细菌运动的物理机制

袁军华

staff.ustc.edu.cn/~jhyuan

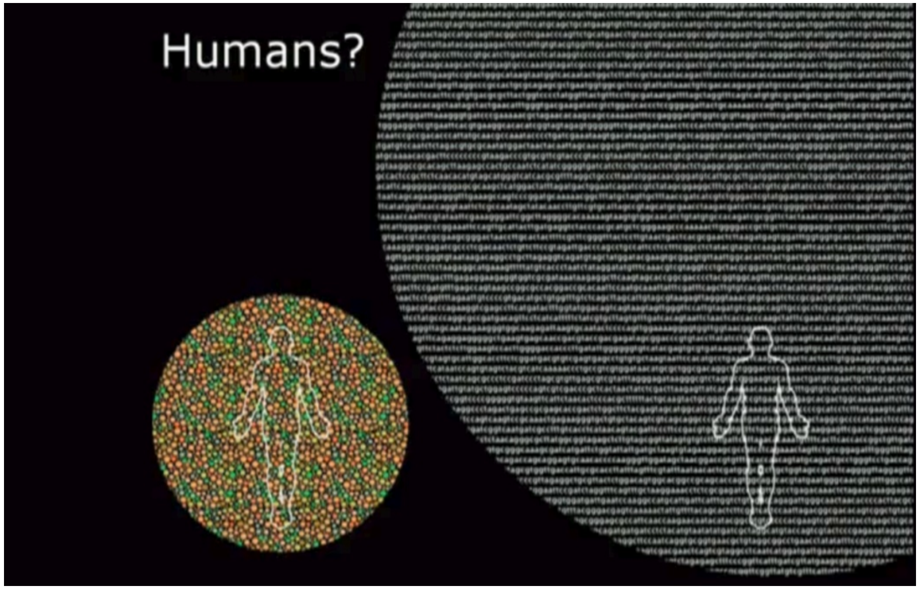
中国科学技术大学物理系

Why bacteria?

Anything found to be true of *E. coli* must also be true of elephants.

– Jacques Lucien Monod (1910–1976),
1965 Nobel Laureate

Diversity & abundance in the bacterial kingdom



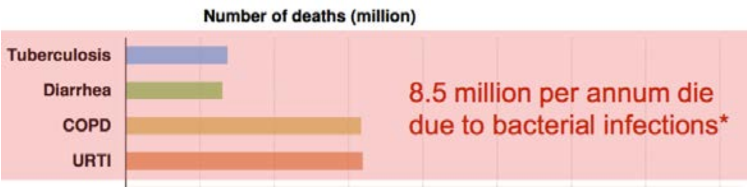
10x more bacterial cells
100x more bacterial genes

(image via B. Bassler)

