The bacterial DNA segregation complexes ParB*S* display a twofold phase separation

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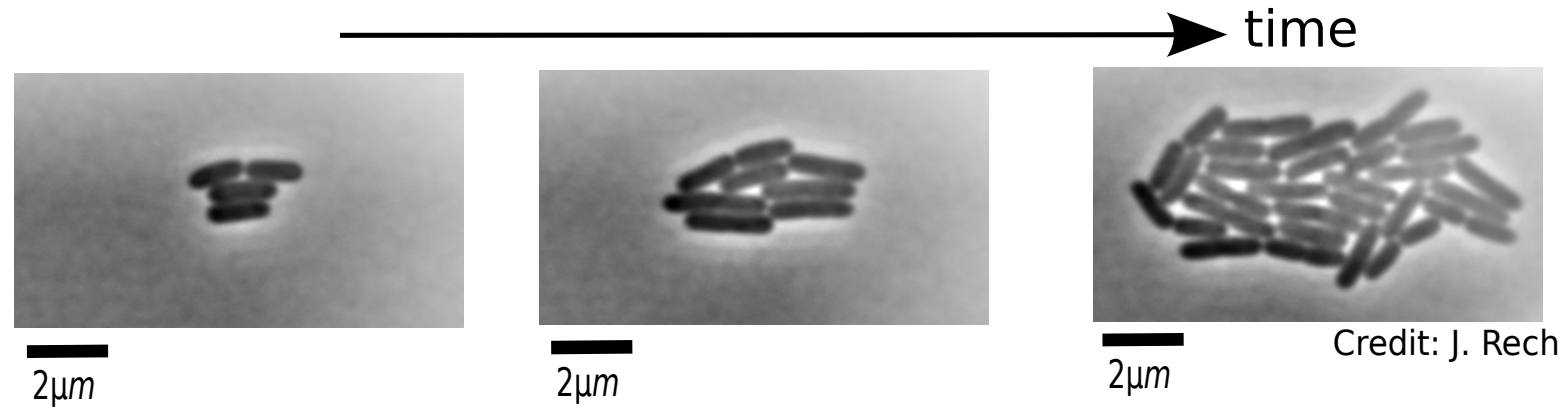
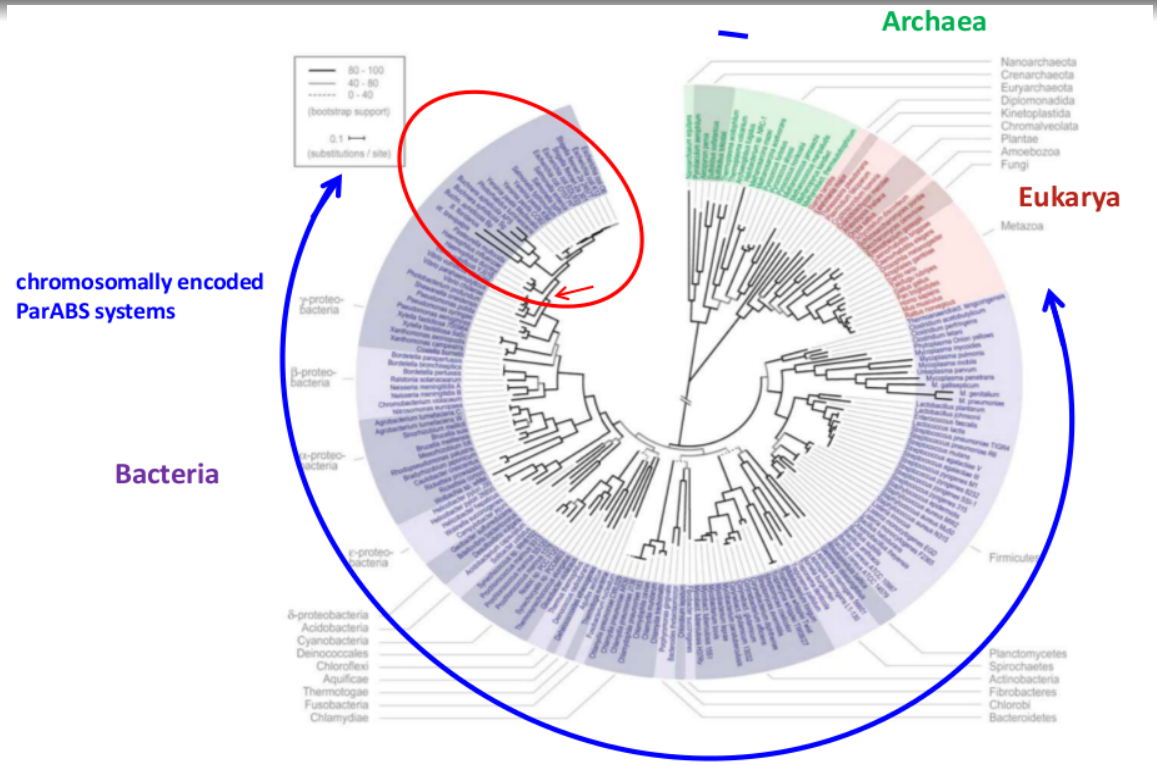
Bacterial DNA segregation



How is the bacterial genome segregated ?

Bacterial DNA segregation: the ParAB*S* system

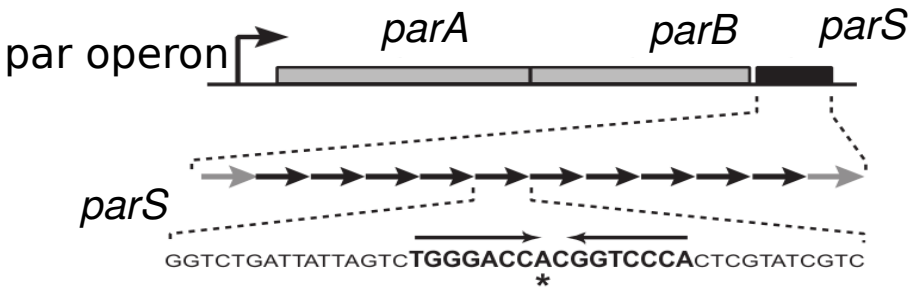


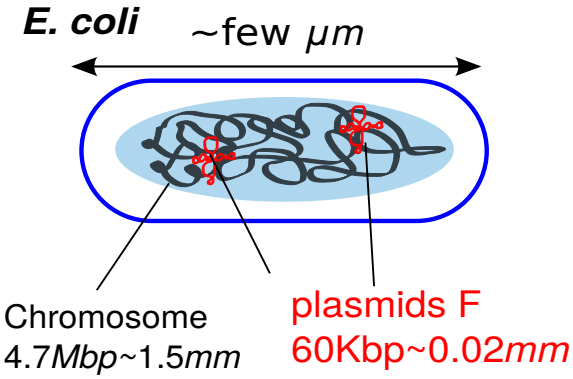
Credit: J-Y. Bouet

→ Chromosomal ParAB*S* is strongly conserved

→ Ancient mechanism of liquid-liquid phase separation (**LLPS**)

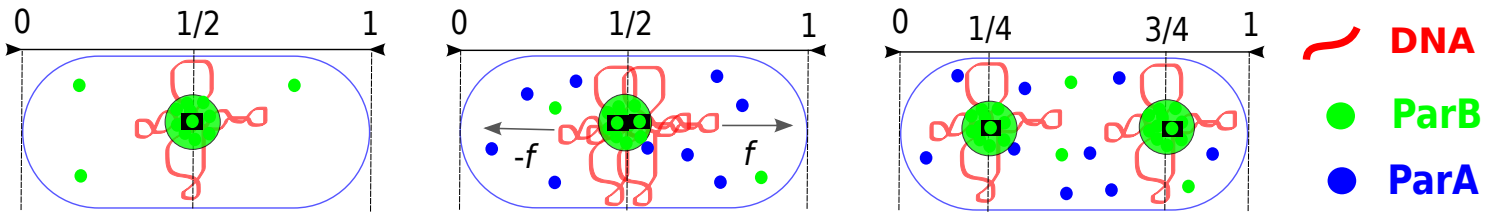
The ParABS system: the molecular actors





- ParA: “motor” protein (ATPase)
- ParB: binding protein (specific or non-specific binding)
- *parS*: specific DNA sequence

