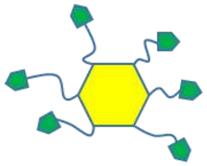
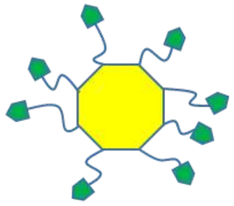
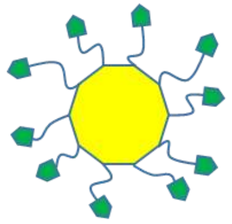
- S. A. Safran (Weizmann Institute)
- E. D. Levy (Weizmann Institute)

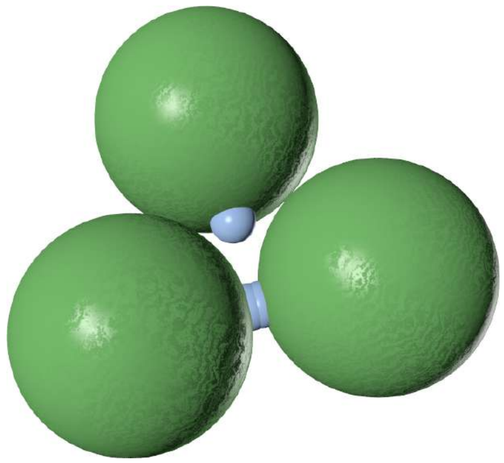


The building blocks

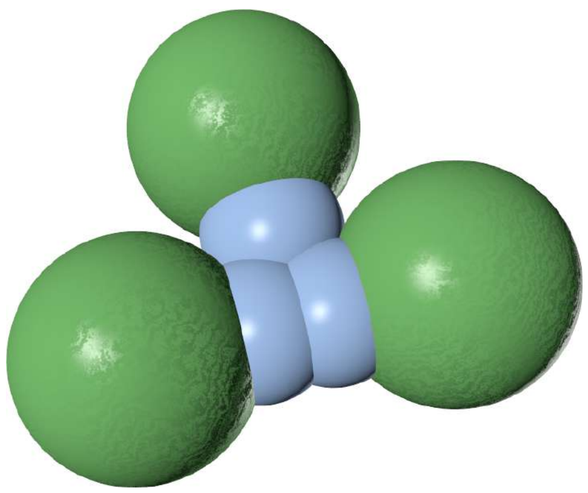
Symmetry	C2	D2	D3	D4	D5
Building blocks					
PDB ID	4LTB	1AEI	3BEY	4B4K	1VPX
Cartoon					

Extending the model

Small patches $\rightarrow$ single-bond-per-patch but slow equilibration



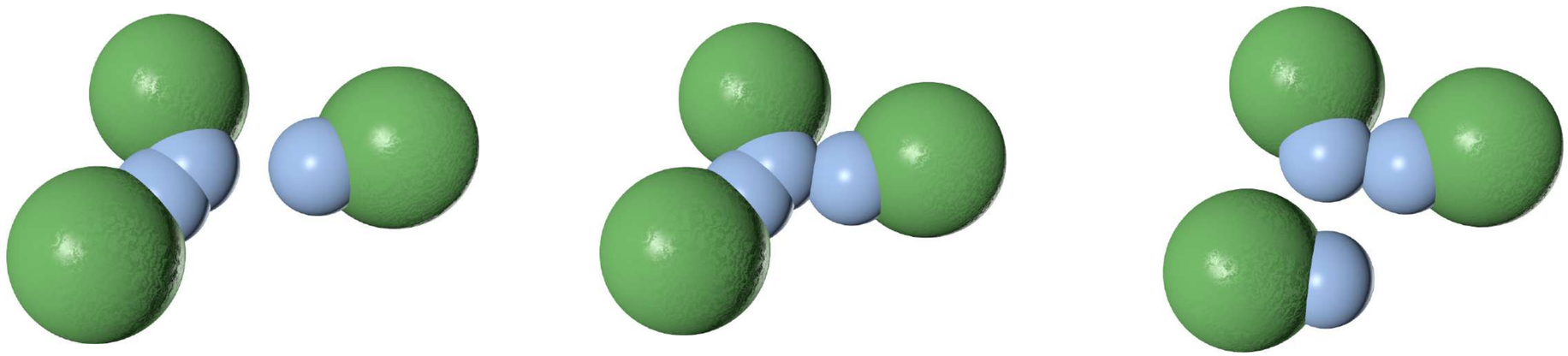
Big patches $\rightarrow$ easier to find partners but multiple bonding possible