5×10^{30} bacteria on Earth, biomass > all plants+animals

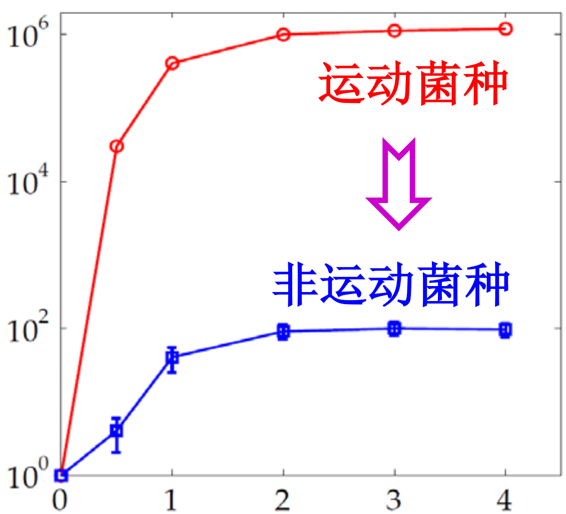
研究意义（细菌运动）

抑制细菌感染



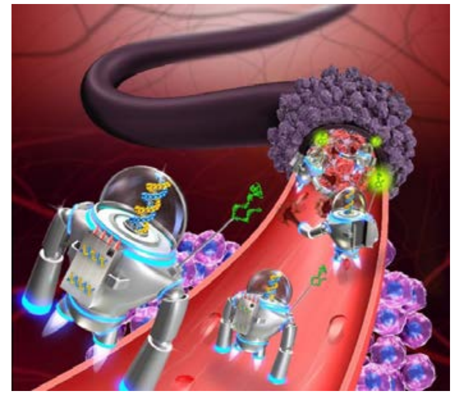
WHO: 每年850万人死于细菌感染

感染细菌数量

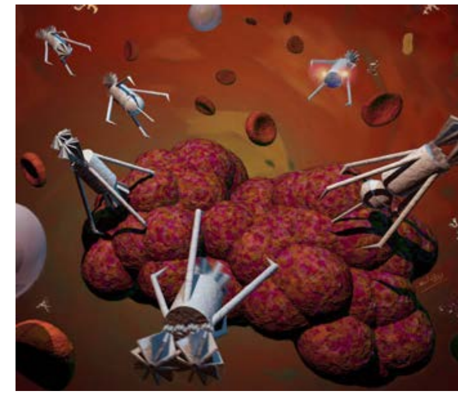


时间（小时）

人工微纳机器



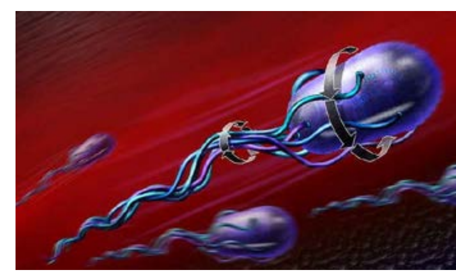
血管疏通



药物输运

基本物理要求：自驱动、运动方向可控

人工：扩散

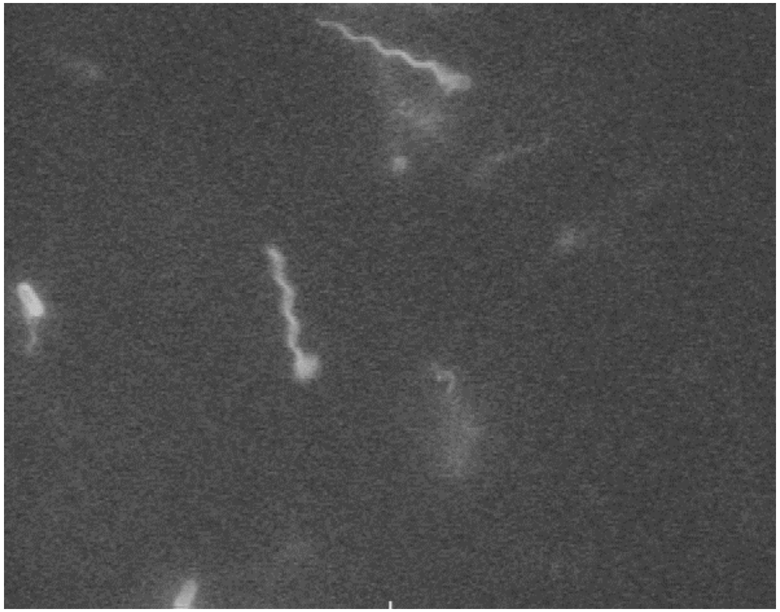


细菌

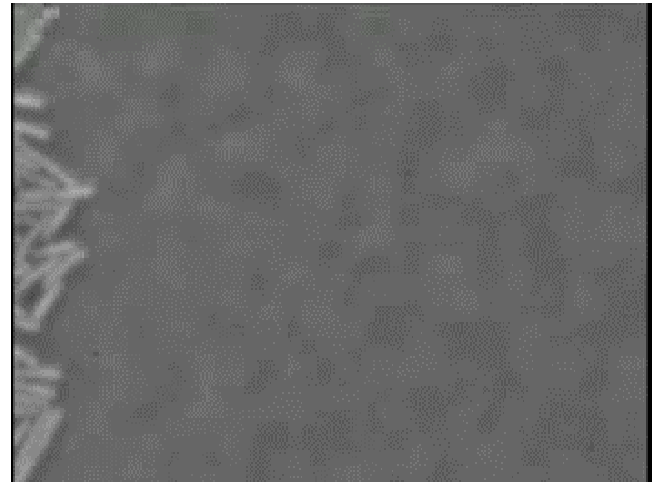
⇒ 提供抗菌新思路

⇒ 启发未来的人工微纳机器

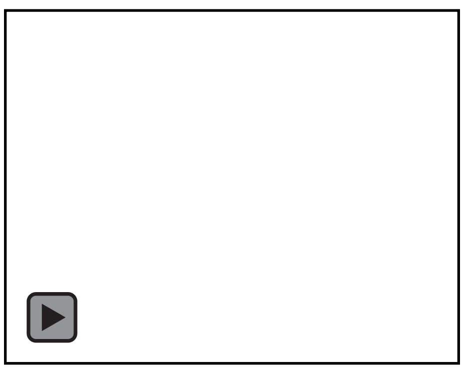
Different ways of bacterial movement



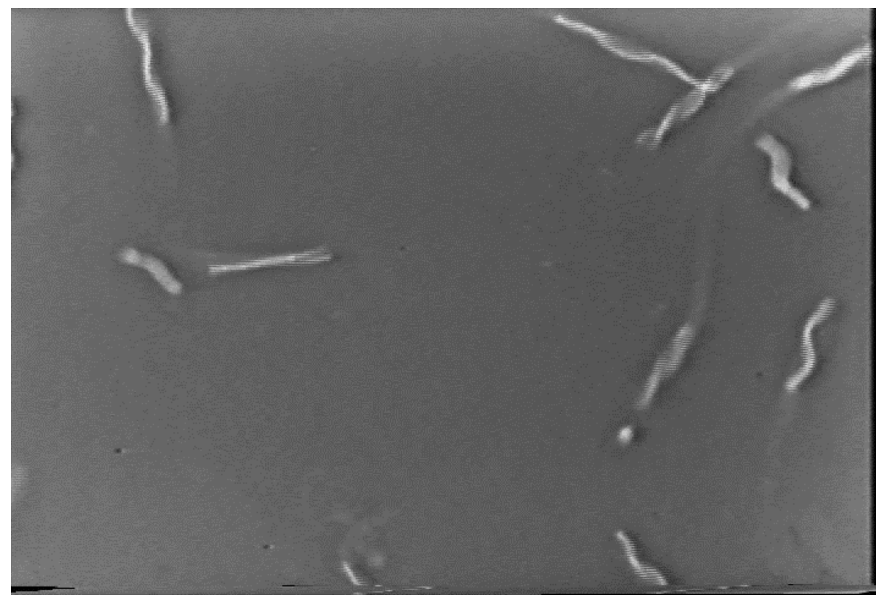
Swimming *E. coli*



Swarming *E. coli*



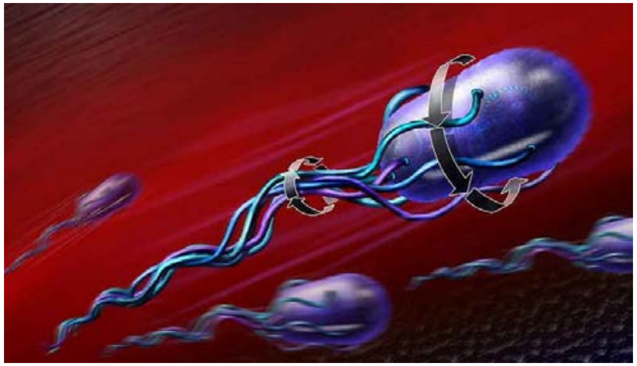
Twitching *pseudomonas*



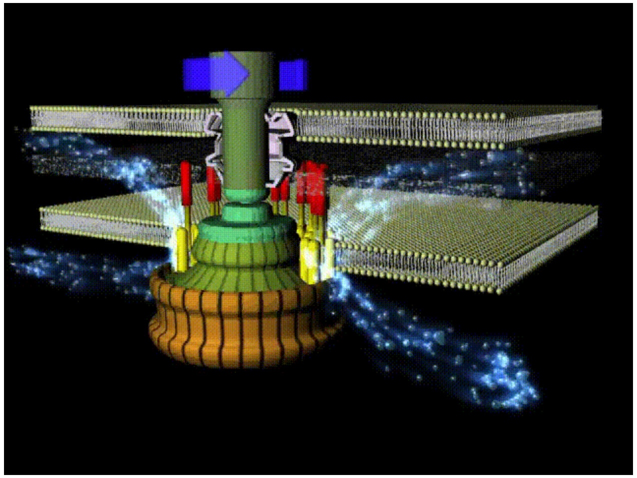
Swimming *S. volutans*

http://www.rowland.harvard.edu/labs/bacteria/index_movies.html

E. Coli & Flagella-based motility

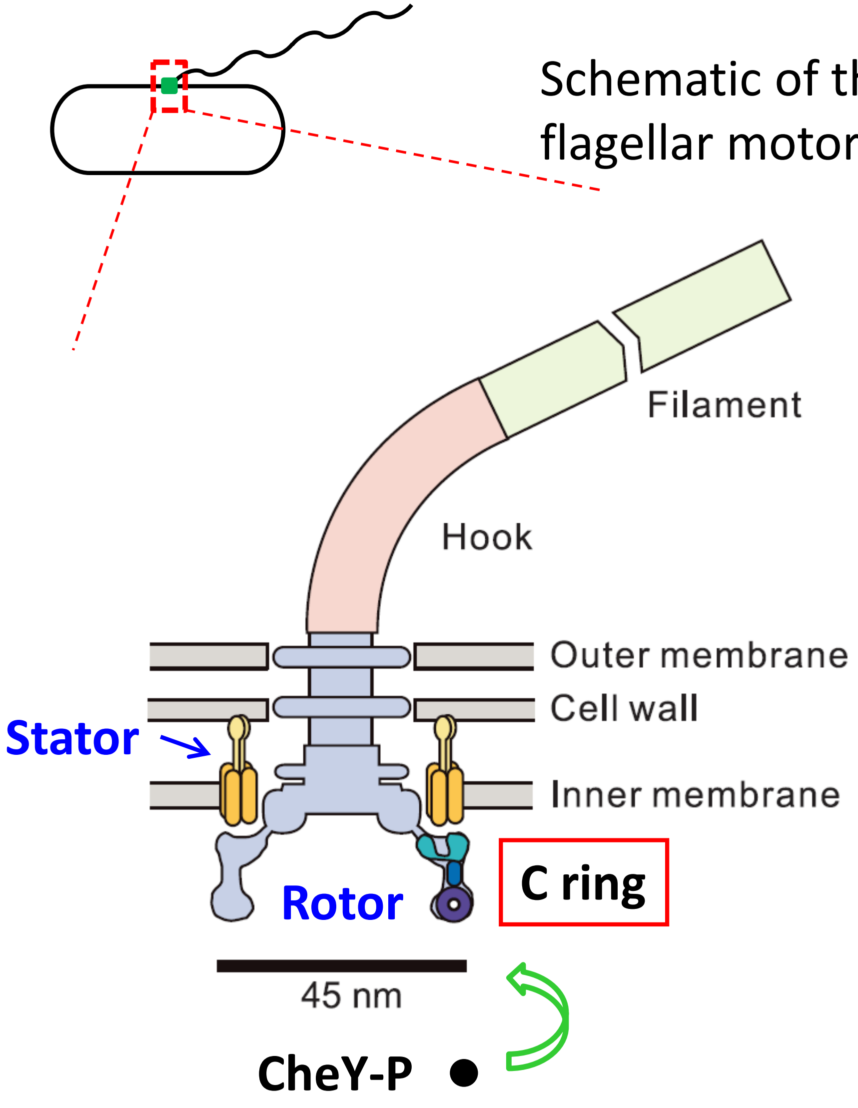


E. coli



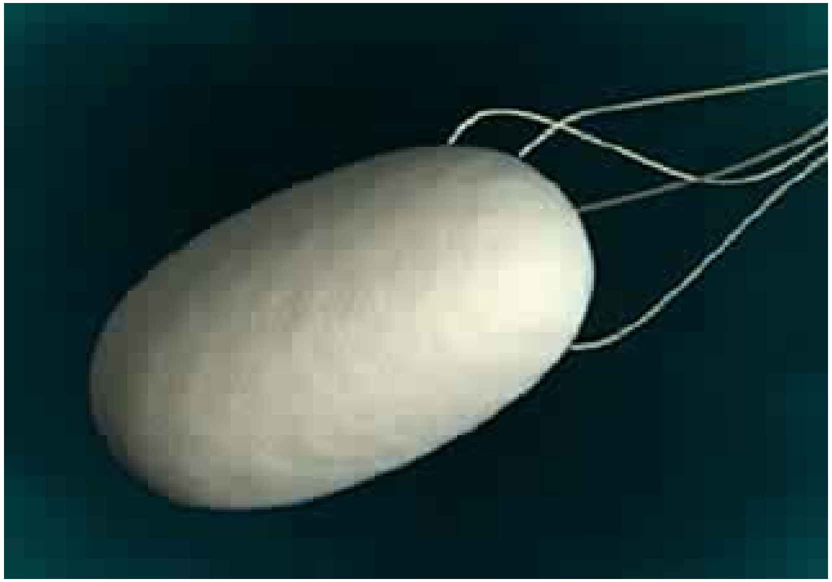
PMF:
Proton motive force

Schematic of the flagellar motor

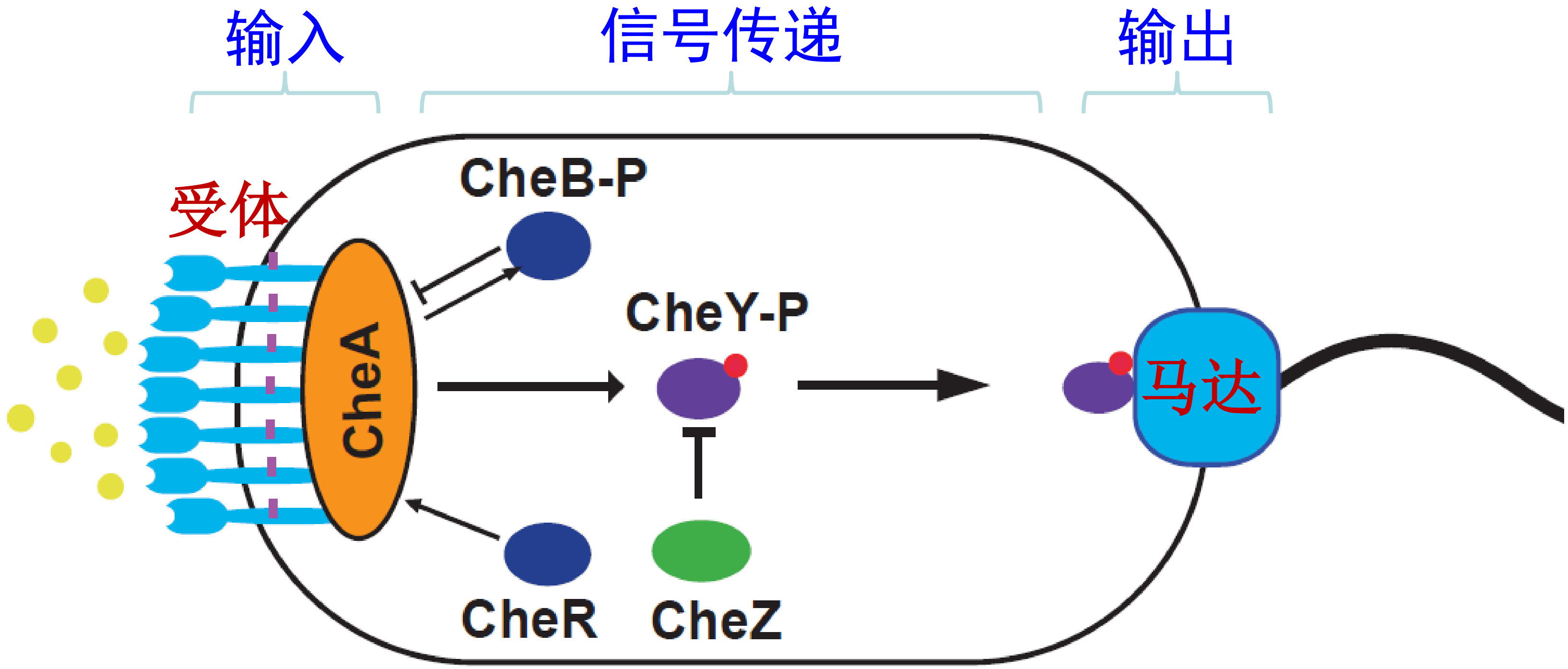


An analogy for torque-generation

鞭毛马达自组装



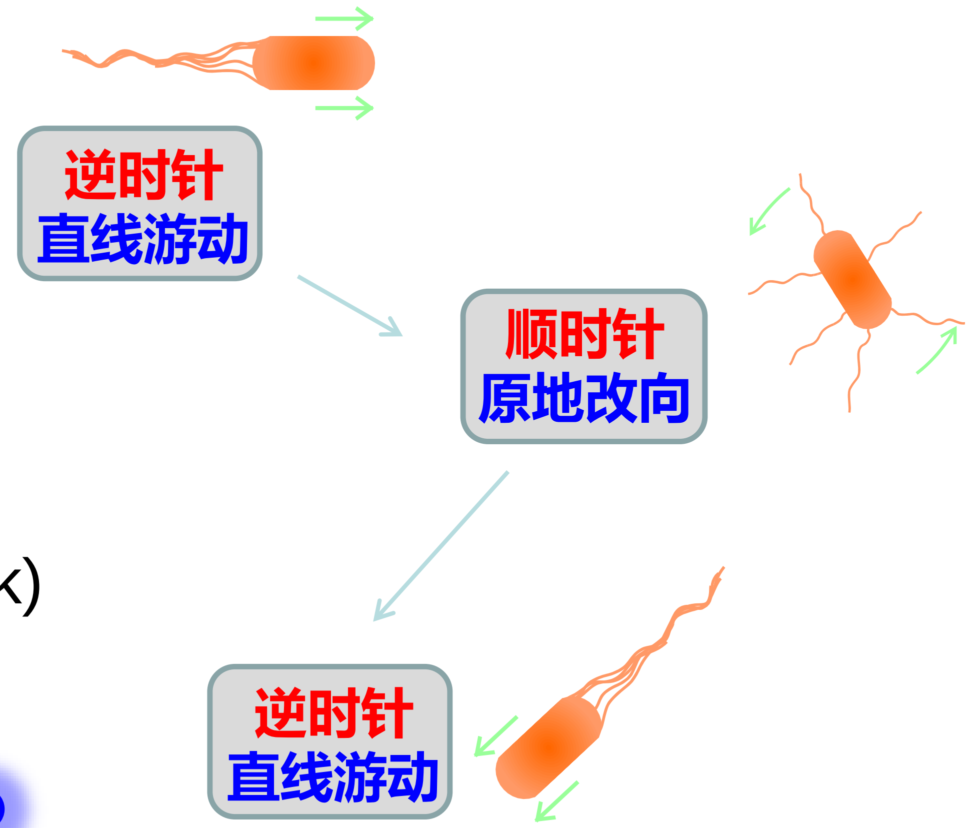
研究背景：大肠杆菌趋化运动



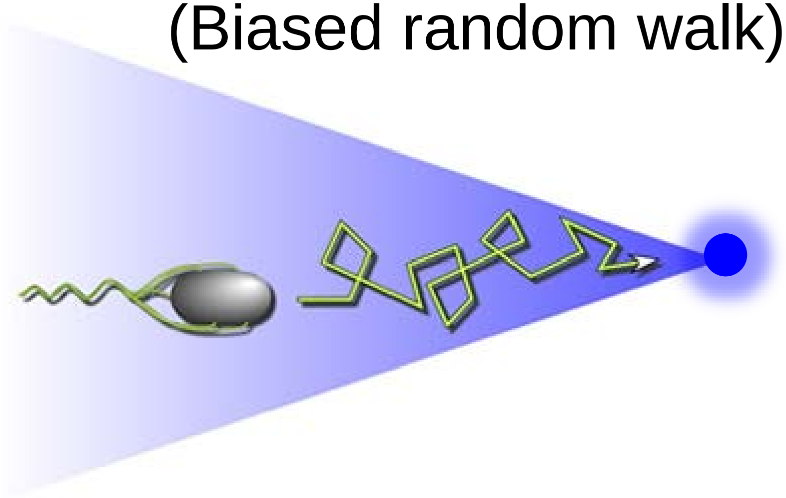
趋化信号转导系统（示意图）

研究背景：大肠杆菌趋化运动

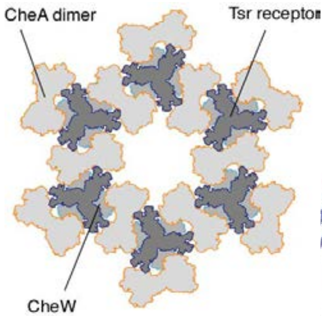
马达转动方向：
细菌游动状态：



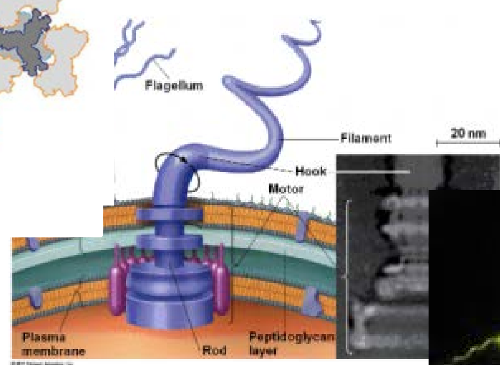
有偏向性的三维随机行走
(Biased random walk)



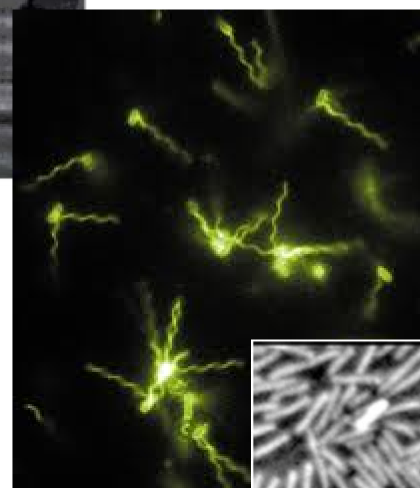
主要研究方向：细菌运动行为的多尺度研究



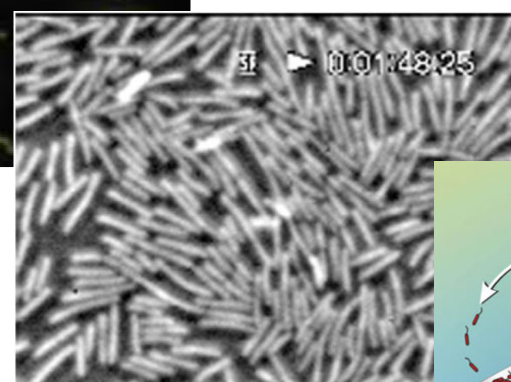
受体蛋白分子水平



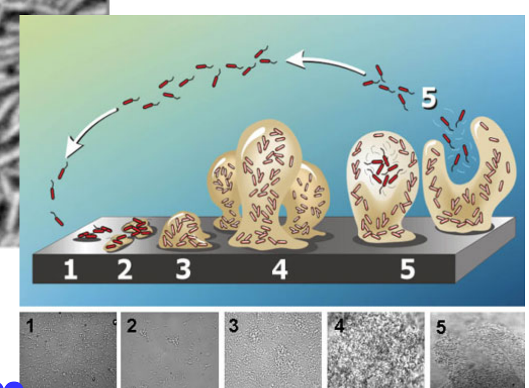
单马达水平



单细菌水平

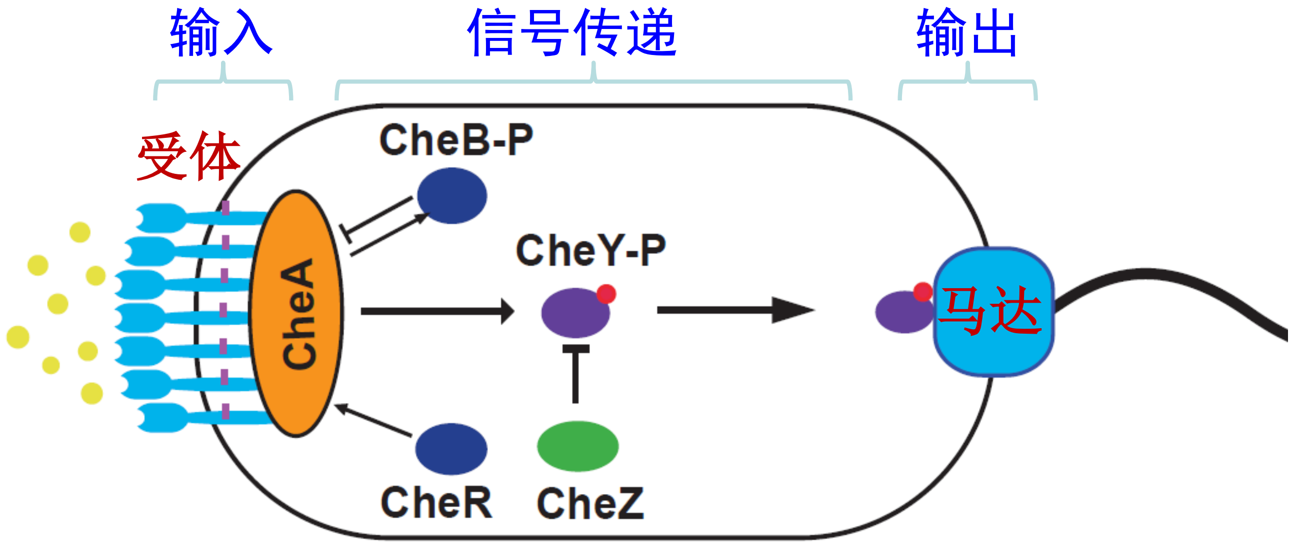


细菌群体



Biofilm

科学问题



② 马达转向如何调控?

转向改变

信号 ⟹ 马达

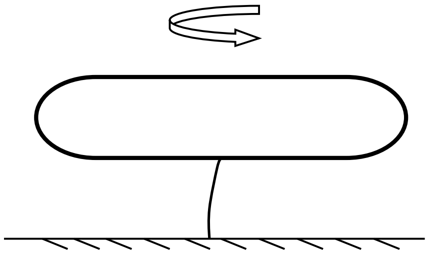
力矩

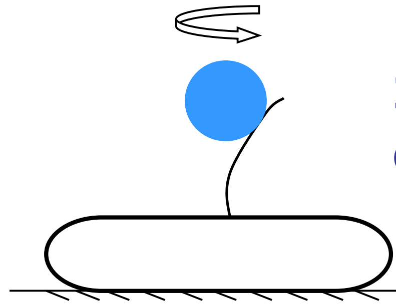
① 信号如何可靠地传递到马达?
（输入输出如何鲁棒耦合）

③ 马达力矩如何产生?

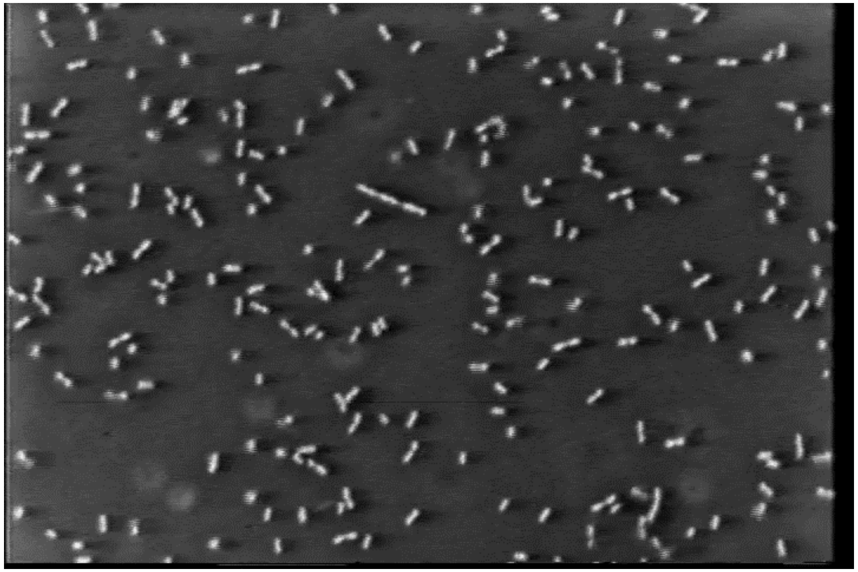
常用实验手段

单马达标记

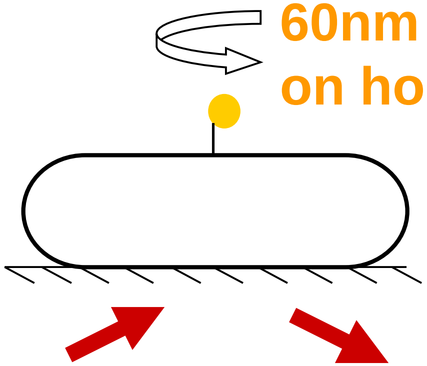




1 to 0.35μm latex beads
on a filament stub

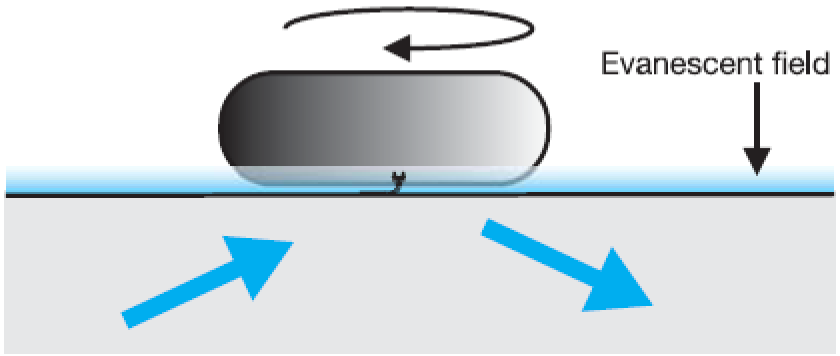


60nm dia. gold bead
on hook



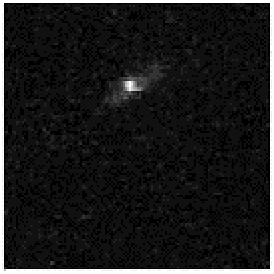
激光暗场

各种荧光技术

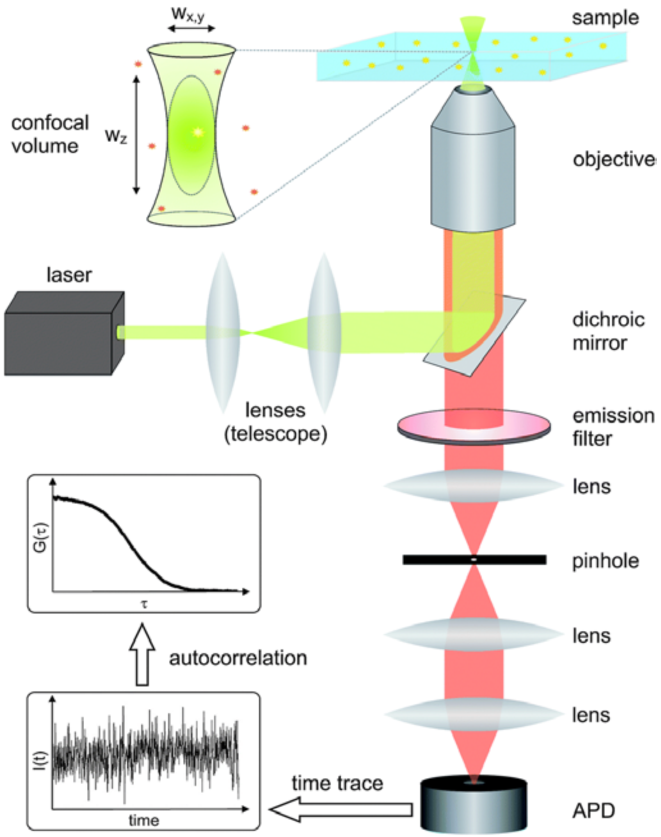


全内反射荧光(TIRF)

