Supporting Information

The bacterial cytoplasm has glass-like properties and is fluidized by metabolic activity

Bradley R. Parry, Ivan V. Surovtsev, Matthew T. Cabeen, Corey O'Hern, Eric Dufresne and Christine Jacobs-Wagner

Table of contents

Supplementary figure legends **3**
Figure S1. Crescentin-GFP structure tracking method 3
Figure S2. Cellular metabolism affects the mobility of storage granules in *C. crescentus* and of plasmids outside the nucleoid in *E. coli*. 3
Figure S3. Mobility of GFP-µNS particles in cells treated with various drugs 3
Figure S4. Nucleoid area in wild-type and *dnaC2* cells following DNP treatment 4
Figure S5. Estimation of the precision of our single-particle tracking method 4
Figure S6. Intensity distributions, binning, size determination and subdiffusion parameters of GFP-µNS particles 4
Figure S7. GFP-µNS particle displacements under untreated and DNP-treated conditions 5
Supplementary movie legends **5**
Movie S1. Movement of detached crescentin-GFP structures ceases when cell growth stops. 5
Movie S2. Crescentin-GFP structures sample the cytoplasmic space in metabolically active cells. 5
Movie S3. Crescentin-GFP structure mobility is dramatically reduced in carbon-starved cells. 6
Movie S4. Crescentin-GFP structure mobility is drastically reduced upon energy depletion by addition of 2,4-dinitrophenol (DNP). 6
Movie S5. Mini-RK2 plasmids are highly mobile under metabolically active conditions. 6
Movie S6. Mini-RK2 mobility is dramatically reduced upon energy depletion by DNP treatment. 6
Movie S7. GFP-µNS particle mobility under active and inactive conditions. 7
Supplemental results **7**
Metabolism-dependent motion still occurs when transcription, translation, peptidoglycan synthesis, MreB activity or FtsZ ring formation is inhibited 7
The nucleoid and its dynamics affect particle distribution, but are not responsible for metabolism-dependent motion 8
Estimation of GFP-µNS particles size 9
Subdiffusive behavior is size-dependent 9
Table 1. Strains used in the study **11**

Extended experimental procedures **12**
Light microscopy 12
Strain construction 13
Titratable modulation of GFP-μNS synthesis 13
Measurements of the diffusion coefficients of GFP-μNS particles in solution 14
Image analysis for single-particle tracking **15**
Crescentin-GFP structure tracking 15
Tracking of Mini-RK2 plasmids, PhaZ-mCherry-labeled granules and GFP-μNS particles 15
Measurement of GFP-μNS particle fluorescence intensity 15
Determination of nucleoid and cytoplasm area 16
Trajectory analysis **16**
Single-particle tracking 16
Double-particle tracking 16
MSD definitions 17
Parameter γ 17
Radius of gyration of the trajectory 18
Non-Gaussian parameter 18
Displacement correlations 18
Tracking precision estimation 18
References **19**

Supplementary figure legends

Figure S1. Crescentin-GFP structure tracking method, related to Figure 1.
MicrobeTracker cell outlines are drawn on phase contrast (A) and epifluorescence images (B). (C) Band-pass filtering removes background noise. (D) A polygon is then circumscribed to the thresholded crescentin-GFP structure. (E) The center of mass is determined for the crescentin-GFP structure and is decomposed into cell coordinates (*l*, *d*) from the cell outline. (F) Schematic showing cell coordinates (*l*, *d*). Scale bar is 1 μm. (G) Crescentin-GFP structures have similar lengths in all tested experimental conditions. Apparent lengths of crescentin-GFP structures from all experimental conditions were determined from the lengths of the polygons drawn around crescentin-GFP structures.

Figure S2. Cellular metabolism affects the mobility of storage granules in *C. crescentus* and of plasmids outside the nucleoid in *E. coli*, related to Figures 1 and 2.
(A) MSDs of PhaZ-mCherry-labeled PHA granules in *C. crescentus* cells (CJW4055) with (*n* = 415 trajectories) or without (*n* = 653 trajectories) DNP treatment. In *C. crescentus*, the nucleoid spreads throughout the cell. (B) MSD of mini-RK2 plasmids in *E. coli dnaC2* cells at the restrictive temperature (37ºC) under metabolically active (untreated, *n* = 104 trajectories) and energy-depleted (+DNP, *n* = 26 trajectories) conditions. Only plasmids outside of the nucleoid (visualized by DAPI staining) were considered.

Figure S3. Motion of GFP-μNS particles in cells treated with various drugs, related to Figure 2.
(A) *E. coli* cells (CJW4617) after 2 h of induction of GFP-μNS synthesis. A representative image is shown as an overlay of GFP-fluorescence (yellow) and phase contrast (blue). The scale bar is 2 μm. (B) Mean squared displacements (MSD) of GFP-μNS particles in *E. coli* cells (CJW4617) treated with MP265 (50 μM) to inhibit MreB polymerization (*n* = 195 trajectories). MSDs for untreated cells (*n* = 729 trajectories) and DNP-treated cells (*n* = 643 trajectories) are shown for comparison. (C) Same as (B) but with chloramphenicol (Cam, 50 μg/ml) to inhibit translation (*n* = 265 trajectories). (D) With rifampicin (Rif, 25 μg/ml) to inhibit transcription (*n* = 210 trajectories). (E) With ampicillin (Amp, 100 μg/ml) or mecillinam (Mcl, 10 μg/ml) to inhibit peptidoglycan synthesis (*n* = 185 and 213 trajectories, respectively).

Figure S4. Nucleoid area in wild-type and *dnaC2* cells following DNP treatment, related to Figure 2.
(A) DNP treatment causes nucleoid expansion. *E. coli* cells (CJW5123) were stained with DAPI under untreated and DNP-treated conditions. Representative images are shown as an overlay of phase and DAPI-channel fluorescence. The scale bar is 1 μm. (B) Nucleoid-free area in individual wild-type *E. coli* cells (CJW5123) under untreated conditions (n = 541 cells) and DNP treatment (n = 498 cells) was quantified as the difference between cytoplasmic and nucleoid areas, measured as the area occupied by cytoplasmic mCherry and DAPI staining, respectively. (C) Nucleoid-free area in individual wild-type *E. coli* cells (CJW5123) under untreated conditions (n = 541 cells) and with rifampicin (Rif, n = 292) or DNP (n = 498) treatments. The nucleoid-free area was quantified as the difference between cytoplasmic and nucleoid areas, measured as the areas occupied by cytoplasmic mCherry and DAPI staining, respectively. (D) Representative images of DAPI-stained *E. coli dnaC2* cells (CJW5124) after 2 h of growth at their restrictive temperature (37°C) with and without DNP are shown as an overlay of phase and DAPI-channel fluorescence. Scale bar is 1 μm. (E) Histogram of nucleoid-free area in *E. coli dnaC2* cells (CJW5124) after 2 h of growth at their restrictive temperature (37ºC) with DNP (n = 467 cells) and without DNP (n = 1069 cells), and quantified as in (B).