ParB*S* displays a twofold phase separation



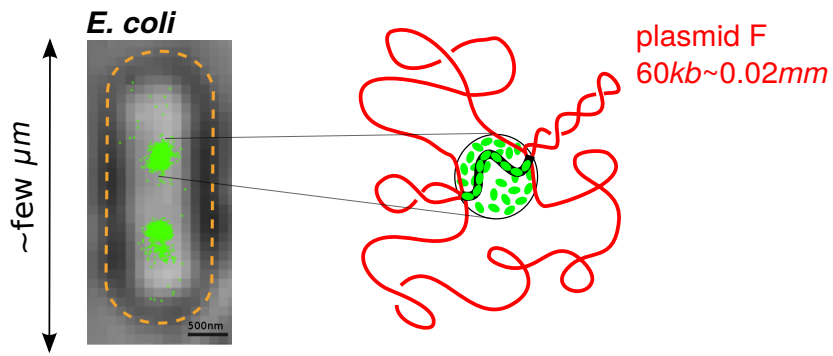
Step 1. Formation of ParB*S*

Equilibrium LLPS

Step 2. Separation of DNA copies

Step 3. Equipositioning

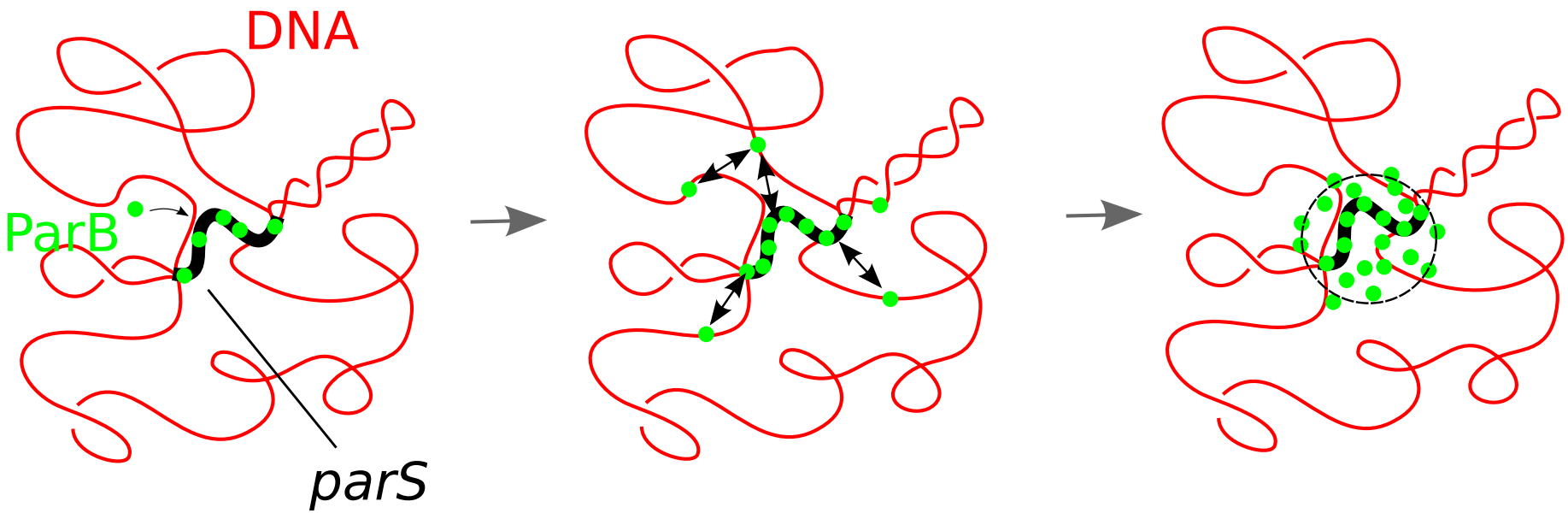
Non-eq. phase separation (ATP)



- Membraneless compartments ParB*S* splitted by active phase separation

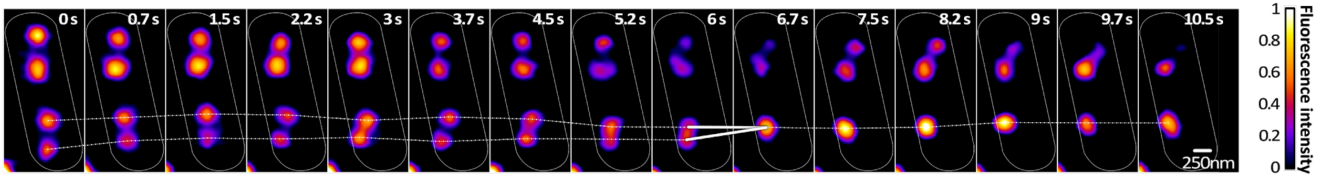
  → atypical phase separation at- and out of- equilibrium

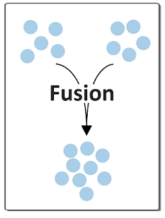
Formation of the ParB*S* complex: a liquid-liquid phase separation ?

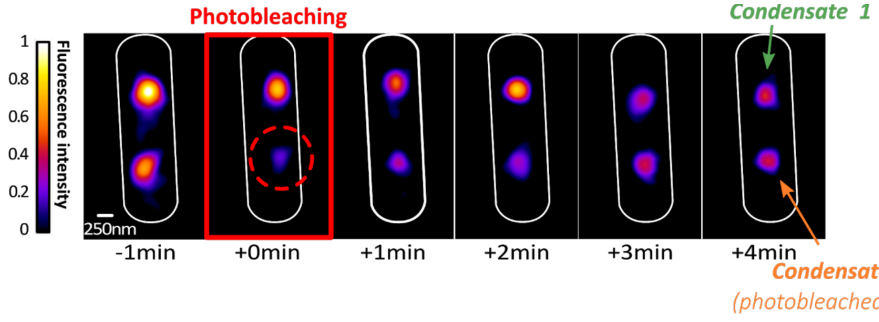


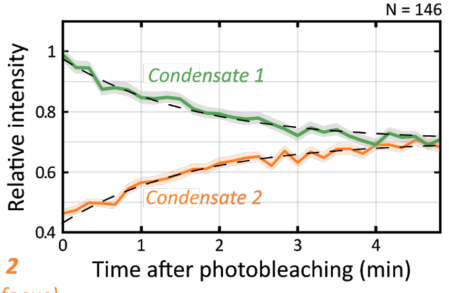
1. Does the partition complex ParB*S* display a LLPS ?
2. Clusters of $\sim$ 300 ParB $\rightarrow$ LLPS possible ?
3. *parS*: 10 binding sites $\rightarrow$ role in the specificity ?

