**Supplementary Information**

**MATERIALS AND METHODS**

Bacterial strains: *E. coli* strains with the GFP-ParB/parS detection system were provided by Stuart Austin (NIH). These MG1655-derived strains have P1 parS inserted at one of six positions around the chromosome. GFP-Δ30ParB was expressed from plasmid pALA2705 with no IPTG induction, as described by Nielsen et al [1]. Ido Golding (University of Illinois) provided a DH5αPRO host strain carrying two plasmids for RNA detection: pIG-BAC(P_lac/ara-mRFP1-96bs)-V and pIG-K133(2cTG) [2]. Lucy Shapiro (Stanford) provided *Caulobacter* strains with a lacO array inserted at one of four sites in the chromosome and LacI-CFP under control of the endogenous xylX promoter [3]. Joe Pogliano (UCSD) provided JP872 (MC4100 Δ714 background), carrying the RK2 plasmid pZZ6 containing a lacO array and pGAP60 expressing GFP-LacI [4].

**Growth Conditions:** *E. coli* strains were grown overnight at 37°C in LB medium with the appropriate antibiotics (100 µg/mL ampicillin for GFP-ParB/parS strains; 30 µg/mL kanamycin and 25 µg/mL chloramphenicol for RNA-protein particle strain; 100 µg/mL ampicillin and 25 µg/mL chloramphenicol for JP872). Cultures were diluted 1:100 into M9 minimal medium and grown to an OD_{600} of ~0.3-0.5. No induction was required to see chromosomal loci. RNA-protein particles were induced with 100 ng/mL anhydrotetracycline and 1 mM IPTG for ~15 min. To visualize the RK2 plasmid, GFP-LacI was induced with 0.15% arabinose for 15 min, followed by 0.2% glucose for 15 min.

*Caulobacter* were grown at 30°C overnight in PYE medium containing 2 µg/mL kanamycin, 5 µg/mL streptomycin and 25 µg/mL spectinomycin. Cultures were diluted 1:100 into M2G minimal medium and grown to an OD_{600} of ~0.2-0.4. Fluorescence was induced by addition of 0.03% xylose for 1 hr.

For biological perturbations, antibiotics were added 30 min prior to imaging at the following concentrations: rifampin (100 µg/mL); chloramphenicol (25 µg/mL); A22 (10 µg/mL); novobiocin (200 µg/mL); azide (0.01%) and deoxyglucose (1 mM).

**Microscopy:** Two microliters of media containing cells were placed on a 1% agarose pad made with the appropriate minimal media. *E. coli* were imaged on a Zeiss Axioplan 2 upright microscope; *Caulobacter* were imaged on a Nikon Diaphot 300 inverted microscope. Both species were viewed with a 60X objective lens. Images were collected on a cooled CCD camera (Princeton Instruments, Trenton, NJ) using MetaMorph software (Molecular Devices, Sunnyvale, CA). Time-lapse movies were taken for 100 frames at 1, 2, or 5 s intervals with a 200 ms exposure time.

**Data analysis:** Movies were analyzed with custom software in MatLab (Mathworks, Natick, MA). The position of *E. coli* movies were analyzed with custom software in MatLab (Mathworks, Natick, MA). The position of we simulated movies of diffusing particles with varying signal-to-noise (S/N) ratios to confirm that the ensemble-averaged mean square displacement was calculated for each data set, pooling trajectories from multiple (typically 3-6) movies from different fields of the same slide: \( \langle r^2(\tau) \rangle_{ens} = \frac{1}{N} \sum_n [r_n(\tau) - r_n(0)]^2 \), where N is the number of loci/particles. The time-averaged mean square displacement was calculated from a single time-series for each locus/particle: \( \langle r_n^2(\tau) \rangle = \frac{1}{T} \sum_t [r_n(t + \tau) - r_n(t)]^2 \), where T is the number of time steps in the trajectory. The velocity autocorrelation function was calculated for a single time-series as: \( C_\delta(v(t + \tau) \cdot v(t)) \), where \( v(t) = \frac{1}{\delta} [r(t + \delta) - r(t)] \) and \( \delta = 1 \) s.

**Data simulations:** We simulated movies of diffusing particles with varying signal-to-noise (S/N) ratios to confirm that the subdiffusive motion observed in vivo was not an experimental artifact. To generate random walks (diffusive; \( \alpha = 1 \)), a step size \( \delta x \) was chosen from a Gaussian distribution with an experimentally determined standard deviation (\( \sigma = 0.3154 \mu \text{m} \)). Time-series were constructed such that \( x(t) = x(t-1) + \delta x \), and similarly for \( y(t) \). A 2-dimensional Gaussian intensity profile was placed at each position \( [x(t), y(t)] \) on top of background noise. Shot noise was introduced by adding a uniformly distributed random number, weighted by the square-root of the intensity at each pixel.