Figure S5. Estimation of the precision of our single-particle tracking method, related to Figure 2.
(A) Histogram of localization errors (difference between tracking results and actual spot position) for simulated fluorescence images. MSD (B) and displacement distributions (C) of simulated Brownian particles with varied diffusion coefficients. (D) A comparison of GFP-μNS and PopZ-YFP MSDs with and without DNP treatment.

Figure S6. Intensity distributions, binning, size determination and subdiffusion parameters of GFP-μNS particles, related to Figure 3.
(A) Histogram of GFP-μNS particle fluorescence intensities, *SI*, for all measured trajectories in *E. coli* cells (CJW4617) under untreated conditions. Each color corresponds to a single bin used in the analysis. (B) Same as (A) but after conversion to relative particle size, $d_{rel} = (SI)^{1/3}$. (C) Plot showing the diffusion coefficients (D) of GFP-μNS particles in cell lysates as a function of their relative size (defined as $(SI)^{1/3}$). D was determined for two distinct experiments (Exp. 1 and Exp. 2) by linear fitting of two-dimensional MSDs (circles) or by fitting a Gaussian to the displacement distributions (squares). Curves are best fits of Einstein-Stock's relationship to each dataset ($D = C/d_{rel}$, where the fitting parameter $C = kT/(3\pi\eta)$ and d_{rel} is the relative size of GFP-μNS particles). The shift between experiments 1 and 2 can be explained by a difference in viscosity between the two cell lysates based on the difference in diffusion coefficients of 110-nm beads (D_{bead} = 2.24 μm^2/s and 2.04 μm^2/s for experiments 1 and 2,

respectively). (D) Plot showing the relationship between absolute and relative sizes of GFP-μNS particles. Lines are the Einstein-Stock best fits (panel E) whose average was used to obtain a calibration curve between relative sizes in arbitrary units and absolute sizes in nanometers. (E) MSD of GFP-μNS particles of varying sizes in wild-type *E. coli* cells (CJW4617) under normal conditions of growth (M9G medium). All trajectories were binned by particle size, and particle size increases with increasing bin numbers. MSDs for selected bins (1, 3, 5, 7, 9) are shown. Lines are the best fit of $MSD(t) \sim Dt^{\alpha}$. (F) Plot showing apparent diffusion coefficient D as a function of particle size obtained from the MSD fitting in (E). Bars represent confidence intervals of fitting. (G) Plot showing exponent α as a function of particle size obtained from the MSD fitting in (E). Bars represent confidence intervals of fitting.

Figure S7. GFP-μNS particle displacements under untreated and DNP-treated conditions, related to Figure 3.

Histogram of GFP-μNS particle displacements in *E. coli* cells (CJW4617) under untreated and DNP-treated conditions for all size bins. Line width indicates Poisson counting error, and the gray shading delineates the estimated tracking error. Displacements were measured over 15-sec intervals.

Supplementary movie legends

Movie S1. Movement of detached crescentin-GFP structures ceases when cell growth stops, related to Figure 1.

The movie shows a time-lapse sequence of GFP-labeled crescentin structures in filamenting (FtsZ-depleted) *C. crescentus* cells (CJW3130). These cells carry a *wbqL* mutation that results in detachment of crescentin structures from the membrane (Cabeen et al., 2010). In the movie, the detached GFP-labeled crescentin structures display motion as cells are growing on a pad containing M2G medium. However, crescentin structure motion suddenly stops when growth ceases for an unknown reason. Phase contrast (blue) and fluorescence (red) images are overlaid. The scale bar is 5 μm. The time stamp shows h:min.

Movie S2. Crescentin-GFP structures sample the cytoplasmic space in metabolically active cells, related to Figure 1.

The movie shows a time-lapse sequence of a crescentin-GFP structure moving in a *C. crescentus* cell (CJW1265) growing on an agarose pad containing M2G medium. The cell outline was determined from phase contrast images by MicrobeTracker. The cyan 'x' indicates the center of mass of crescentin-GFP structure intensities. The scale bar is 1 μm.

Movie S3. Crescentin-GFP structure motion is dramatically reduced in carbon-starved cells, related to Figure 1.

C. crescentus cells (CJW1265) from an M2G culture were washed and resuspended in M2 buffer for 3 h to deplete cells of glucose (sole carbon source). Cells were then spotted on an agarose pad containing M2 buffer (and no glucose) for imaging. The movie shows a representative time-lapse sequence of a crescentin-GFP structure in a carbon-starved cell. The cell outline was determined from phase contrast images by MicrobeTracker. The cyan 'x' indicates the center of mass of crescentin-GFP structure intensities. The scale bar is 1 µm.

Movie S4. Crescentin-GFP structure mobility is drastically reduced upon energy depletion by addition of 2,4-dinitrophenol (DNP), related to Figure 1.

C. crescentus cells (CJW1265) from an M2G culture were spotted on an agarose pad containing M2G and 500 µM DNP. The movie shows a representative time-lapse sequence of a crescentin-GFP structure in a DNP-treated cell. The cell outline was determined from phase contrast images by MicrobeTracker. The cyan 'x' indicates the center of mass of crescentin-GFP structure intensities. The scale bar is 1 µm.

Movie S5. Mini-RK2 plasmids are highly mobile under metabolically active conditions, related to Figure 1.

Mini-RK2 plasmids were visualized with plasmid-encoded GFP-LacI in *E. coli* cells (strain JP924). GFP-LacI synthesis was induced on an arabinose concentration gradient pad to identify cells with low GFP-LacI expression levels to minimize plasmid clustering. Cells were imaged 60 min later at 37°C. The cell outline was determined from phase contrast images by MicrobeTracker. The cyan 'x' indicates mini-RK2 plasmid position. The scale bar is 1 µm.

Movie S6. Mini-RK2 mobility is dramatically reduced upon energy depletion by DNP treatment, related to Figure 1.

Mini-RK2 plasmids were visualized with plasmid-encoded GFP-LacI in *E. coli* cells (strain JP924). GFP-LacI synthesis was induced on an arabinose concentration gradient pad to identify cells with low GFP-LacI expression levels, which minimized plasmid clustering. After 30 min of incubation on the gradient pad to allow for GFP-LacI expression, capillary action was used to expose cells to DNP. This was followed by time-lapse imaging. The cell outline was determined from phase contrast images by MicrobeTracker. The cyan 'x' indicates mini-RK2 plasmid position. The scale bar is 1 µm.