宽场荧光

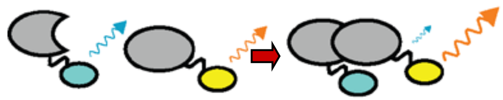


全内反射荧光

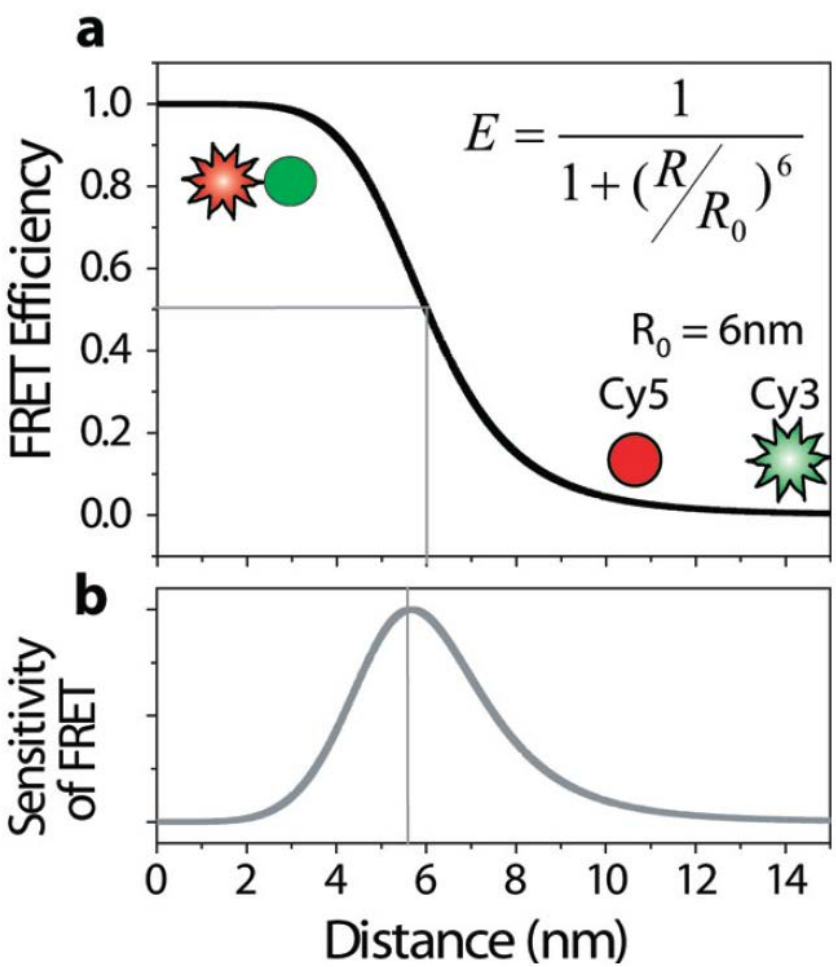


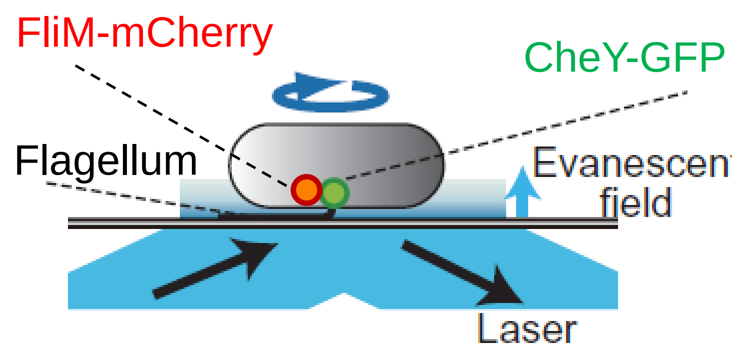
荧光相关光谱(FCS)

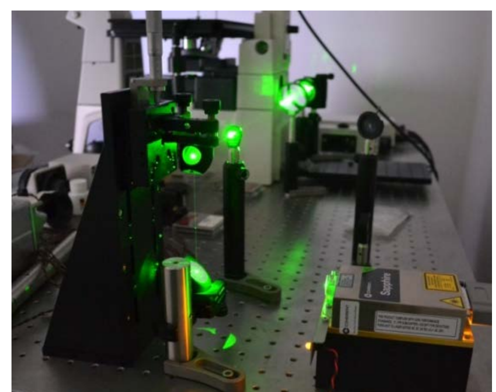
单分子荧光共振能量转移(smFRET)



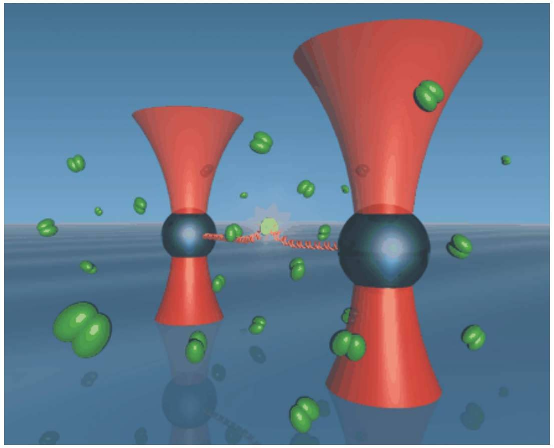
GFP & mCherry FRET
Cy3 & Cy5 FRET

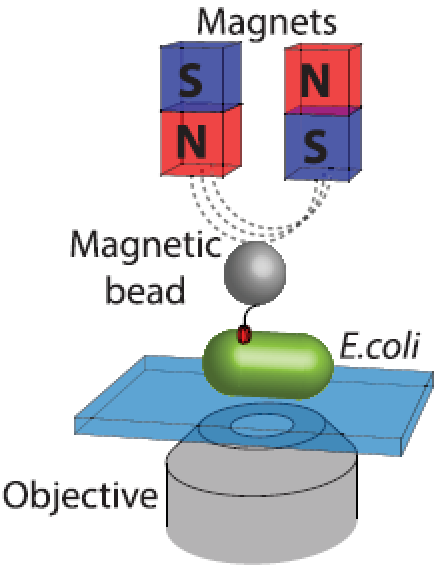


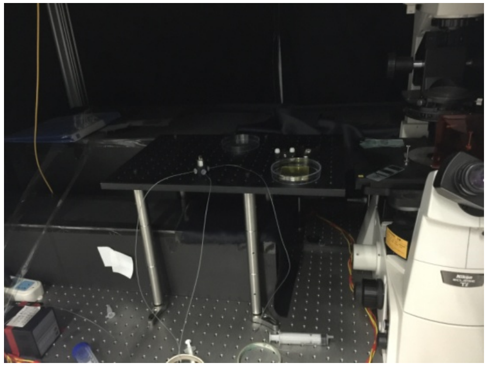


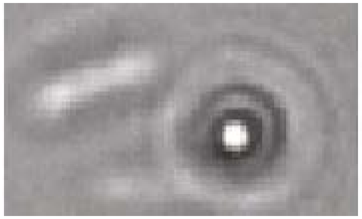


光镊、磁镊









超分辨成像

光学荧光超分辨
（分辨率10纳米）

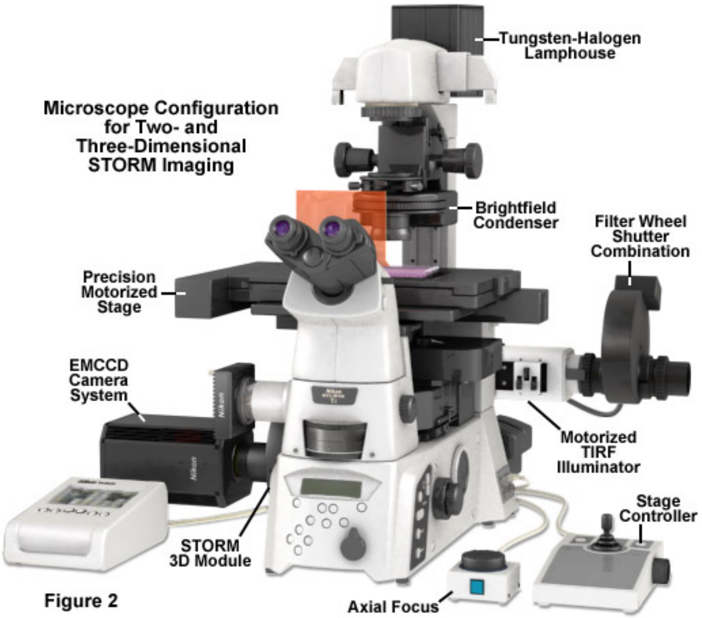
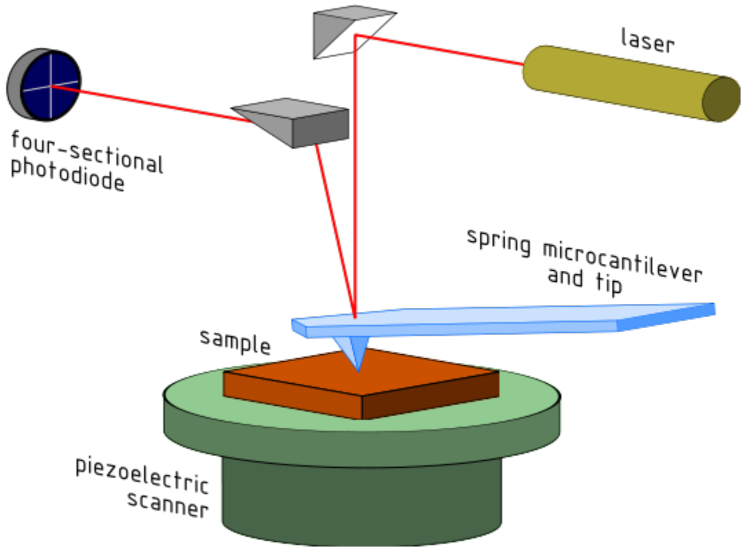


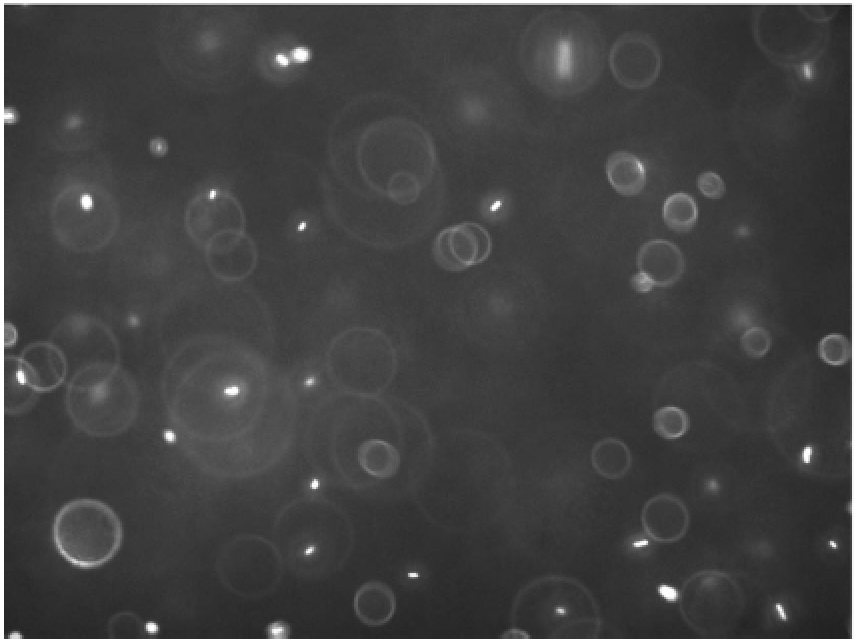
Figure 2

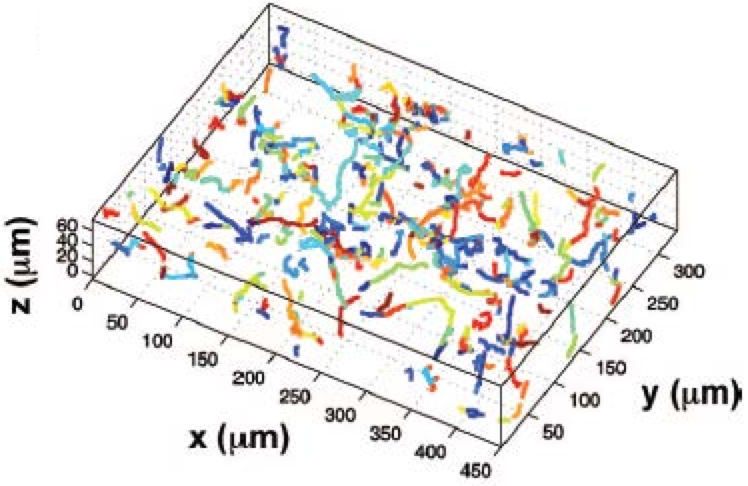
原子力显微镜
（分辨率1纳米）



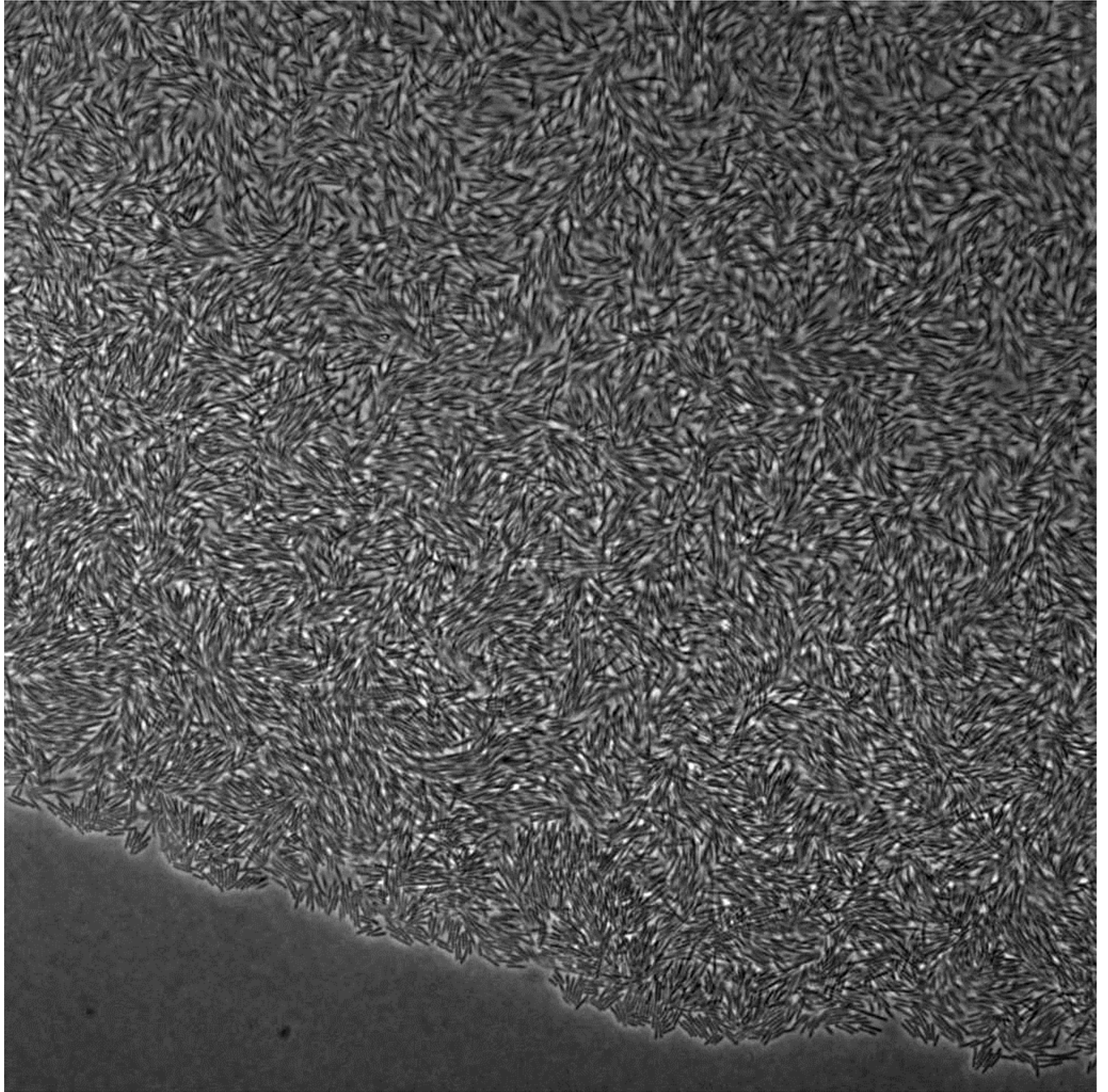
细菌运动追踪

3-d tracking:

