Architecture of a bacterial DNA segregation apparatus: active caging of ParB by stochastic self-assembly nucleated from the centromere

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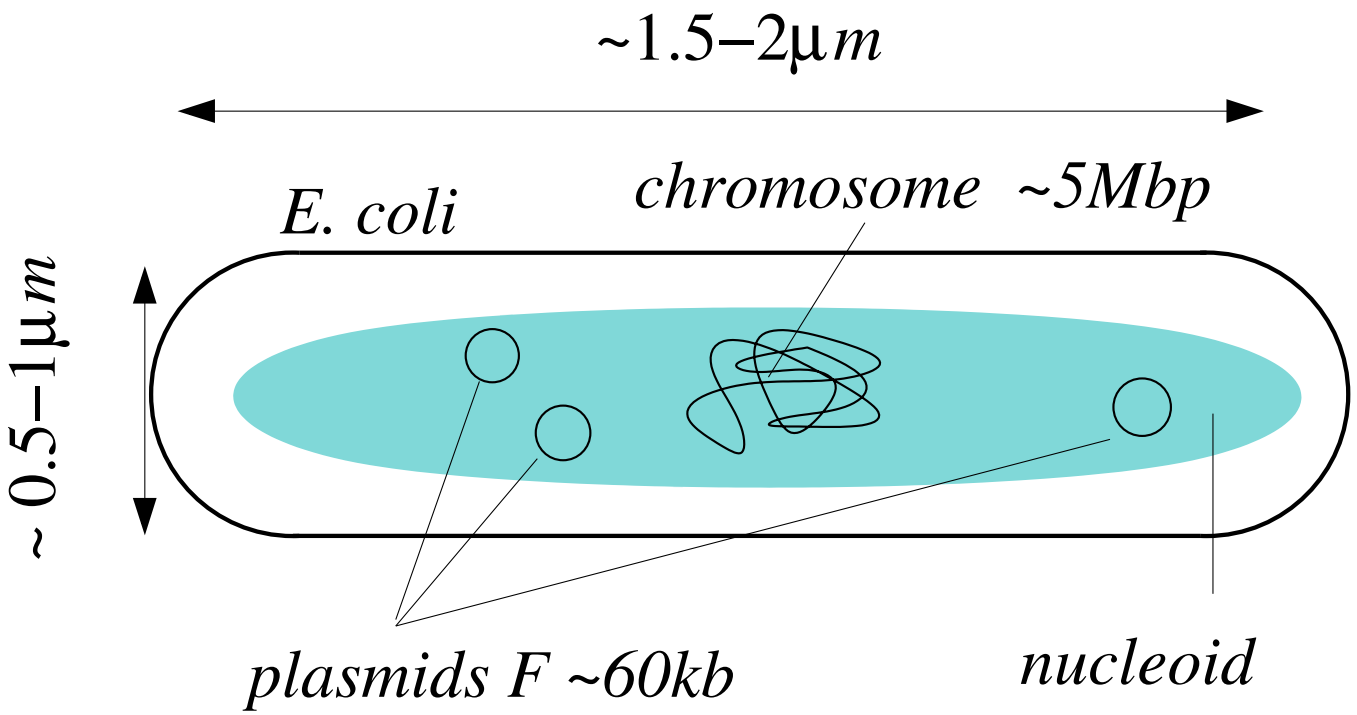
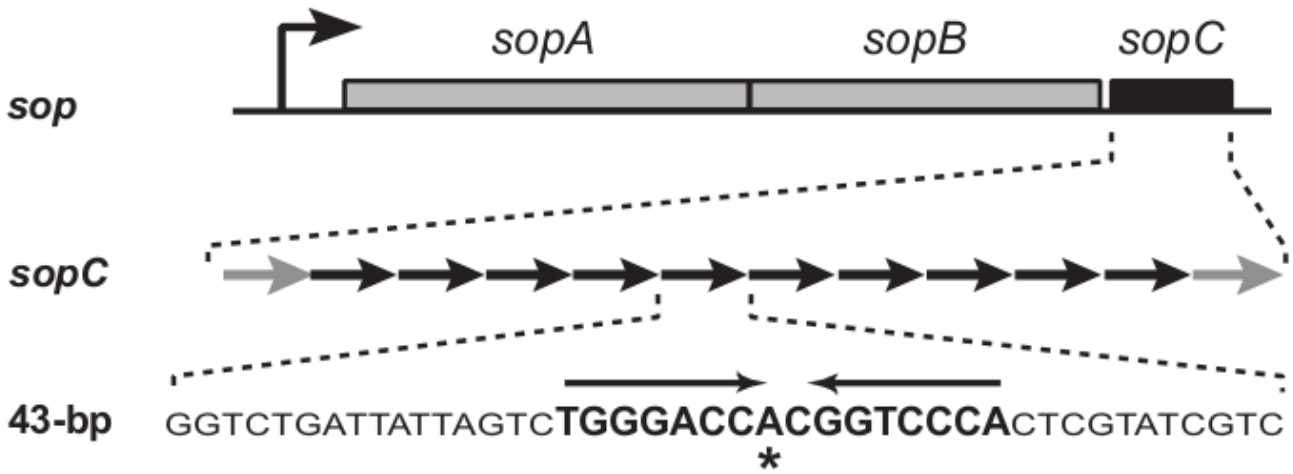
Intro: DNA segregation & Active partition system



