Physical modeling of active bacterial DNA segregation

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Harvard University, USA
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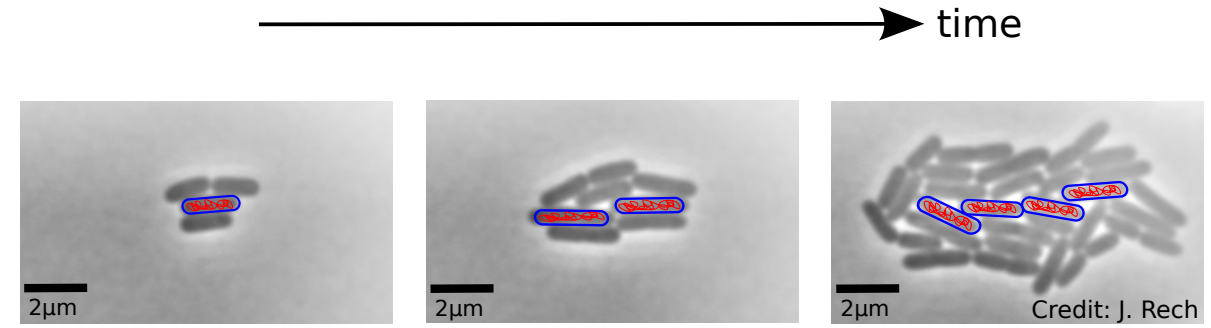
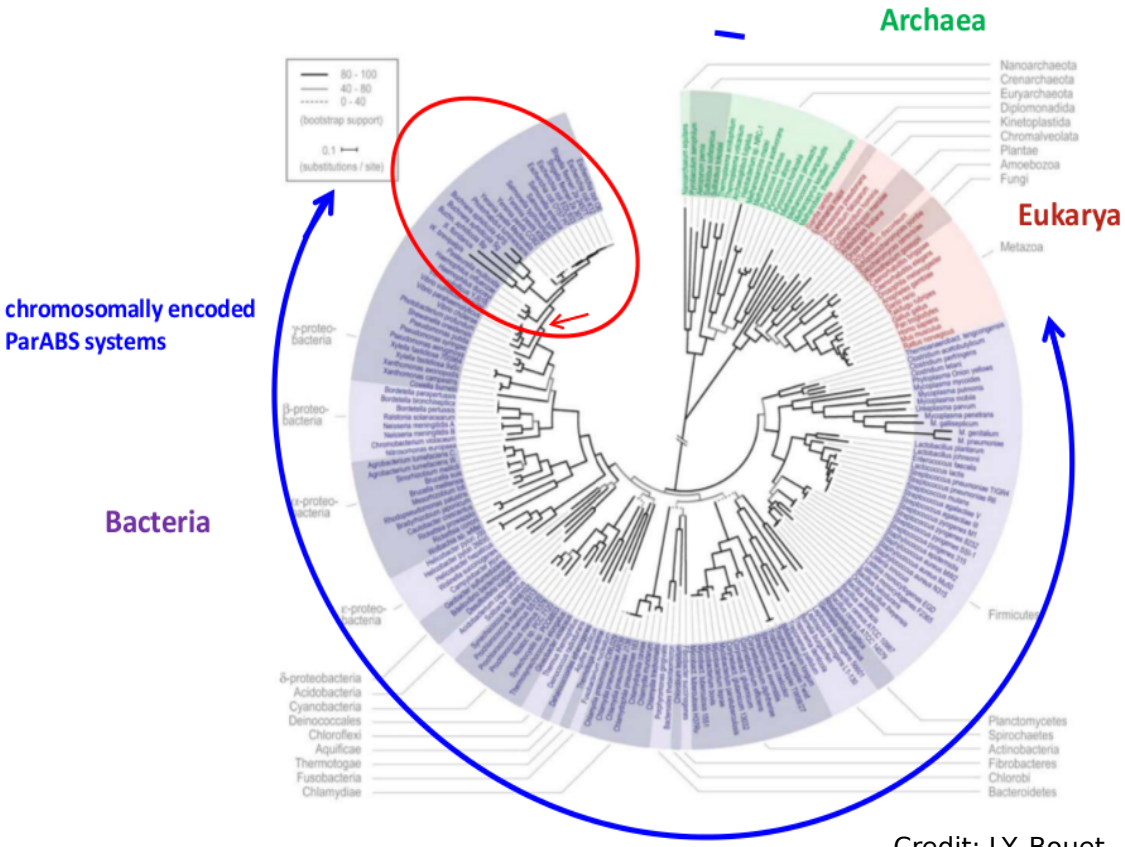
Segregation of bacterial DNA



How is the bacterial genome segregated ?

Segregation of bacterial DNA: the ParAB*S* system

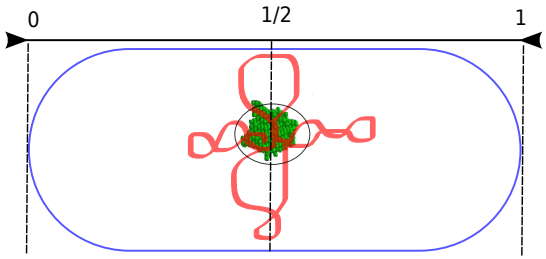


Credit: J-Y. Bouet

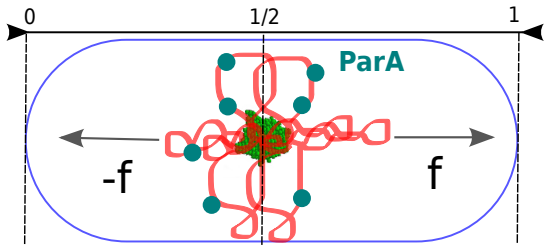
Partition system ParABS is strongly conserved

How does ParAB*S* work ?

Step 1. Formation of the partition complex

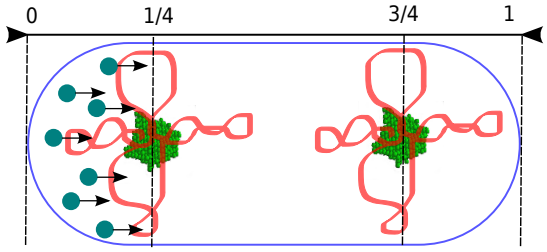


Step 2. Separation of the copies of DNA



opposite forces

Step 3. Positioning



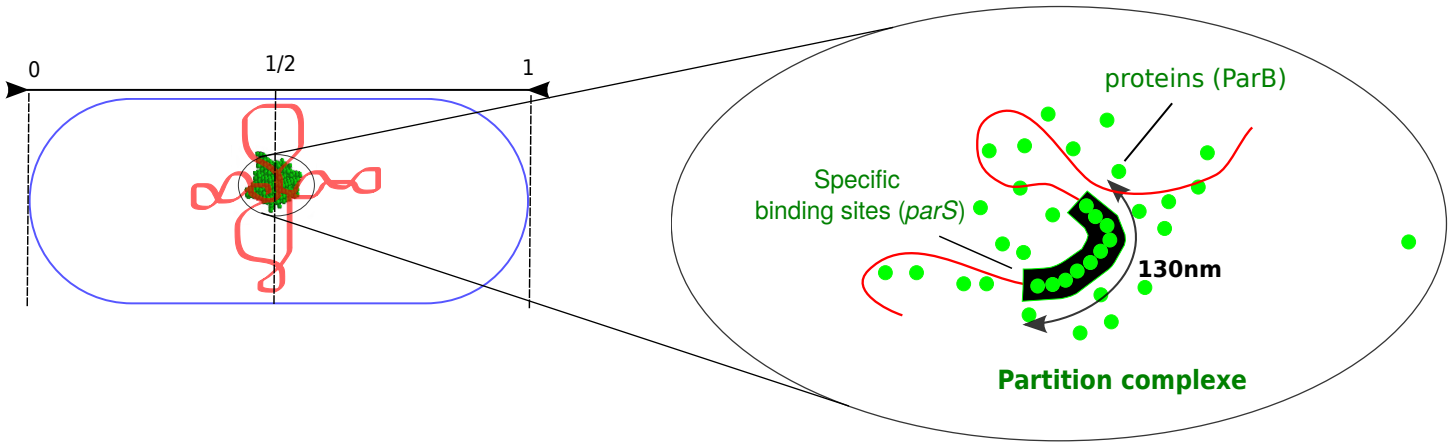
oscillations of ParA

3 components:
a) 2 proteins (ParA & ParB)
b) specific binding sites (*parS*)

"Reaction-Diffusion" or "Filament pulling" mechanisms

How does ParAB*S* work ?