The best of both worlds

The FS™ trick

Configuration



V_2	$-\epsilon$	-2ϵ	$-\epsilon$
V_3	0	$\lambda\epsilon$	0

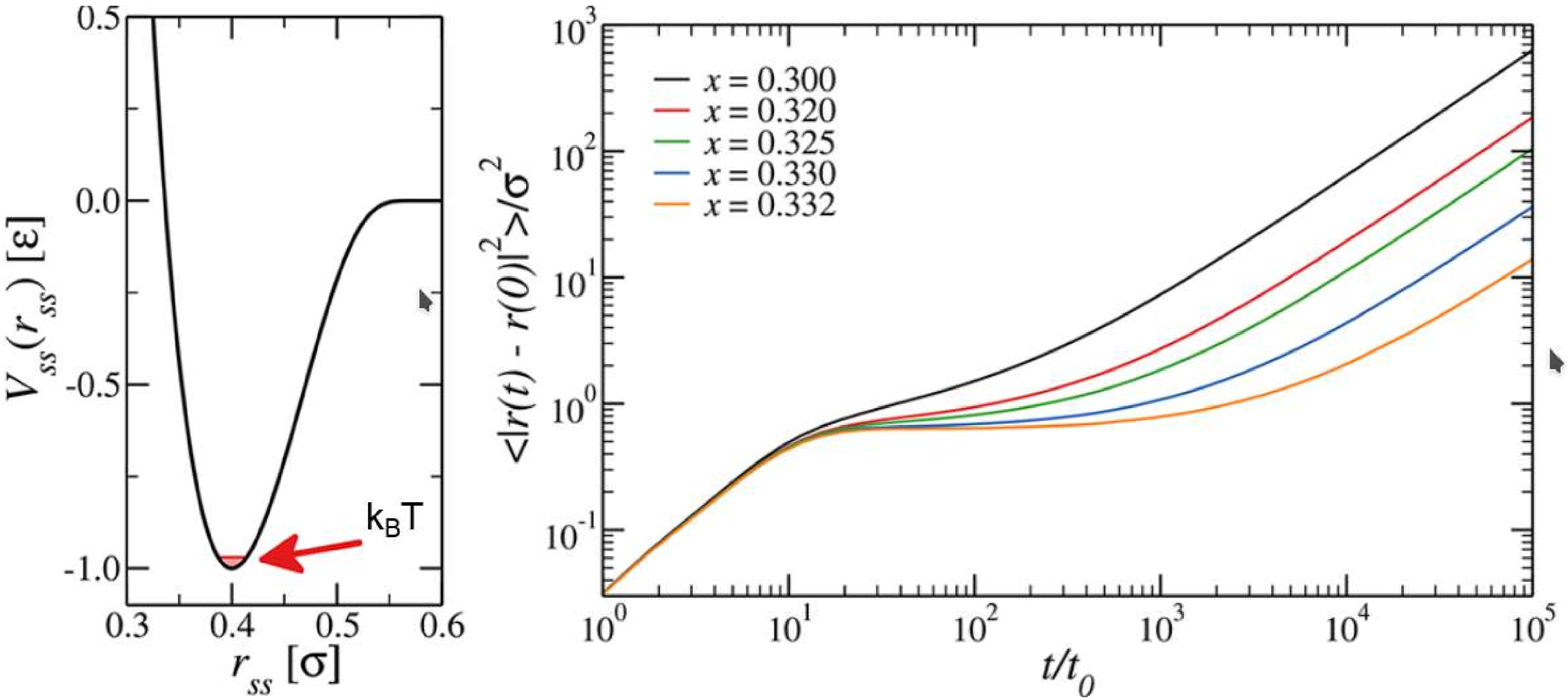
The value of λ controls the behaviour

- $\lambda \geq 1 \rightarrow$ single-bond-per-patch
- $\lambda = 1 \rightarrow$ free swapping!