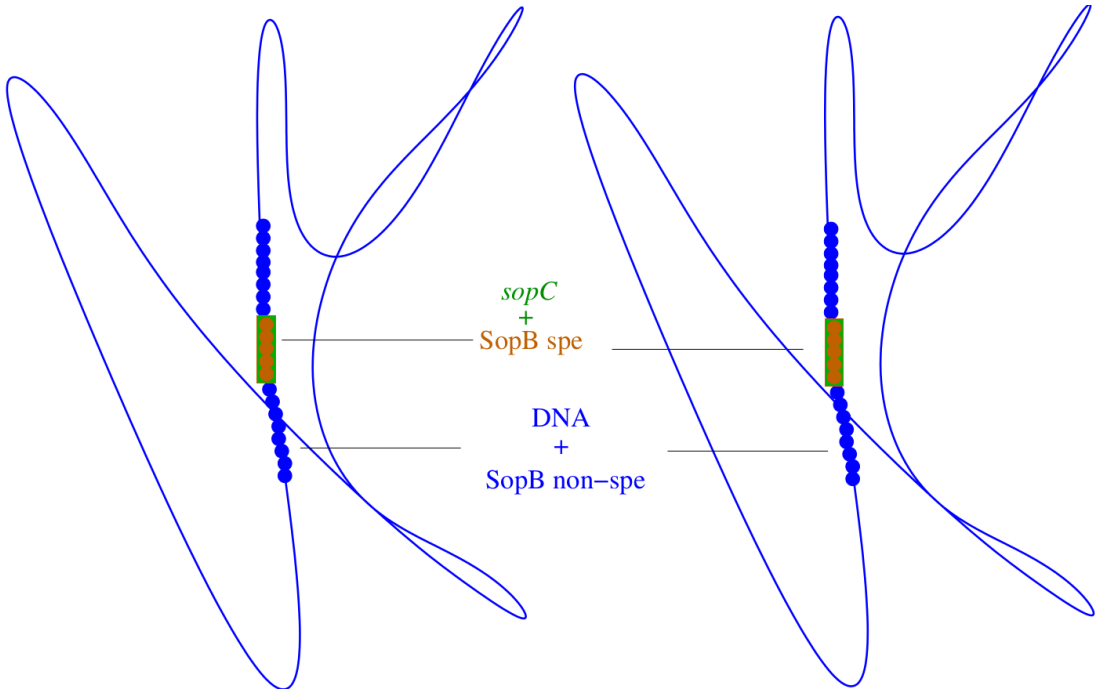
- Plasmid F: active partition system ParABS (=SopABC)
- Only type present on bacterial chromosome
- Most prevalent in low copy plasmids

The active partition system ParABS (SopABC)



