



# An Ultrasensitive Bacterial Motor Revealed by Monitoring Signaling Proteins in Single Cells

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# Outline

Introduce the Bacterial  
Motor/Signaling protein



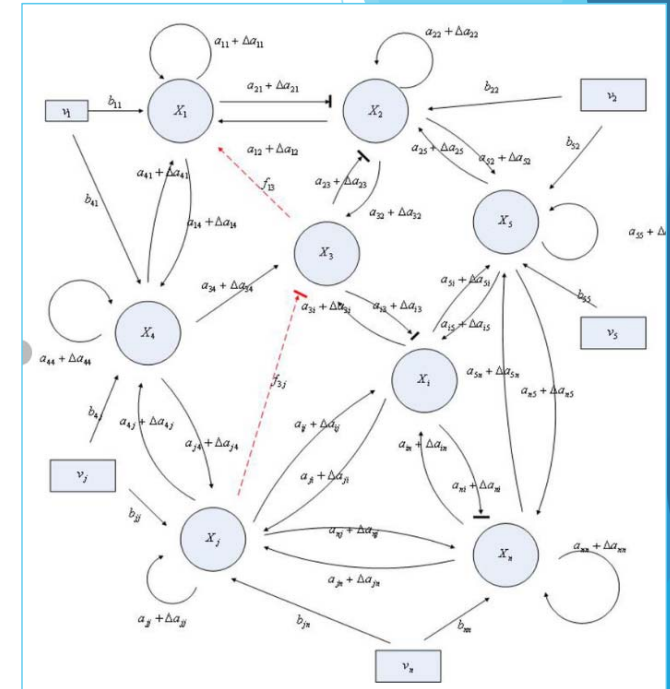
Breakdown Fluorescence  
Correlation Spectroscopy



Compare results/discuss  
implications

# Introduction - Why care?

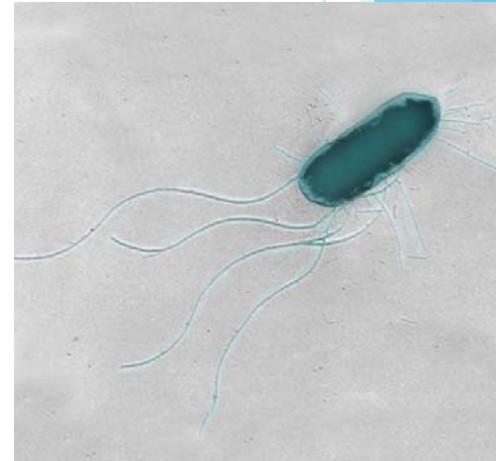
- ▶ Biochemical networks are the CPU's of cell life
- ▶ Current understanding of these networks relies mainly on data collected from cell populations
- ▶ This study presents an experimental method to study such biochemical networks at the single-cell level



[https://www.researchgate.net/figure/Linear-biochemical-network-of-n-molecules-with-intrinsic-parameter-fluctuation-1a-and\\_fig1\\_3481318](https://www.researchgate.net/figure/Linear-biochemical-network-of-n-molecules-with-intrinsic-parameter-fluctuation-1a-and_fig1_3481318)

# E. coli

- ▶ Flagella can either rotate clockwise or counter clockwise
  - ▶ CW = tumbling, CCW = swimming smoothly
- ▶ Chemotactic signaling protein Chey-P is produced in response to outside stimuli
- ▶ Resulting motion leads E.coli away from danger

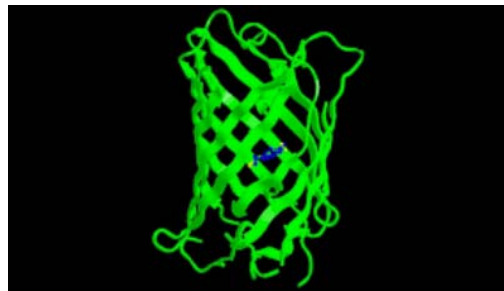


# Objective and Methods

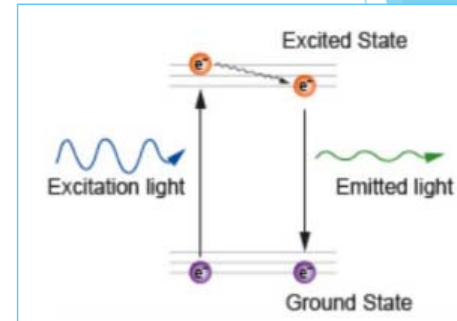
- ▶ Observe the input-output relation between Chey-P and flagellar motion in a single *E. coli*
  - ▶ Record counter clockwise vs clockwise motion bias
- ▶ Control and measure Chey-P Concentration
  - ▶ Done with Fluorescence Correlation Spectroscopy (FCS)
- ▶ Compare these results to studies involving cell populations
  - ▶ Previously, Chey-P was ruled out as the signaling protein of CW bias due to weak correlation


