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 *ara*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^{o}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^{o}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 chromosomal loci and RNA-protein particles was determined by non-linear least squares fitting to a 2-dimensional Gaussian function. These positions were projected onto the long (x) and short (y) axes of the cell to generate a time-series, $r(t) = (x(t), y(t))$, for each locus/particle. 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: $C_v(\tau) = \langle v(t+\tau)\cdot v(t)\rangle$, 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 δx was chosen from a Gaussian distribution with an experimentally determined standard deviation ($\sigma = 0.3154$ μm). 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 I, 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 is small. Indeed, Martin *et al* [5] predict a scaling of 0.7-0.9 for our error and diffusion coefficient, while we calculate $\alpha = 0.94$. Furthermore, our experimental data exhibit the same scaling for almost 3 decades of time ($1 - 10^3$ s). If noise were

responsible for the apparent subdiffusion, then we would expect to see a crossover to a larger α at longer times.

We also simulated movies of varying lengths - between 10^0 and 10^4 time steps - to investigate the distribution of apparent diffusion coefficients. Our results match those of Saxton, who previously showed that this distribution is very sensitive to the number of time steps in a trajectory [6].

Polymer simulations:

SUPPLEMENTAL FIGURE 1

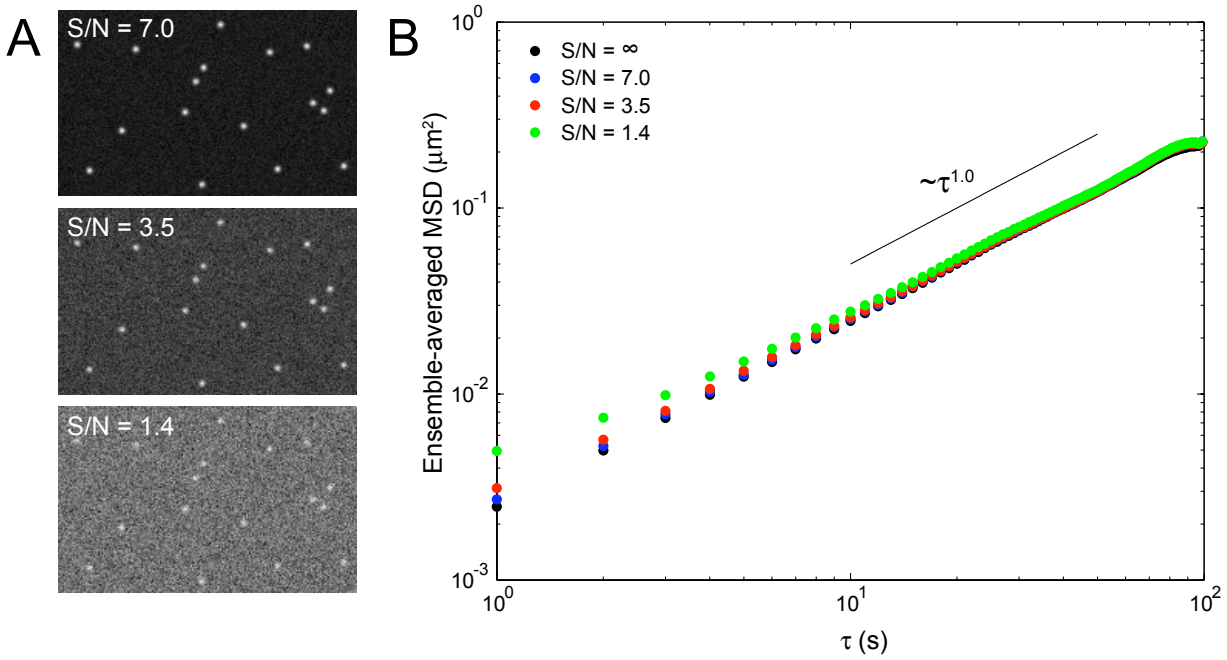

FIG. 1: (A) Snapshots of simulated data with different signal-to-noise ratios. (B) A log-log plot of the ensemble-averaged MSD for data simulated with each S/N ratio. The experimental S/N ratio was ~3.5.

SUPPLEMENTAL TABLE I

TABLE I: Power laws were fitted to the ensemble-averaged MSD for each S/N ratio.

S/N	σ (μm)	$2\sigma^2/4D$ (s)	α
7.0	0.01	0.083	0.99
3.5	0.02	0.333	0.94
1.4	0.04	1.33	0.87

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