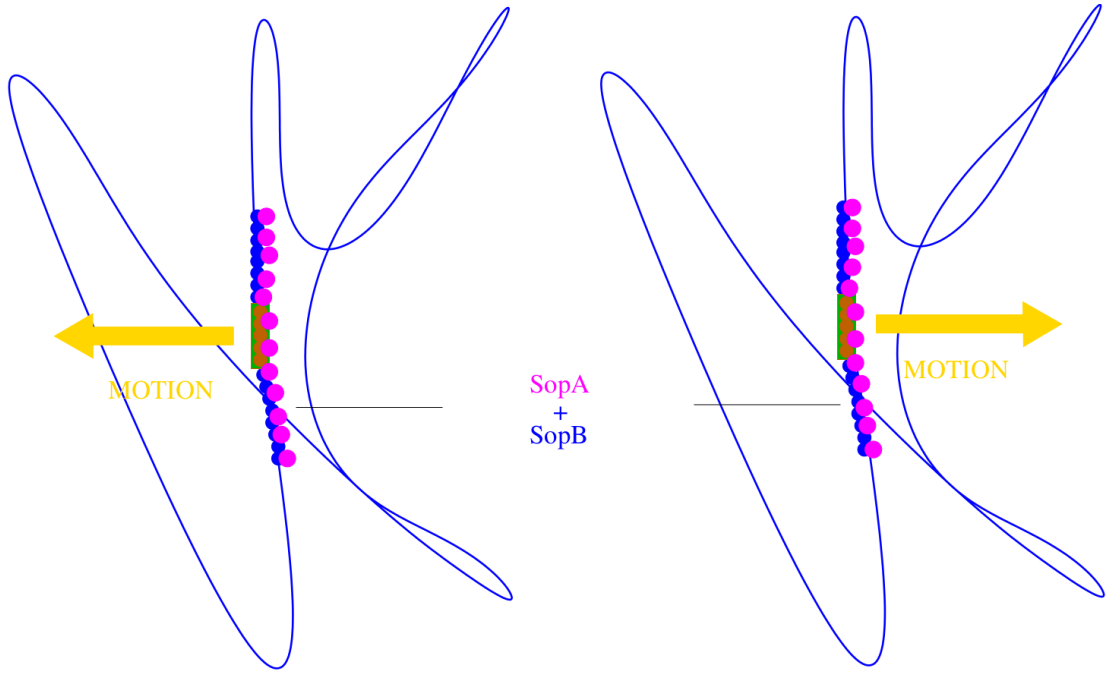
- SopA (ParA): motor protein
- SopB (ParB): binding protein
- *sopC (parS)*: centromere-like DNA sequence

The partition system ParABS (SopABC)



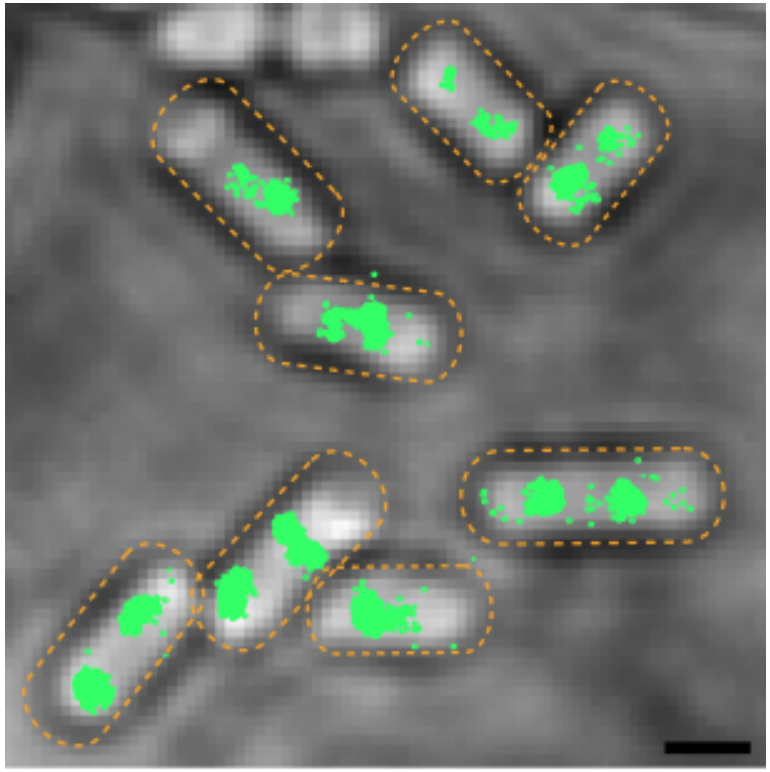
Step 1: formation of the partition complex on the two replicas (during/after replication)