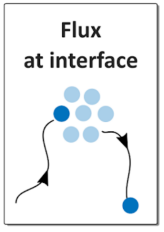
Liquid-like behaviour of ParB*S* complexes












Baptiste Guilhas


Antoine Le Gall


Marcelo Nollmann

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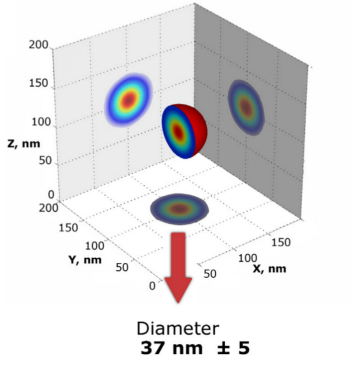
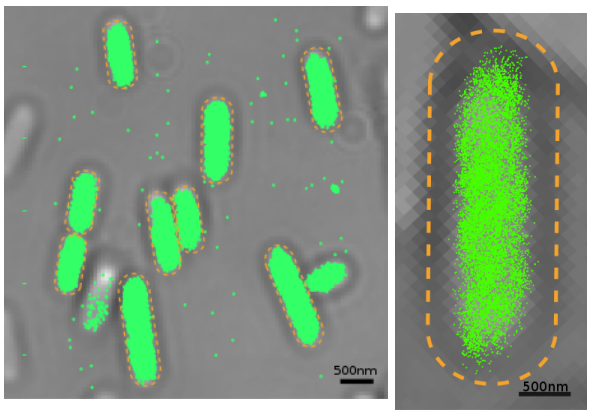


Diameter
37 nm ± 5

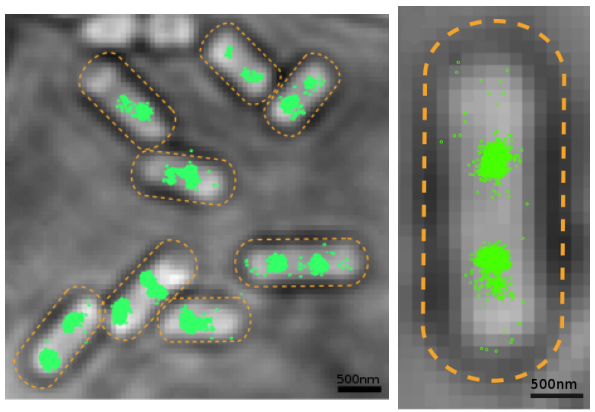
Guilhas, JCW,...Le Gall, Nollmann **ATP-driven separation of liquid phase condensates in bacteria** *Mol. Cell* '20

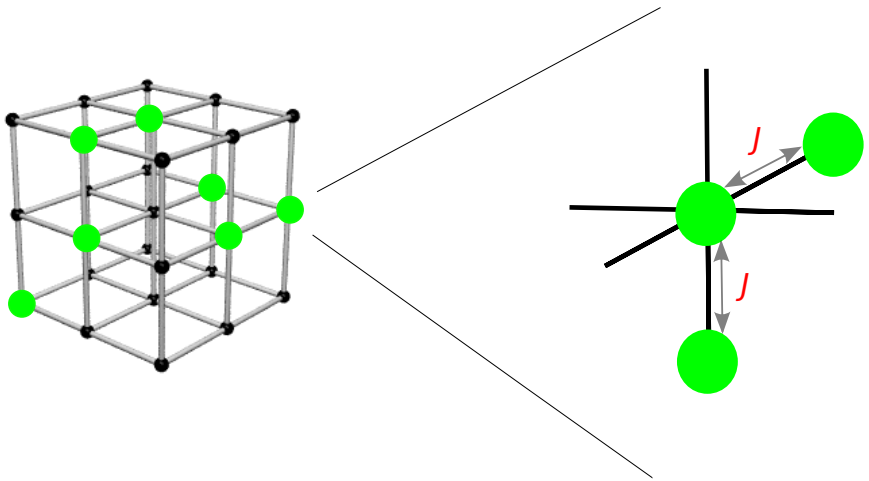
Liquid-like behaviour of ParB*S* complexes

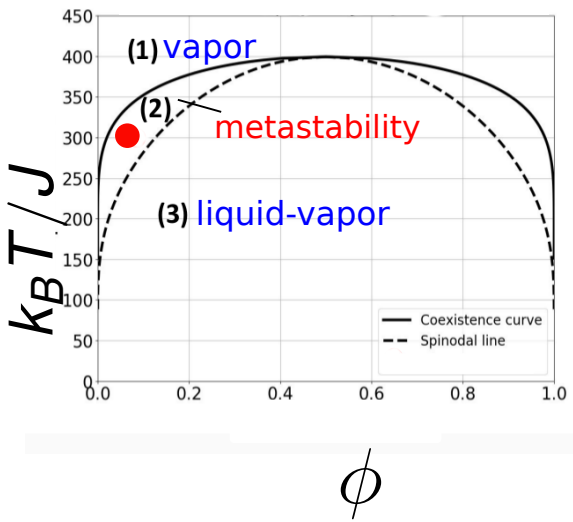


specific binding

ParB/*parS*

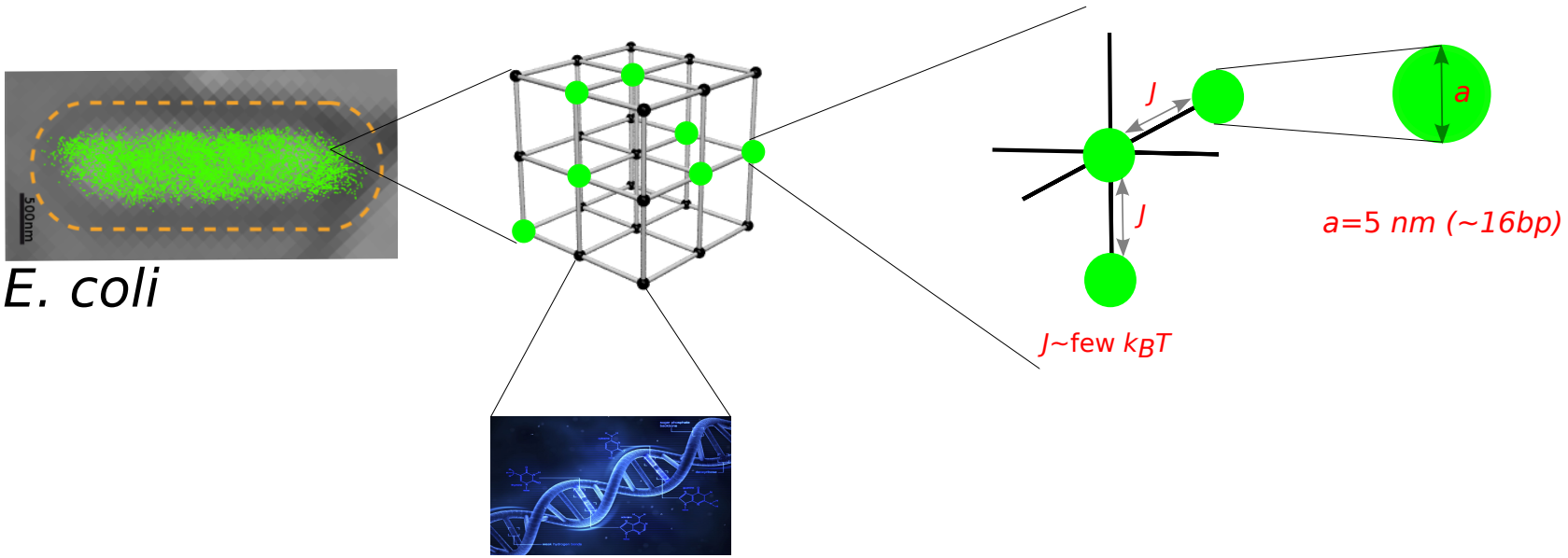






Guilhas, JCW,...Le Gall, Nollmann **ATP-driven separation of liquid phase condensates in bacteria** *Mol. Cell* '20