# FCS - Fluorescence

- ▶ Fluorescence is the emission of light by a substance that has absorbed EM radiation
- ▶ Green Fluorescent Protein (GFP) is used as our source of fluorescence



[https://proteopedia.org/wiki/index.php/Green\\_Fluorescent\\_Protein](https://proteopedia.org/wiki/index.php/Green_Fluorescent_Protein)



 The Nobel Prize in Chemistry 2008  
Osamu Shimomura, Martin Chalfie, Roger Y. Tsien

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## The Nobel Prize in Chemistry 2008



Photo: U. Montan  
**Osamu Shimomura**  
Prize share: 1/3



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**Roger Y. Tsien**  
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The Nobel Prize in Chemistry 2008 was awarded jointly to Osamu Shimomura, Martin Chalfie and Roger Y. Tsien *"for the discovery and development of the green fluorescent protein, GFP"*.

Photos: Copyright © The Nobel Foundation

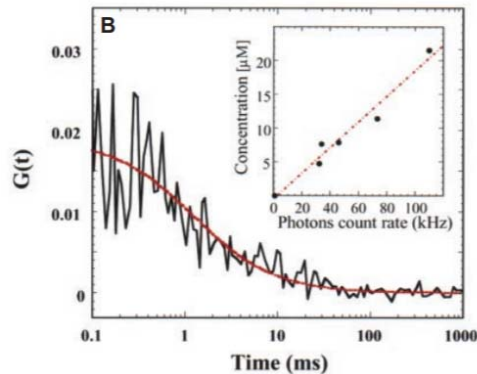
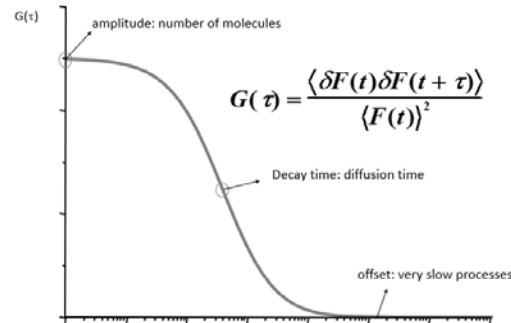
# FCS - Correlation

▶ General Autocorrelation function

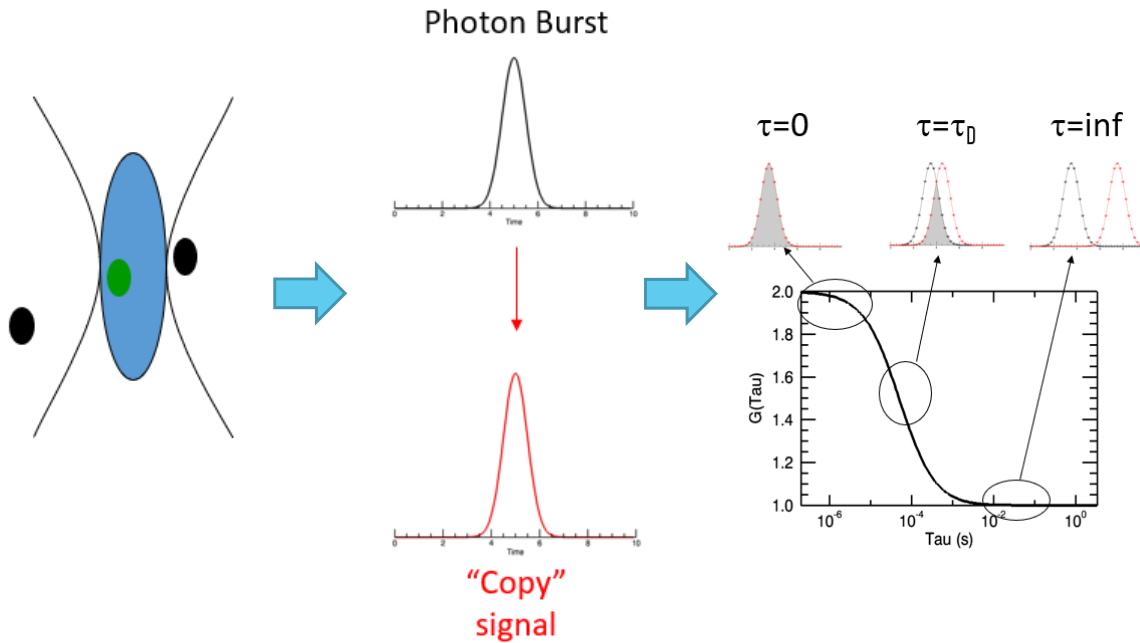
- ▶ With  $F(t) = \kappa Q \int d\mathbf{r} W(\mathbf{r}) C(\mathbf{r}, t)$ 
  - ▶ Q is the quantum yield
  - ▶ W(r) describes the observation volume
  - ▶ C(r,t) describes fluorophore concentration