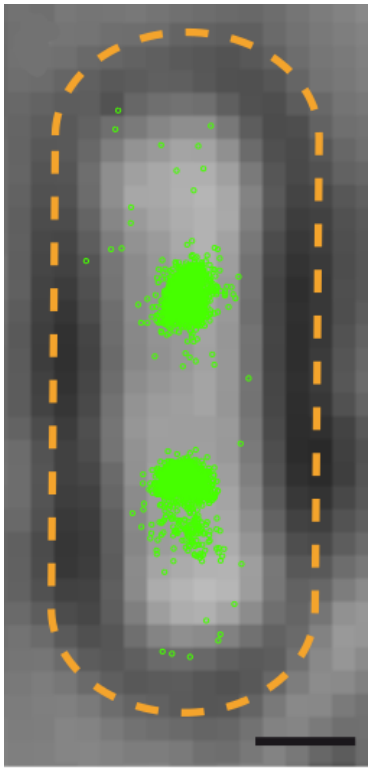
The partition system ParABS (SopABC)



Step 2: Separation of the two replicas.

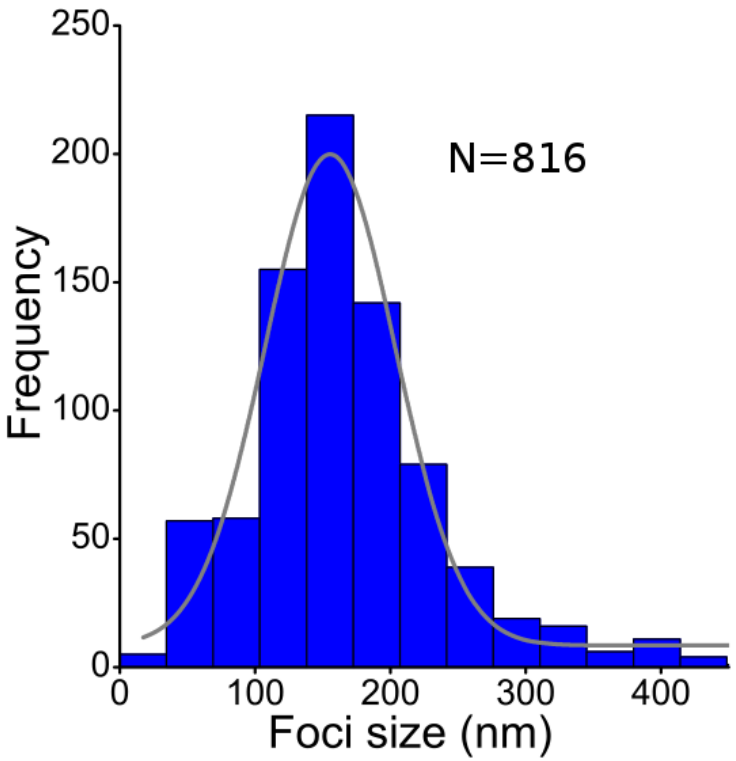
SopB is highly confined in foci

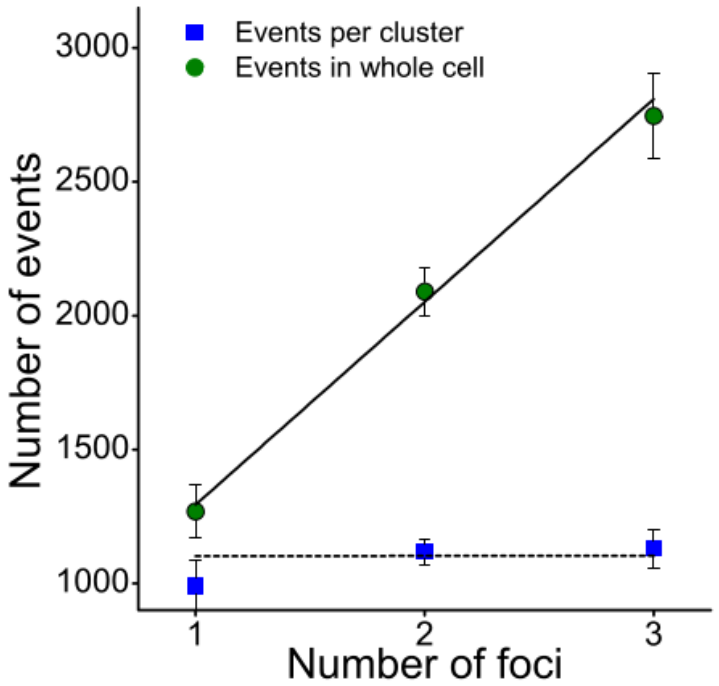




D. Cattoni & M. Nollmann, Single Molecule Localization Microscopy (PALM)

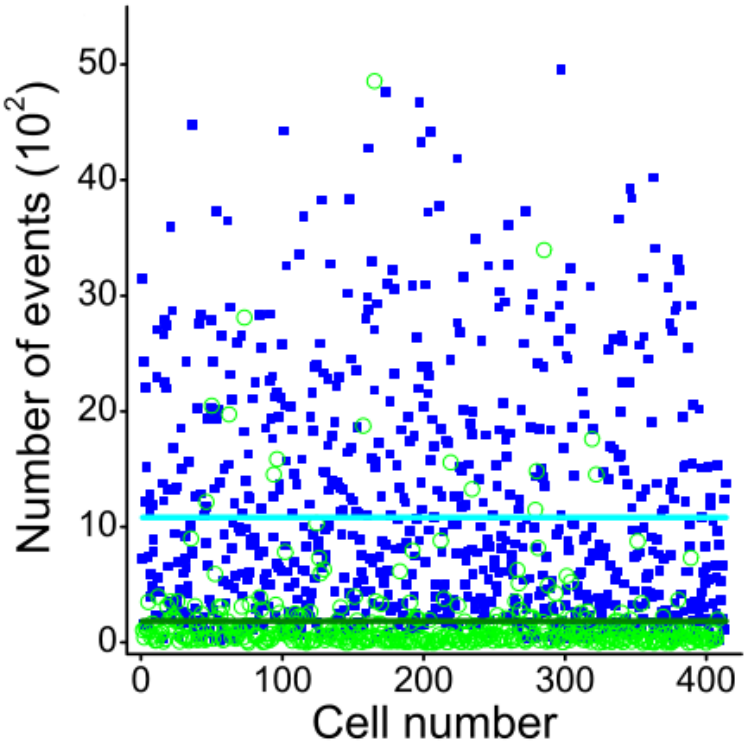
SopB is highly confined in foci