Step 1. Formation of the partition complex



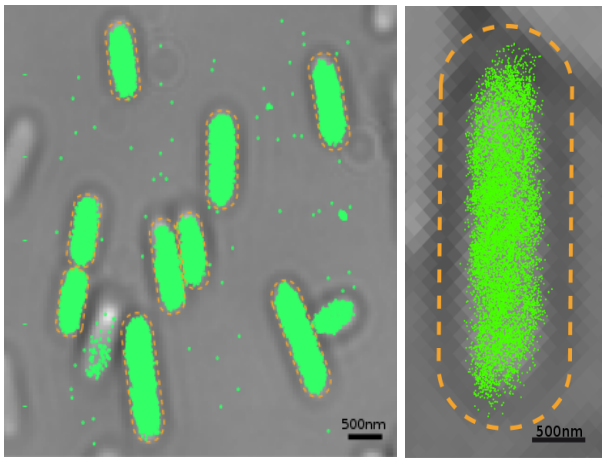
What is the architecture of the partition complex ?

- **Stochastic self-assembly of ParB proteins at centromeres builds bacterial DNA segregation apparatus**, A. Sanchez, D. Cattoni, J-C. Walter, J. Rech, A. Parmeggiani, M. Nollmann & J-Y. Bouet, *Cell Systems* (2015).

Super-resolution microscopy: Spatial distribution of ParB

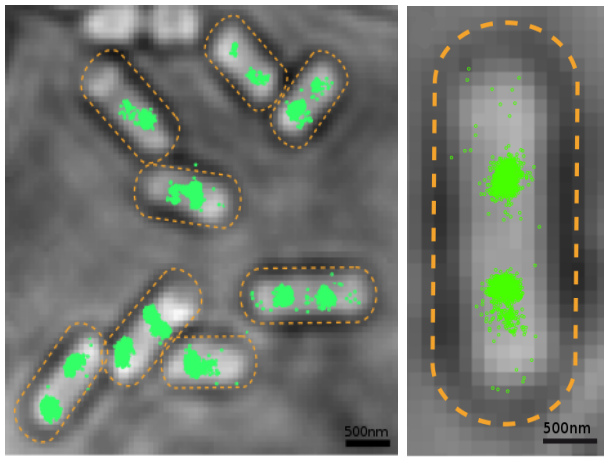
Foci are nucleated by *parS*

Super-Resolution microscopy (PALM)
D. Cattoni, A. Le Gall, M. Nollmann (Centre de Biochimie Structurale, Montpellier)



specific binding

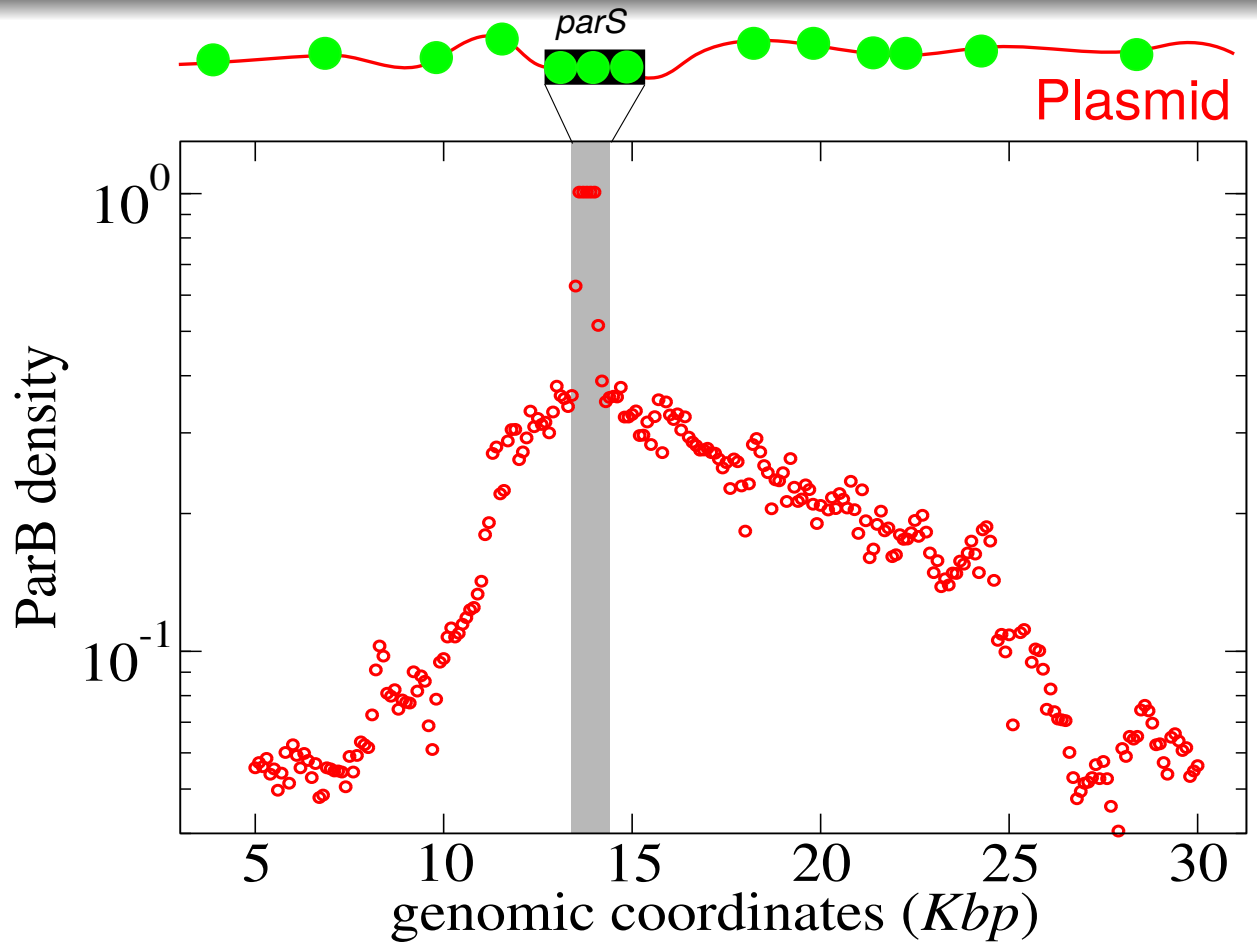
ParB/*parS*



- Focus diameter (upper bound) $150 \pm 20nm$
- Fixed number of ParB in a focus [≈300 ParB dimers/focus]
- Most of the ParB (≈ 90%) are located in the foci