F. Sciortino, Eur. Phys. J. E (2017), L. Rovigatti *et al., Macromolecules* (2018)

Bond swapping $\rightarrow$ no arrest

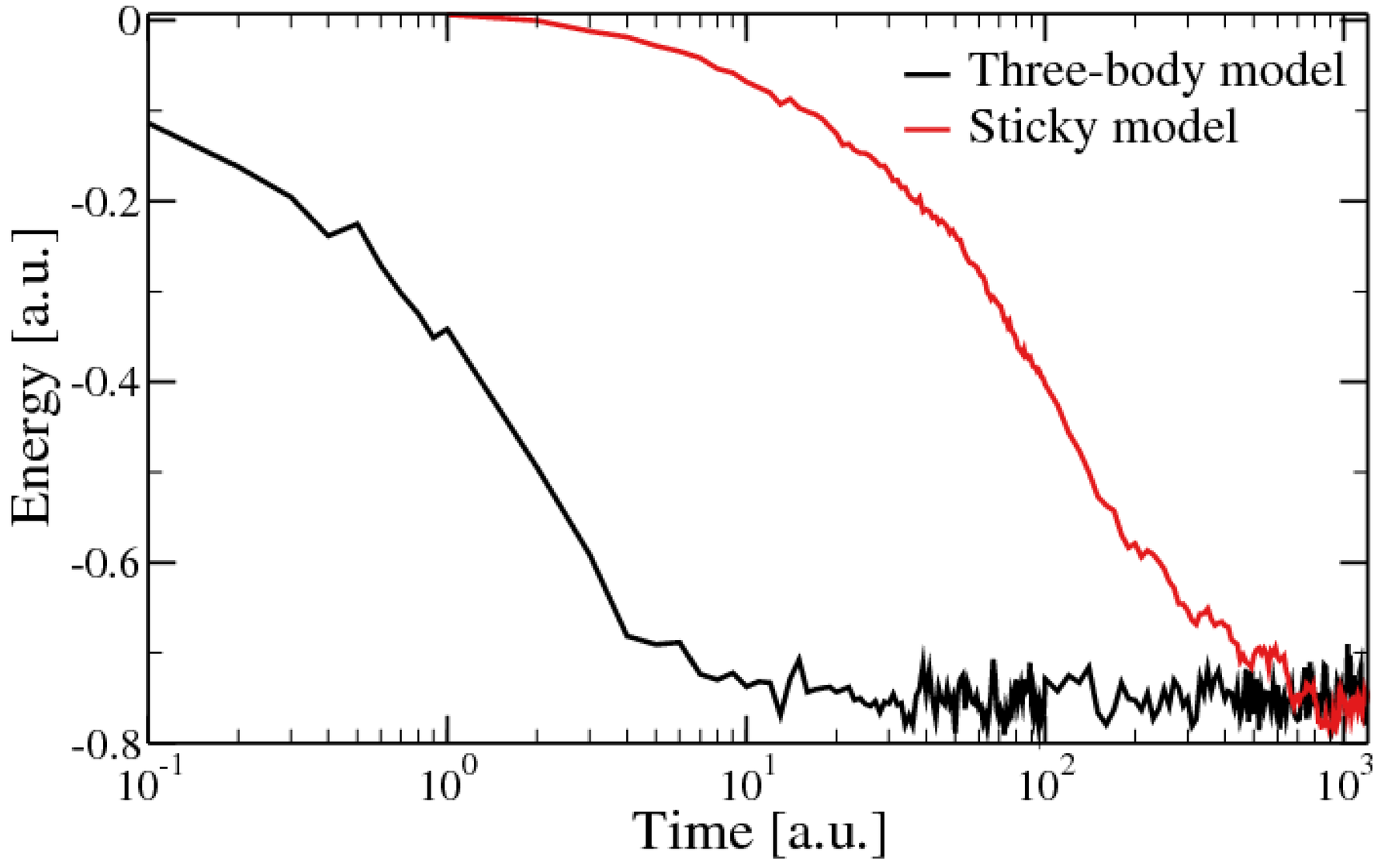
Systems can be equilibrated down to $T \rightarrow 0$