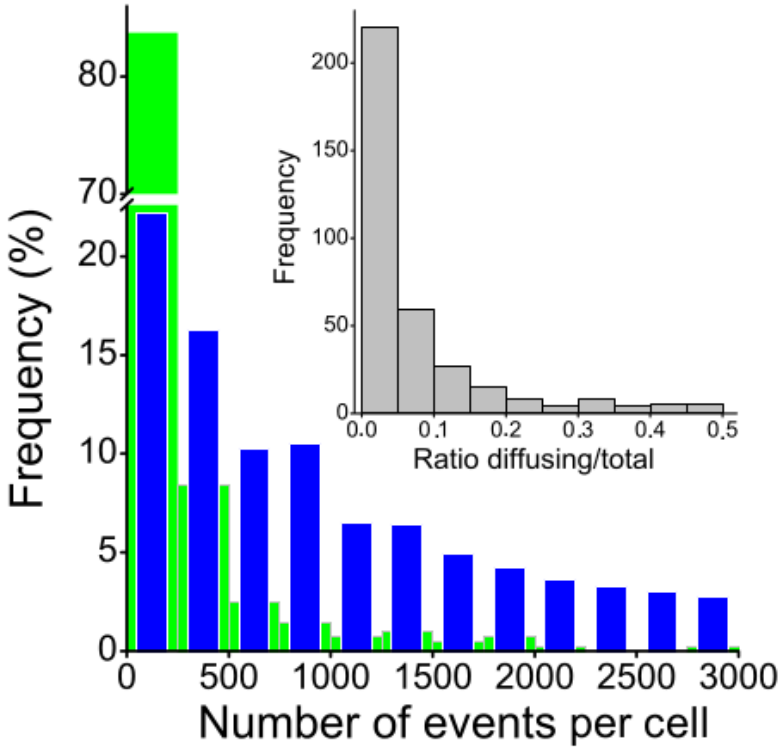




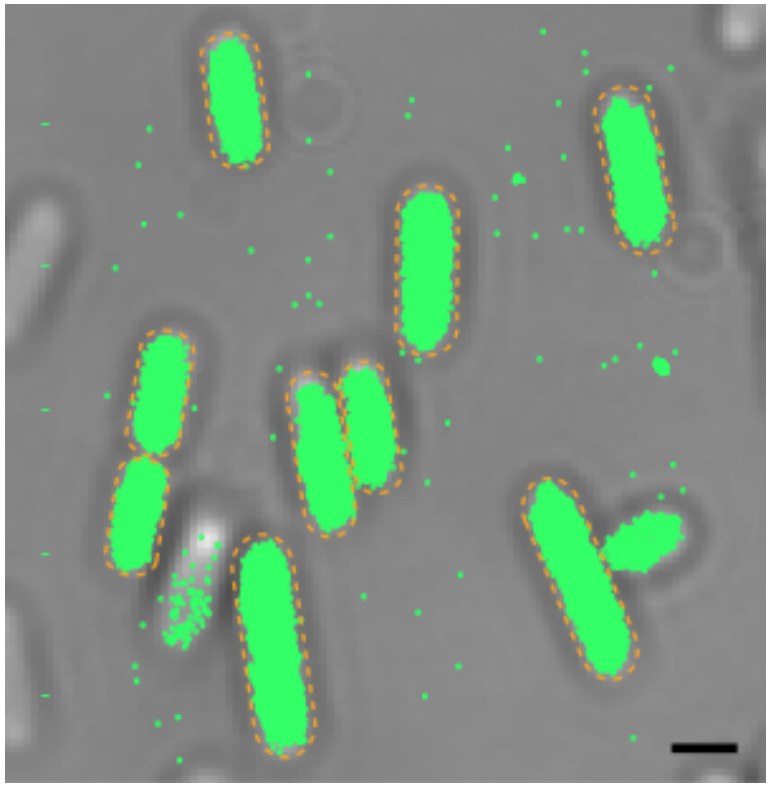
Foci diameter $150 \pm 20nm$

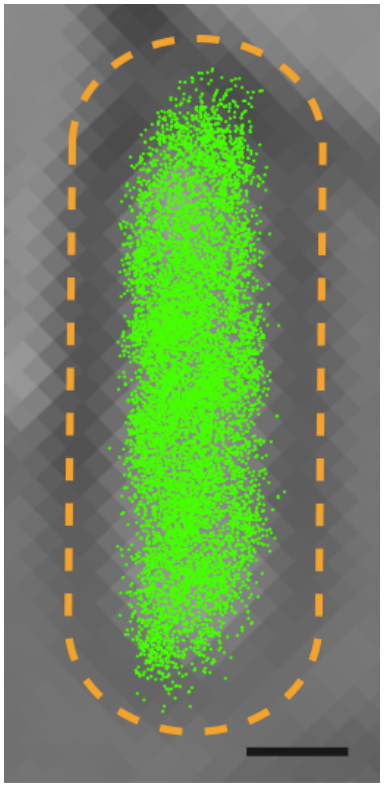
SopB is highly confined in foci



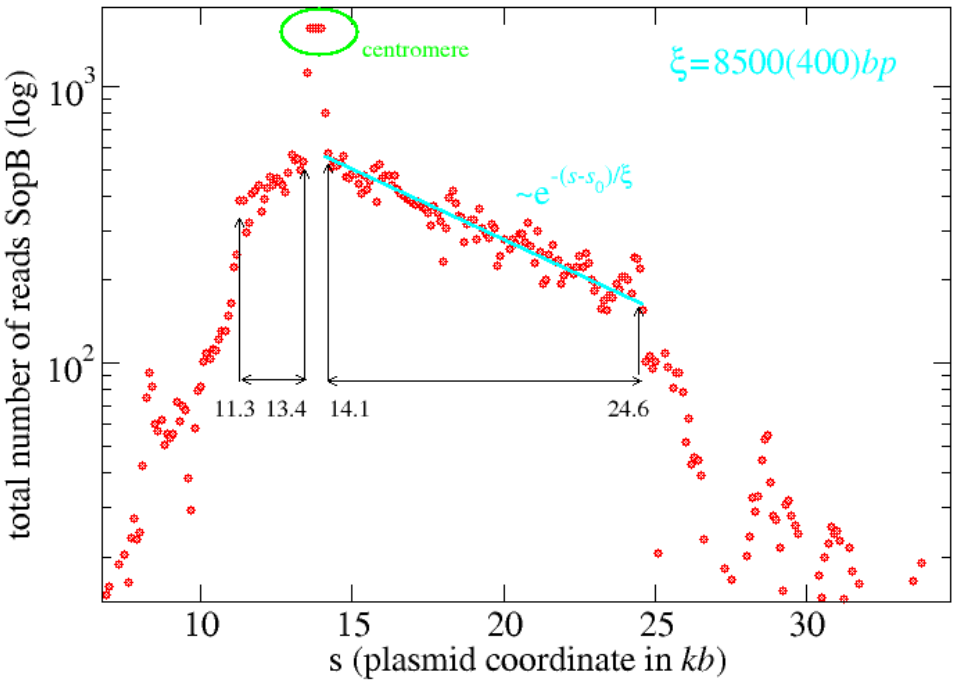


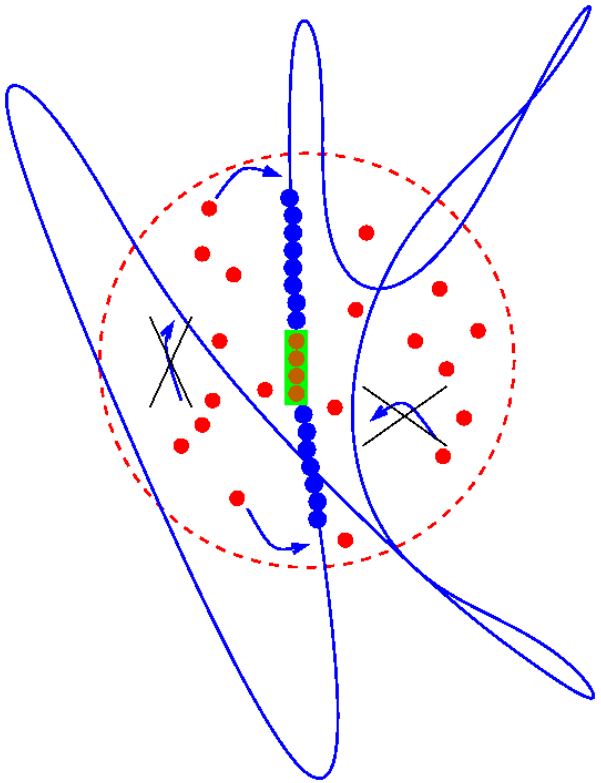
sopC is necessary to form foci





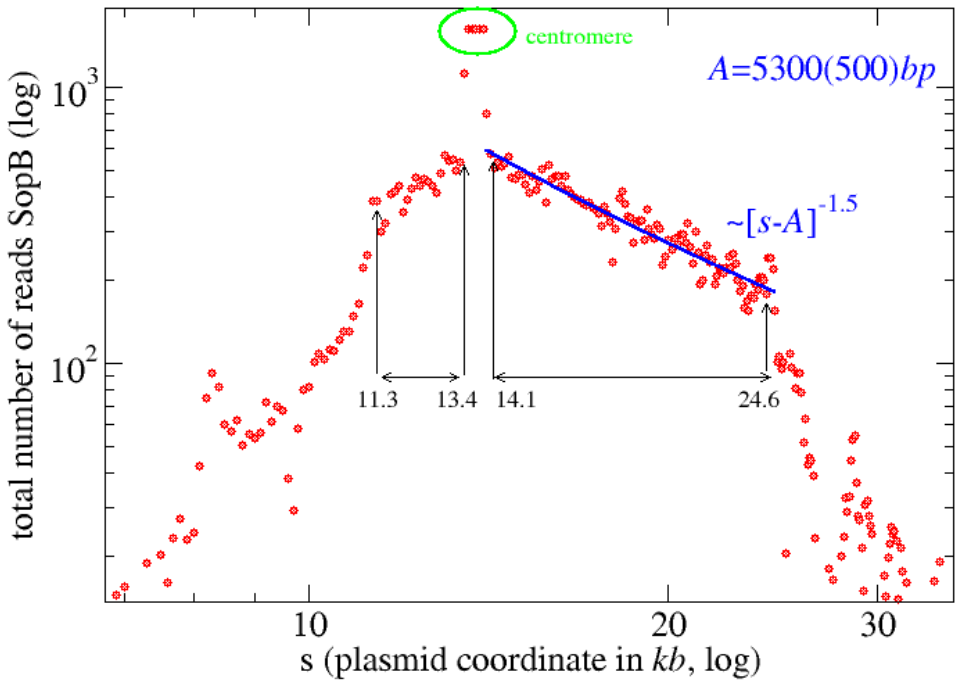