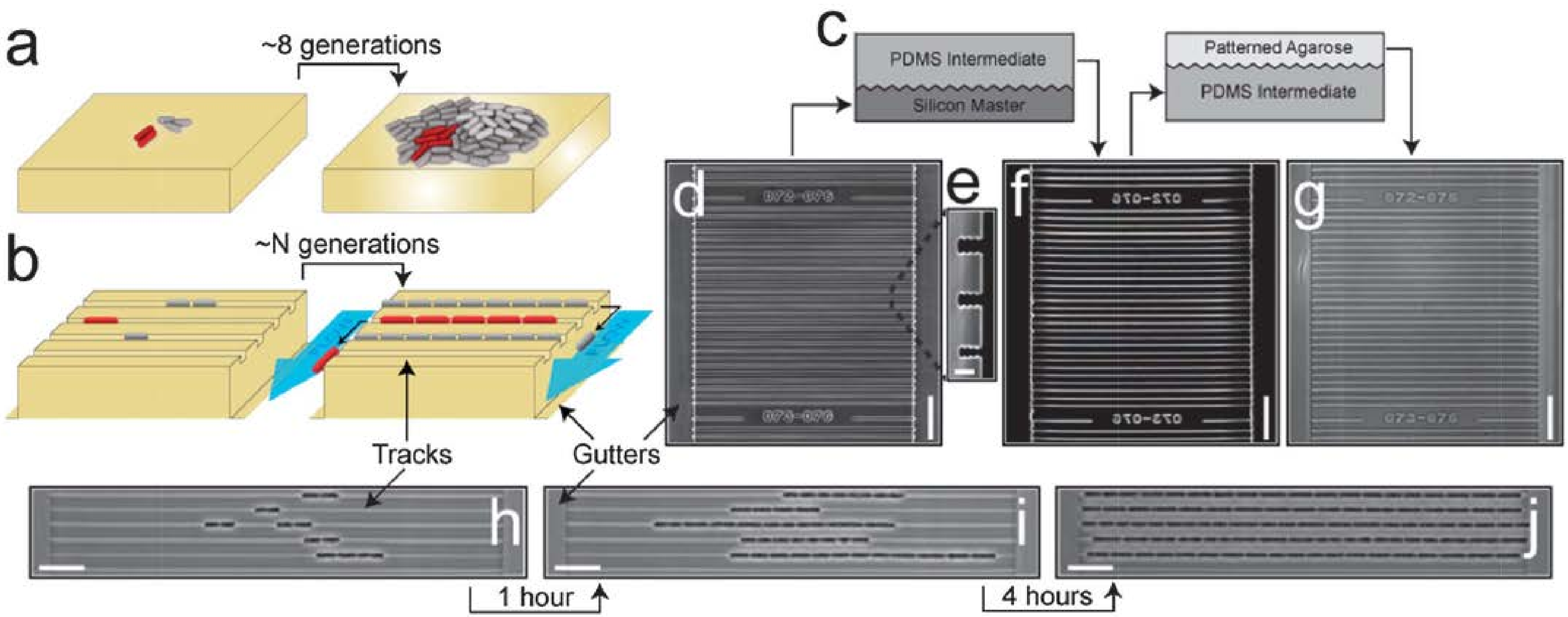




拥挤环境下的追踪：



微流控



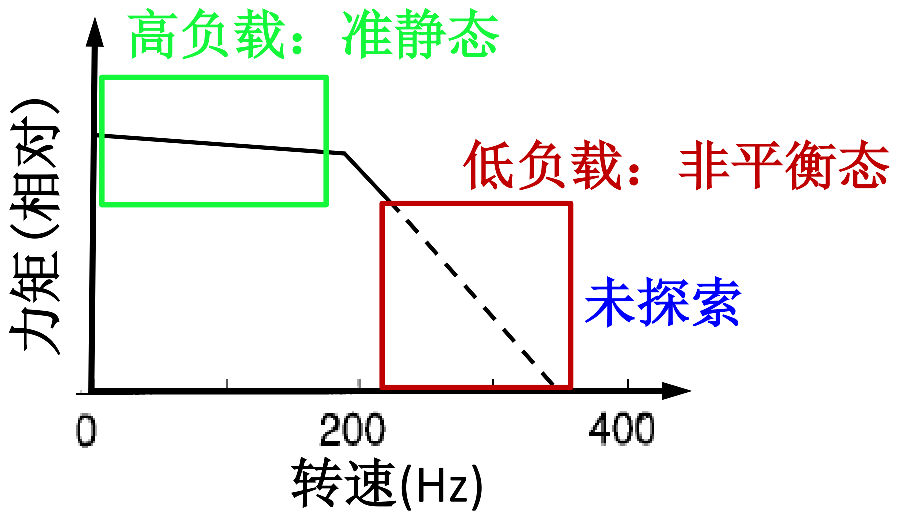
a
~8 generations
b
~N generations
c
PDMS Intermediate
Silicon Master
Patterned Agarose
PDMS Intermediate
d
e
f
g
Tracks
Gutters
h
i
j
1 hour
4 hours

