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# PRECISE MEASUREMENT OF MIN PROTEIN OSCILLATIONS IN BACTERIAL CELLS USING TIRF MICROSCOPY

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**B**iological systems have many intricate structures and mechanisms that are truly fascinating examples of Nature's nanotechnology. A casual look through the classic textbook "Molecular Biology of the Cell"<sup>[1]</sup> provides many examples that inspire much of the current efforts in biological physics or quantitative biology. One striking example is the self-assembly of a variety of proteins within bacterial cells that control cell division, both spatially and temporally. In the rod-shaped bacterium *Escherichia coli*, an important part of the cell division process is the oscillation of Min proteins along the major axis of the cell<sup>[2]</sup>. This system has been the focus of much experimental<sup>[3-5]</sup> and theoretical<sup>[6,7]</sup> work during the past decade, since it can be studied using fluorescence microscopy by tagging the Min system with fluorescent proteins, and using simplified reaction-diffusion models that can reproduce many aspects of the system dynamics. By oscillating from pole to pole of the bacterial cell, a family of Min proteins (MinC, MinD and MinE) prevent the FtsZ protein division septum from forming anywhere except for the midpoint of the cell. This ensures that an equal amount of genetic material is transferred to the two daughter cells. Of particular interest is the protein MinD, which inhibits local formation of the FtsZ ring<sup>[3]</sup>. The MinD oscillation period is approximately 40 s for healthy *E. coli* cells<sup>[2]</sup> but it is highly sensitive to different stresses on the cell<sup>[4,5]</sup>. Because of this sensitivity of the oscillation period to changes in the environment of the bacterial cell, the MinD oscillation period is a good metric of cell viability.

## SUMMARY

We use high resolution TIRF microscopy to make precision measurements of MinD protein oscillations in *E. coli* as an *in-vivo* metric of cell viability.

In our laboratory, we have constructed a custom experimental setup that allows the measurement of changes in the MinD oscillation due to different stresses on a large number of bacterial cells belonging to the same population. The heart of the experiment is a custom-built,

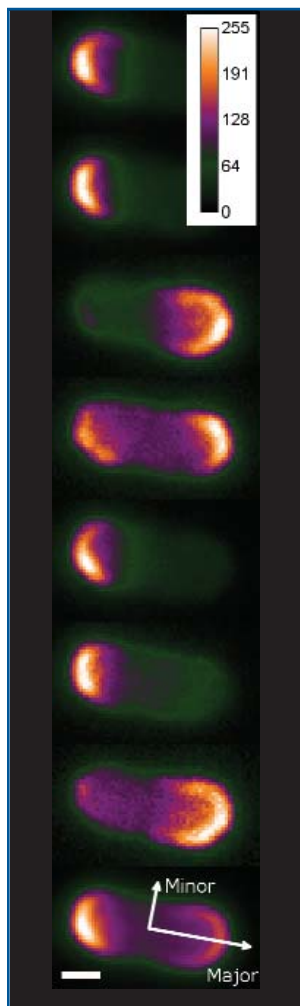


Fig. 1 Representative frames from a time series of TIRF microscopy images of an *E. coli* cell. The fluorescence is observed within the range 520 – 535 nm from the GFP-MinD fusion protein, and therefore indicates the local concentration of MinD. The frames are separated by 10 s and the scale bar shown is 500 nm in length. The color scale used to represent the intensity values in the images is shown as an inset. The pole-to-pole MinD oscillation is clearly visible, along with a characteristic "horse-shoe"-shaped localization of fluorescent intensity at the poles of the cell that is not clearly resolved when using a standard 60× TIRF objective lens.