L. Rovigatti *et al., Macromolecules* (2018)

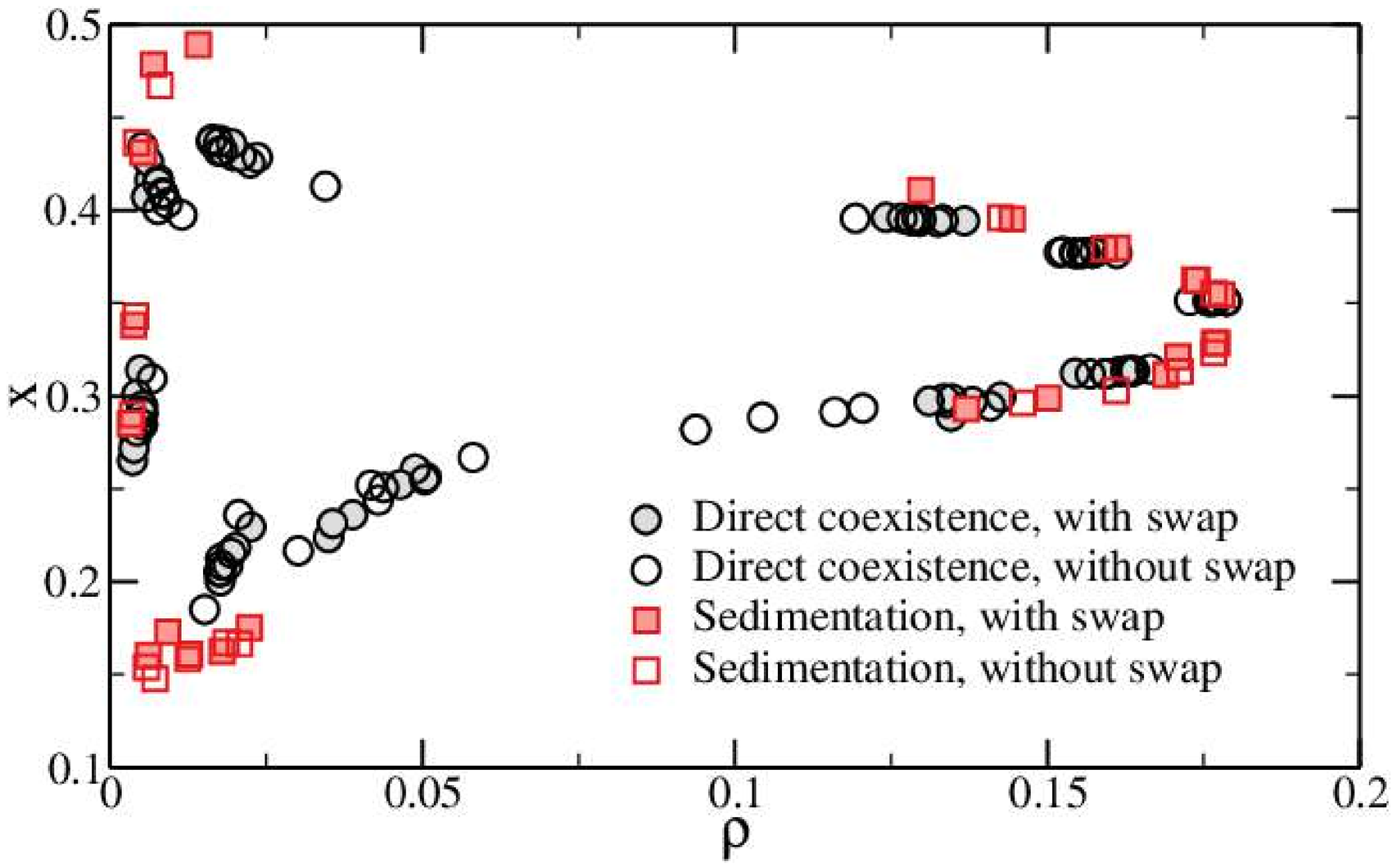
Free lunch, for once

Faster equilibration for $\lambda = 1$



What is the influence of λ?