ChIP-sequencing: ParB distribution along the plasmid

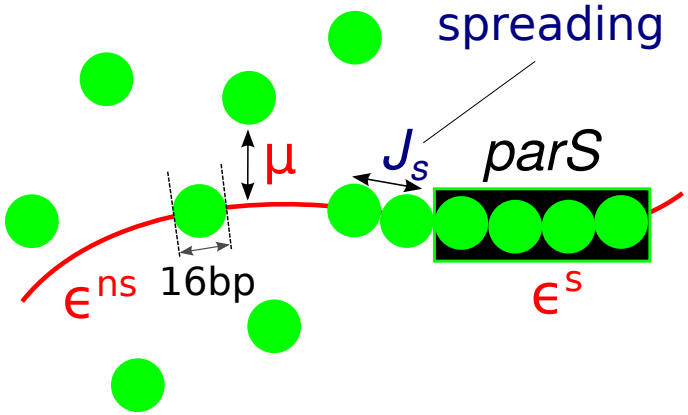
ParB density along the plasmid

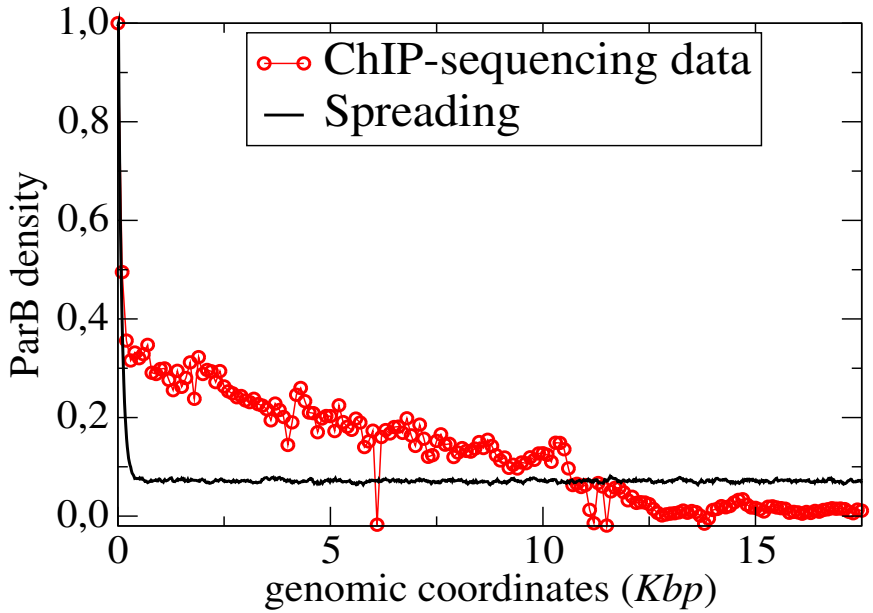


ChIP-sequencing: A. Sanchez, R. Diaz & J-Y. Bouet (LMGM, Toulouse, France)

Modeling of the partition complex

Spreading model

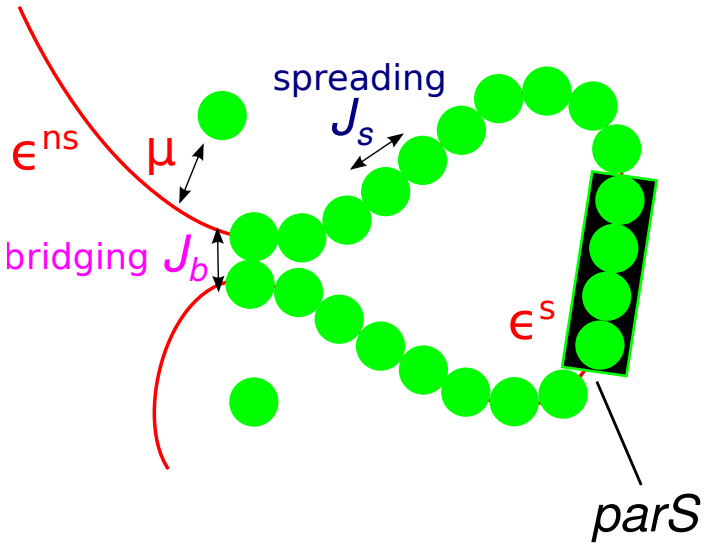


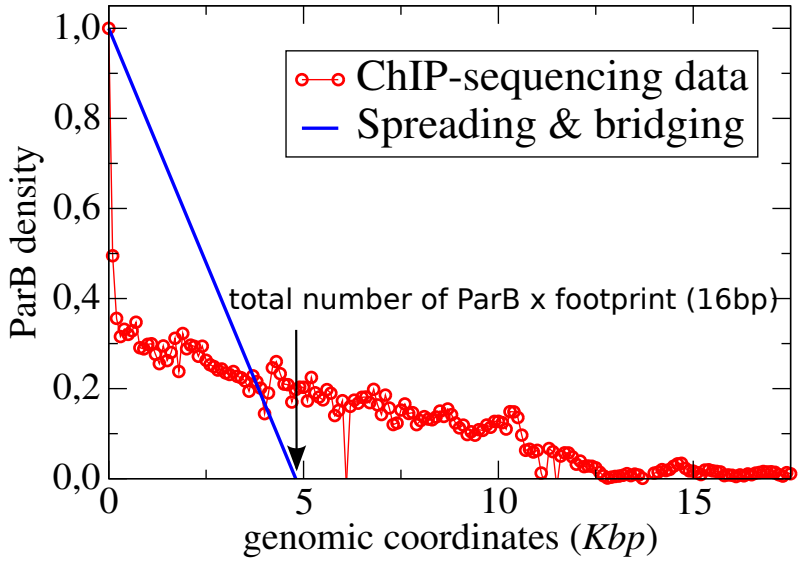


$$\mathcal{H} = -J_s \sum_i \phi_i \phi_{i+1} - \sum_i (\mu + \epsilon_i)\phi_i$$

- $\epsilon_i = \epsilon^s$ and $\epsilon_i = \epsilon^{ns}$ for specific and non-specific sites, respectively.
- Monte Carlo simulations: $J_s = 6kT$, $\epsilon^{ns} = 6kT$, $\epsilon^s = 15kT$ and $\mu = -12.17kT$ (300 particles).

Spreading & bridging model

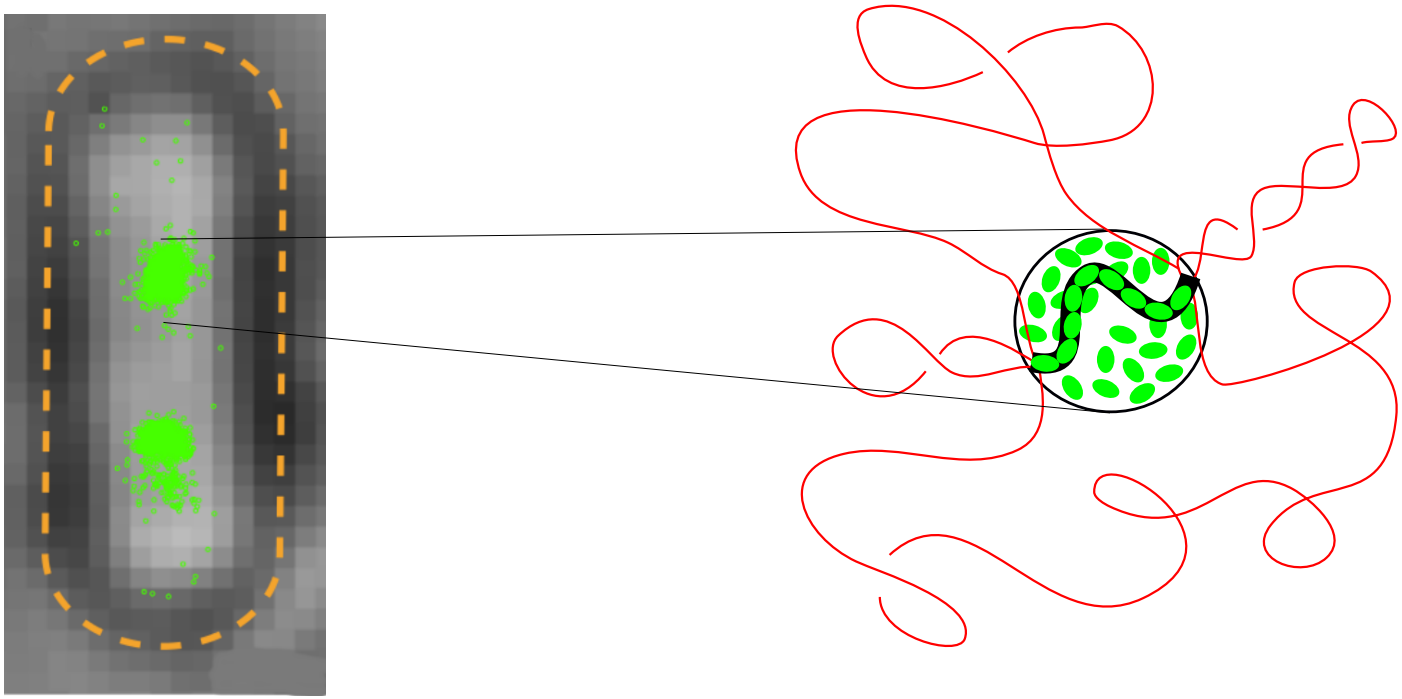




C.P. Broedersz, X. Wang, Y.M. Meir, J.J. Loparo, D.Z. Rudner & N.S. Wingreen, PNAS (2014).