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temperature controlled flow cell which allows us to flow through various reagents as well as bacterial cells. The flow region of the cell is defined by a glass coverslip coated with a thin layer of the cationic polymer poly-L-lysine (PLL). The flowing of bacteria suspended in phosphate buffered saline (PBS) results in adhesion of the negatively-charged bacterial cells to the positively-charged PLL layer on the glass coverslip. We have used an *E. coli* strain PB103/ $\lambda$ DR122, which has been genetically modified to express GFP-tagged MinD proteins [2]. This allows the MinD oscillation to be visualized using fluorescence microscopy. We image the cells using total internal reflection fluorescence (TIRF) microscopy using an Olympus IX71 inverted optical microscope with a 150 $\times$  magnification TIRF objective lens. Fluorescence in the GFP-tagged MinD is excited with a Spectra Physics Cyan Laser System (20 mW power,  $\lambda = 488$  nm), and images are captured with a high sensitivity, 1.4 megapixel CCD camera (Retiga EXi). By using TIRF, we excite only fluorophores that lie within a small distance ( $\sim 100$  nm) of the coverslip surface within the flow cell, resulting in fluorescence only from bacterial cells that are adhered to the coverslip surface. This small excitation depth significantly increases the contrast relative to that in epifluorescence techniques, in which all fluorophores within the sample are excited. To reduce photobleaching of the GFP fluorophores, we limited the exposure time to 200 ms and attenuated the laser light intensity by a factor of four using neutral density filters. A time sequence of representative images is shown in Fig. 1.

The MinD oscillation periods were determined by analyzing a time series of images using a custom program that is written using MATLAB<sup>®</sup>. In the image analysis procedure, bacterial

cells are selected in each image by choosing well-adhered cells with a length of the order of 2  $\mu\text{m}$  that is characteristic of the late exponential phase for *E. coli* bacteria. For each selected bacterium, the position of the “centre of mass” of the fluorescence intensity is tracked. After determining the orientation of the cell within the sample plane, the trajectory is decomposed into its major and minor axis components. The major axis component of the intensity oscillation is fitted to a general sinusoidal function:

$$y(t) = A \cos\left(\frac{2\pi}{\tau}t - \phi\right) \quad (1)$$

where  $A$  is the amplitude of the oscillation,  $\tau$  is the oscillation period and  $\phi$  is a phase shift. A least squares fit of the major axis intensity oscillation to Eq. 1 allows the precise determination of the oscillation period  $\tau$ . A typical data set and the corresponding least squares fit to the data is shown in Fig. 2.

Typically, many bacterial cells are observed within each image. For each experimental condition, the oscillation periods  $\tau$  of many cells are measured. A typical distribution of measured  $\tau$  values is shown in Fig. 3. We characterize the distribution by the average value of  $\tau$  and its standard error  $\sigma_\tau = \sigma/\sqrt{N}$ , where  $\sigma$  is the standard deviation and  $N$  is the number of cells that are included in the distribution, such that  $\sigma_\tau$  decreases with increasing  $N$ .

As an example of the data that we can obtain with our custom experiment and analysis software, we show the measured temperature dependence of the MinD oscillation period in Fig. 4. We measure the relative change in the oscillation period by normalizing all values to the value at 25.°C. A dramatic

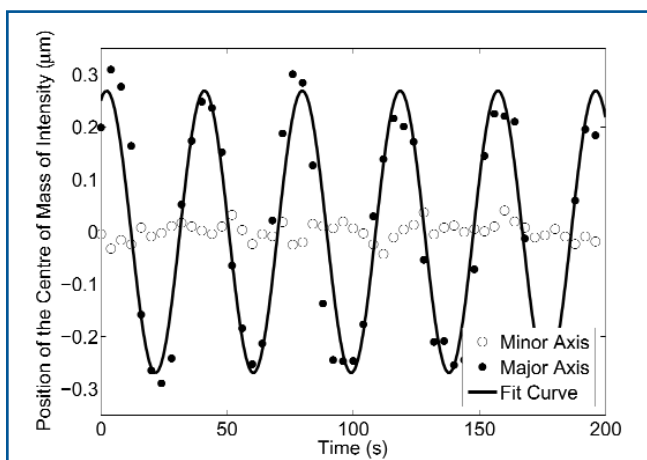


Fig. 2 A plot of the position of the center of mass of the intensity of the fluorescence signal versus time within an *E. coli* bacterial cell. Closed and open circles indicate the components of the trajectory along the major and minor axes, respectively. The solid line is a least-squares fit of the major axis component of the trajectory to Eq. 1. The pole-to-pole nature of the oscillation is evident in the major axis component, and the fit allows the determination of the MinD oscillation period  $\tau$ .