举例：发展新技术观测马达行为

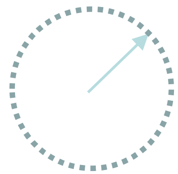
目标：

① 观测马达在极低负载下行为。

② 准确观测马达动力学行为。



挑战：

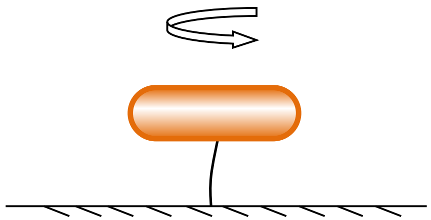


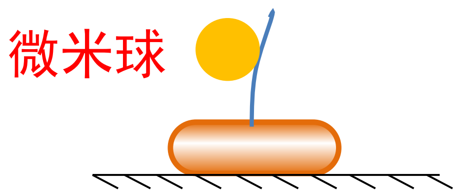
轨迹半径 < 50纳米

转速 ~ 350转/秒

传统技术

传统技术的缺点：



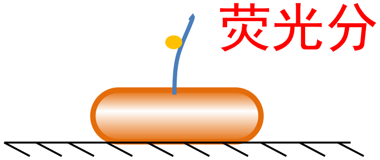


①高负载。
②低通滤波。

旋转粘滞阻力 ∝ 小球直径的立方

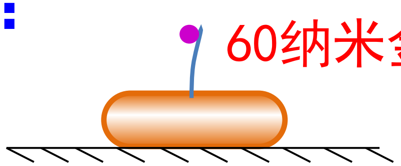
新技术观测马达行为

尝试：



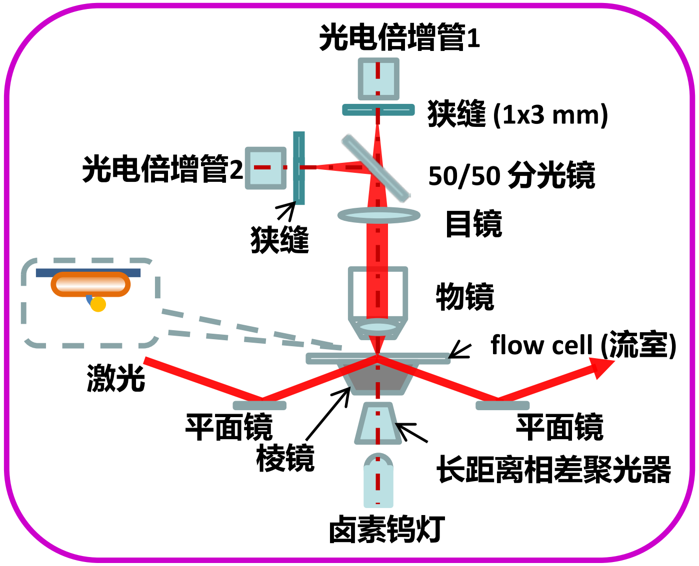
光强不够，不可行

新技术：

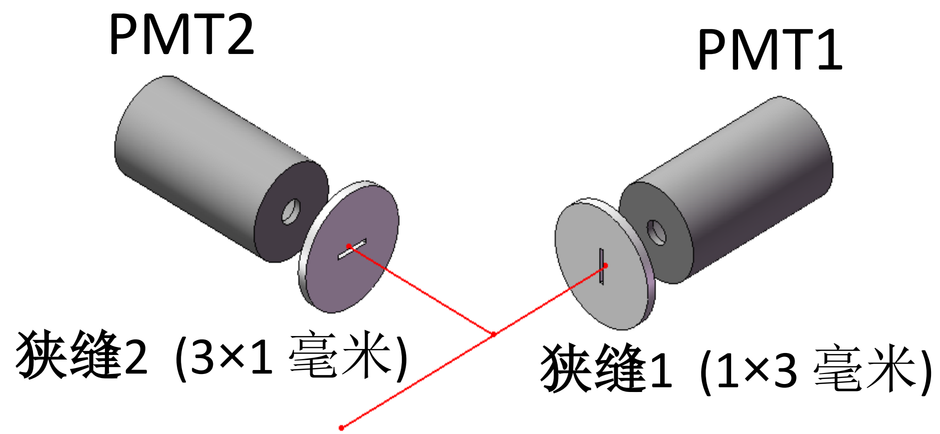


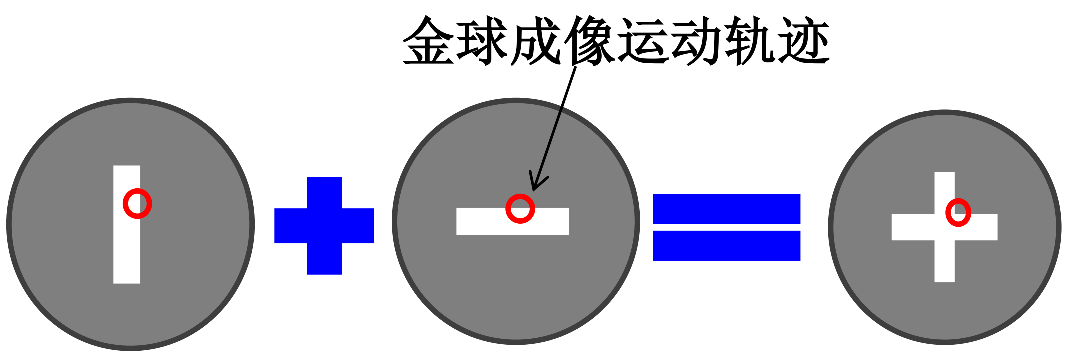
表面等离基元效应 ⇨ 散射光极强

背散射激光暗场

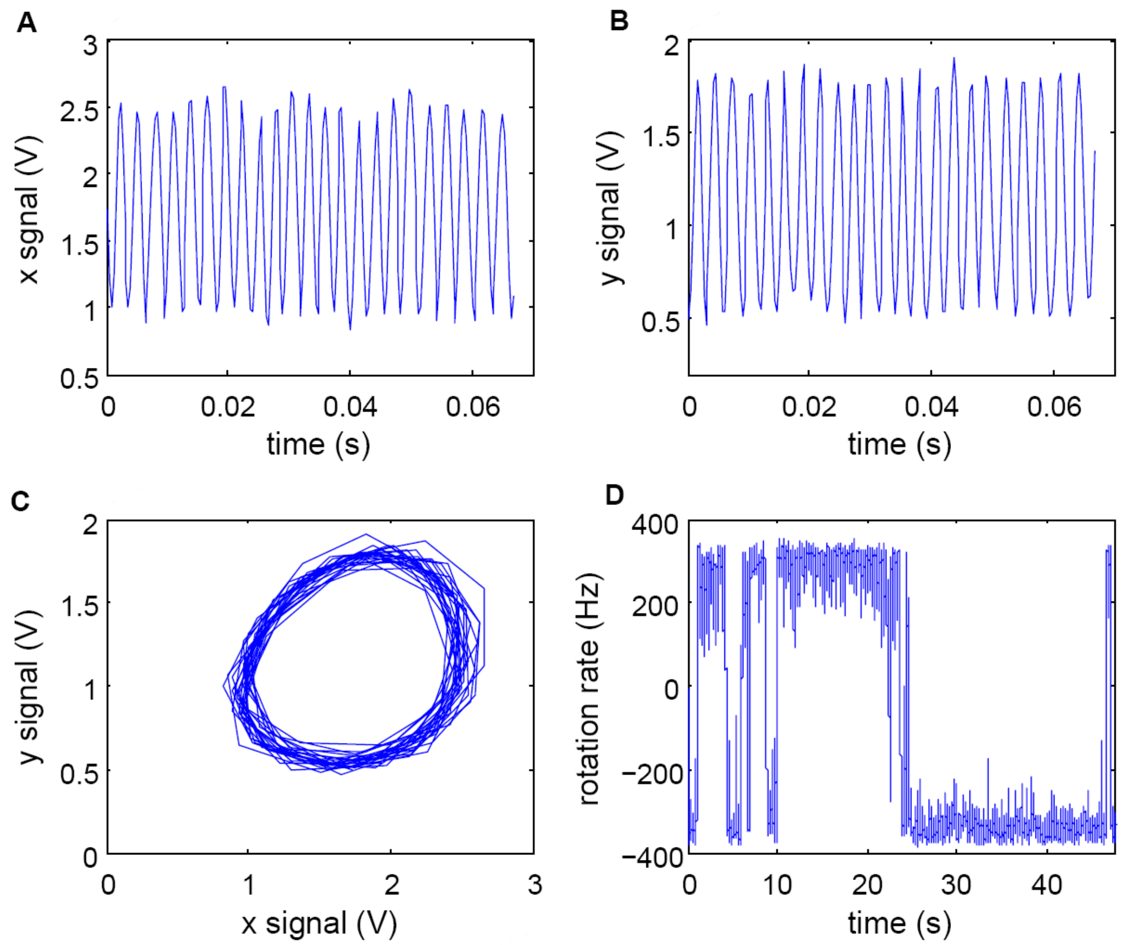


PMT对金球定位





信号举例

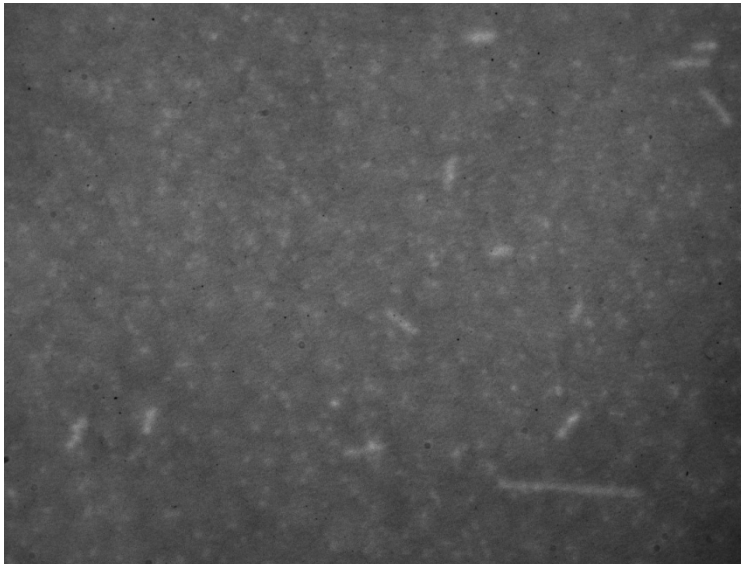


时间分辨：100kHz
空间分辨：0.5nm

信号对比

传统技术：

细菌 + 0.5微米乳胶小球



相衬成像

新技术：

细菌 + 60纳米金球

激光暗场

新技术将信噪比提高 > 10^4

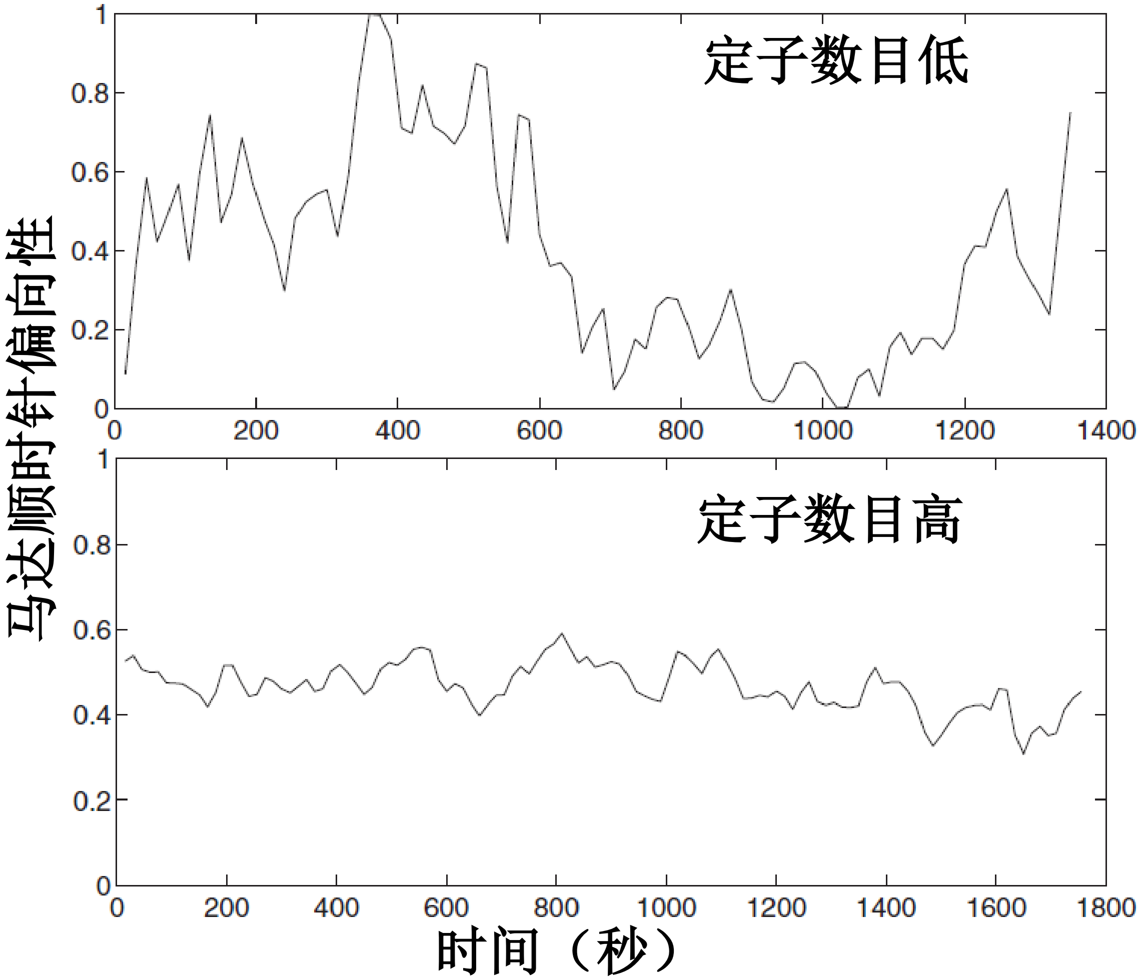
时间分辨：100kHz

空间分辨：0.5nm

使准确观测马达行为成为可能

新技术观测马达行为

以高时空分辨，准确观测马达动力学。



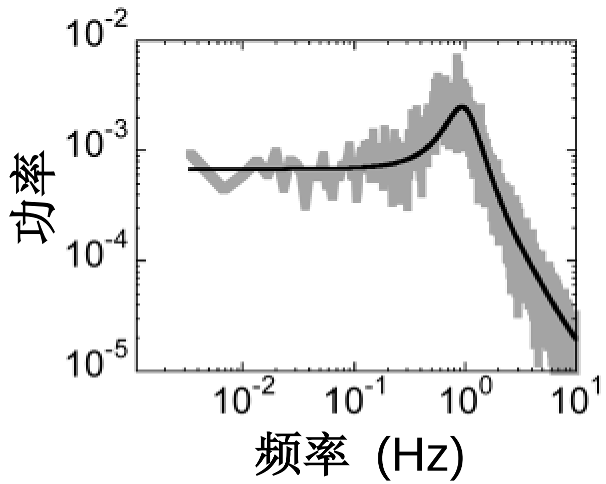
⇨ **马达转向偏向性随时间波动。**

定子也影响转向。

Wang, ***Yuan****, Berg* ***PNAS*** 111:15752(2014)

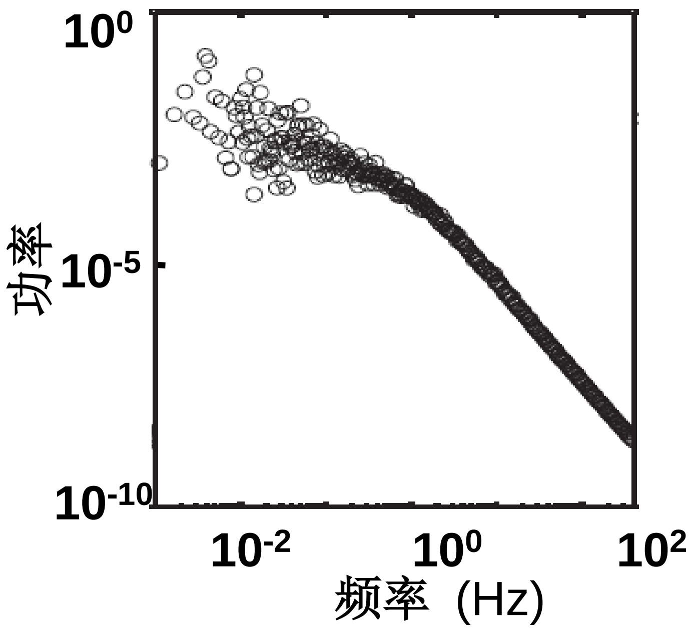
新技术观测马达行为

马达转动的频谱：



哈佛大学Cluzel组的实验结果

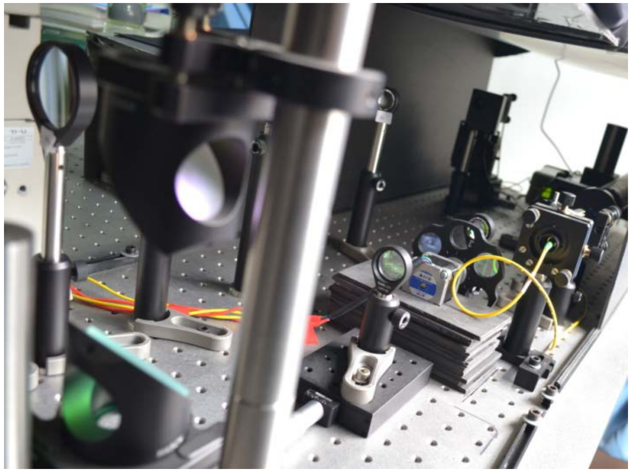
Korob *et al*, **Phys. Rev. Lett** 96:058105(2006)

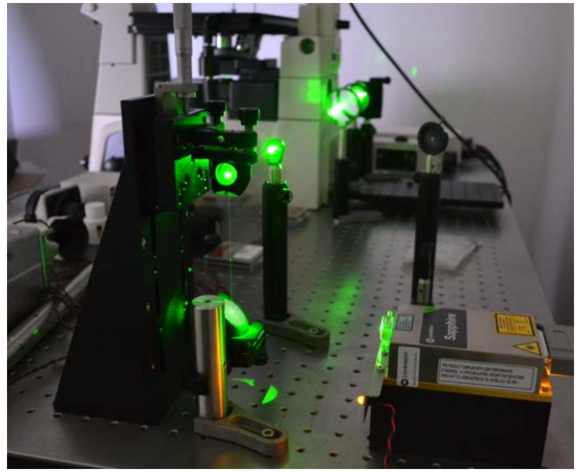


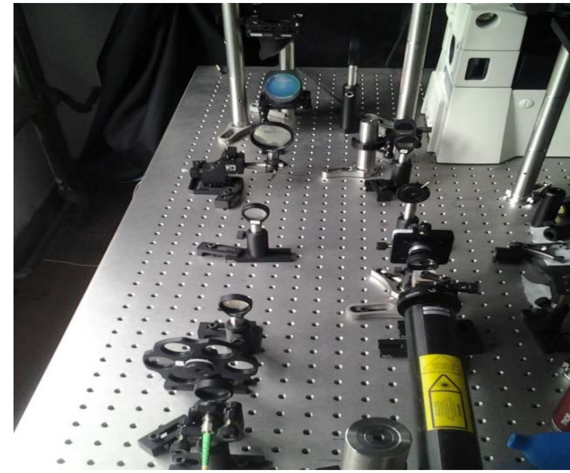
我们的实验结果

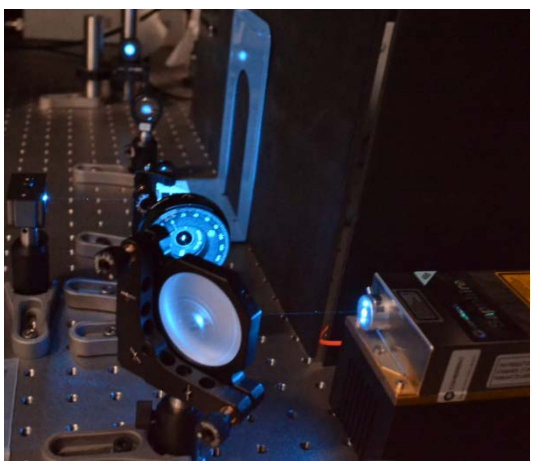
Wang, ***Yuan****, Berg* ***PNAS*** 111:15752(2014)

实验平台

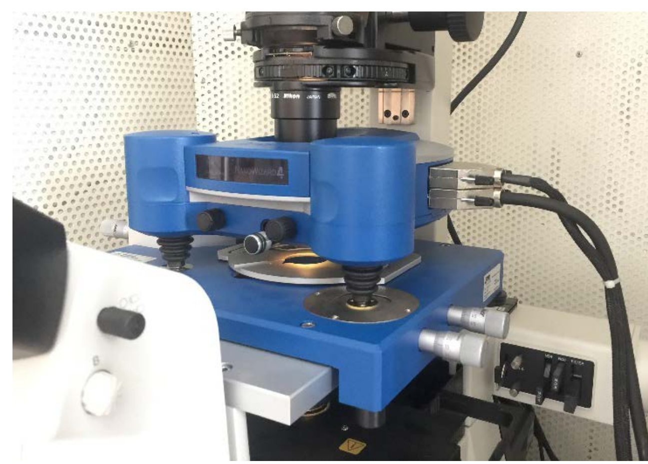

激光暗场与光镊集成


荧光共振能量转移

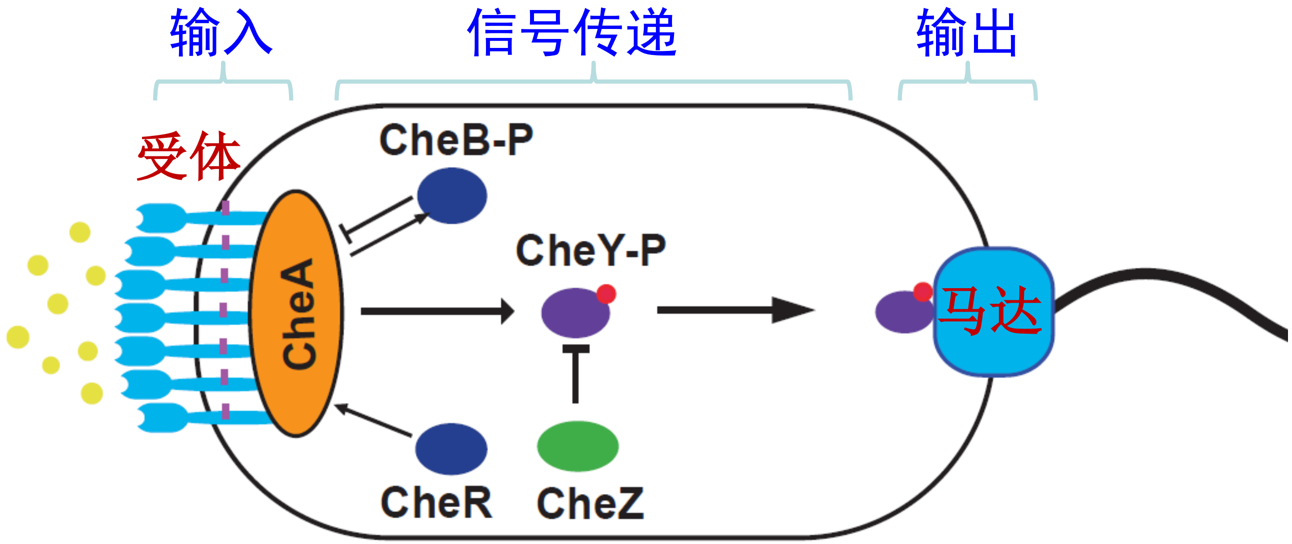

单分子荧光


光控质子泵


超分辨成像


原子力与荧光集成

科学问题



信号 ⟹ 马达

① 信号如何可靠地传递到马达?
（输入输出如何鲁棒耦合）

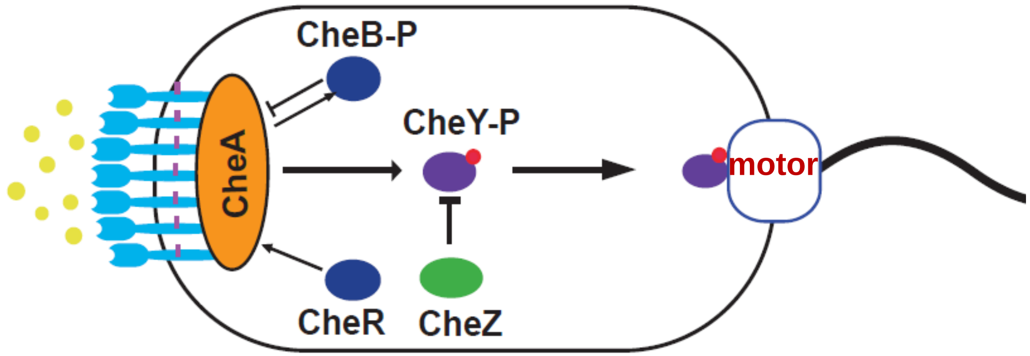
② 马达转向如何调控?

转向改变

力矩

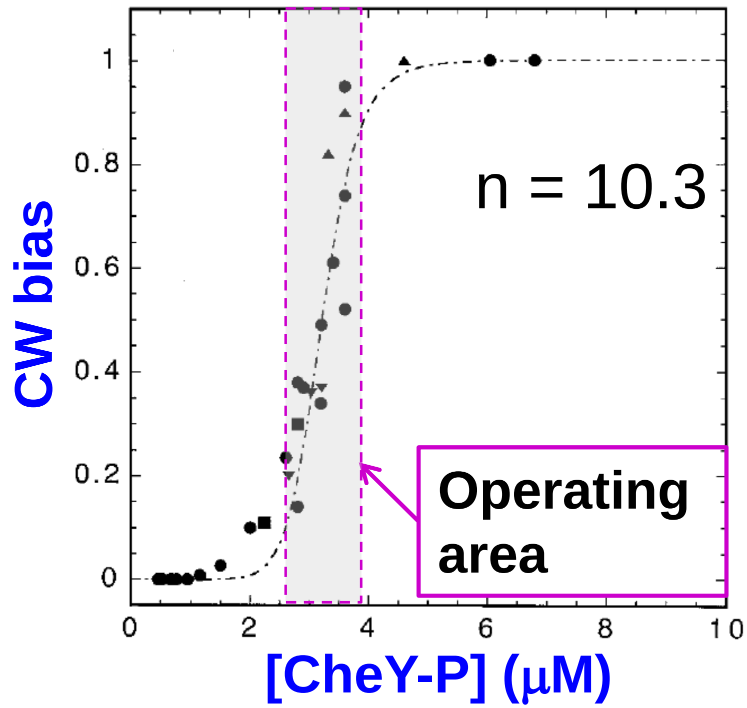
③ 马达力矩如何产生?