Movie S7. GFP-μNS particle mobility under active and inactive conditions, related to Figure 2. The movie shows a representative time-lapse sequence of a GFP-μNS particle moving in an *E. coli* cell (CJW4617) at 30°C under untreated (left movie panel) and DNP treated (right movie panel) conditions. GFP-μNS synthesis was induced by the addition of 200 μM IPTG for 2 h. Cells were then washed of IPTG and imaged on an agarose pad containing M9G medium. The time-lapse sequence is shown as an overlay of fluorescence images (yellow) with the corresponding cell outline (green) determined from phase contrast images. The scale bar is 1 μm. The time stamp corresponds to min:sec.

Supplemental results

Metabolism-dependent motion still occurs when transcription, translation, peptidoglycan synthesis, MreB polymerization or FtsZ ring formation is inhibited

ATP-dependent diffusive behavior, known as "active diffusion", has been reported in eukaryotic cells (Brangwynne et al., 2009). This active diffusion arises from the activity of molecular motors (such as myosins, dyneins and kinesins) that agitate the elastic cytoskeletal network. While bacteria lack comparable motors or a cytoskeletal network that crisscrosses the cytoplasm, many bacteria like *E. coli* and *C. crescentus* contain the actin homolog MreB. However, inactivation of MreB by MP265 treatment (Takacs et al., 2010) had no effect on GFP-μNS mobility (Fig. S3B). In cells blocked for DNA replication initiation (*dnaC2* cells at the restrictive temperature), ring formation of the FtsZ tubulin homolog is inhibited; yet metabolism-dependent motion of GFP-μNS particles and plasmids still occurred (Fig. 2D-E and Fig. S2B).

Still, other macromolecular complexes exhibit motor-like behavior in metabolically active cells. Both ribosomes and RNA polymerases exert force as they displace their substrates or themselves during elongation. We therefore considered the possibility that their work cycles may introduce energy-dependent motion in the cytoplasm. However, when we inhibited translation by treatment with chloramphenicol, we did not observe a significant change in the MSD, aside from a very subtle up-shift at later time points (Fig. S3C). While transcription inhibition through rifampicin treatment diminished GFP-μNS mobility on average, the mobility reduction was too small compared to the DNP effect (Fig. S3D) to explain metabolism-dependent motion (see also below). Inhibition of peptidoglycan synthesis with mecillinam or ampicillin had no measurable effect on GFP-μNS mobility (Fig. S3E).

The nucleoid and its dynamics affect particle distribution, but are not responsible for metabolism-dependent motion

In *E. coli*, the chromosomal DNA condenses into a central region known as the nucleoid, creating DNA-free regions at the cell poles (Fig. S4A). GFP-μNS particles spent most of their time in cell pole regions (Fig. S3A), with 76% of them exhibiting a polar localization at a given time. Thus, while GFP-μNS foci can move from pole to pole in metabolically active cells (Fig. 2A, untreated), they avoid the nucleoid region. Because of this nucleoid exclusion effect, we asked whether DNA and/or its dynamics may drive the dramatic reduction in mobility that we observed in DNP-treated cells.

This was important to consider as DNP treatment results in a significant expansion of chromosome (nucleoid) (Fig. S4A-B), raising the possibility that mobility reduction may simply be due to smaller DNA-free areas under DNP treatment. Rifampicin, which is well known to expand the nucleoid (Cabrera and Jin, 2003; Dworsky and Schaechter, 1973), also reduces the DNA-free area by a similar to DNP degree (Fig. S4C). However, the reduction in GFP-μNS mobility under rifampicin treatment was small compared to DNP (Fig. S3D). Thus, the DNP-induced reduction in the DNA-free area cannot alone explain the dramatic effect of DNP on the GFP-μNS motion. To test this further, we sought to increase the size of DNA-free regions in the cytoplasm under DNP treatment by tracking our probe in the temperature-sensitive *dnaC2* mutant (Carl, 1970; Withers and Bernander, 1998). When shifted to its restrictive temperature, this mutant cannot initiate new rounds of DNA replication; it blocks cell division but continues to grow. As a result, filamentous cells are obtained with only one chromosome (Withers and Bernander, 1998). After 2 h at the restrictive temperature, we observed large DNA-free regions (Fig. S4D), with an area of 2.6 ± 2.3 μm^2 (n = 467 cells; Fig. S4E). Even after these cells were treated with DNP, the DNA-free regions remained large (Fig. S4D), with an area of 2.0 ± 1.3 μm^2 (n = 1069 cells; Fig. S4E). In fact, because of their cell filamentous phenotype, DNP-treated *dnaC2* cells tended to have more DNA-free space than untreated wild-type cells (2 vs. 1.5 μm^2 on average). Still, GFP-μNS motion remained confined in DNP-treated *dnaC2* cells (Fig. S4F-G), even though the large majority (84%) of GFP-μNS particles were found in DNA-free regions under both untreated and DNP-treated conditions. Additionally, when we tracked mini-RK2 plasmids and nucleoids simultaneously in the *dnaC2* strain, we found that the mobility of plasmids outside of the nucleoid (i.e., in the large DNA-free regions) dropped dramatically under DNP treatment (Fig. S2B).

In eukaryotic nuclei, the mobility of relatively large components such as mRNA-protein complexes (mRNPs) can become passively trapped in narrow regions of confinement within chromatin following depletion of ATP (Shav-Tal et al., 2004; Vargas et al., 2005). However, the metabolism-dependent motion in the large DNA-free regions of *dnaC2* cells excludes the possibility of probe entrapment within the dense DNA meshwork. It also reinforces the notion that transcription cannot alone

explain metabolism-dependent motion, as transcription occurs within the nucleoid. From these results, we conclude that, although the chromosomal DNA has an effect on the spatial distribution of GFP-µNS through DNA exclusion, neither the DNA nor its dynamics can explain the dependence of particle motion on cellular energy.

Estimation of GFP-µNS particles size

To determine the relationship between fluorescence intensity (*SI*) of GFP-µNS particles and their sizes (*d*), we first calculated their relative size (d_{rel}) as:

$$d_{rel} = (SI)^{1/3},$$

assuming that GFP-µNS particles have constant density.