$$\mathcal{H} = \mathcal{H}_{DWLC} - J_s \sum_i \phi_i \phi_{i+1} - J_b \sum_{\langle i,j \rangle_{3D}} g_{ij} \phi_i \phi_j - \sum_i (\mu + \epsilon_i) \phi_i$$

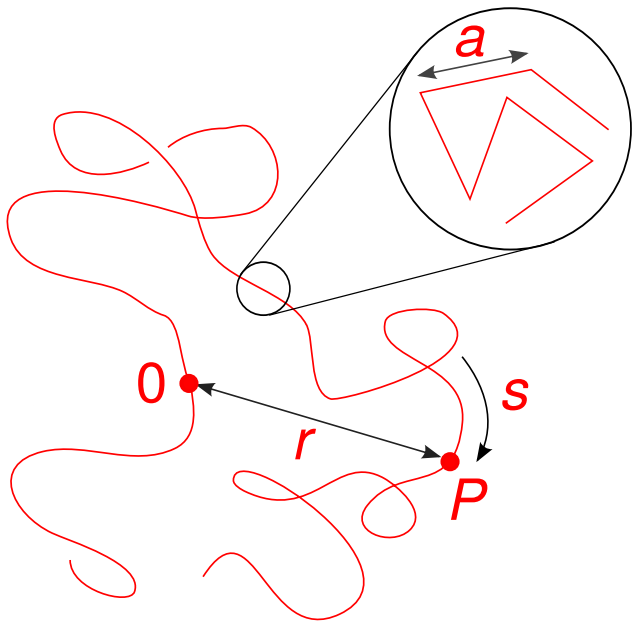
The stochastic binding model



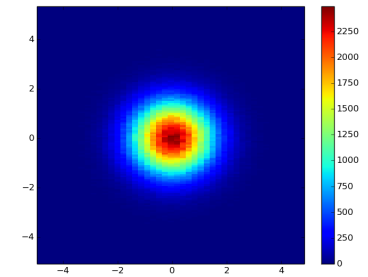
The stochastic binding model: polymer conformation

Freely-jointed chain:

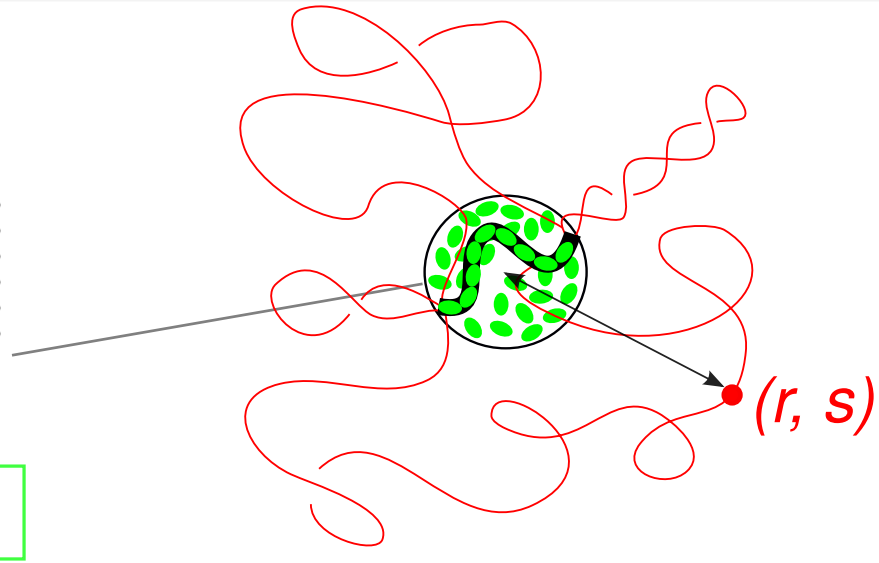
$$P(r,s) \sim \frac{1}{s^{3/2}} e^{-\frac{3r^2}{2R(s)^2}} \qquad \text{where} \quad R(s) = \sqrt{as}$$