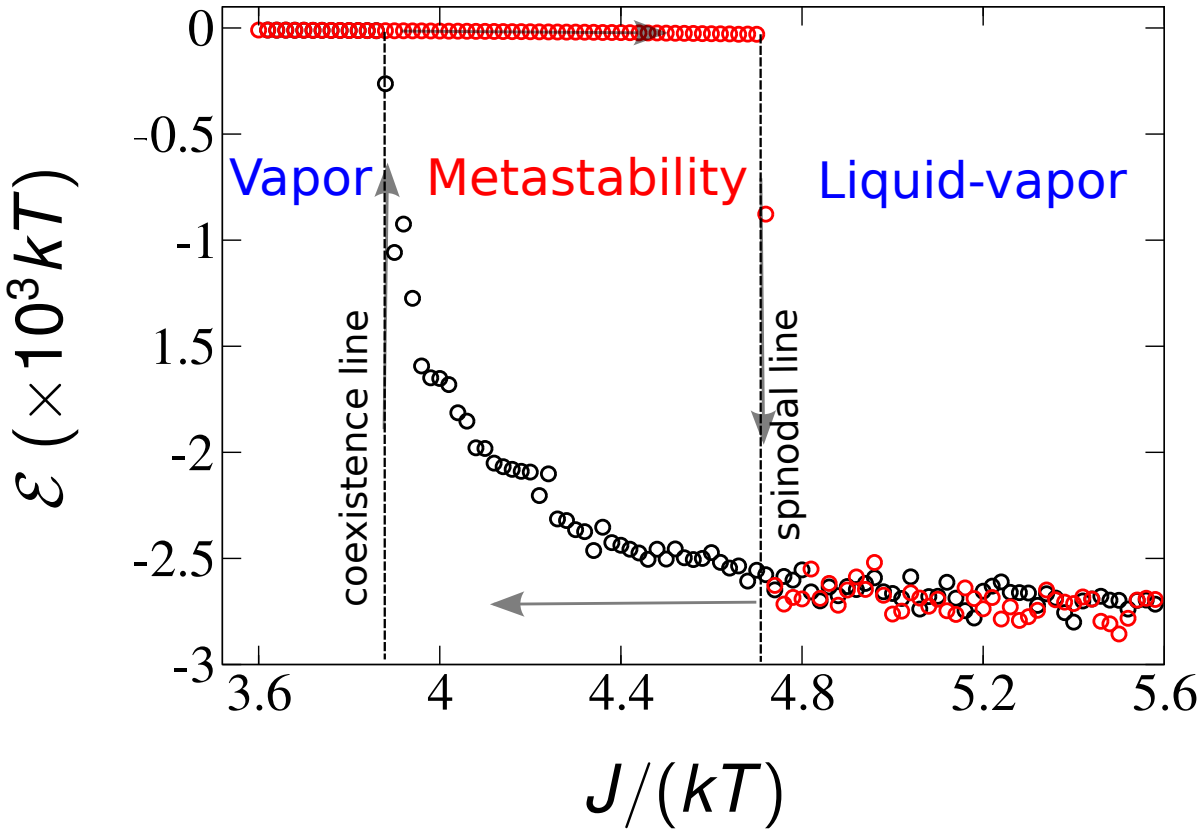
The ParB proteins as a Lattice Gas



$$\mathcal{E} = -J \sum_{\langle i,j \rangle} \phi_i \phi_j - \varepsilon_{parS} \sum_i \phi_i$$

- Diffusion $D \sim 1 \mu m^2.s^{-1}$ & Interaction energy J

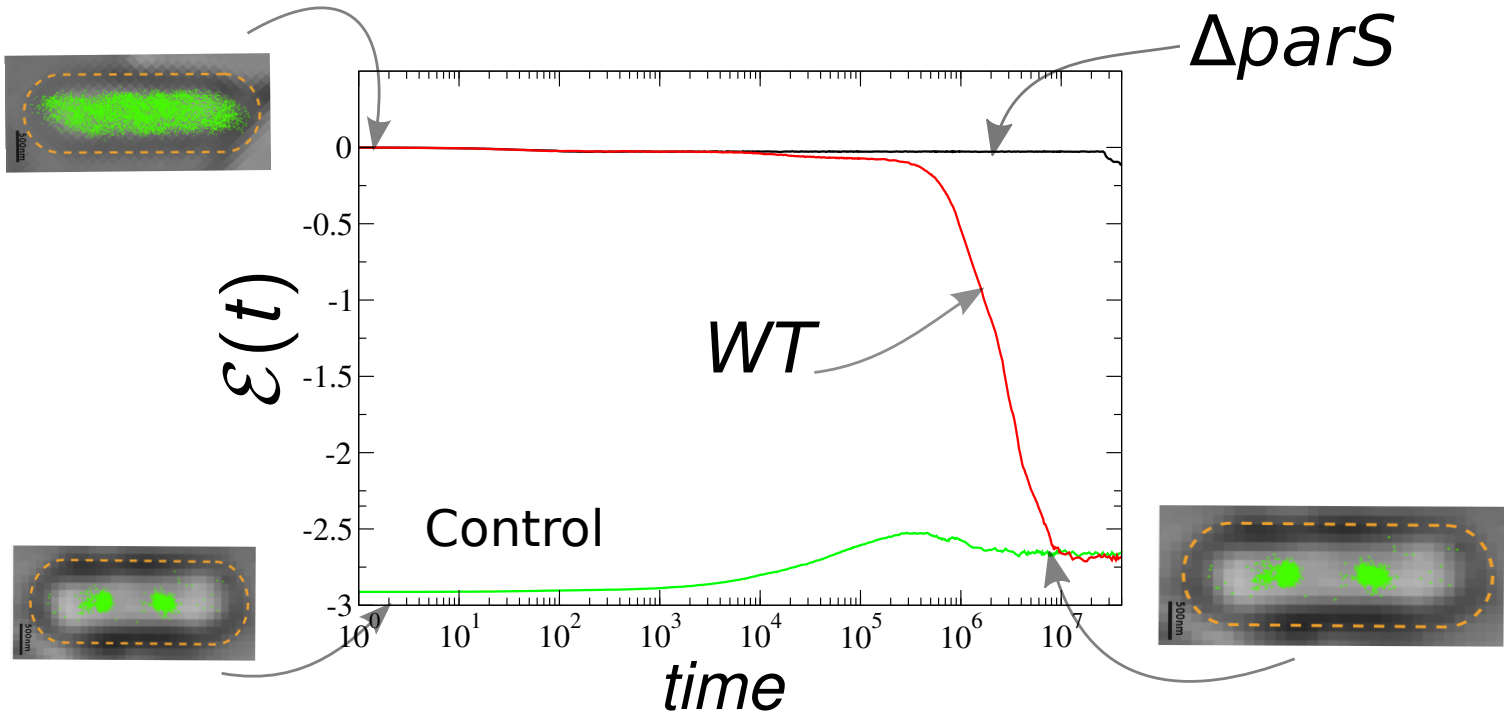
Phase diagram of the ParB*S* system



1. 1st order phase transition (jump in the energy & hysteresis)
2. Metastable region for $J \sim$ few $k_B T$

Guilhas, JCW,...Le Gall, Nollmann **ATP-driven separation of liquid phase condensates in bacteria** *Mol. Cell* '20