To estimate the absolute size of GFP-µNS particles, we measured their diffusion coefficients in solution following cell lysis (see Extended Experimental Procedures below for details). We tracked the motion of the particles and deduced diffusion coefficients in two ways: 1) by fitting the MSDs and 2) by fitting the displacement distributions. Both calculations resulted in similar values and provided an estimate for the accuracy of the measurements. Fig. S6C shows two experimental datasets of diffusion coefficients plotted as a function of relative particle size. Since the viscosity (η) of the cell lysates likely differs from water, we added 110-nm fluorescent beads to the lysates and measured their diffusion, and obtained diffusion coefficients D_{bead} = 2.24 µm^2/s and 2.04 µm^2/s for experiment 1 and 2, respectively. The diffusion coefficients of GFP-µNS particles were fitted with the Einstein-Stokes relationship ($D = C/d_{rel}$, where fitting parameter C is $kT/(3\pi\eta)$) (Fig. S6C). The absolute sizes of GFP-µNS particles were obtained as $d = d_{bead}\, D_{bead} / D$, where d_{bead} = 110 nm (Fig. S6D). Each Einstein-Stokes fitting provides a means to convert sizes in arbitrary units to sizes in nanometers. We used the average line as a calibration curve (d = 685nm $(SI)^{1/3}$).

Subdiffusive behavior is size-dependent

The size-dependence of glassy properties suggests that cytoplasmic components of different sizes perceive the cytoplasm differently. Small components (e.g., proteins and metabolites) perceive the environment as a liquid while larger (ca. > 30 nm) components perceive the same environment as glassy. Consistent with this notion, small fluorescent proteins like Kaede and mEos2 have been reported to display normal diffusion (Bakshi et al., 2011; English et al., 2011), while larger components such as RNA-protein aggregates exhibit subdiffusive behavior (Golding and Cox, 2006; Weber et al., 2010). Analysis of a probe's diffusive behavior is often limited to the examination of how an MSD scales with time ($MSD(t) \sim D\, t^{\alpha}$), where D is the apparent diffusion coefficient and α is the scaling exponent (α = 1 for normal diffusion). For RNA-protein aggregates, $\alpha \approx 0.7$ (Golding and Cox, 2006; Weber et al., 2010),

which is indicative of subdiffusion (α <1). Interestingly, a recent study reported a scaling exponent α close to 1 for large protein aggregates (Coquel et al., 2013), suggesting normal diffusion. We propose that the discrepancy between α values arises from differences in particle sizes. When examining the MSDs of GFP-µNS particles, we found that not only the apparent diffusion coefficient *D*, but also the scaling exponent α is size-dependent (Fig. S6E-G). *D* declines with size (Fig. S6F), as expected, but surprisingly, α increases with size (Fig. S6G), perhaps because of an increasing nucleoid exclusion effect. The protein aggregates examined in Coquel et al (2013) had *D* values smaller than the *D* values we report for GFP-µNS particles or reported by Golding and Cox (2006) for RNA/protein aggregates, suggesting that particles in the work of Coquel et al were the largest. Coquel et al (2013) estimated the diffusion and size of misfolded protein aggregates simultaneously by fitting experimental MSDs with the results of simulations of confined Brownian particles. Based on the fitting, they concluded that the diffusion coefficients of aggregates obey Stokes-Einstein relationship $D = C/r$, with $C = kT/(6\pi\eta) \approx 5 \cdot 10^4$ nm^3/s , where *D* is the diffusion coefficient, *k* and *T* are the Boltzmann constant and temperature, η is the viscosity and *r* is the particle radius. If we extrapolate their relationship $D = (5 \cdot 10^4 \text{ nm}^3 \text{ s}^{-1})/r$ to GFP for which r = 2 nm (Busch et al., 2000), we obtain $D_{GFP} \approx 3 \cdot 10^{-2}$ µm^2/s, which is ~200-300 times smaller than experimentally determined *D* values (6-10 µm^2/s) for GFP (Elowitz et al., 1999; Konopka et al., 2006; Slade et al., 2009; van den Bogaart et al., 2007). This suggests that the relationship $D = (5 \cdot 10^4 \text{ nm}^3\text{s}^{-1})/r$ observed in Coquel et al (2013) does not extrapolate to objects of protein size. This highlights the importance of performing additional quantitative analyses (displacement distribution and correlation, α_2, etc.) beyond the MSD to dissect particle motion.

Table 1. Strains used in the study

Strain name	Relevant genotype or features	Description	Reference
***C. crescentus* strains**			
CB15N	Synchronizable variant of wild-type CB15, also named NA1000	used as a wild-type strain	(Evinger and Agabian, 1977)
CJW1265	CB15N *creS*::pHL32creS-mgfp	Replaces endogenous *creS* gene with *creS-mgfp*	This study
CJW2245	CB15N *popZ*::pBGent-popZ-YFP	Has chromosomal *yfp-popZ* under native promoter	(Ebersbach et al., 2008)
CJW3130	CB15N *ftsZ*: :pXMCS7ftsZ *creS*::pHL23creS-mgfp *wbqL*::pBGENT-KO	Replaces endogenous *creS* gene with *creS-mgfp* in cells with inducible *ftsZ* expression and with deleted *wbqL*	(Cabeen et al., 2010)
CJW4055	CB15N *xylX*::*phaZ-mcherry*	Has inducible *phaZ-mcherry* fusion at the *xylX* locus	(Werner et al., 2009)
***E. coli* strains**			
CJW4386	MG1655 *dnaC2*/pGAP500/ pGAP60	Mini-RK2 tracking system (see JP924) in MG1655 *dnaC2*	This study
CJW4617	MG1655 Δ*(lacZYA)*::*gfp-muNS*	Replaces part of *lac* operon with *gfp-μNS*	This study
CJW4619	MG1655 *dnaC2* Δ(*lacZYA)*::*gfp-muNS*	Same as CJW4617 but in *dnaC2* background	This study
CJW5123	MG1655 pBlueScript-*mcherry*	MG1655 producing free cytoplasmic mCherry	This study
CJW5124	MG1655 *dnaC2* pBlueScript-*mcherry*	MG1655 *dnaC2* expressing free cytoplasmic mCherry	This study
JP924	MC4100 *ara*Δ714/pGAP500/pGAP60	Carries mini-RK2 plasmid containing a 10-kb *lacO* array (pGAP500) and a compatible plasmid for arabinose-inducible *gfp-lacI* expression (pGAP60)	(Derman et al., 2008)
MG1655	F-lambda- *ilvG- rfb*-50 *rph*-1	used as a wild-type strain	(Jensen, 1993)
MG1655 *dnaC2*	MG1655 *dnaC2*	Temperature-sensitive mutant. At the restrictive temperature, DNA replication is blocked.	(Withers Bernander 1998)

Extended experimental procedures

Light microscopy

For experiments involving *C. crescentus*, agarose pads contained 1 or 1.2% agarose in M2G medium except where noted. Stationary phase culture supernatant was used for crescentin-GFP tracking under stationary phase conditions, and was obtained through 40 h of culture ($OD_{660} \geq 1.7$), centrifugation (1 min 10,000 × g) and supernatant filtration (0.22 µm filters). For glucose depletion experiments, M2 salt buffer was used, and cells from M2G cultures were washed into M2 salt buffer and incubated on a glucose-free M2 pad for 3 h prior to imaging.