The stochastic binding model

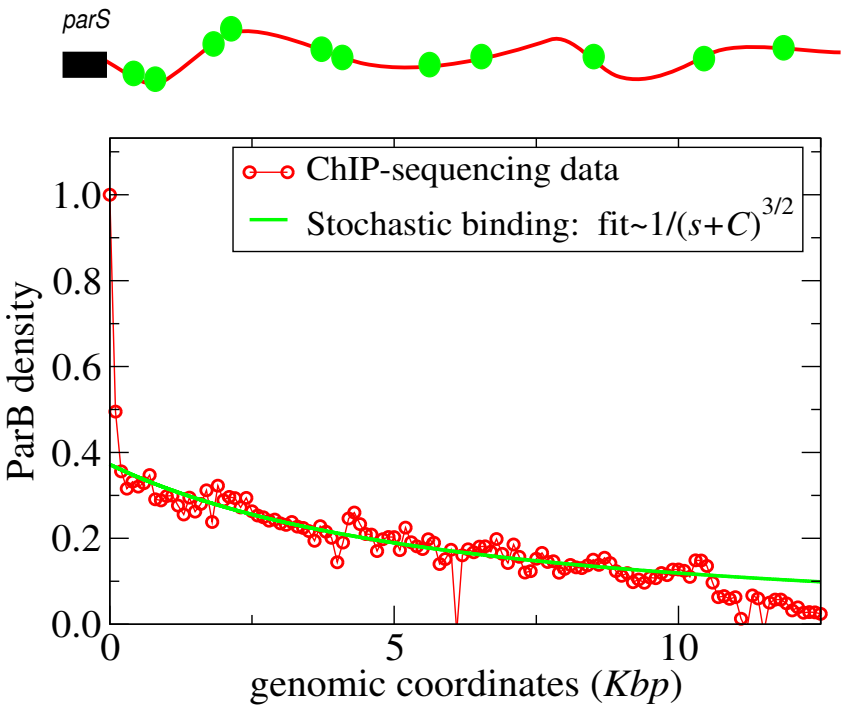


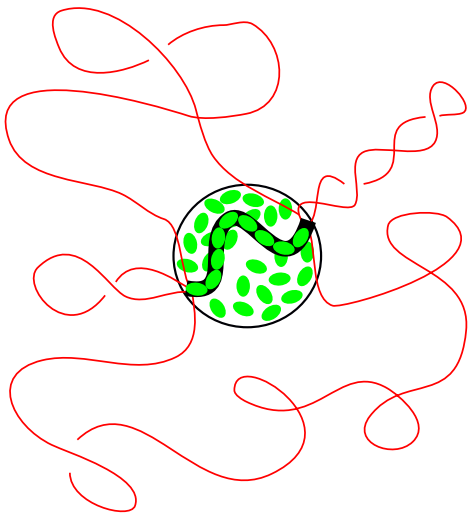
$C(r) \sim e^{-r^2/(2\sigma^2)}$



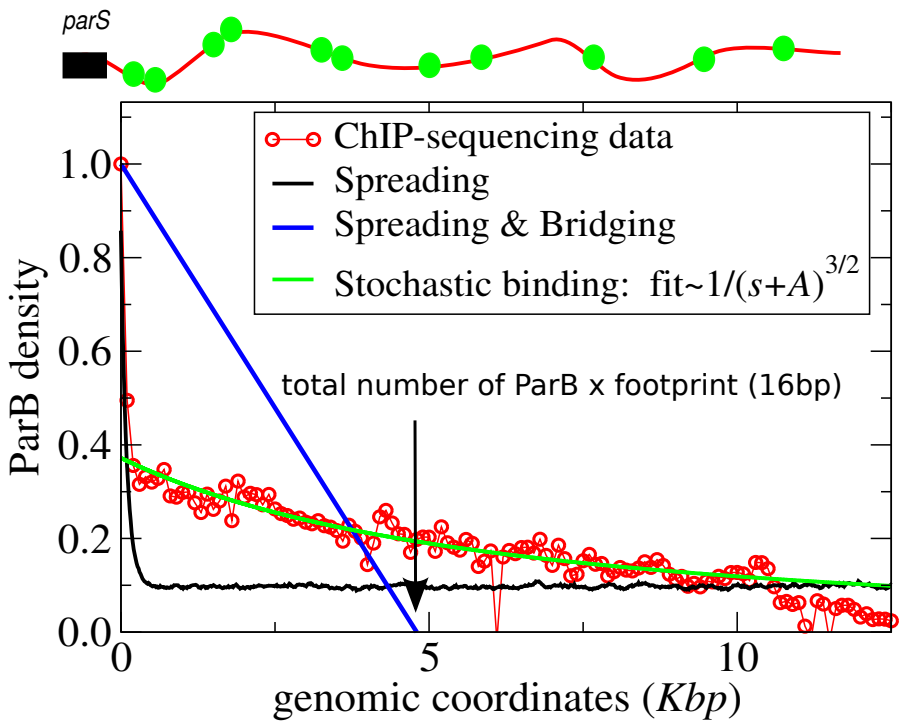
$$
\begin{aligned}
P_{binding}(s) &= \int_V d^3\vec{r}\; P(r,s)\, C(r)\,, \\
&\sim \int_V d^3\vec{r}\; \frac{1}{s^{3/2}}\, e^{-\frac{3r^2}{2R(s)^2}}\, e^{-\frac{r^2}{2\sigma^2}}\,, \\
&\sim \frac{1}{(s+C)^{3/2}} \quad \text{where } C = 3\frac{\sigma^2}{a}
\end{aligned}
$$

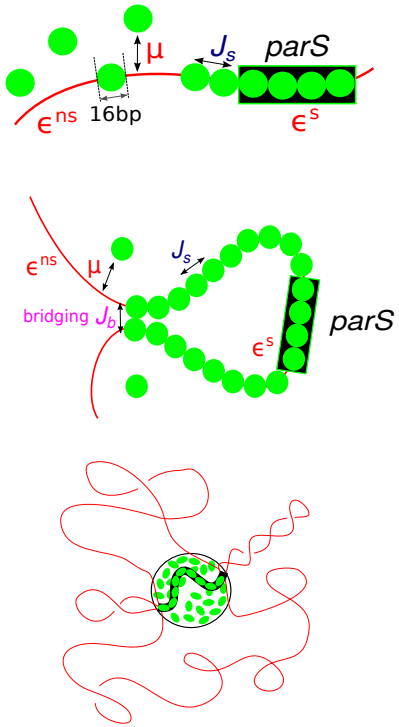
The stochastic binding model





The stochastic binding model





Summary

- Combination of approaches: Super-resolution microscopy, ChIP-sequencing & physical models: decipher the architecture of the partition complex.
  - ParB organized spatially in foci,
  - Linear density: freely fluctuating plasmid in a focus of ParB.
- Stochastic binding in better agreement vs. previous models for the plasmid F.
- General mechanism potentially useful in other biological processes.
- Perspectives: modeling of the dynamical phase with ParA.

ChIP-sequencing

A. Sanchez
R. Diaz
J. Rech
J-Y. Bouet

Super-resolution microscopy PALM

D. Cattoni
A. Le Gall
M. Nollmann

Physical modeling

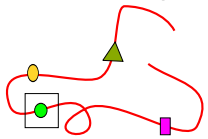
J. Dorignac
F. Geniet
V. Lorman
J. Palmeri
A. Parmeggiani

ChIP-sequencing

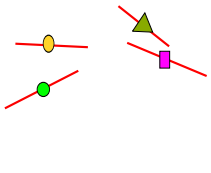
ChIP-sequencing: A. Sanchez, R. Diaz, J. Rech & J-Y. Bouet
Laboratoire de Microbiologie et Génétique Moléculaires, Toulouse, France