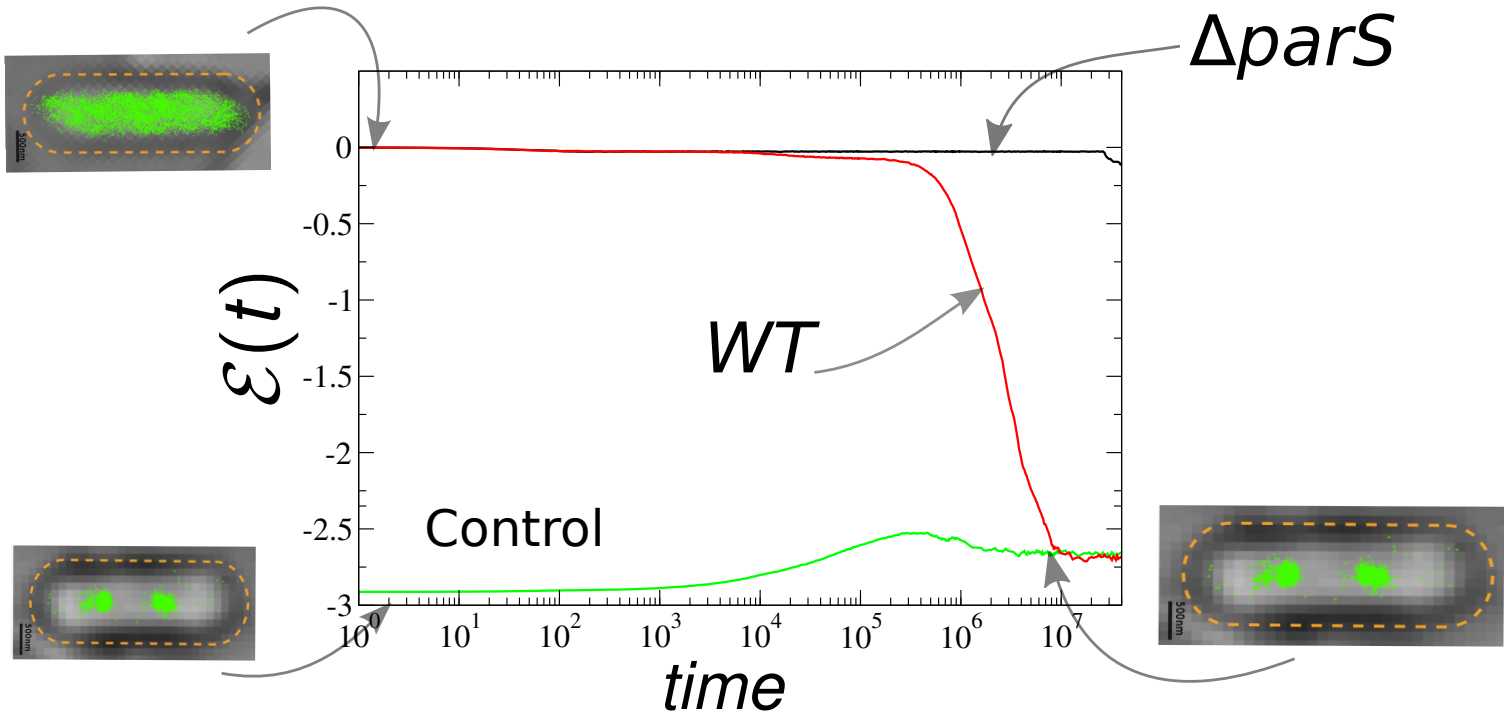
Kinetic of ParB*S* nucleation



- Monte Carlo dynamics at $J = 4.5kT$

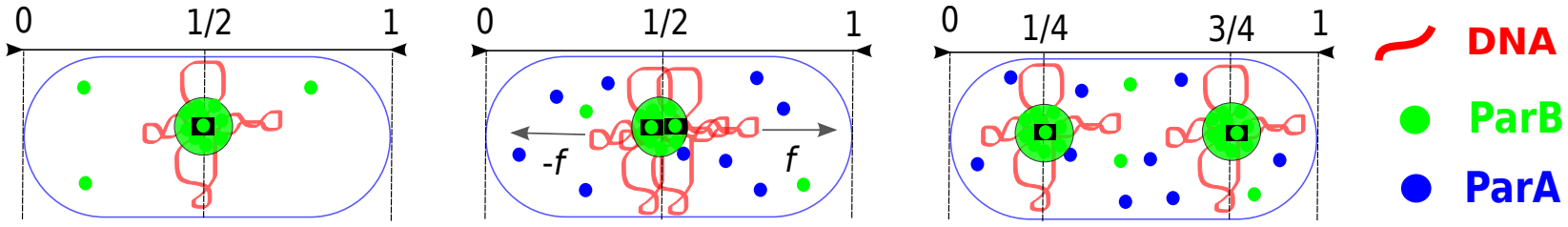
Guilhas, JCW,...Le Gall, Nollmann **ATP-driven separation of liquid phase condensates in bacteria** *Mol. Cell* '20

Kinetic of ParB*S* nucleation



1. *parS* is needed to catalyze LLPS *in vivo*
2. Nucleation is lost for cryptic sequences with affinities $< J$

ParB*S* displays a twofold phase separation



Step 1. Formation of ParB*S*

Equilibrium LLPS

Step 2. Separation of DNA copies

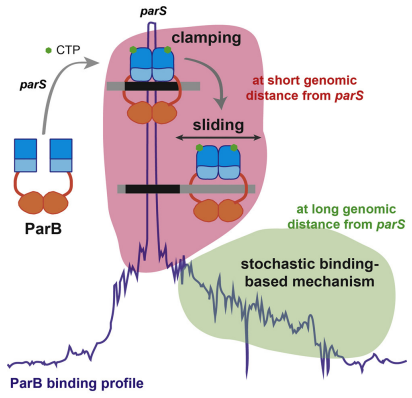
Step 3. Equipositioning

Non-eq. phase separation (ATP)

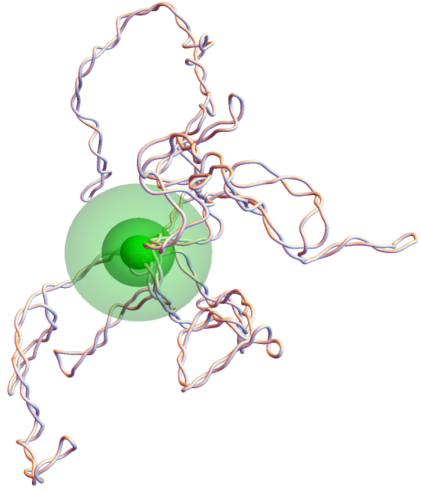
1. The partition complexe ParB*S* displays a LLPS (300 ParB)
   $\rightarrow$ metastable regime: response increased
   $\rightarrow$ *parS* catalyzes LLPS: high specificity at *parS*
2. ParA subsequently phase separates actively the condensates

JCW et al **Surfing on protein waves: proteophoresis as a mechanism for bacterial genome partitioning** *Phys. Rev. Lett.* 2017

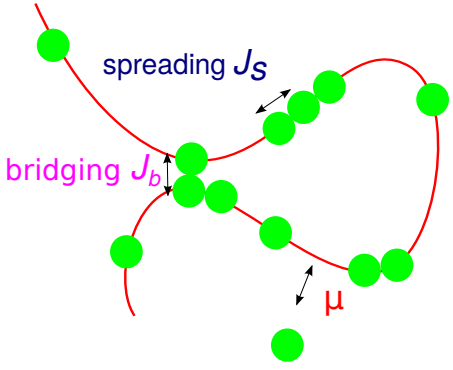
Perspectives: Role of other mechanisms in the LLPS



JCW,..., Bouet **Physical modeling of a sliding clamp mechanism for the spreading of ParB at short genomic distance from bacterial centromere sites** *Iscience* 2020