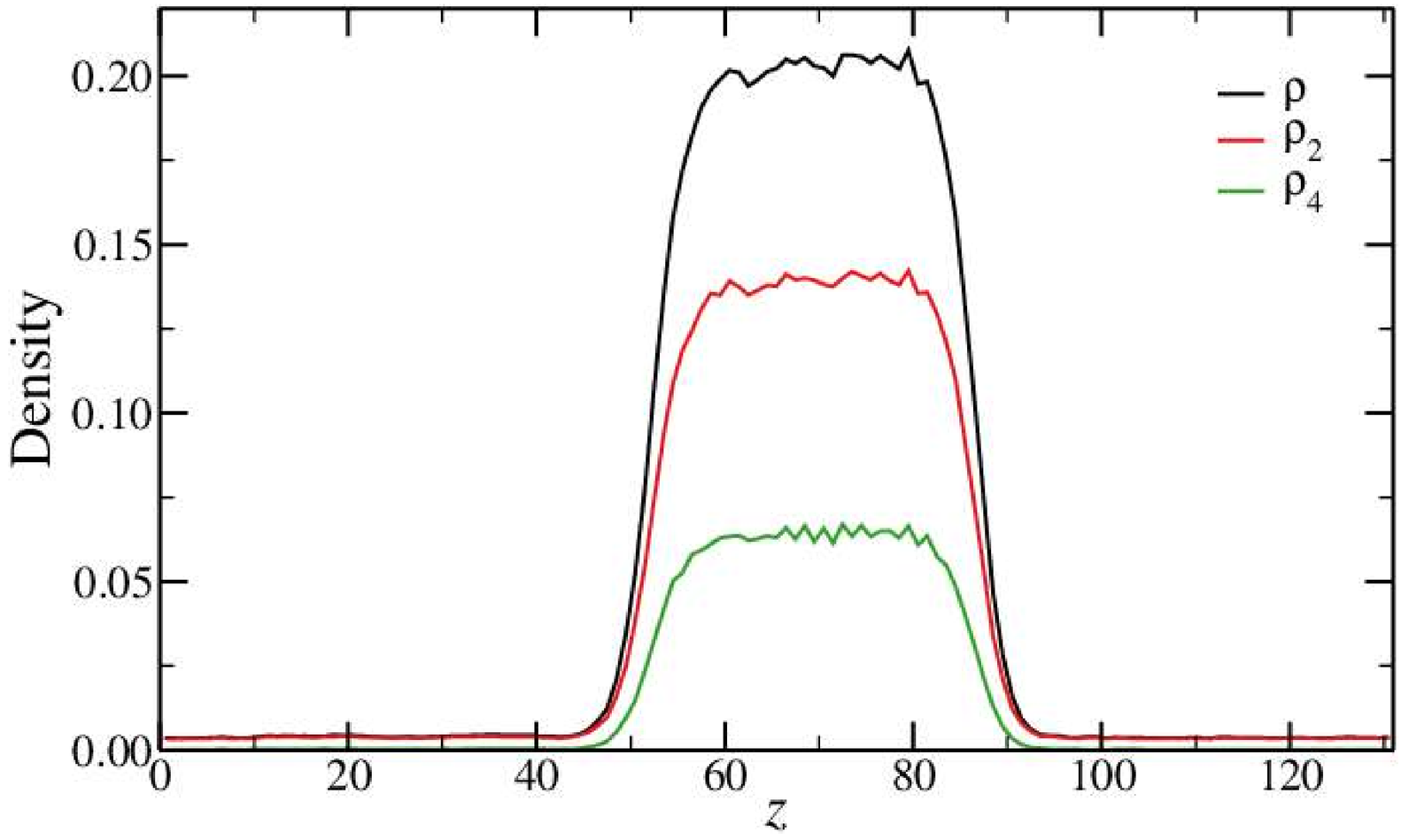
Systems with $\lambda = 1$ and 10 have the same phase diagrams