▶ For this study,  $G(t) = \frac{1}{N} \left[ 1 + \frac{4Dt}{\omega^2} \right]$

- ▶ N is the number of molecules of GFP
- ▶ D is the diffusion constant
- ▶  $\omega = 0.3$  micro meters



# Fluorescence Correlation Spectroscopy

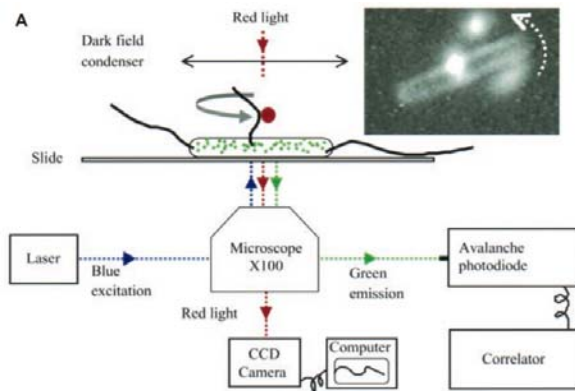


Animation from <https://www.zeiss.com/content/dam/Microscopy>



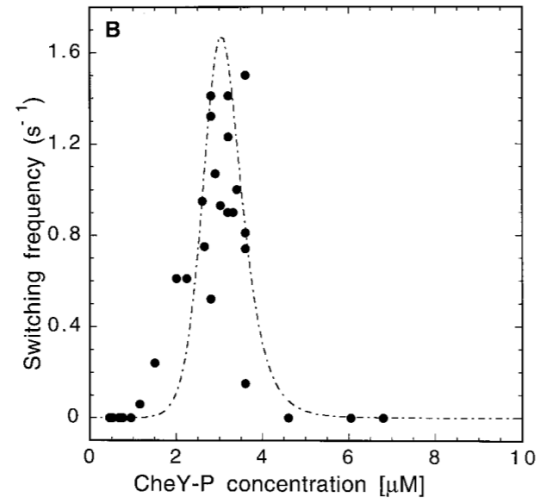
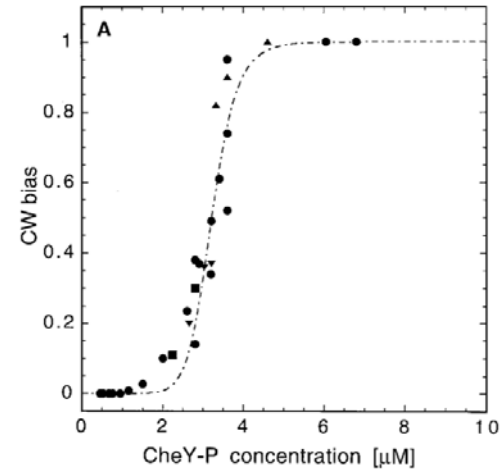
# Experimental Setup