The simulated movies were analyzed in MatLab with the same software used to analyze experimental movies. As shown in Supplemental Figure 1 and Supplemental Table 1, apparent subdiffusion can arise at short times from errors in position measurements in noisy images. However, for our experimental S/N ratio (~3.5), the effect was small. Indeed, Martin et al [5] predicted a scaling of 0.7-0.9 for our error and diffusion coefficient, while we calculated \( \alpha = 0.94 \). Furthermore, our experimental data exhibited the same scaling for almost 3 decades of time (\( 1 - 10^3 \) s). If noise

we performed two sets of simulations of a polymer composed of subdiffusive monomers; the
results of these simulations appear in Fig. 4 of the main text. The polymer chain in these simulations was modeled
as a discrete Gaussian chain [7], with \( M + 1 \) beads, Kuhn length \( b \), number of Kuhn segments per bead \( g \), thermal
energy \( k_B T \), and monomer drag coefficient \( \xi \). The first simulation had monomer segments that experience transient
pause events, thus behaving as a continuous time random walk (CTRW). Our model for the time distribution in the
diffusive state was

\[
S_{\text{diff}}(t)dt = \frac{1}{t_{\text{diff}}} \exp \left( -\frac{t}{t_{\text{diff}}} \right) dt,
\]

and our model for the time distribution in the pause state was

\[
S_{\text{pause}}(t)dt = \begin{cases} 
\frac{1}{\alpha+1} t_{\text{pause}}^{-\alpha} dt, & t < t_{\text{pause}} \\
\frac{1}{\alpha+1} \left( t_{\text{pause}}^{-\alpha} \right)^{\alpha+1} dt, & t \geq t_{\text{pause}}
\end{cases}
\]

A detailed discussion of this CTRW model is found in Ref. [8]. The simulations whose results appear in Fig. 4A
of the main text had parameter values: \( M = 99 \), \( b = 0.5477 \), \( g = 1 \), \( k_B T = 1 \), \( \xi = 1 \), \( t_{\text{diff}} = 1 \), \( t_{\text{pause}} = 1 \), and \( \alpha = 0.7 \). This choice of dimensionless parameters was equivalent to rescaling the time by \( 10(\xi b)^2/(3k_B T) \) and all lengths by \( b\sqrt{10}g/3 \), resulting in a strongly confined polymer chain for small confinement radii \((r < 6)\). The second simulation contained monomers that have a power-law memory kernel, consistent with fractional Langevin motion (FLM). A detailed discussion of this FLM model is found in Ref. [8]. Translating this model to a simulation required the equations of motion to be converted to a discrete-time representation. The discrete-time fluctuation dissipation theorem was written as

\[
\langle \vec{F}_i^{(B)} \vec{F}_i^{(B)} \rangle = \frac{2\xi k_B T}{\delta^{2-\alpha}} I, \quad \langle \vec{F}_i^{(B)} \vec{F}_j^{(B)} \rangle = \frac{2\xi k_B T}{\delta^{2-\alpha}} \left[ \left( |i-j| - 1 \right)^{2-\alpha} + \left( |i-j| + 1 \right)^{2-\alpha} - 2|i-j|^{2-\alpha} \right] I,
\]

where \( \vec{F}_i^{(B)} \) is the discrete-time Brownian force (averaged from \( t = i\delta - \delta/2 \) to \( t = i\delta + \delta/2 \)). Implementing this model into a numerical simulation required a numerical approximation to the fractional derivative representation of the particle velocity

\[
\xi \frac{d\vec{R}(t)}{dt} = \frac{1}{\Gamma(3-\alpha)} \alpha D_1^{1-\alpha} \vec{F}(t) = \frac{1}{\Gamma(3-\alpha)\Gamma(\alpha)} \frac{d}{dt} \left[ \int_0^t dt' \left( \frac{\vec{F}(t')}{t-t'} \right)^{1-\alpha} \right],
\]

where \( \vec{F}(t) \) is the total force (potential and Brownian forces) on the particle at time \( t \). We defined the discrete-time total force \( \vec{F}_j \), such that \( \vec{F}_0 = \vec{F}(t) \), \( \vec{F}_1 = \vec{F}(t-\delta) \), and \( \vec{F}_{j+2} = \vec{F}(t-\delta-(j+1)\Delta) \). With this definition, we derived a modified Grünwald definition of the fractional derivative

\[
\alpha D_1^{1-\alpha} \vec{F}(t) = \frac{1}{\Delta^{1-\alpha}} \sum_{j=0}^{n_{\text{cut}}} A_j + 1 \vec{F}_j
\]

where

\[
A_1 = \frac{\Gamma(\rho)\Gamma(2-\alpha)}{\Gamma(1-\alpha+\rho)}, A_{(j+1)\geq2} = \frac{\Gamma(j-1+\alpha)}{\Gamma(\alpha-1)\Gamma(j+1)} \frac{j}{j+\rho-1},
\]

\( \rho = \delta/\Delta \), and \( n_{\text{cut}} \) is a cutoff in the number of memory terms. As simulation time progresses, the memory terms in the simulation had to be re-discretized due to limitations in computer memory. This process of re-discretization removed half the data by averaging the forces of adjacent memory terms, increasing the memory step size from \( \Delta \) to \( 2\Delta \). This procedure resulted in memory inaccuracies at very long times; however, our results in Fig. 4B of the main text are sufficiently accurate to achieve close agreement with our analytical results. The simulations whose results appear in Fig. 4B of the main text had parameter values: \( M = 99 \), \( b = 0.5477 \), \( g = 1 \), \( k_B T = 1 \), \( \xi = 1 \), and \( \alpha = 0.7 \).
FIG. 1: (A) Snapshots of simulated data with different signal-to-noise ratios. (B) A log-log plot of the ensemble-averaged MSD for data simulated with each S/N ratio. The experimental S/N ratio was ~3.5.

SUPPLEMENTAL TABLE I

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<th>S/N</th>
<th>$\sigma$ (µm)</th>
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