Direct coexistence

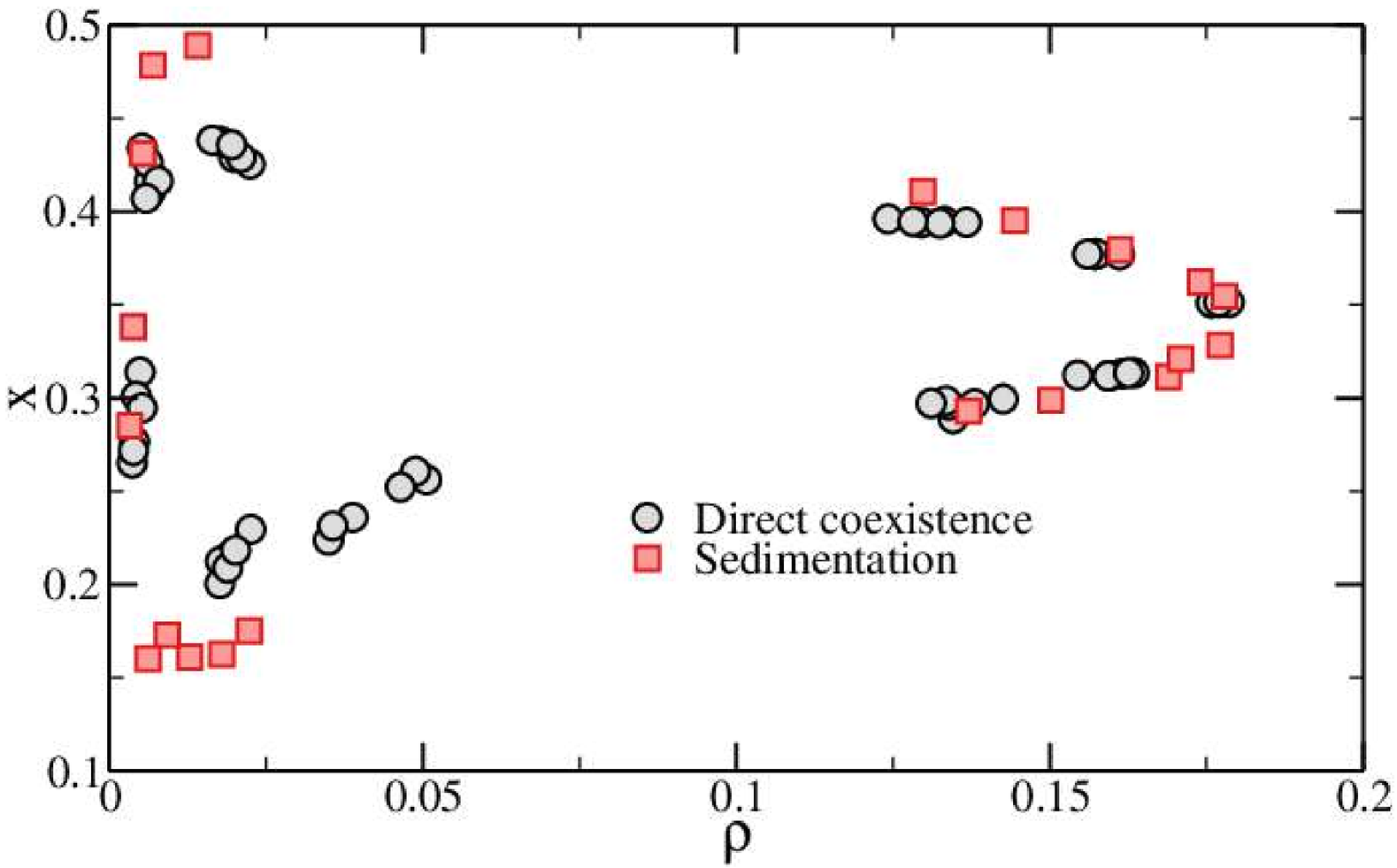
The recipe

1. Equilibrate at small pressure (the specific value is not important)
2. Enlarge the box along one direction (here z)
3. Compute the density profile and extract the coexisting densities