For *E. coli* experiments, agarose pads contained glycerol-based medium (M9G), except where noted. For mini-RK2 imaging, L-arabinose gradient agarose pads were used to minimize plasmid clustering (Derman et al., 2008). Gradient pads were constructed from two adjacent agarose pads, one containing 0.2% L-arabinose and a second without L-arabinose. L-arabinose was allowed to diffuse into the pad lacking L-arabinose, creating an L-arabinose concentration gradient. The field selected for imaging was chosen as the dimmest region (corresponding to low GFP-LacI levels) still containing discernible foci.

In all drug treatments, cells from a culture were spotted on the agarose pad with appropriate medium and concentration of chemicals: DNP - 2 and 0.5 mM for *E. coli* and *C .crescentus*, respectively; chloramphenicol - 50 µg/ml; rifampicin - 25 µg/ml; MP265 - 50 µM; ampicillin - 100 µg/ml; mecillinam - 10 µg/ml. For mini-RK2 experiments only, cells were incubated on the gradient pad for 30 min at 30°C in the absence of DNP. Then, 2-5 µL of a 200 mM DNP solution was added to L-arabinose gradient pads by capillary action between slide and cover glass. After DNP diffused throughout the pad (determined by the near uniform distribution of yellow color coming from the DNP solution), imaging was performed. Imaging started 5-7 min after first contact of the cells with the antibiotics unless stated otherwise. For nucleoid visualization, 100-500 ng/mL of DAPI was added to the pad.

An objective heater maintained cells immobilized on agarose pads at 26°C, 30°C or 37°C, as noted, during image acquisition. Images were acquired with a 100X phase contrast objective on either a Nikon Eclipse Ti-U (Hamamatsu Orca-II ER LCD camera), or a Nikon E80i (Hamamatsu Orca-ER LCD or Andor iXon^{EM} + CCD cameras), or a Nikon Ti-E (Hamamatsu Orca R2 or Andor iXon^{EM}X3 DU897 CCD cameras). Micromanager (Edelstein et al., 2010), Metamorph (Molecular Devices) or NIS-Elements (Nikon) software was used for microscope control. Images were taken every 2 min (crescentin-GFP structures), 30 sec (plasmids) or 15 sec (GFP-µNS, PhaZ-mCherry), except where noted.

Strain construction

CJW1265: EcoRI/NcoI digestion removed the 3' half of *creS* from pKScreS-gfp (Cabeen et al., 2010), which was triple ligated along with the NcoI/NotI fragment carrying *mgfp* from pKSmgfp (Cabeen et al., 2010) and EcoRI/NotI-digested pHL32 (Cabeen et al., 2010) to create pHL32creS-mgfp. This construct was electroporated into *E. coli* S17-1 and mobilized into *C. crescentus* CB15N by conjugation.

CJW4617 and **CJW4619:** pER12 (pBAD322A-gfp-muNS) plasmid (kind gift from Dr. A. Janakiraman, City College of New York), which encodes an N-terminal GFP fusion to residues 471-721 of μNS (Broering et al., 2005), was used as a template. The *gfp-muNS* fragment was amplified by PCR (using primers ATATTGGTACCATGTCGAAAGGAGAAGAA and ATTATCTCGAGCTACAGCTCATCAG TTGGAAC) and then digested by KpnI/XhoI. The *nptI* gene was amplified from pKD4 plasmid and digested by XhoI/SmaI. Then, *gfp-muNS* and *nptI* fragments were triple ligated into the pKS plasmid. The resulting plasmid was used as a PCR template to generate a *gfp-muNS-nptI* fragment with flanking regions complementary to *lacI* and *cynX*, respectively (using primers GCCGATTCATTAATGCAGCT and GGCCTGATAAGCGCAGCGTATC). This fragment was used to replace (*lacZYA*) by one-step λ-recombination (Datsenko and Wanner, 2000). The resulting *gfp-muNS-nptI* replacement of *lacZYA* was transduced by P1-phage into MG1655 and MG1655 *dnaC2* strains, producing strains CJW4617 and CJW4619, respectively.

CJW4386: pGAP500 and pGAP60 plasmids were electroporated into MG1655 *dnaC2.*

CJW5123 and CJW5124: pBlueScript-mCherry (kind gift from Dr. Manuel Campos, Yale University) was electroporated into MG1655 and MG1655 *dnaC2* to produce strains CJW5123 and CJW5124, respectively.

Titratable modulation of GFP-μNS synthesis

In strains CJW4617 and CJW4619, GFP-μNS synthesis is under the control of the chromosomal IPTG-inducible promoter *lac*. In these strains, part of the *lac* operon, which includes the *lacY* gene encoding the lactose/IPTG permease, is deleted (see Strain construction above). The absence of this permease disrupts a positive feedback loop in promoter activation, and changes the IPTG-inducible *lac* promoter from an all-or-none promoter to a titratable promoter with a gradual response to IPTG concentration (Marbach and Bettenbrock, 2012). Without the LacY permease, IPTG is still able to passively cross the cytoplasmic membrane of *E. coli* (Marbach and Bettenbrock, 2012).

Measurements of the diffusion coefficients of GFP-µNS particles in solution

The sizes of GFP-µNS particles were estimated by measuring their diffusion coefficients in solution following cell lysis by T7 phage treatment. Fluorescent beads (FluoSpheres F8803, Invitrogen, OR, USA) of known size (110 nm) were simultaneously tracked to measure sample viscosity.