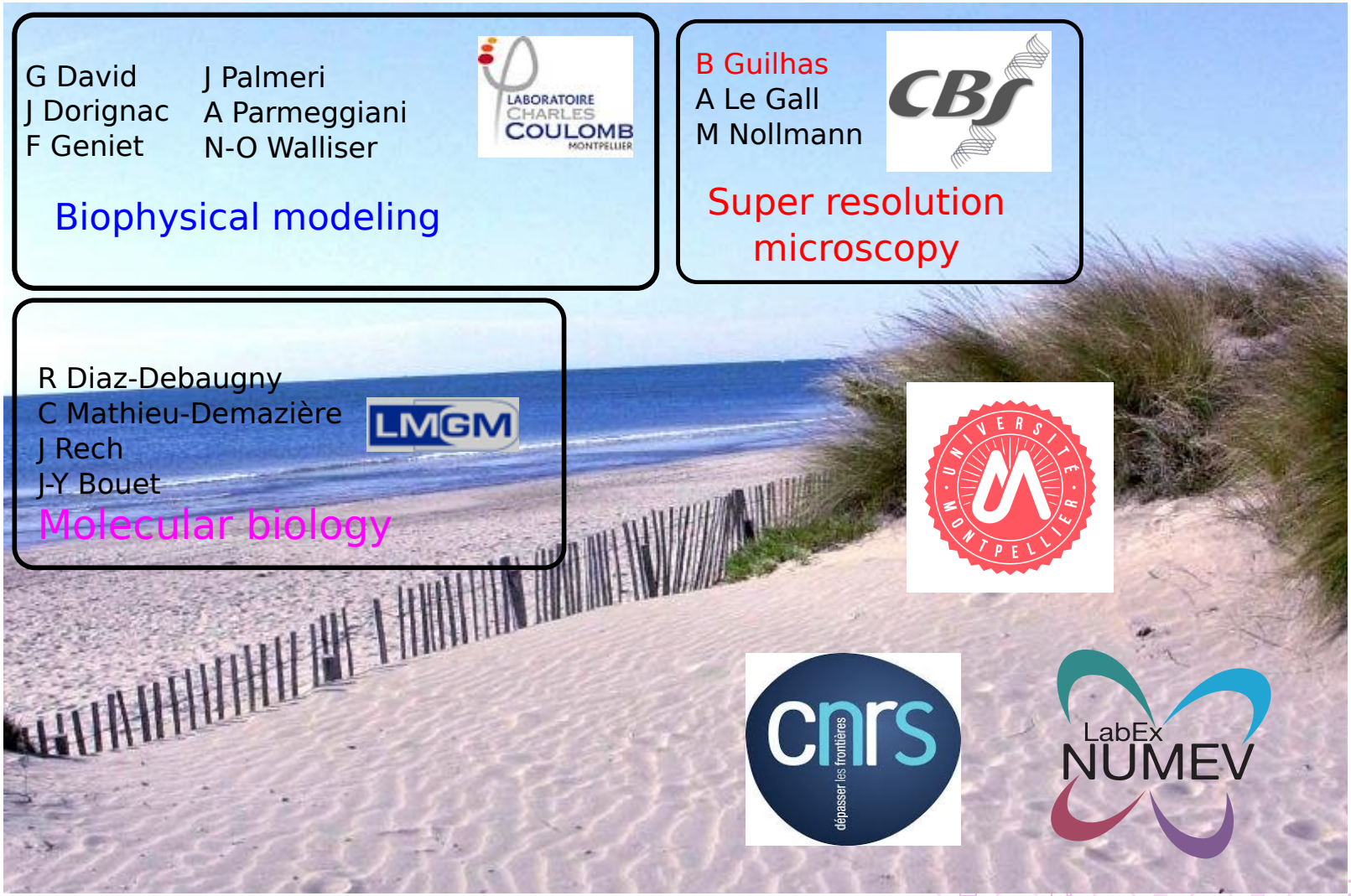


JCW, ..., Bouet, Junier **Supercoiled DNA and non-equilibrium formation of protein complexes: A quantitative model of the nucleoprotein ParBS partition complex** *PLOS Comp. Biol.* 2021

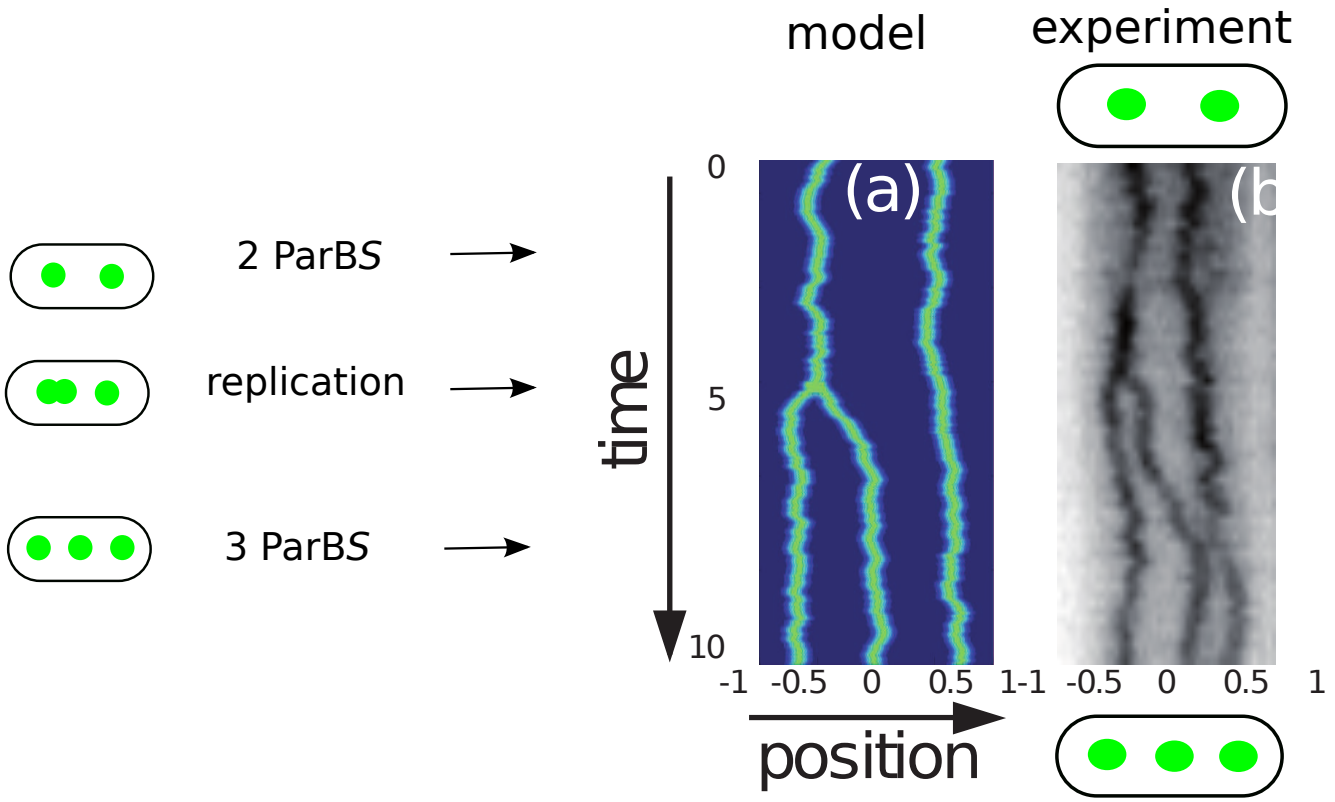


David, JCW, ..., Palmeri **Phase separation of polymer-bound particles induced by loop-mediated one dimensional effective long-range interactionsx** *Phys. Rev. Res. 2020*

Thank you for your attention!