New adaptation mechanism



Hill function: $$Y = \frac{X^n}{X^n + K_{1/2}^{\ n}}$$

Motor ultrasensitivity



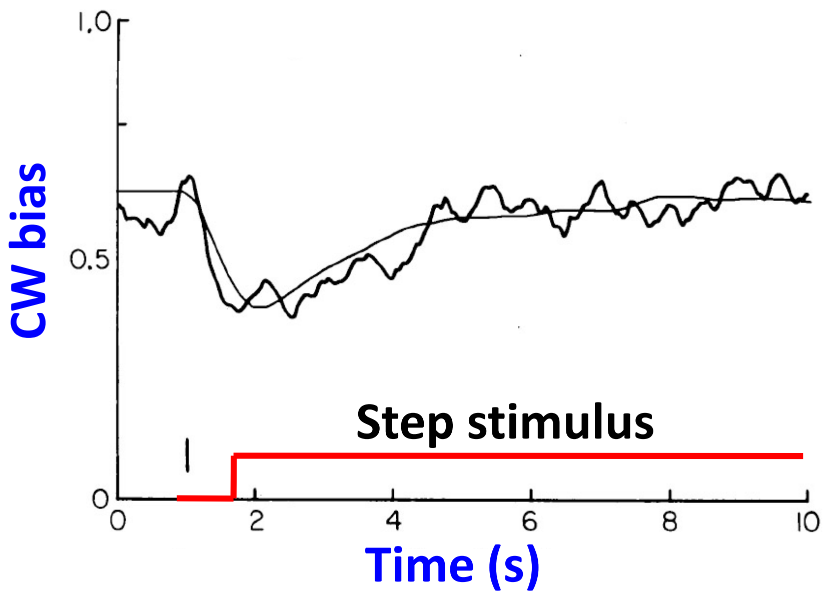
Cluzel *et al*, ***Science*** 287:1652(2000)

How is signal relayed reliably:
(input & output robustly coupled) maintain [CheY-P] in the operating area?

Discovery of the new adaptation mechanism will solve the prob.

Adaptation by CheR/CheB

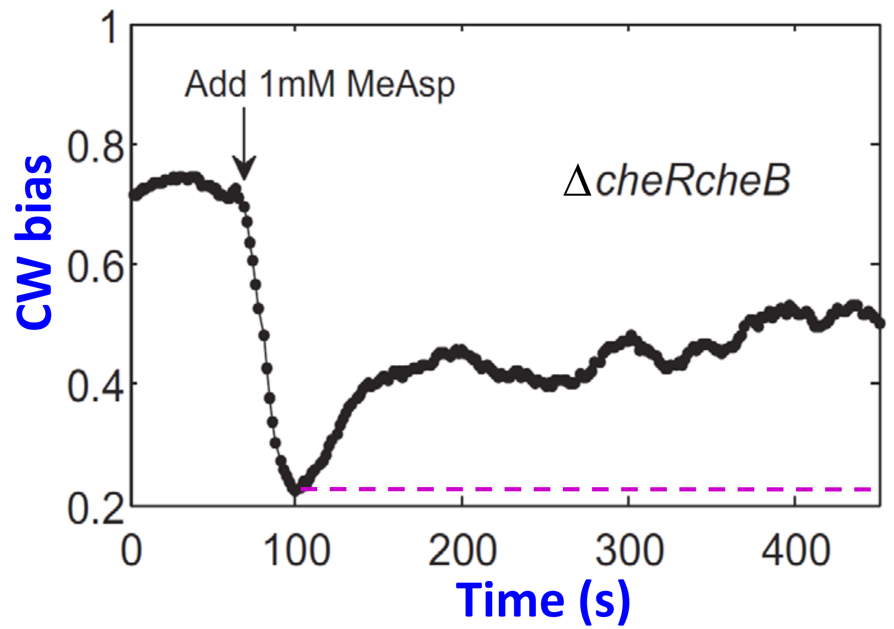
Perfect adaptation：(wild type)



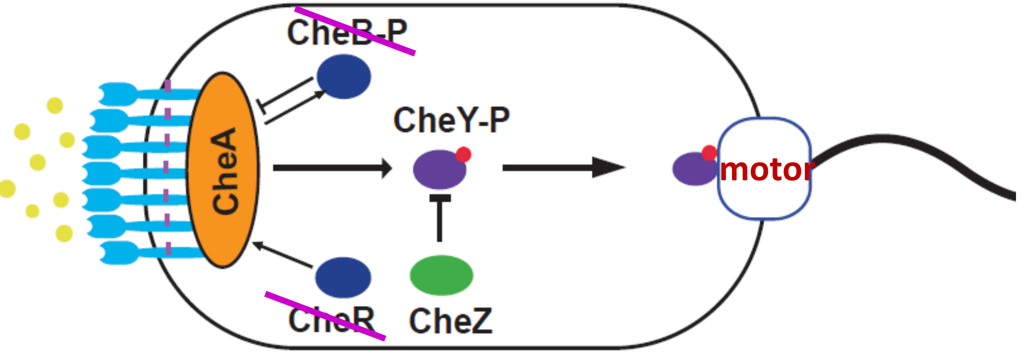
Segall *et al*, ***PNAS*** 83:8987(1986)

Partial adaptation in Δ*cheRcheB* cells

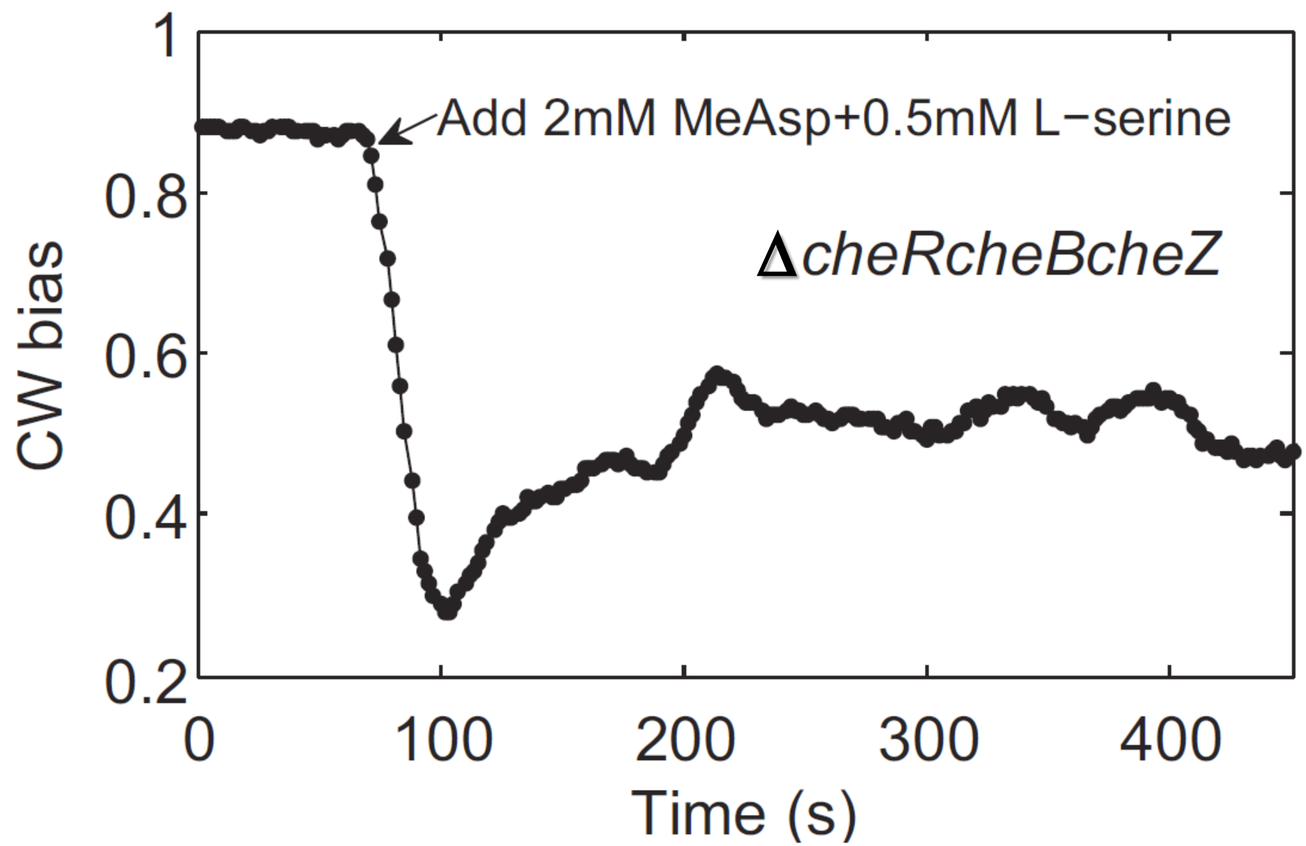
Partial adaptation： (mutant)

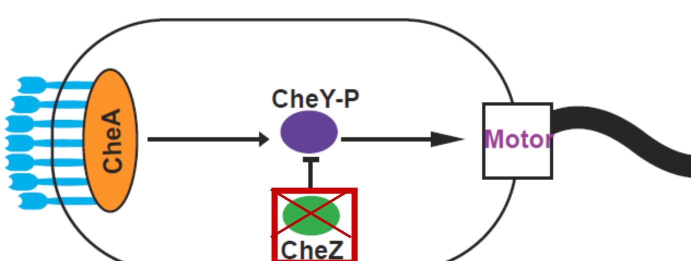


Unkown adaptation mech.！



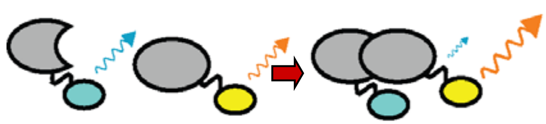
Partial adaptation is independent of CheZ





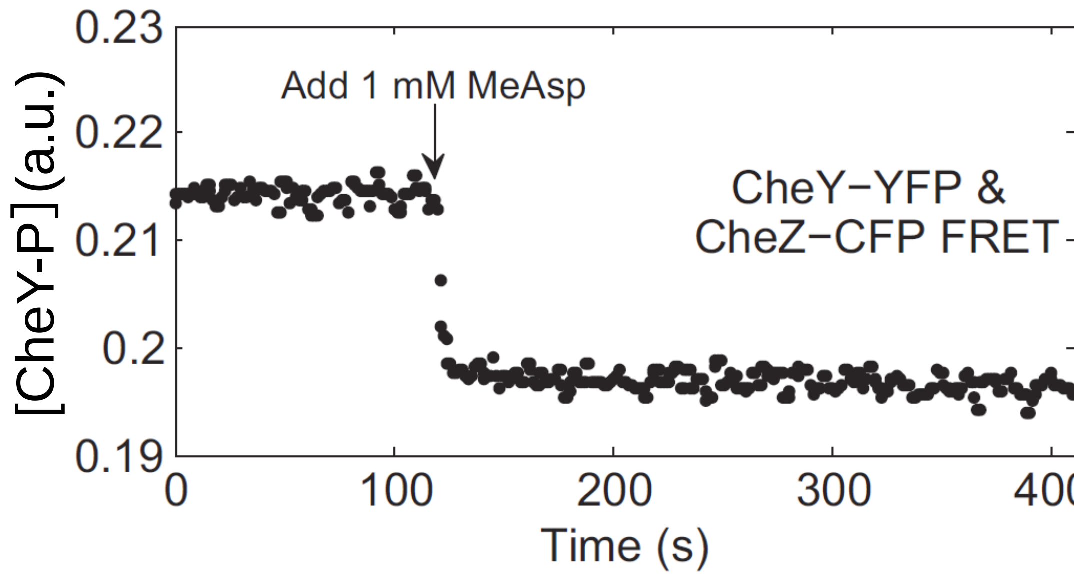
No adaptation in [CheY-P] in Δ*cheR cheB* cells

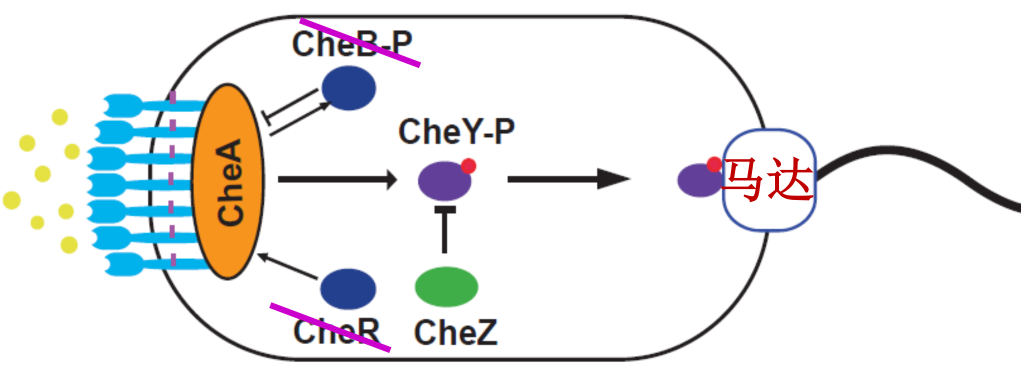
FRET



Detect [CheY-P]

No adaptation in [CheY-P]:

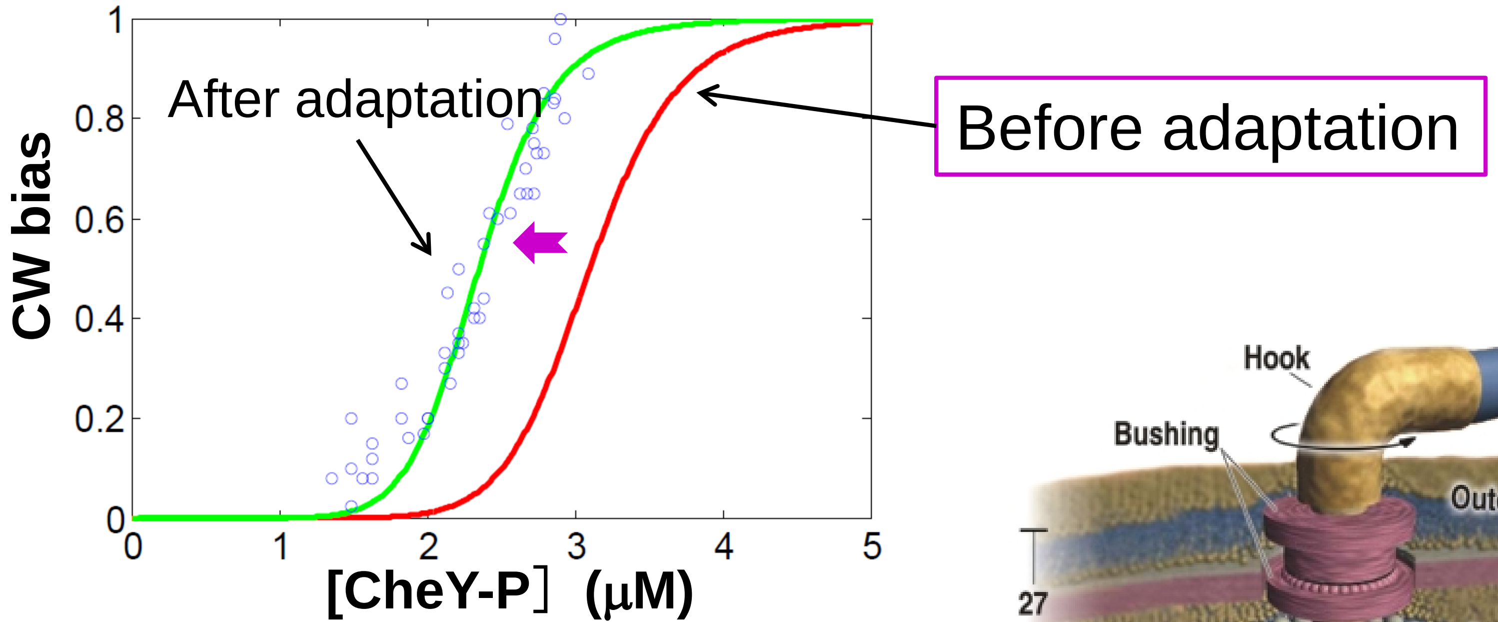




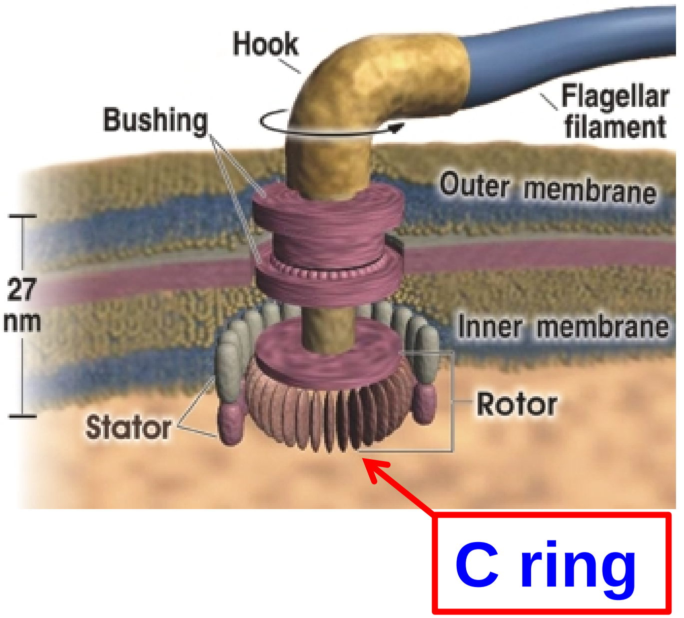
Adaptation @ motor！

Bias Vs. [CheY-P] curve for the adapted motor

Motor response curve shifts：



$$y = \frac{1}{1 + L\left(\frac{1 + x/(KC)}{1 + x/K}\right)^{N}}$$

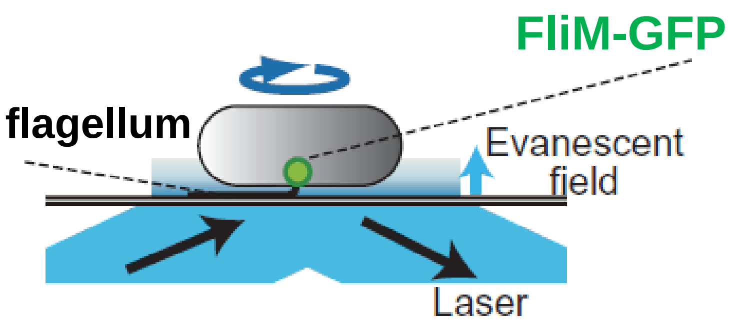


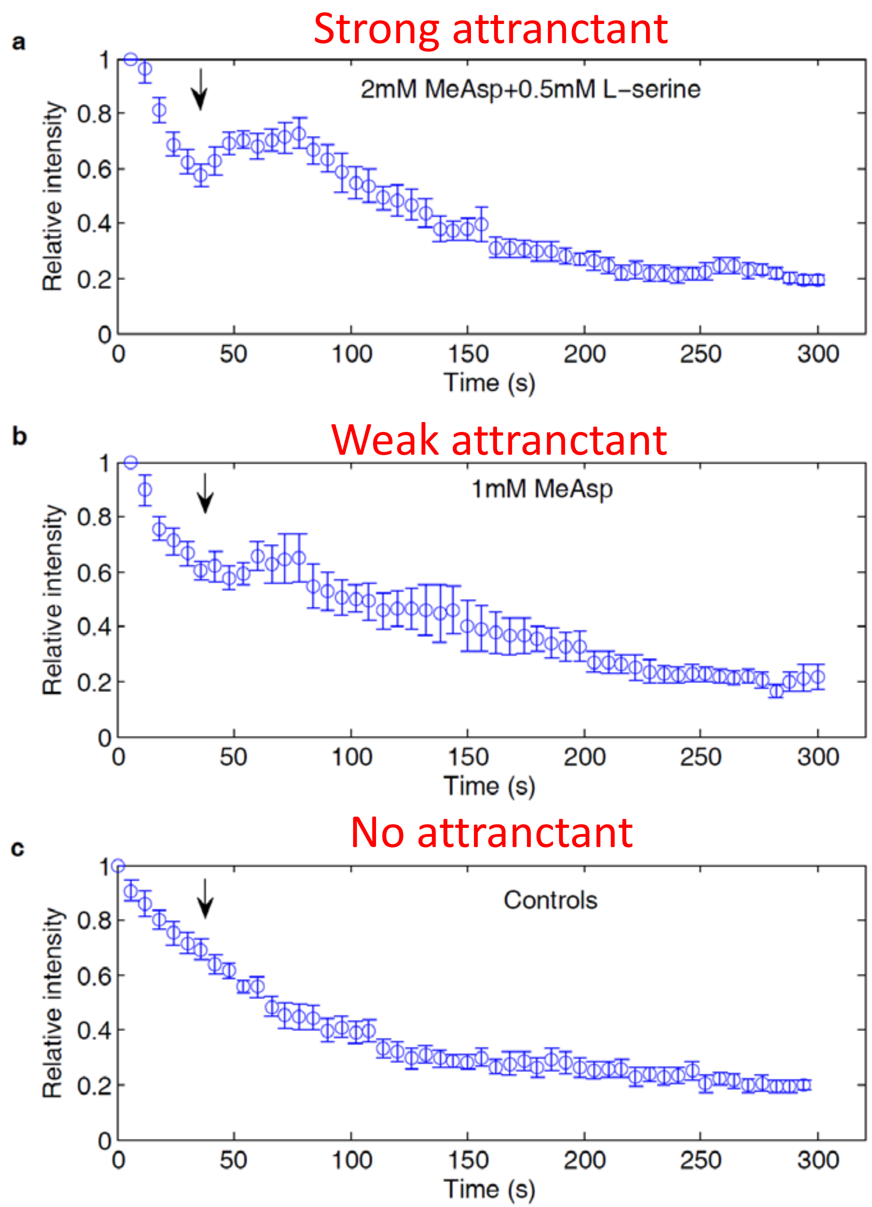
N: 34 → 42

Motor adapts by adjusting N

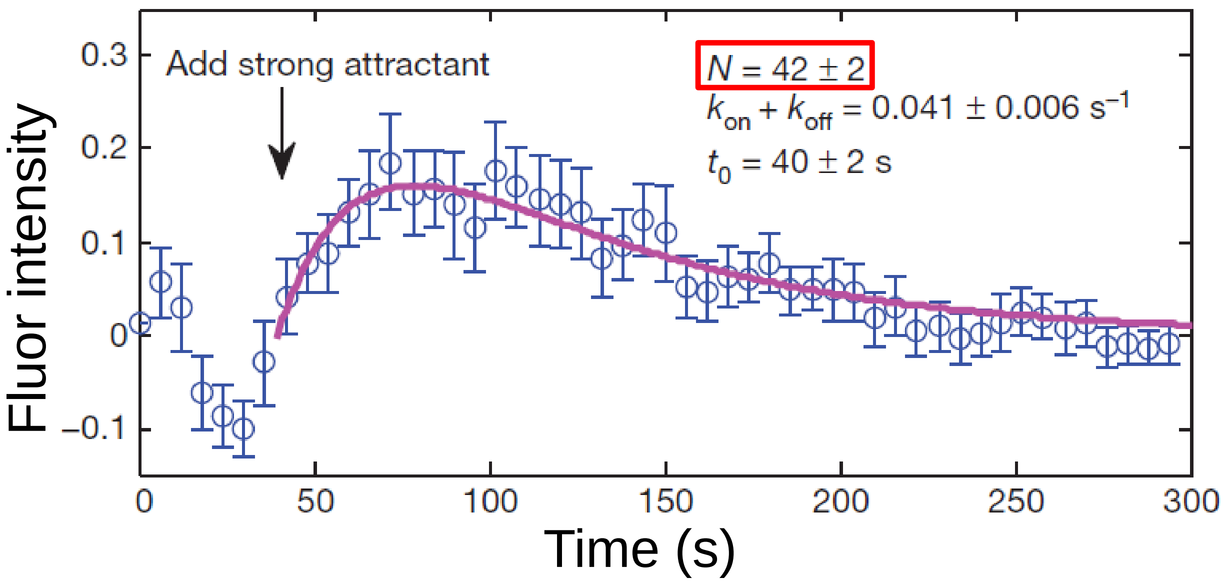
Adaptation by changes in # of FliM units

TIRF：





Quantitative analysis



Motor intensity：

$$f \propto (N-34)(e^{-\lambda t} - e^{-(k_{on}+k_{off})t})$$

***N*: 34 ⟶ 42**

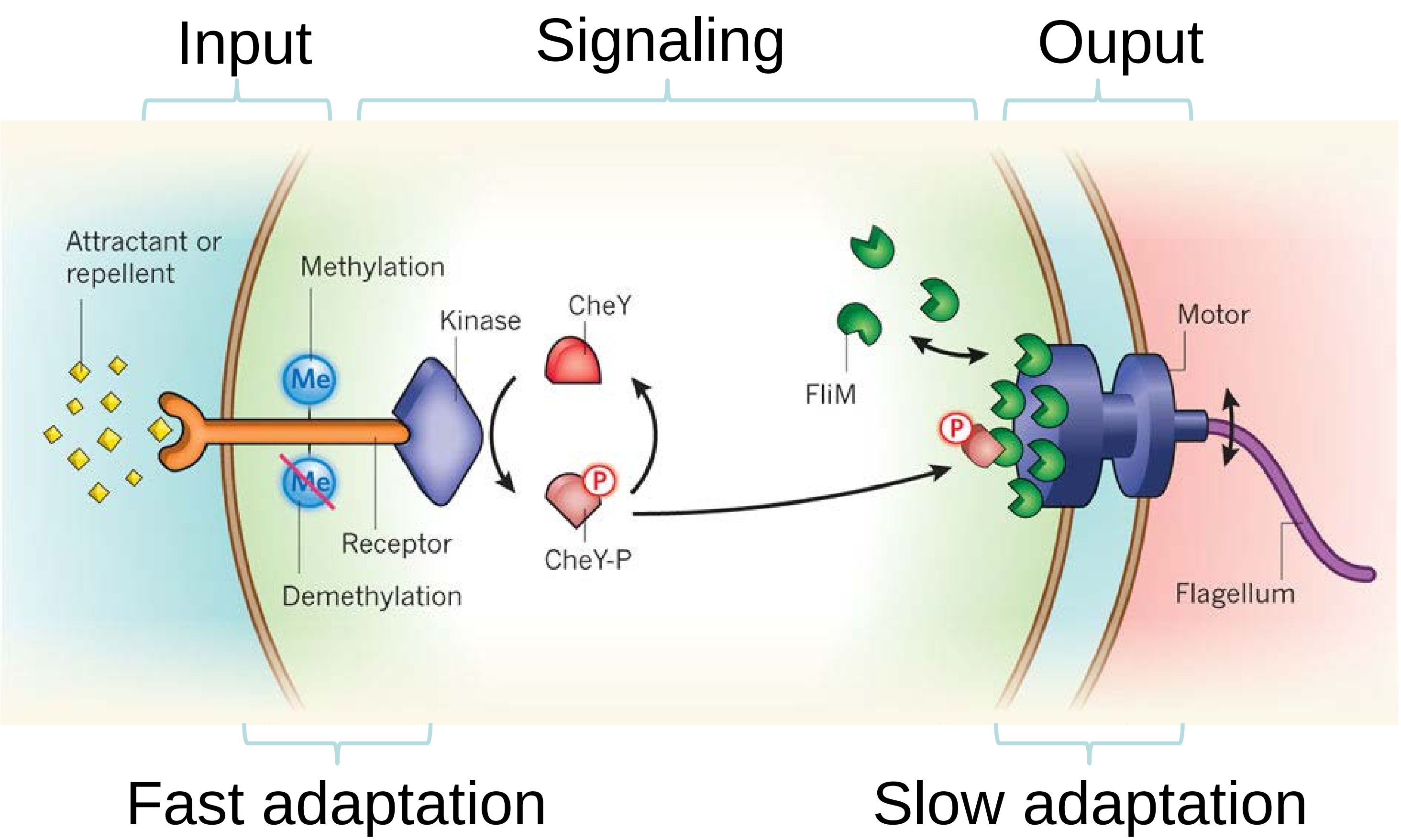
Agrees with the previous result

Yuan et al. ***Nature*** 484:233 (2012)

Yuan et al. ***J. Mol. Biol.*** 425:1760 (2013)

new adaptation mechanism

Motor remodels to adapt to the environment:

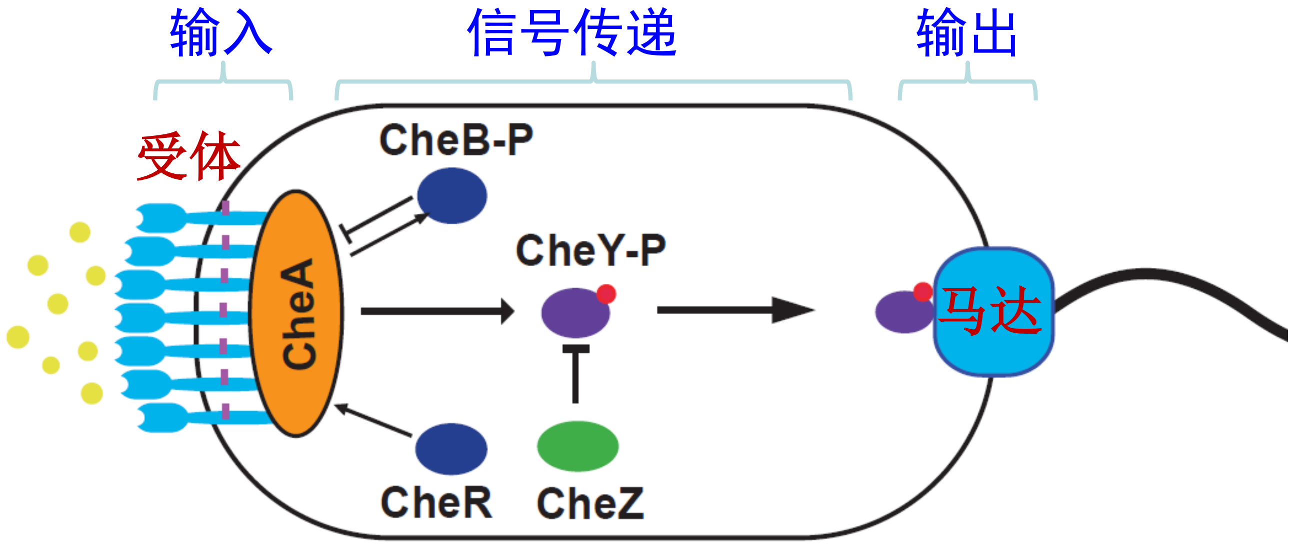


"Adaptive remodeling" ***Yuan et al., Nature*** 484:233 (2012)

Summary

- Discovered a new level of adaptation mechanism for chemotaxis: motor level;
- proposed “adaptive remodeling” as a general working mechanism for molecular machines.

科学问题



② 马达转向如何调控?

转向改变

信号 ⟹ 马达

① 信号如何可靠地传递到马达?
（输入输出如何鲁棒耦合）

力矩

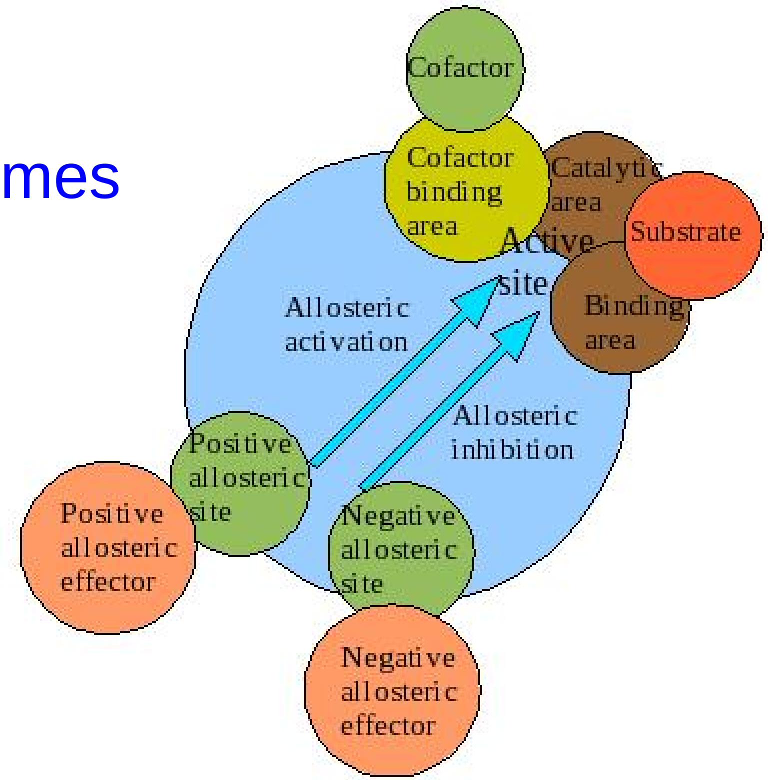
③ 马达力矩如何产生?