Specifically, synthesis of GFP-µNS in CJW4617 cells was induced for 2 (Experiment 1, Exp1) or 18 (Experiment 2, Exp2) h with 0.2 mM ITPG (Exp1) or 1 mM IPTG (Exp2) in M9G medium at 30°C. Stronger induction in Exp2 was used to generate a wide distribution of GFP-µNS particles sizes by increasing the fraction of larger particles. After induction, cells were washed of IPTG following centrifugation and transferred into M9 glucose at 37°C for 25 min. Then, cells were infected with T7 phages. After incubation of infected cells for 12 min (phage-induced lysis occurs ~ 20 min after infection in M9 glucose medium at 37°C), the cells were concentrated ~25-fold by centrifugation and incubated at 37°C for an additional 18 min. The cell lysate containing the released GFP-µNS particles was then centrifuged at 4,000 g for 5 min to remove cell debris. In each experiment, two samples (with and without addition of the beads) were placed on a multi-well slide, sealed and imaged on a microscope (using 100X phase contrast objective on either a Nikon Eclipse Ti-U with 3D-STORM lenses or a Nikon E80i and Andor iXon^{EM}X3 DU897 or Andor iXon^{EM} + CCD cameras, respectively) at room temperature. The focal plane was established between top and bottom of the imaging well (height ~ 30 µm from top to bottom), and a sequence of images was acquired in a streaming mode (frame rate of 46 ms for Exp1 and 22 ms for Exp2). Before each new acquisition, a new field of view was selected.

Images were analyzed using spotFinder (Sliusarenko et al., 2011) to identify fluorescent spots and to measure their intensities. The spots were tracked using a custom-made Matlab script: two spots were connected between consecutive frames if their inter-frame distance was less than 10 pixels. If more than one connection was possible, the spots with the smallest inter-frame displacement were linked. Reliable determination of particle intensities requires measurement of spot intensity when the particle is in the focal plane. Thus, only traces in which the particle crossed the focal plane during acquisition (based on maximum values allowed for width, relative square error and perimeter variance of 2D-Gaussian fit to the spot) were analyzed. For each trace, particle fluorescence intensity was determined at a frame with the minimal relative square error. We found from control experiments with fluorescent beads of varied sizes that beads with small sizes could not be reliably measured due to their fast movement and weak fluorescent signal. Therefore, we considered only GFP-µNS particles with intensities > 0.2 A.U., which were binned by their intensities into 3 (Exp1) or 4 (Exp2) bins.

For each bin, the diffusion coefficients of GFP-µNS particles were calculated in two different ways: 1) by linear fitting of two-dimensional MSDs ($MSD(t) = 4\,D\,t$, where D is a fitting parameter) to the first

3-4 time points, and 2) by fitting a Gaussian to the displacement distribution ($dP dx = 2 \exp(-dx^2/a) / (\pi a)$, where fitting parameter $a = 4 D dt$).

Image analysis for single-particle tracking

For particle tracking experiments, cell outlines were determined by MicrobeTracker (Sliusarenko et al., 2011) from phase contrast images and were used to define cell coordinates, *l* and *d*, along the long and short axes of the cell, respectively. Particle motion was quantified using these cellular coordinates.

Crescentin-GFP structure tracking

As crescentin-GFP forms filamentous structures with an occurrence of one per cell, single-particle tracking methods were adapted to quantify its motion. Cell outlines generated from phase contrast images (Fig. S1A) were used to delimit regions of interest in epifluorescence images (Fig. S1B). Individual regions of interest corresponding to single cells were band-pass filtered (Fig. S1C). Crescentin-GFP structures were outlined by the smallest convex polygon that could be drawn around the fluorescence signal (Fig. S1D). The polygon's center was determined as the center of mass of raw pixel values from pixels identified in the band-pass filtered image after thresholding (Fig. S1E). The center of the structure in image coordinates was decomposed into cellular coordinates (*l*, *d*) (Fig. S1F).

Tracking of Mini-RK2 plasmids, PhaZ-mCherry-labeled granules and GFP-μNS particles

GFP-LacI-labeled mini-RK2 plasmids, PhaZ-mCherry-labeled PHA granules and GFP-μNS particles appear as a diffraction-limited spots. Their positions were determined relative to cell coordinates (*l*, *d*) by a 2D-Gaussian fit to the raw fluorescent image of the spot.

Measurement of GFP-μNS particle fluorescence intensity

SpotFinder (Sliusarenko et al., 2011), which calculates the volume of the best-fit 2D-Gaussian to the raw fluorescence image of the spot, was used to measure single-particle fluorescence intensity. To compare the fluorescence intensity of GFP-μNS foci across different experiments, we used green fluorescent beads (100-nm FluoSpheres F8803, Invitrogen, OR, USA) as standards. Fluorescence intensities of all GFP-μNS particles were normalized to the median intensity of the beads, which was taken as a unit value. Particle intensities can fluctuate from frame to frame for a variety of reasons (fluctuations in illumination, blurring caused by particle motion during image acquisition, particle movement out of the focal plane, photobleaching, etc.). To avoid ambiguity caused by these factors, we only measured intensities of diffraction-limited (with width <225nm, i.e. no blurring) spots and used the median of the first five observations (to minimize effects caused by photobleaching) as a measurement of

the particle fluorescence intensity. We confirmed that these measurements did not introduce biases by comparing cumulative histograms of fluorescence intensities of all trajectories under untreated and DNP-treated conditions, and finding that they were essentially the same (data not shown). To test that DNP treatment did not change particle size, we induced GFP-μNS synthesis as usual, washed out the inducer, split the culture into two samples, placed one sample on a regular M9G containing pad and the other on an M9G + DNP pad, and measured fluorescence intensities of GFP-μNS particles. We observed no differences in the fluorescence distribution between the two samples (data not shown).

Determination of nucleoid and cytoplasm area

Sub-pixel outlines of non-diffraction-limited image features were identified with a second derivative-based method following image filtering and interpolation (complete details will soon be submitted for publication elsewhere). A region's area was determined from its outline.

Trajectory analysis

Single-particle tracking

All particles were tracked in 2D (relative to the long (*l)* and short (*d*) axes of the cell) as described above, whereas all quantitative analyses in this work were performed in the dimension of the cell's long-axis, *l*, unless otherwise stated. When cell division occurred, the tracking of the particle was restarted as a new trajectory in the daughter cell that inherited the particle. Only cells with one GFP-μNS focus (except for the double-particle tracking experiments) and only traces with a minimal length of 5 frames were analyzed. Displacement length distributions for all probes were determined from their displacements between consecutive frames (2 min for crescentin-GFP structures, 1 min for PHA granules, 30 sec for mini-RK2 plasmids, 10 sec for mini-RK2 plasmids in the *dnaC2* strain, and 15 sec for GFP-μNS). To take cell growth into account, we used relative cell positions (then back-calculated to absolute distances) to calculate displacements between two frames (assuming that the relative cell position of non-moving particles remains unchanged). Cell growth did not have any significant effect on displacement length distributions, as cell growth was minimal over inter-frame time intervals.