1. Liquid culture

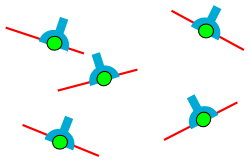
2. Formaldehyde cross-links



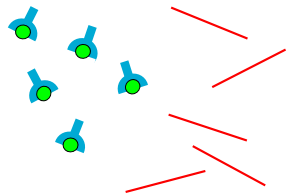
3. Sonication



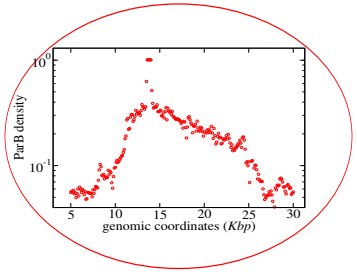
4. Immunoprecipitation



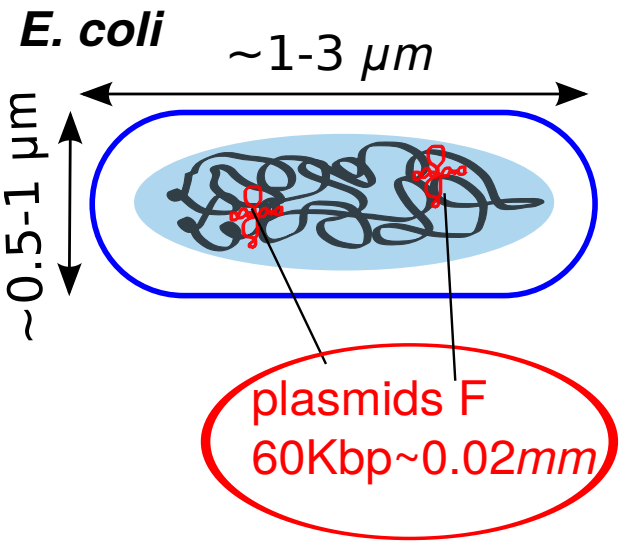
5. Reverse cross-links

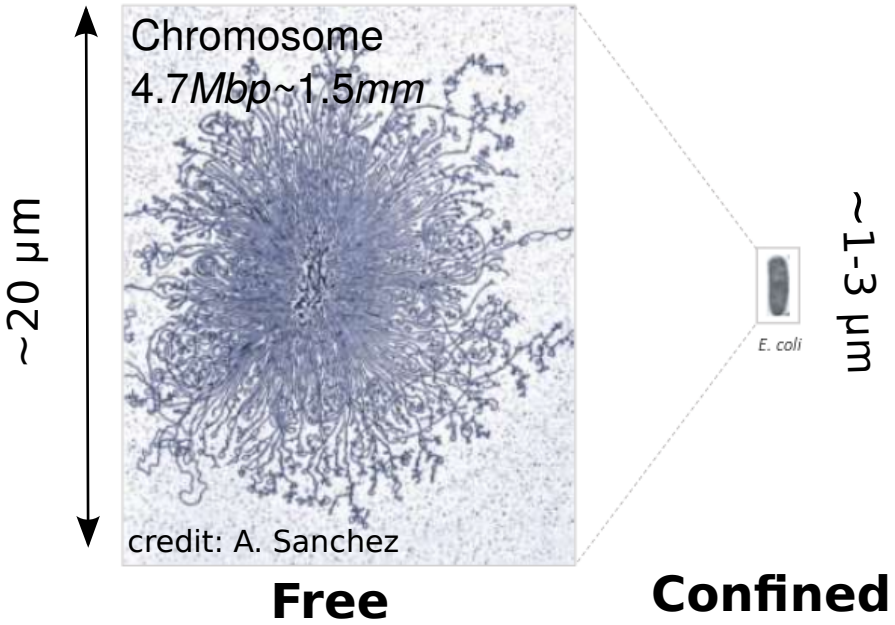


6. DNA sequencing and Analysis

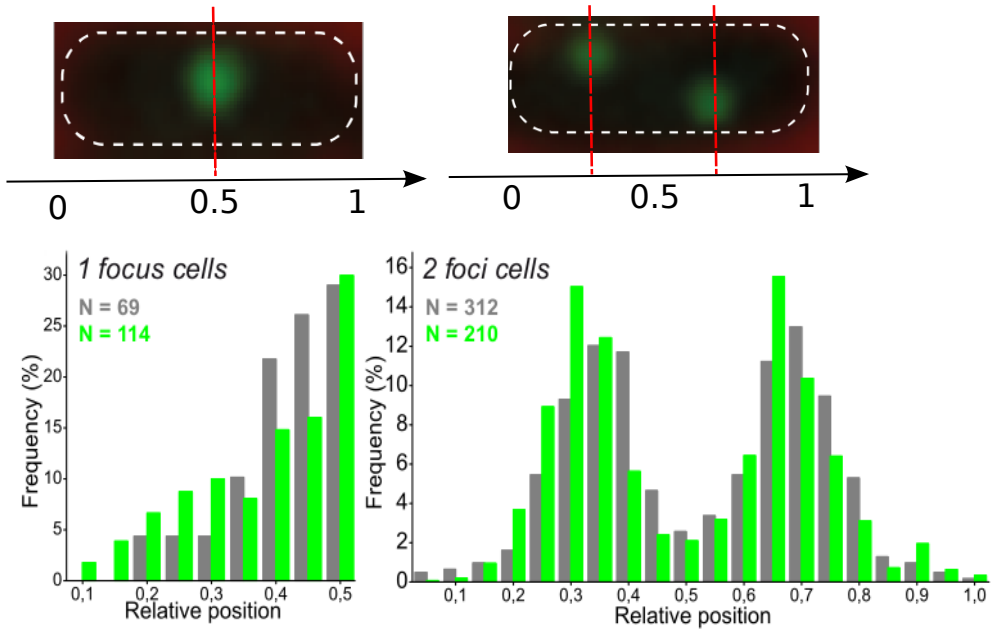


Physical dimensions of bacteria



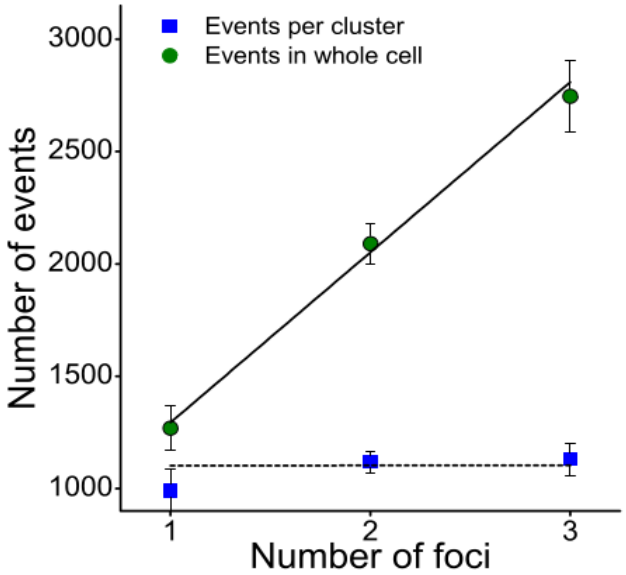


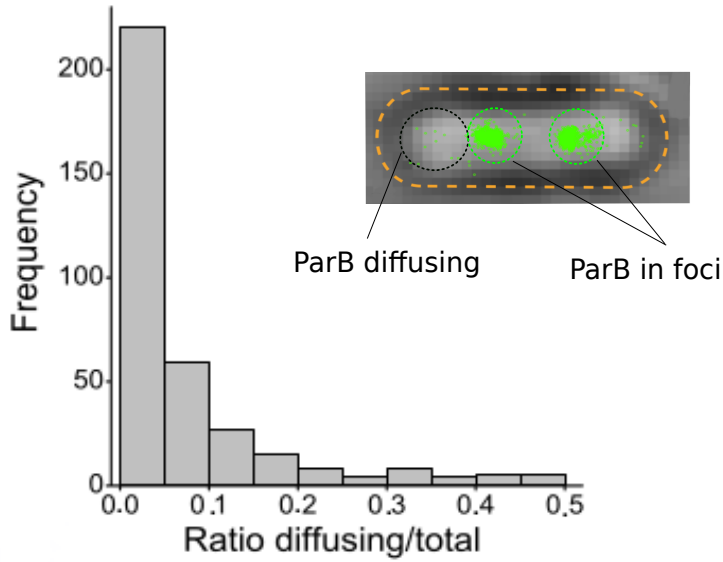
Position of the foci in the cell



D. Cattoni & M. Nollmann, Single Molecule Localization Microscopy (PALM)

Characteristics of foci





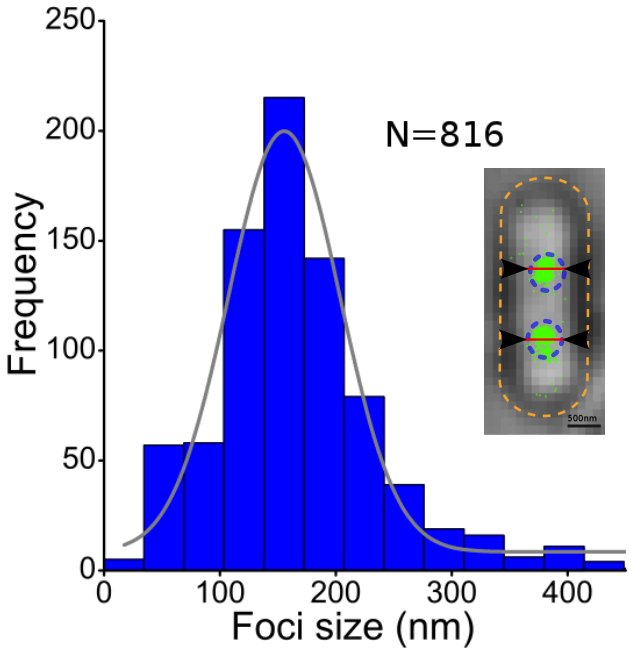
D. Cattoni & M. Nollmann, Single Molecule Localization Microscopy (PALM)

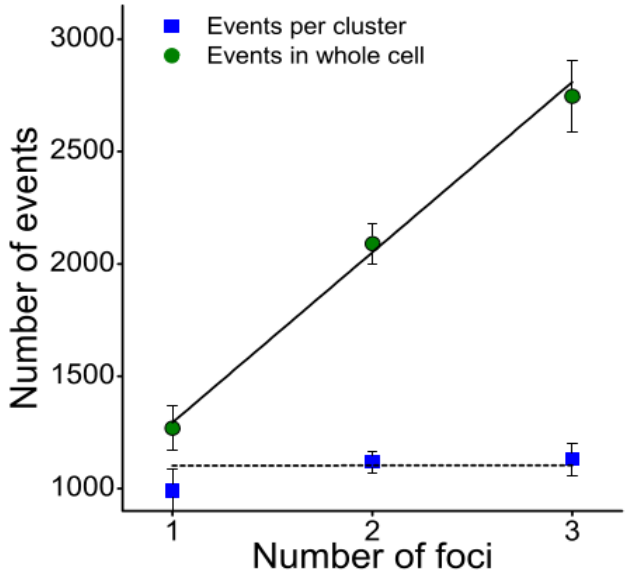
- Constant value of ParB in a focus

  [≈300 ParB dimers/focus, Bouet *et al* '05, molecular biology methods]