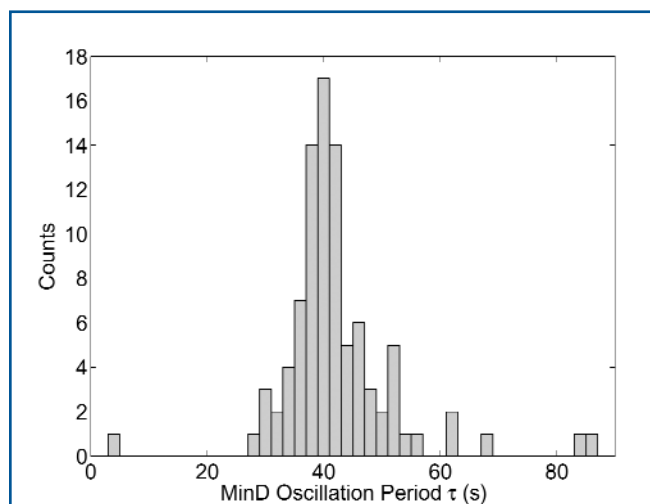


Fig. 3 A typical distribution of the MinD oscillation period  $\tau$  for *E. coli* cells, obtained from a time series of TIRF images. For this distribution,  $N = 91$ , the average value of  $\tau$  is 42 s, and the standard error  $\sigma_\tau$  is 1 s.

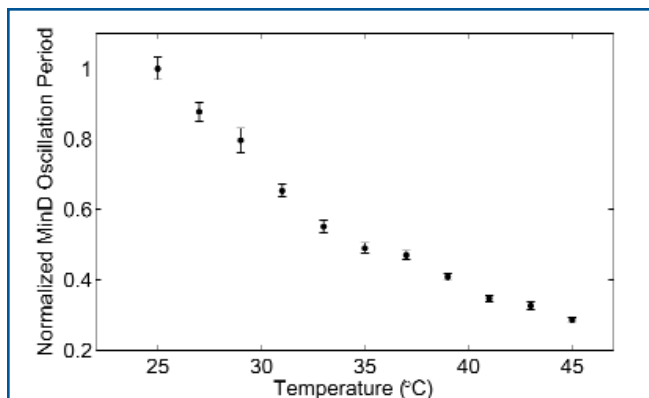


Fig. 4 The temperature dependence of the *E. coli* MinD oscillation period  $\tau$  values, which are normalized to the value measured at 25°C ( $49 \pm 1$  s). The error bars indicate the standard error  $\sigma_{\tau}$  of the measured  $\tau$  values.

decrease in the oscillation period is observed with increasing temperature, as reported by Touhami *et al.*<sup>[5]</sup>, which highlights the need for precise temperature control during the experiment.

Because our 150x TIRF objective lens provides high spatial resolution in the captured images, we are developing more sophisticated methods to extract more detailed information from the images. We are also measuring the effects of cationic antimicrobial peptides on the MinD oscillation, with the hope of elucidating their mechanism of action.

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#### REFERENCES

1. B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter, *Molecular Biology of the Cell, Fifth Edition*, Garland Science: New York, 2007.
2. D.M. Raskin, P.A.J. de Boer, "Rapid pole-to-pole oscillation of a protein required for directing division to the middle of *Escherichia coli*", *Proc. Natl. Acad. Sci. USA*, **96**, 4971-4976 (1999).
3. J. Lutkenhaus, "Assembly dynamics of the bacterial MinCDE system and spatial regulation of the Z ring", *Annu. Rev. Biochem.*, **76**, 539-562 (2007).
4. B.P.B. Downing, A.D. Rutenberg, A. Touhami, M. Jericho, "Subcellular Min oscillations as a single-cell reporter of the action of polycations, protamine, and gentamicin on *Escherichia coli*", *PloS One*, **4**, e7285 (2009).
5. A. Touhami, M. Jericho, A.D. Rutenberg, "Temperature dependence of MinD oscillation in *Escherichia coli*: running hot and fast" *J. Bacteriol.*, **188**, 7661-7667 (2006).
6. K. Kruse, M. Howard, W. Margolin, "An experimentalist's guide to computational modelling of the Min system", *Mol. Microbiol.*, **63**, 1279-1284 (2007).
7. K.C. Huang, Y. Meir, N.S. Wingreen, "Dynamic structures in *Escherichia coli*: spontaneous formation of MinE rings and MinD polar zones", *Proc. Natl. Acad. Sci. USA*, **100**, 12724-12728 (2003).