Double-particle tracking

For double-particle tracking experiments, synthesis of GFP-μNS was induced with a higher concentration of IPTG (1mM) for 2 h at 30°C. Most cells still exhibited one GFP-μNS spot per cell, but occasionally cells with two spots were observed. Image analysis was performed as described above, and trajectories were constructed by minimizing the total inter-frame displacements. To verify this approach,

we compared displacement distributions from single-particle tracking (in cells with only one GFP-μNS spot) and double-particle tracking (in cells with two spots) in the same experiment and did not observe significant distinctions between the two sets of trajectories (data not shown).

MSD definitions

Unless otherwise stated, we used an ensemble-averaged mean square displacement (*MSD*) defined as:

$$MSD(t) = \frac{1}{n}\sum_{i=1}^{n}(x_i(t) - x_i(0))^2, \quad [1]$$

where $x(t)$ is the coordinate of a given particle at moment t, $x(0)$ is the coordinate of this particle at the beginning of the trajectory, and n is a number of all trajectories.

MSD_τ was calculated as:

$$MSD_\tau(t) = \frac{1}{n}\sum_{i=1}^{n}\frac{1}{m}\sum_{j=0}^{m-1}(x_i(\tau_j + t) - x_i(\tau_j))^2, \quad [2]$$

where the time-averaged MSD of each trajectory is computed and then averaged for all trajectories.

To examine whether the finite number of trajectories or their limited duration causes deviations between MSD and MSD_τ, we simulated a Brownian particle confined within a cell (cell length l = 3μm) and compared MSD and MSD_τ for 250 simulations of 600-min trajectories and did not find significant deviations (data not shown). In addition, we simulated a particle undergoing independent non-Gaussian displacements (with a distribution corresponding to experimental displacements for bin 6 under DNP treatment) confined within a cell (cell length l = 3μm, 250 simulations of 600-min trajectories). The deviation between MSD and MSD_τ in the simulation was small compared to the experimental data (data not shown).

Parameter γ

When comparing *MSD* and MSD_τ to examine whether the system is ergodic or not, we assume that the time evolution of the probe displacement is a stationary process, i.e. the average physical properties of the cellular environment do not change with time. As a measure of the difference between MSD and MSD_τ, we calculated total relative deviation γ as

$$\gamma = \frac{1}{m}\sum_{t=0}^{t=t_{max}}\frac{MSD(t) - MSD_\tau(t)}{MSD(t)}. \quad [3]$$

where m is the total number of experimental points per MSD curve.

Radius of gyration of the trajectory

The radius of gyration of a trajectory is a time-averaged metric of the space explored by a particle. We calculated R_g in one dimension as

$$R_g = \left[\sum_{i=0}^{m-1} \frac{1}{m}(x(t_i) - x_0)^2)\right]^{1/2}, \quad [4]$$

where $x(t_i)$ is the coordinate of a particle at i-th observation, m is the total number of observations per trajectory, and x_0 is the mean coordinate of a particle:

$$x_0 = \frac{1}{m}\sum_{i=0}^{m-1} x(t_i). \quad [5]$$

Non-Gaussian parameter

The non-Gaussian parameter α_2 was calculated from experimental distributions of particle displacements as:

$$\alpha_2 = \frac{<dx^4>}{3<dx^2>^2} - 1, \quad [6]$$

which is the fourth moment of the distribution relative to its second moment. Here, dx is a displacement and <> denotes averaging over the distribution. The non-Gaussian parameter measures how much the tail of the distribution deviates from Gaussian, and is zero for a Gaussian distribution. Broader distributions (but with the same standard deviation) result in higher values of α_2.

Displacement correlations

To calculate averaged next displacements as a function of step size, all displacements dx (at variable time t) were binned (700 displacements in each bin), then for each bin, corresponding displacements (at time $t+dt$) were averaged (with signs "-" or "+" if the direction of the displacement was the same as or opposite to the initial displacement).

Tracking precision estimation

We estimated the precision of our single-particle tracking with two procedures. First, we simulated images acquired with a CCD-based detector by adding diffraction-limited spots to a background of Poisson noise. Simulated datasets were evaluated with tracking software used for experimental datasets. The signal-to-noise ratio was set to be comparable with experimental data. Initially, the diffraction-limited spots were maintained at fixed positions and we calculated the differences in real spot positions versus identified spot positions. The resulting distribution of localization errors forms a Gaussian distribution (Fig. S5A), and shows that 90% of simulated diffraction-limited spots were identified within a 3.8 nm radius of the real spot location and that 97% of simulated diffraction-limited spots were identified within a 6.7 nm radius of the real spot location. To discern cumulative success of

our tracking algorithms, we converted static diffraction-limited spots into Brownian particles, which were simulated at defined apparent diffusion coefficients as previously described (Saxton, 2007), using Matlab's built-in *randn* function to generate random numbers. The shapes of the resulting MSDs and displacement distributions are metrics to gauge dynamic tracking precision. Since the MSD of a Brownian particle will form a straight line on a log-log plot, we used this comparison and found that our tracking methods have sufficient precision to measure the diffusion of diffraction-limited particles accurately with apparent diffusion coefficients ≥ 3.3 nm^2 per second (Fig. S5B). Then, we calculated one dimensional displacement distributions of the simulated Brownian particles and recovered Gaussian-shaped distributions (Fig. S5C), a distribution predicted for Brownian particles.

Second, we tracked individual diffraction-limited foci formed by the pole-organizing protein PopZ fused with YFP in *C. crescentus*. These fluorescent foci are thought to be immobile as PopZ-YFP localizes at the cell poles (Bowman et al., 2008; Ebersbach et al., 2008). The MSD of PopZ-YFP foci in active (untreated) and inactive (+DNP) cells had an approximately zero slope (Fig. S5D), indicating that our tracking method does not introduce significant errors originating from growth rate correction or noise in cell outline determination. Furthermore, the MSD of PopZ-YFP foci were approximately two orders of magnitude below the MSD of GFP-μNS in DNP-treated wild-type cells (Fig. S5D). By comparison with PopZ localization and precision estimates from simulated data, we concluded that we are able to distinguish small (> 20 nm) displacements.

References

Bakshi, S., Bratton, B.P., and Weisshaar, J.C. (2011). Subdiffraction-limit Study of Kaede diffusion and spatial distribution in live Escherichia coli. Biophys. J. *101*, 2535-2544.

Bowman, G.R., Comolli, L.R., Zhu, J., Eckart, M., Koenig, M., Downing, K.H., Moerner, W.E., Earnest, T., and Shapiro, L. (2008). A polymeric protein anchors the chromosomal origin/ParB complex at a bacterial cell pole. Cell *134*, 945-955.