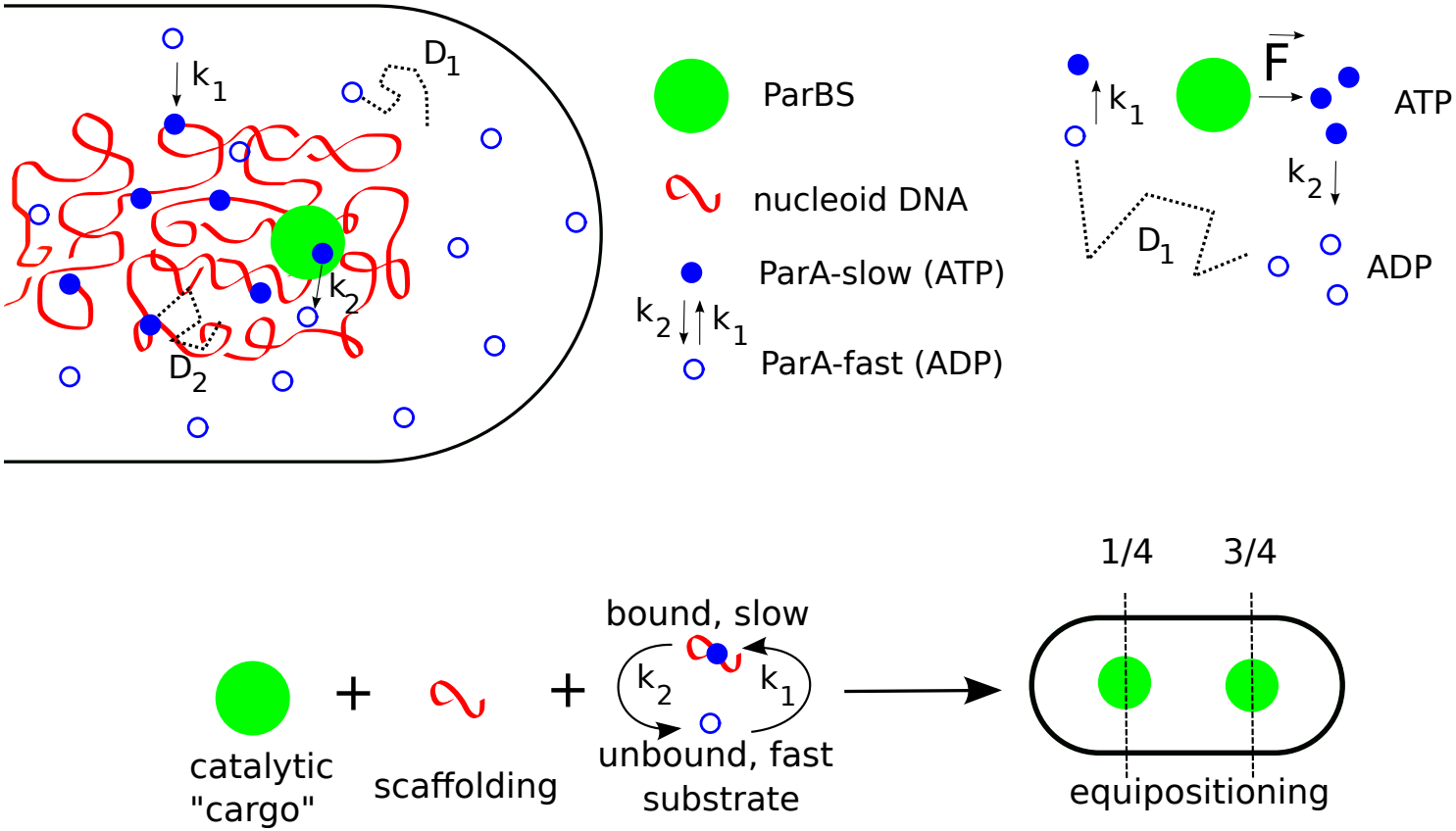


Equipositioning along the cell axis



What are the main physical actors of DNA segregation ?

Reaction-diffusion process: Molecular interactions



Reaction-Diffusion equations

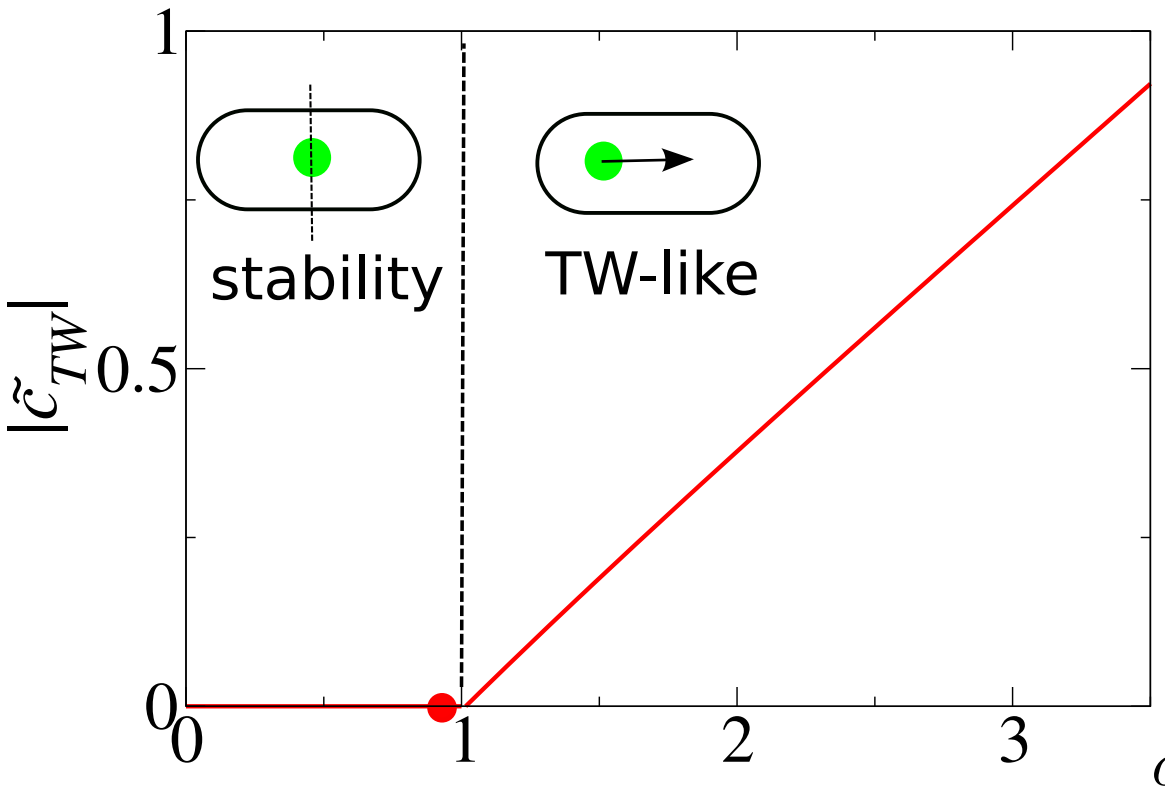
ParA-slow (ATP): $$\frac{\partial v}{\partial t} = D_2\,\Delta v + k_1\,u(\mathbf{r},t) - k_2\,v(\mathbf{r},t)\sum_i S(\mathbf{r}-\mathbf{r}_i(t))$$

ParA-fast (ADP): $$\frac{\partial u}{\partial t} = D_1\,\Delta u - k_1\,u(\mathbf{r},t) + k_2\,v(\mathbf{r},t)\sum_i S(\mathbf{r}-\mathbf{r}_i(t))$$

ParBS: $$m\gamma\frac{d\mathbf{r}_i}{dt}(t) = \varepsilon\int_V \nabla v(\mathbf{r}',t)\,S(\mathbf{r}'-\mathbf{r}_i(t))\,d^3\mathbf{r}'$$

- Feedback between the partition complexes and ParA densities
  $\rightarrow$ Non-linear system with dynamical instability