Spreading of SopB along the plasmid: polymerization

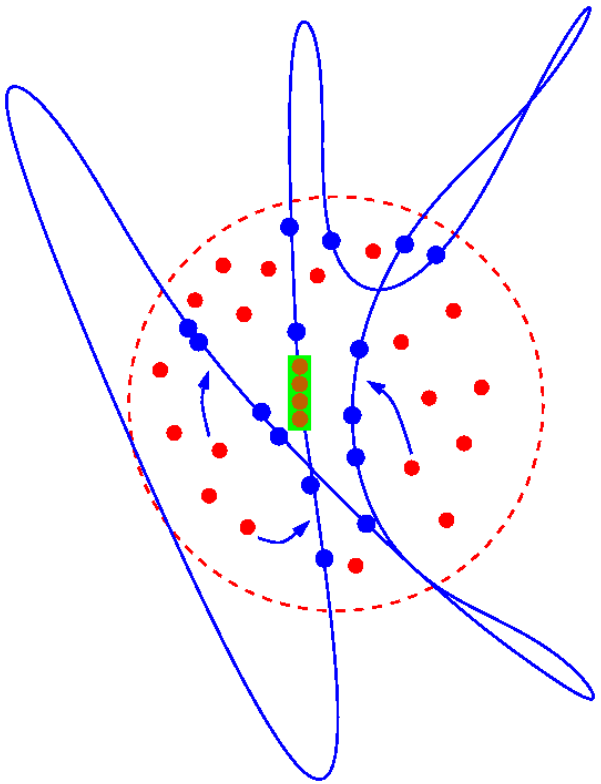




A. Sanchez, J. Rech, J-Y. Bouet, ChIP-sequencing

Spreading of SopB along the plasmid: “stochastic binding”





"stochastic binding": Preliminary quantitative calculation

Plasmid modeled by a Freely-jointed chain of Kuhn length $a = 300bp$

$$P_{binding}(s) = \int_0^\infty d^3\vec{r}\; P(r,s)\, c_{SopB}(r) \propto \frac{1}{[s-A]^{1.5}}$$

where

- $P(r,s) \propto s^{-1.5} e^{-3r^2/(2as)}$
- $c_{SopB}(r) \propto e^{-r^2/2\sigma^2}$
- $A = s_0 - 3\sigma^2/a^2$
- s_0 the position of the centromere

from the fitting of ChIP-sequencing data: foci of size $300nm$

Conclusion

- ParB is highly confined in foci ($\sim 10^3$ ParB per foci)
- Confinement requires spe and non-spe binding
- Propagation of ParB: cannot be explained by polymerization alone
- “Stochastic binding” scheme: conformation of the plasmid (further experiments, includes plectonemes in the model)
- Mechanism of caging of SopB ?

Sanchez A, Cattoni D I, Walter J-C, Rech J, Parmeggiani A, Nollmann M & Bouet J-Y, *submitted*

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Titration of SopB