Brangwynne, C.P., Koenderink, G.H., MacKintosh, F.C., and Weitz, D.A. (2009). Intracellular transport by active diffusion. Trends Cell Biol. *19*, 423-427.

Broering, T.J., Arnold, M.M., Miller, C.L., Hurt, J.A., Joyce, P.L., and Nibert, M.L. (2005). Carboxyl-proximal regions of reovirus nonstructural protein muNS necessary and sufficient for forming factory-like inclusions. J. Virol. *79*, 6194-6206.

Busch, N.A., Kim, T., and Bloomfield, V.A. (2000). Tracer diffusion of proteins in DNA solutions. 2. Green fluorescent protein in crowded DNA solutions. Macromolecules *33*, 5932-5937.

Cabeen, M.T., Murolo, M.A., Briegel, A., Bui, N.K., Vollmer, W., Ausmees, N., Jensen, G.J., and Jacobs-Wagner, C. (2010). Mutations in the Lipopolysaccharide biosynthesis pathway interfere with crescentin-mediated cell curvature in Caulobacter crescentus. J. Bacteriol. *192*, 3368-3378.

Cabrera, J.E., and Jin, D.J. (2003). The distribution of RNA polymerase in Escherichia coli is dynamic and sensitive to environmental cues. Mol. Microbiol. *50*, 1493-1505.

Carl, P.L. (1970). Escherichia coli mutants with temperature-sensitive synthesis of DNA. Mol. Gen. Gen. *109*, 107-122.

Coquel, A.S., Jacob, J.P., Primet, M., Demarez, A., Dimiccoli, M., Julou, T., Moisan, L., Lindner, A.B., and Berry, H. (2013). Localization of protein aggregation in Escherichia coli is governed by diffusion and nucleoid macromolecular crowding effect. PLoS Comp. Biol. *9*, e1003038.

Datsenko, K.A., and Wanner, B.L. (2000). One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. Proc. Nat. Acad. Sci. USA *97*, 6640-6645.

Derman, A.I., Lim-Fong, G., and Pogliano, J. (2008). Intracellular mobility of plasmid DNA is limited by the ParA family of partitioning systems. Mol. Microbiol. *67*, 935-946.

Dworsky, P., and Schaechter, M. (1973). Effect of rifampin on the structure and membrane attachment of the nucleoid of Escherichia coli. J. Bacteriol. *116*, 1364-1374.

Ebersbach, G., Briegel, A., Jensen, G.J., and Jacobs-Wagner, C. (2008). A self-associating protein critical for chromosome attachment, division, and polar organization in caulobacter. Cell *134*, 956-968.

Edelstein, A., Amodaj, N., Hoover, K., Vale, R., and Stuurman, N. (2010). Computer control of microscopes using microManager. Current protocols in molecular biology / edited by Frederick M Ausubel [et al] *Chapter 14*, 14.20.11-14.20.17.

Elowitz, M.B., Surette, M.G., Wolf, P.E., Stock, J.B., and Leibler, S. (1999). Protein mobility in the cytoplasm of Escherichia coli. J. Bacteriol. *181*, 197-203.

English, B.P., Hauryliuk, V., Sanamrad, A., Tankov, S., Dekker, N.H., and Elf, J. (2011). Single-molecule investigations of the stringent response machinery in living bacterial cells. Proc. Nat. Acad. Sci. USA *108*, E365-E373.

Evinger, M., and Agabian, N. (1977). Envelope-associated nucleoid from Caulobacter crescentus stalked and swarmer cells. J. Bacteriol. *132*, 294-301.

Golding, I., and Cox, E.C. (2006). Physical nature of bacterial cytoplasm. Phys. Rev. Lett *96*, 098102.

Jensen, K.F. (1993). The Escherichia coli K-12 "wild types" W3110 and MG1655 have an rph frameshift mutation that leads to pyrimidine starvation due to low pyrE expression levels. J. Bacteriol. *175*, 3401-3407.

Konopka, M.C., Shkel, I.A., Cayley, S., Record, M.T., and Weisshaar, J.C. (2006). Crowding and confinement effects on protein diffusion in vivo. J. Bacteriol. *188*, 6115-6123.

Marbach, A., and Bettenbrock, K. (2012). lac operon induction in Escherichia coli: Systematic comparison of IPTG and TMG induction and influence of the transacetylase LacA. J. Biotechnol. *157*, 82-88.

Saxton, M.J. (2007). Modeling 2D and 3D diffusion. Meth. Mol. Biol. *400*, 295-321.

Shav-Tal, Y., Darzacq, X., Shenoy, S.M., Fusco, D., Janicki, S.M., Spector, D.L., and Singer, R.H. (2004). Dynamics of single mRNPs in nuclei of living cells. Science *304*, 1797-1800.

Slade, K.M., Steele, B.L., Pielak, G.J., and Thompson, N.L. (2009). Quantifying green fluorescent protein diffusion in Escherichia coli by using continuous photobleaching with evanescent illumination. J. Phys. Chem. B *113*, 4837-4845.

Sliusarenko, O., Heinritz, J., Emonet, T., and Jacobs-Wagner, C. (2011). High-throughput, subpixel precision analysis of bacterial morphogenesis and intracellular spatio-temporal dynamics. Mol. Microbiol. *80*, 612-627.

Takacs, C.N., Poggio, S., Charbon, G., Pucheault, M., Vollmer, W., and Jacobs-Wagner, C. (2010). MreB drives de novo rod morphogenesis in Caulobacter crescentus via remodeling of the cell wall. J. Bacteriol. *192*, 1671-1684.

van den Bogaart, G., Hermans, N., Krasnikov, V., and Poolman, B. (2007). Protein mobility and diffusive barriers in Escherichia coli: consequences of osmotic stress. Mol. Microbiol. *64*, 858-871.

Vargas, D.Y., Raj, A., Marras, S.A., Kramer, F.R., and Tyagi, S. (2005). Mechanism of mRNA transport in the nucleus. Proc. Nat. Acad. Sci. USA *102*, 17008-17013.

Weber, S.C., Spakowitz, A.J., and Theriot, J.A. (2010). Bacterial chromosomal loci move subdiffusively through a viscoelastic cytoplasm. Phys. Rev. Lett. *104*, 238102.

Werner, J.N., Chen, E.Y., Guberman, J.M., Zippilli, A.R., Irgon, J.J., and Gitai, Z. (2009). Quantitative genome-scale analysis of protein localization in an asymmetric bacterium. Proc. Nat. Acad. Sci. USA *106*, 7858-7863.

Withers, H.L., and Bernander, R. (1998). Characterization of dnaC2 and dnaC28 mutants by flow cytometry. J. Bacteriol. *180*, 1624-1631.