- ▶ Green fluorescent protein needs to fuse with Chey-P
- ▶ The PS2001 strain of *E. Coli* lacks the *Chey-P* gene entirely
  - ▶ Plasmid pMGS98 (CMR) introduced to give a Chey-GFP expressing gene
- ▶ Concentration of Chey-GFP is observed at the same time as flagella rotation bias
  - ▶ Inducer isopropyl- $\beta$ -D-thigalactoside (IPTG) used to promote Chey-GFP



# Results

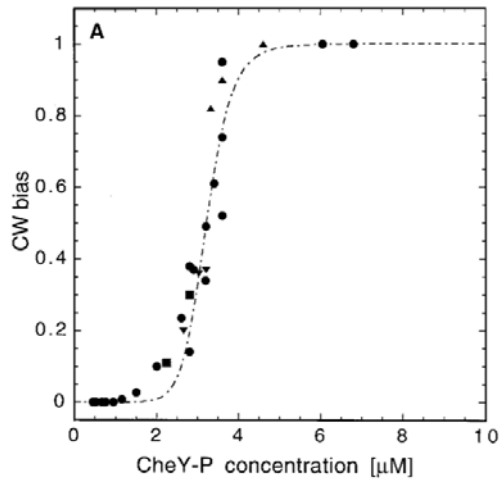
- Strong correlation between CW bias and CheY-P concentration
- Hill coefficient of  $10.3 \pm 1.1$ 
  - Previous studies found to have Hill coefficient between 3.5 - 5.5



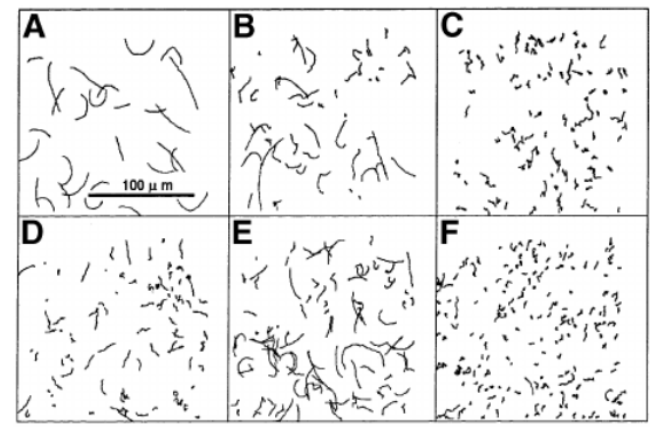
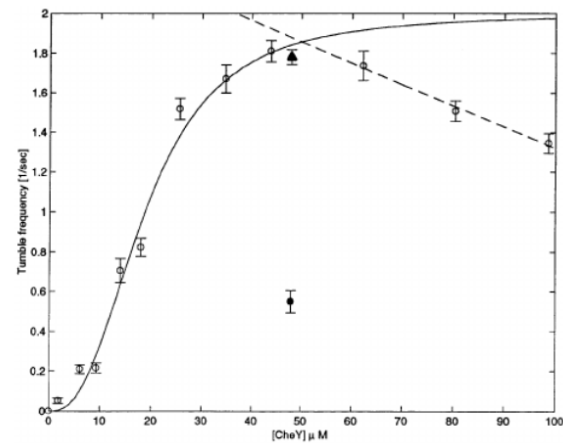
# Comparison to Prior Studies

## ➤ Single Molecule vs Population based

Cluzel *et al.*



U.Alon *et al.*



Images taken from U. Alon *et al.*, EMBO J. 17, 4238 (1998).

# Conclusion

- ▶ A higher Hill coefficient indicates a stronger correlation between motion and Chey-P
- ▶ A strong correlation between chemotactic protein Chey-P and the rotational bias of flagella motors is shown
- ▶ This study demonstrates the indispensable value of single-cell measurements

# References

- ▶ Cluzel et al, Science vol 287 pg 1652 - 1654 (2000)
  - ▶ [https://pdfs.semanticscholar.org/a1cd/607b6d25f03b4974663fca155d8f868229aa.pdf?\\_ga=2.57128942.553286184.1568330894-1190593417.1568330894](https://pdfs.semanticscholar.org/a1cd/607b6d25f03b4974663fca155d8f868229aa.pdf?_ga=2.57128942.553286184.1568330894-1190593417.1568330894)
- ▶ U. Alon et al., EMBO J. 17, 4238 (1998).
  - ▶ <https://www.embopress.org/doi/full/10.1093/emboj/17.15.4238>