- Most of the ParB (≈ 90%) are located in the foci

Characteristics of foci





D. Cattoni & M. Nollmann, Single Molecule Localization Microscopy (PALM)

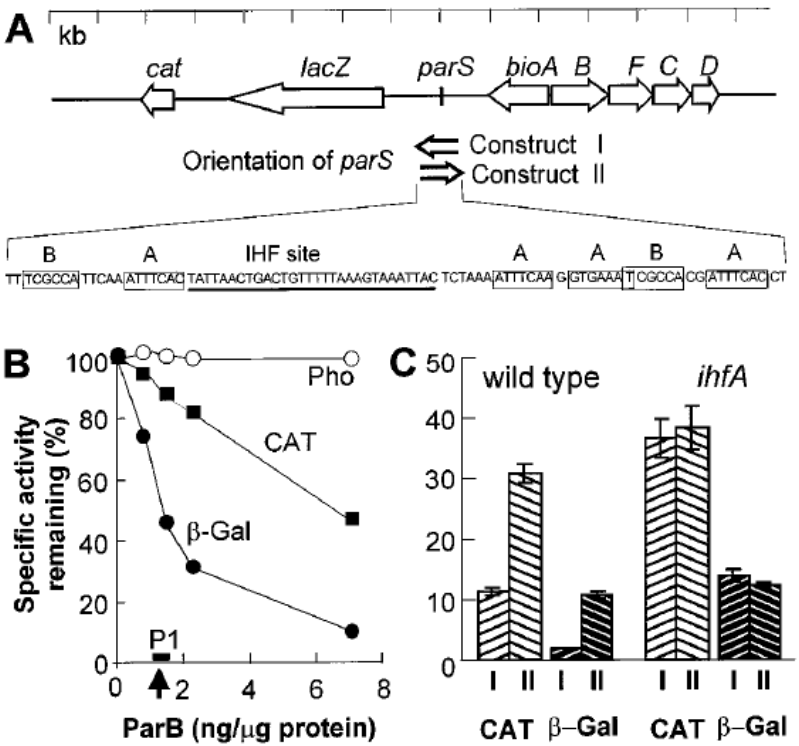
- Focus diameter (upper bound) $150 \pm 20nm$
- Fixed number of ParB in a focus [≈300 ParB dimers/focus]
- Most of the ParB (≈ 90%) are located in the foci

Silencing of genes

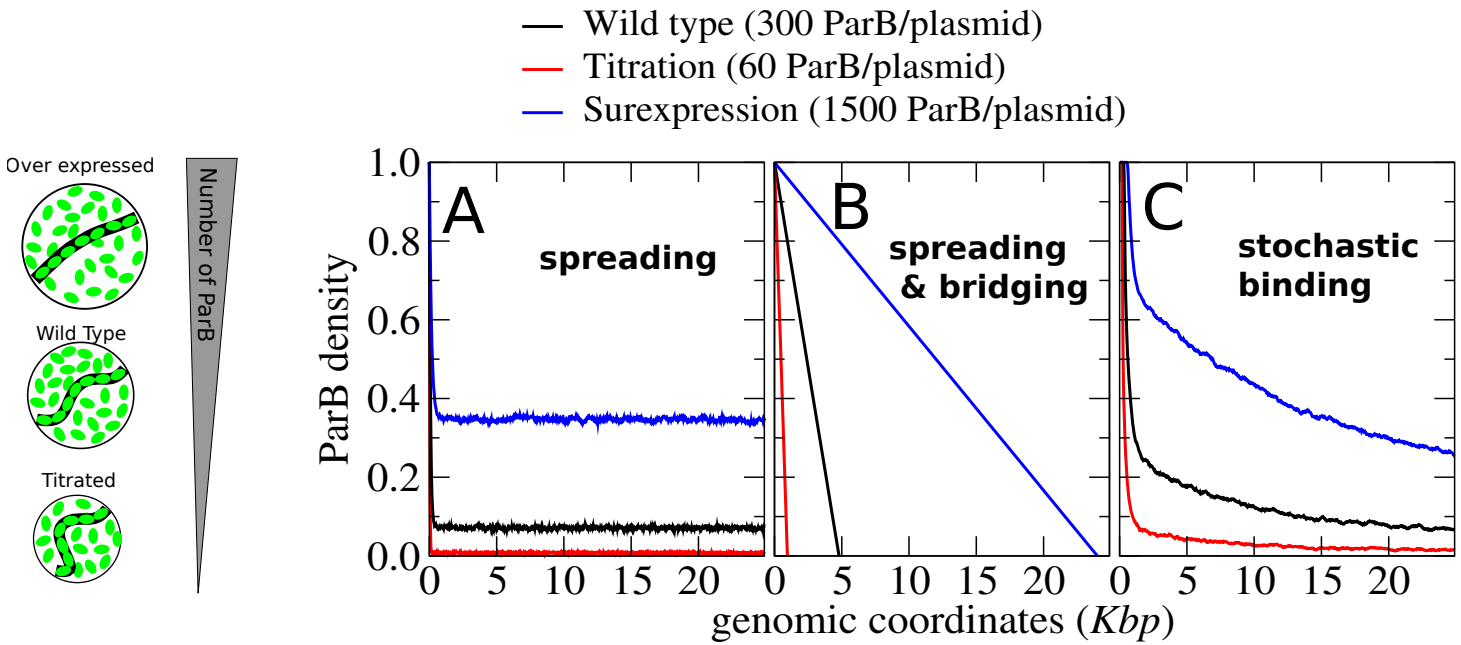
Silencing of Genes Flanking the P1 Plasmid Centromere

Oleg Rodionov, Małgorzata Łobocka,* Michael Yarmolinsky†

22 JANUARY 1999 VOL 283 SCIENCE

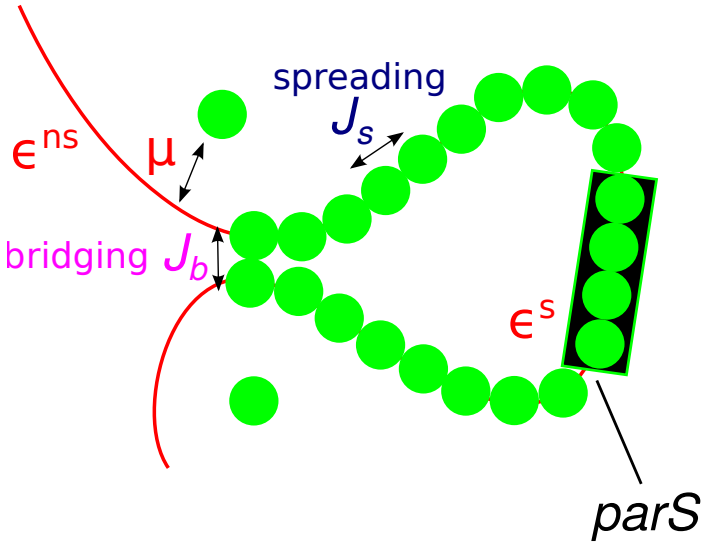


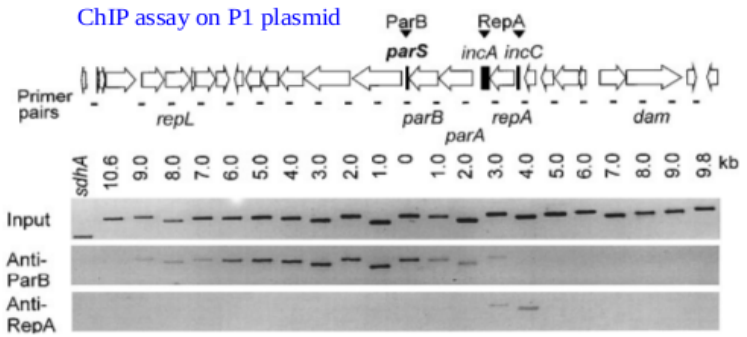
Perspectives: Variation of ParB expression



(B) C.P. Brœdersz, X. Wang, Y.M. Meir, J.J. Loparo, D.Z. Rudner & N.S. Wingreen, *PNAS* (2014)