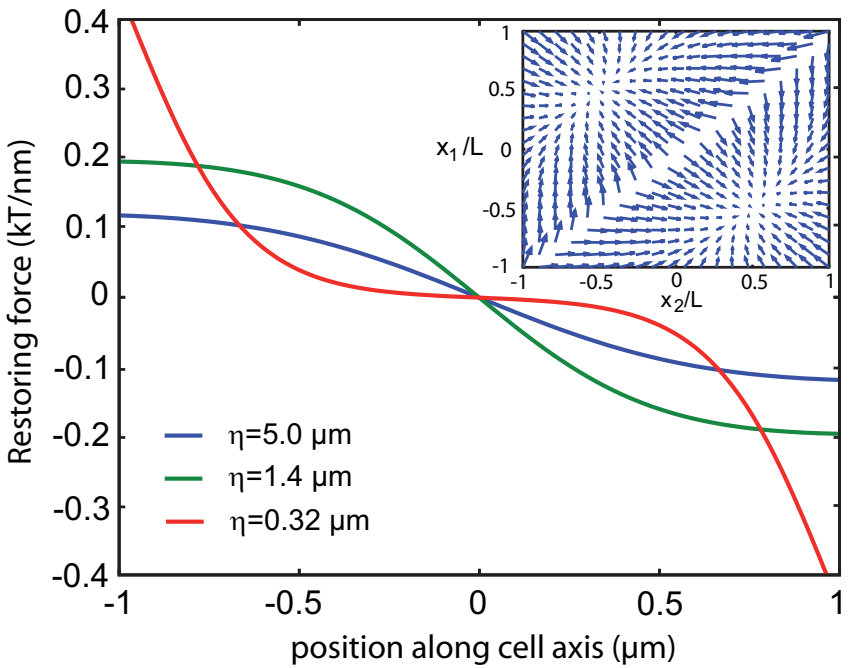
Dynamical instability: bifurcation obtained with Traveling Wave ansatz

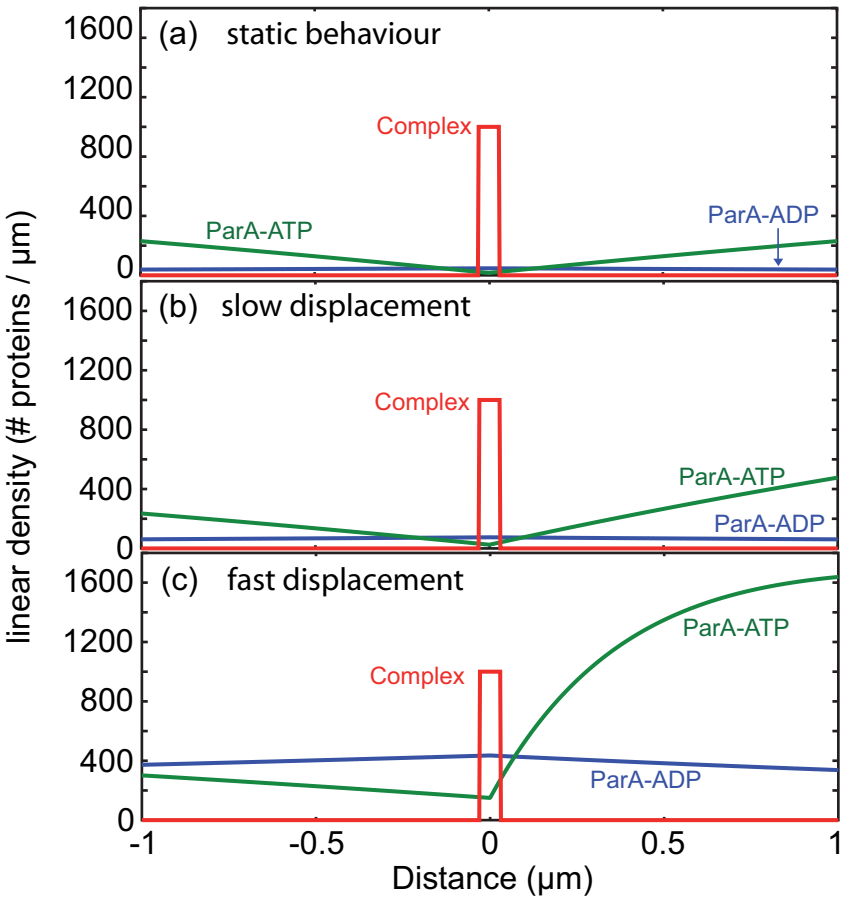


$$\frac{\alpha}{\alpha_c} = \frac{\varepsilon N_p}{m\gamma D_2}$$

Restoring force & gradient of ParA-ATP

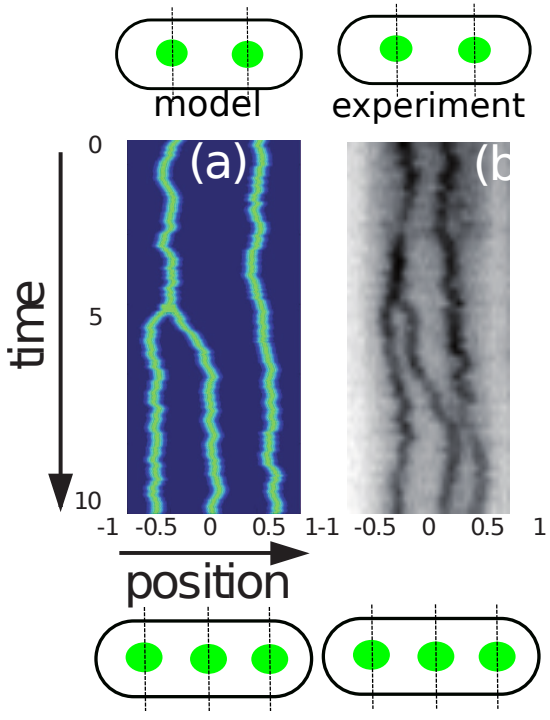
$$\eta = \sqrt{\frac{D_1}{k_1}}$$



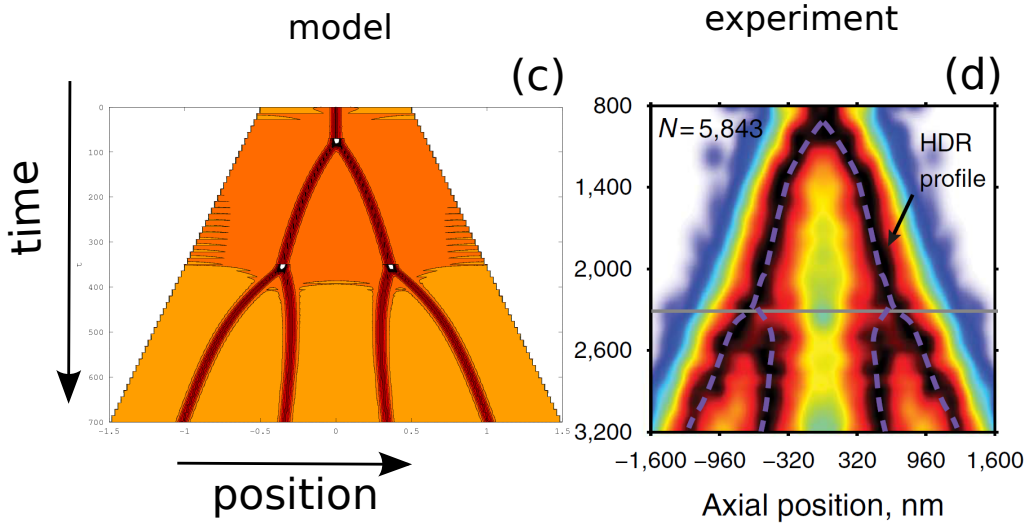


Comparison with experiments

Single cell



Average



Fluorescence microscopy & Superresolution microscopy