Sedimentation *vs.* direct coexistence

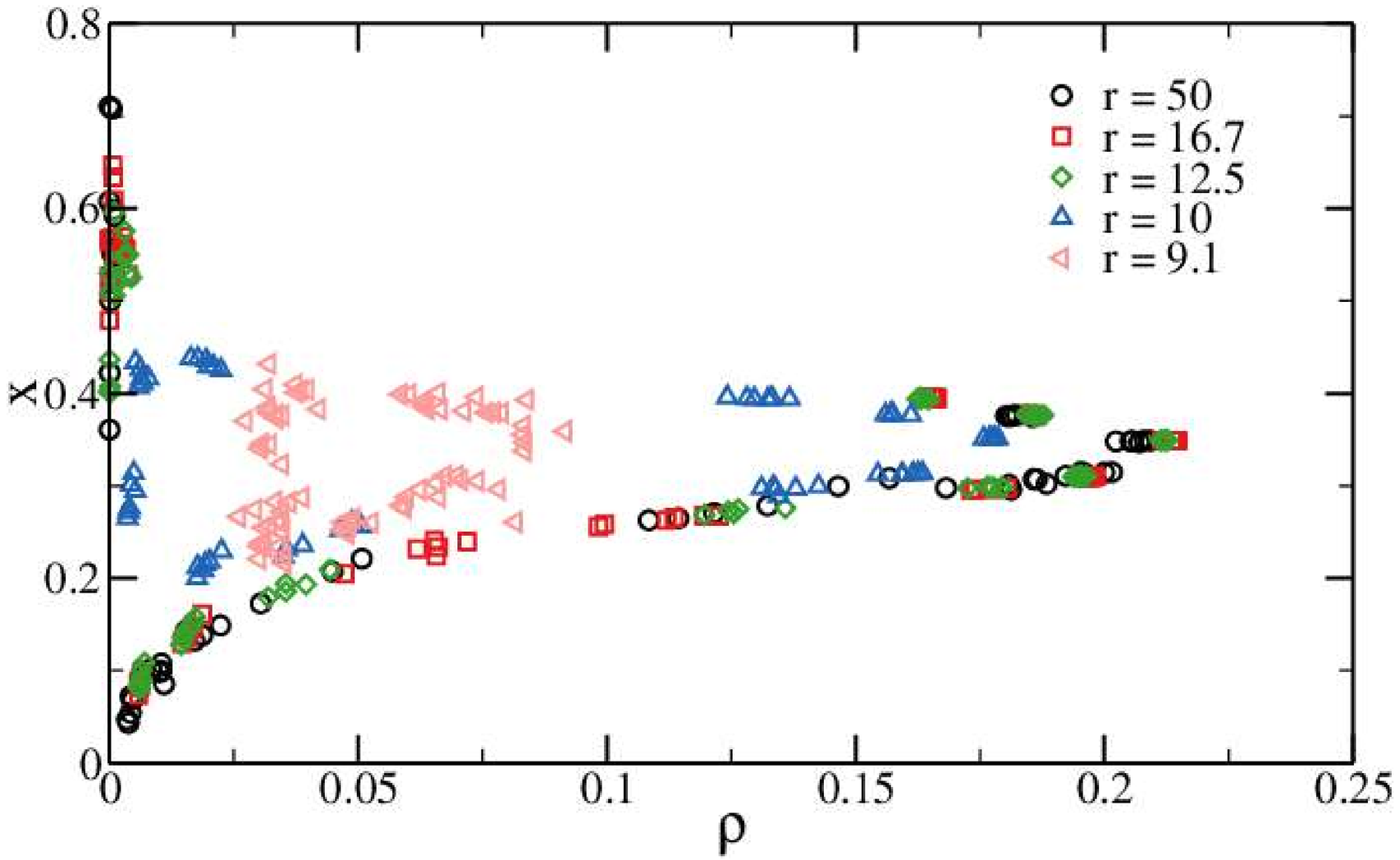
$$x = \frac{\rho_4}{\rho_2+\rho_4}, \rho = \rho_2 + \rho_4$$



Less numerically demanding but no out-of-equilibrium options

Equilibrium phase diagrams

The phase diagram enlarges as the bond strength (affinity) grows, as in the experiments



FRAP pictures