Spreading & bridging model





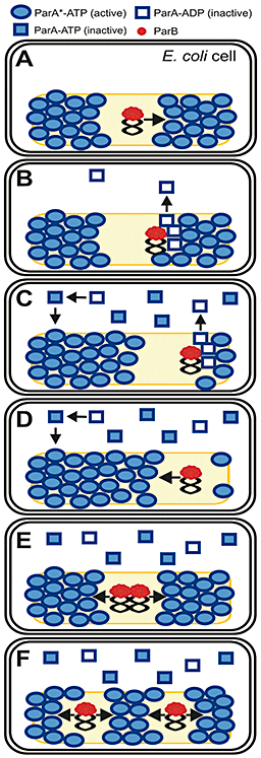
Rodionov, Science 1999

C.P. Broedersz, X. Wang, Y.M. Meir, J.J. Loparo, D.Z. Rudner & N.S. Wingreen, PNAS (2014).

$$\mathcal{H} = \mathcal{H}_{DWLC} - J_s \sum_i \phi_i \phi_{i+1} - J_b \sum_{\langle i,j \rangle_{3D}} g_{ij} \phi_i \phi_j - \sum_i (\mu + \epsilon_i) \phi_i$$

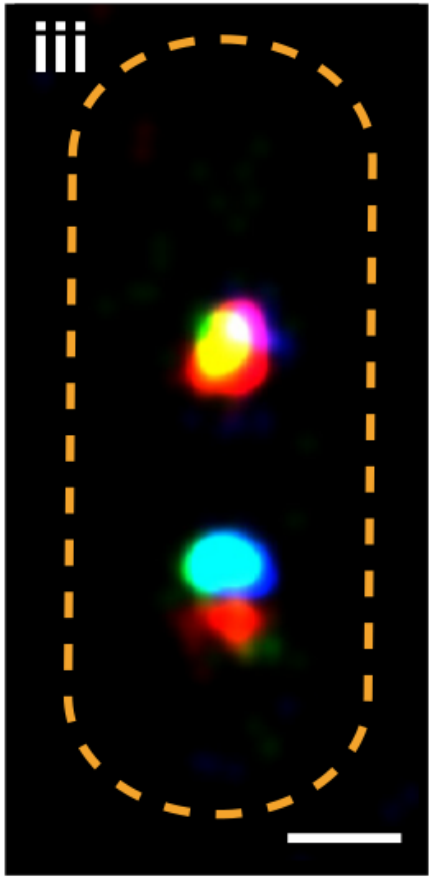
- $\epsilon^{ns} \approx 1kT$, $\epsilon^s = 10kT$, $J_s = 6 - 8kT$

Reaction-Diffusion



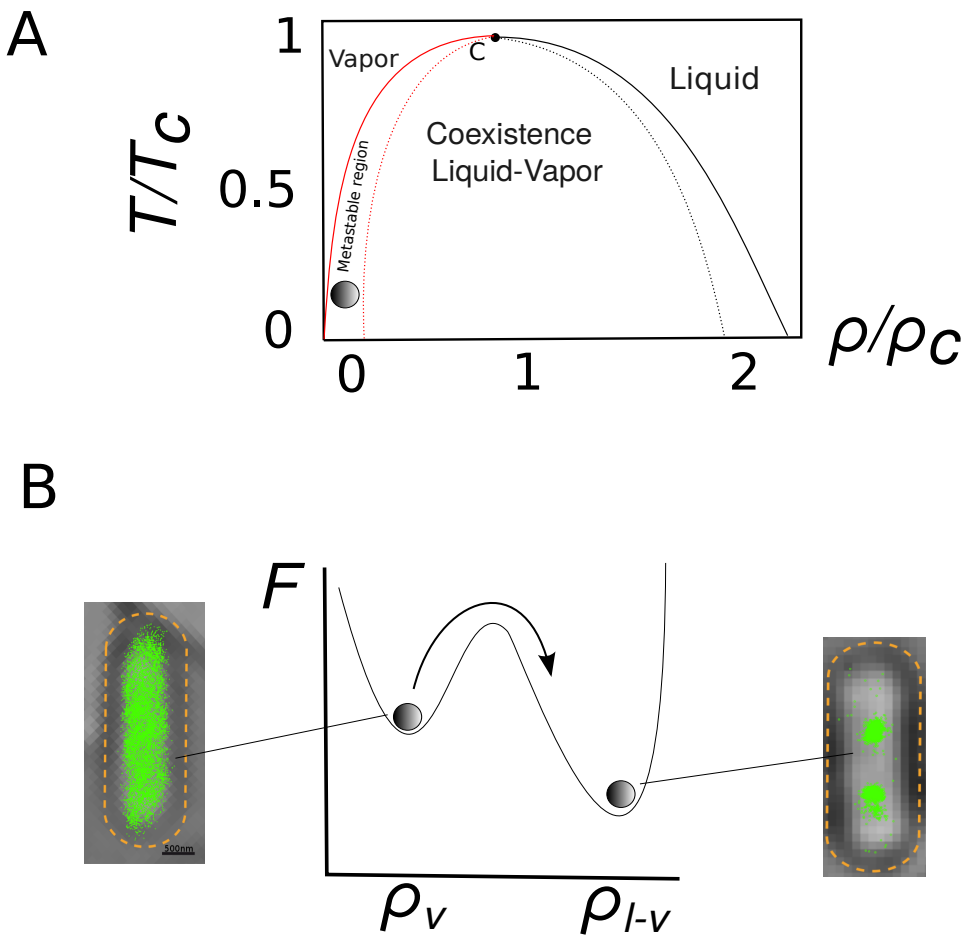
Vecchiarelli et al *Molecular Microbiology* (2010).

ParB is confined in foci

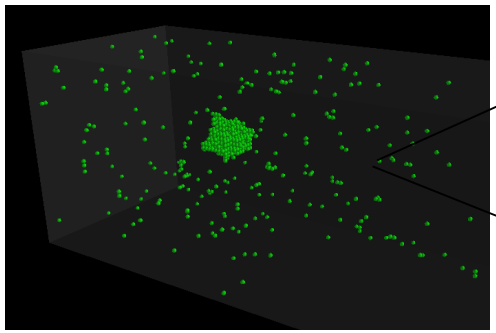


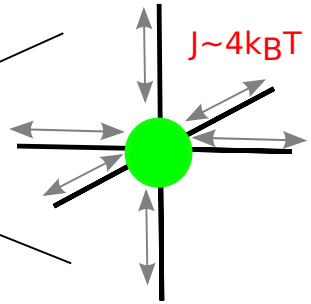
D. Cattoni & M. Nollmann, Single Molecule Localization Microscopy (PALM)

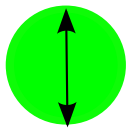
Nucleation theory



The lattice gas (COP Ising model)





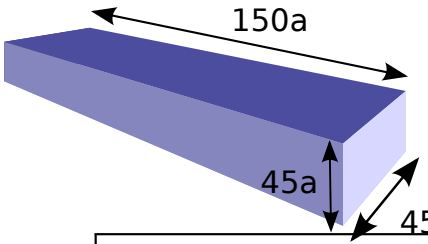


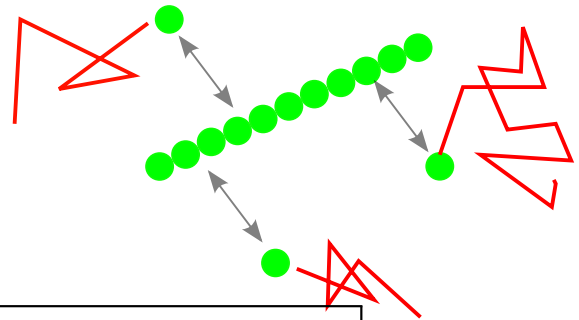
a=5 nm (~16bp)

DNA: chromosome + F-plasmid ~ 5Mbp

5Mbp/16bp~300,000 binding sites

parS: **10 fixed particles on the lattice**





$$E = -J \sum_{\langle i,j \rangle} \phi_i \cdot \phi_j \qquad (\phi_i = 0 \text{ or } 1)$$

Liquid-vapor transition: effect of nucleation