Nonequilibrium effect in the allosteric regulation of the bacterial flagellar switch

转向调控

Allostery

Regulatory Enzymes

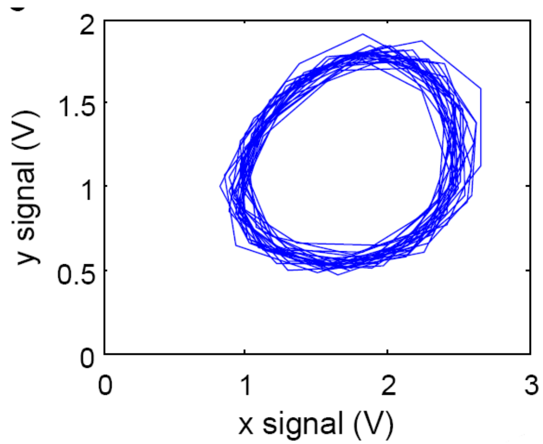


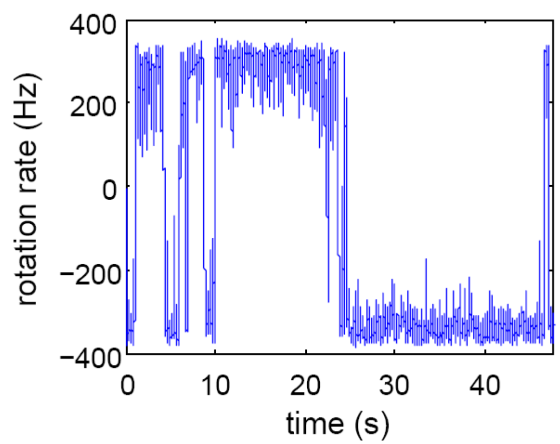
Gene regulation

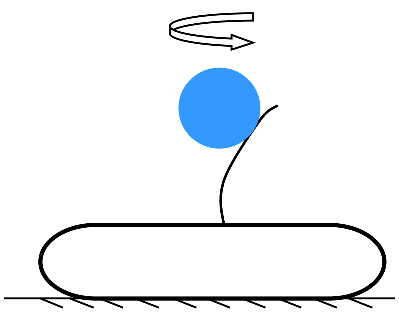
Membrane Proteins: ion channels, receptors

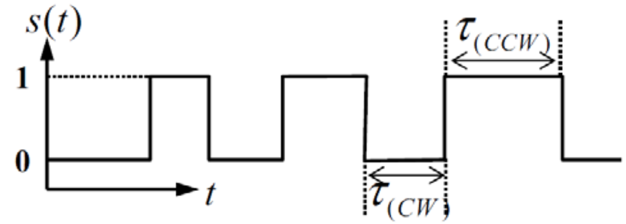
MWC, KNF … models ➡ Ising model

Monitoring motor switching



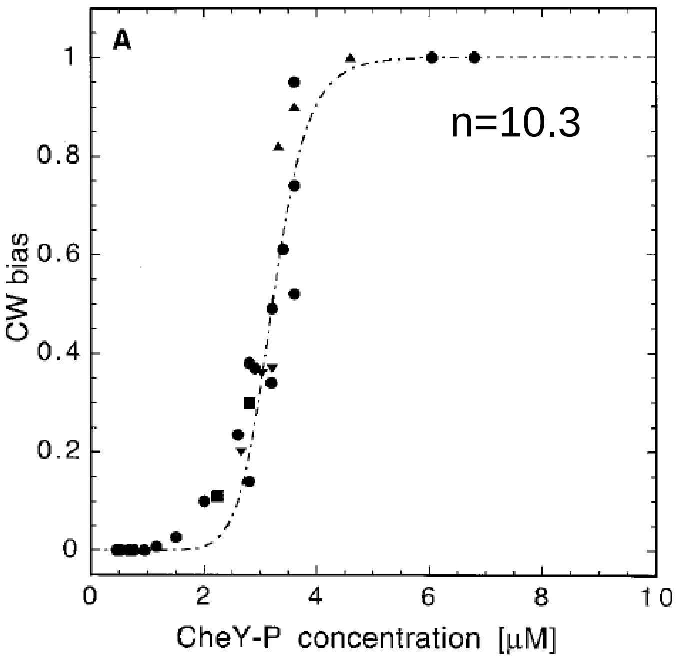


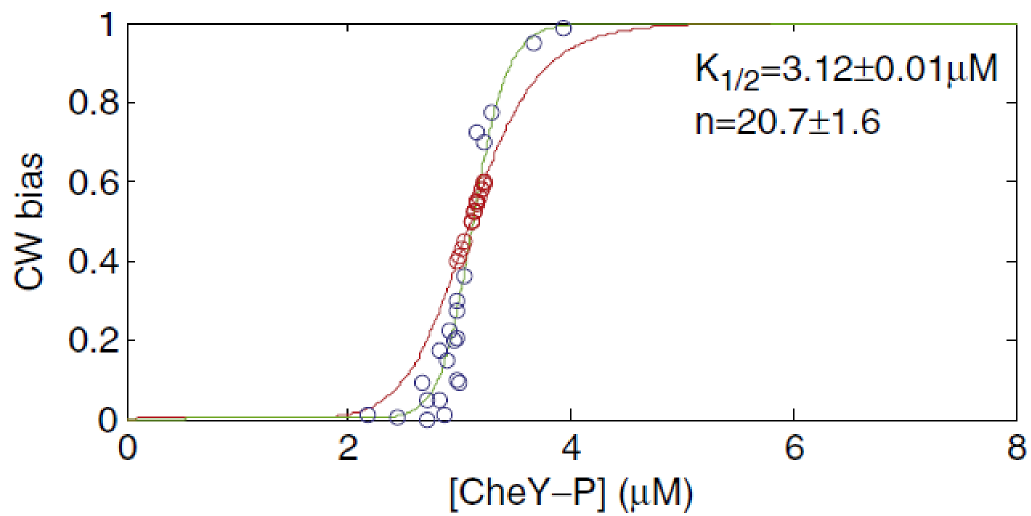




- ➢Bias Vs. [CheY-P]
- ➢Interval distributions

Motor ultrasensitivity (high cooperativity)





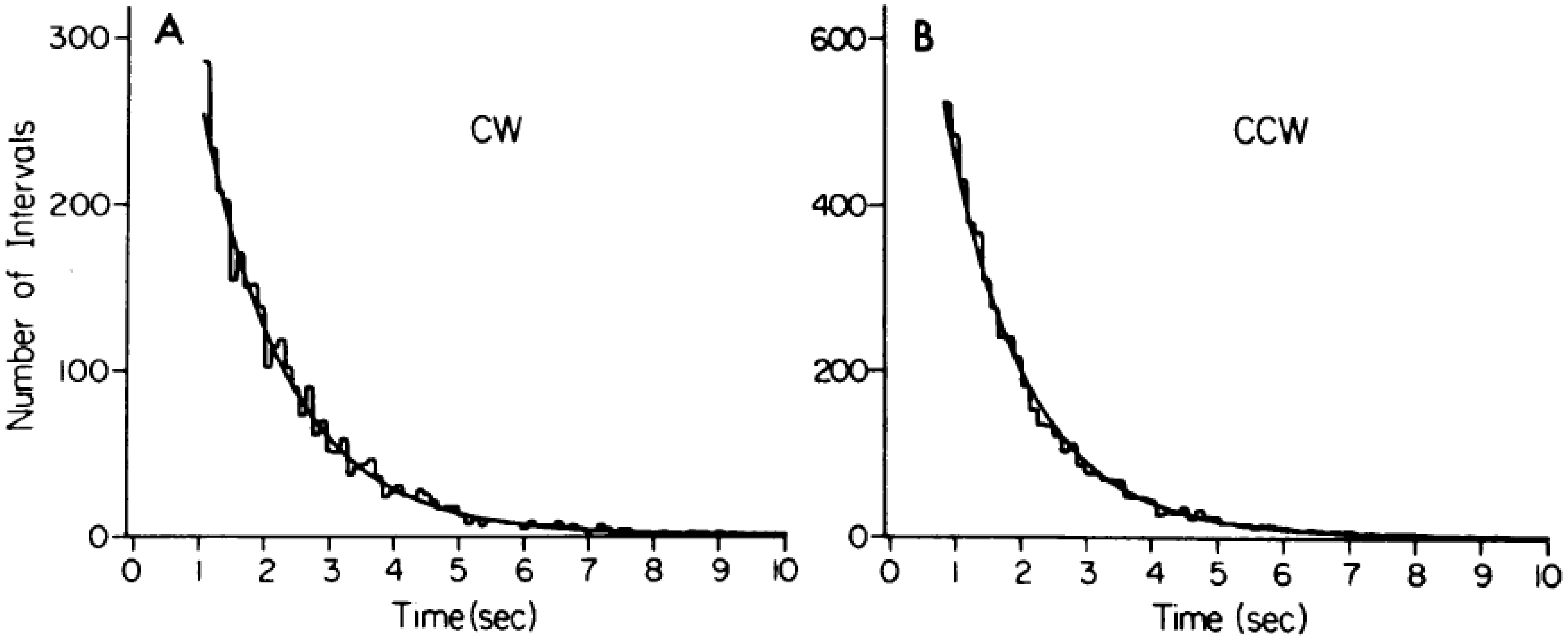
P. Cluzel et al. ***Science*** *287:1652.*

J. Yuan et al. (2012) ***Nature*** *484:233*
J. Yuan et al. (2013) ***JMB*** *425:1760*

Hill function:

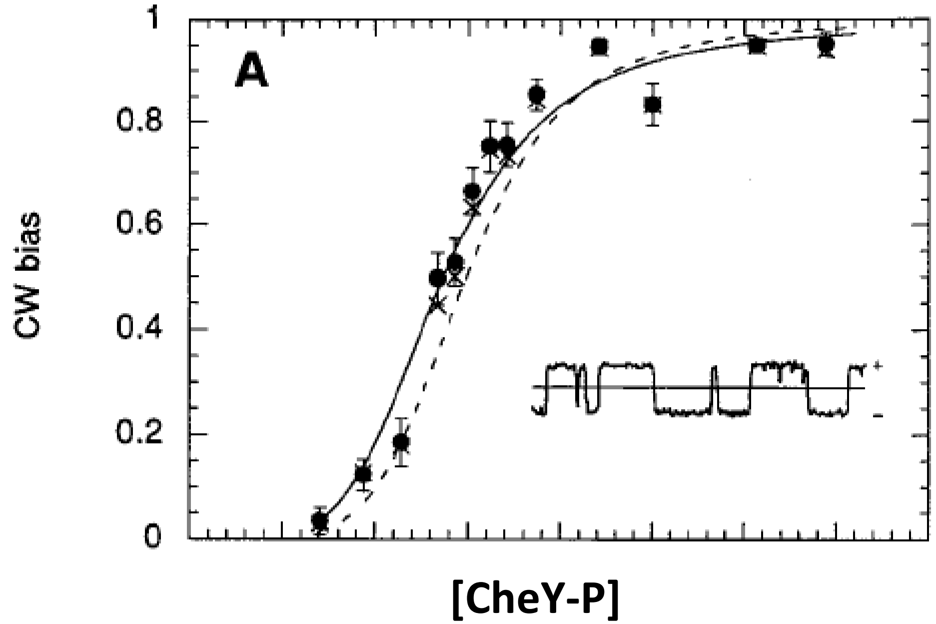
$$Bias = \frac{Y^n}{Y^n + K^n}$$

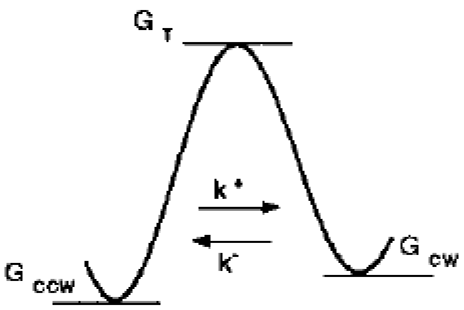
Interval distribution



Block et al. 1983, J. Bacteriol.

Two-state model

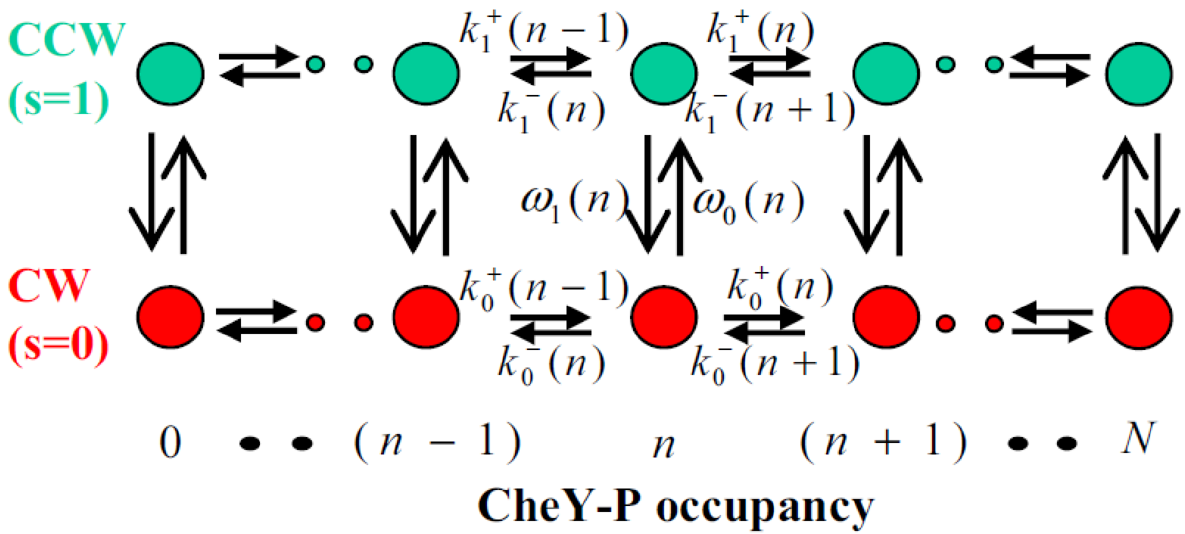




$$Bias = \frac{1}{1 + Ae^{-BY/(Y+K_D)}}$$

B.E. Scharf et al. 1998, PNAS

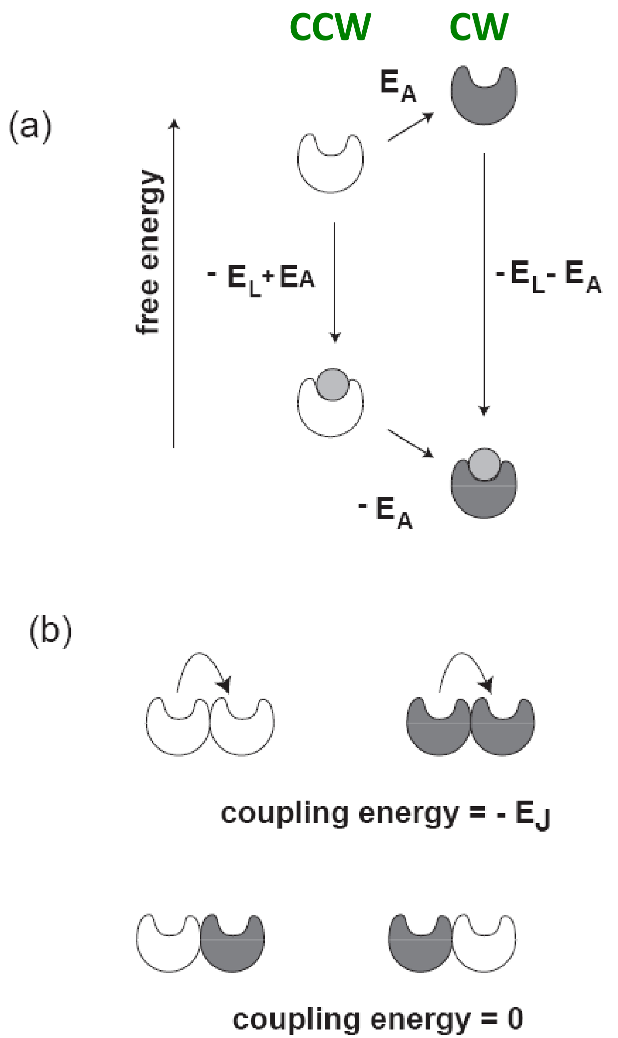
MWC model

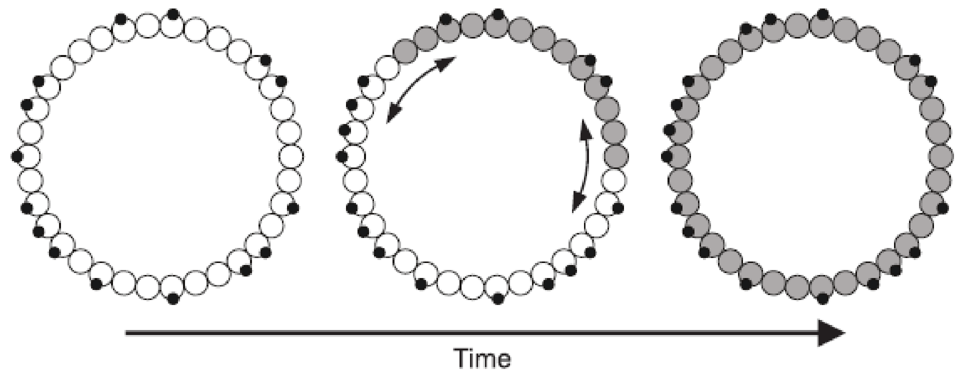


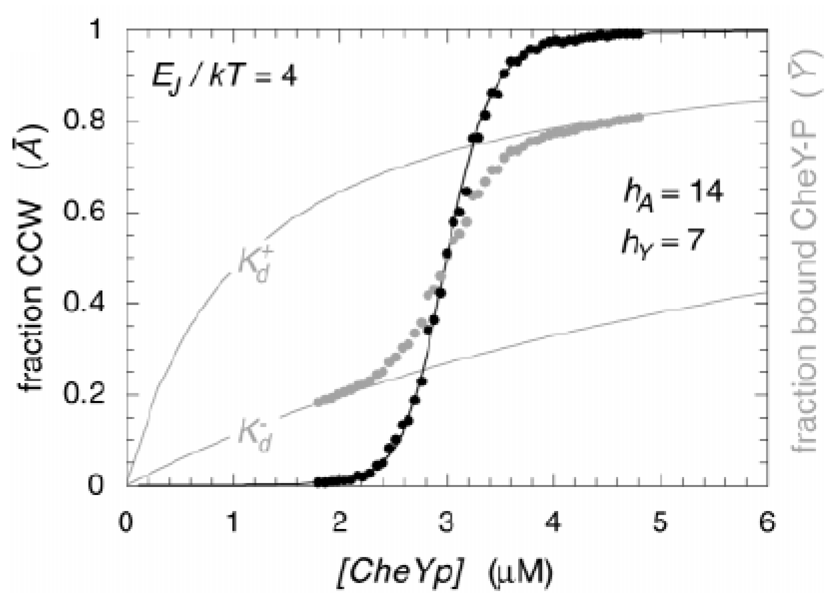
$$Bias = \frac{1}{1 + L\left(\frac{1 + Y/(KC)}{1 + Y/K}\right)^N}$$

Uri Alon et al., EMBO J. 1998

Ising model for the switch



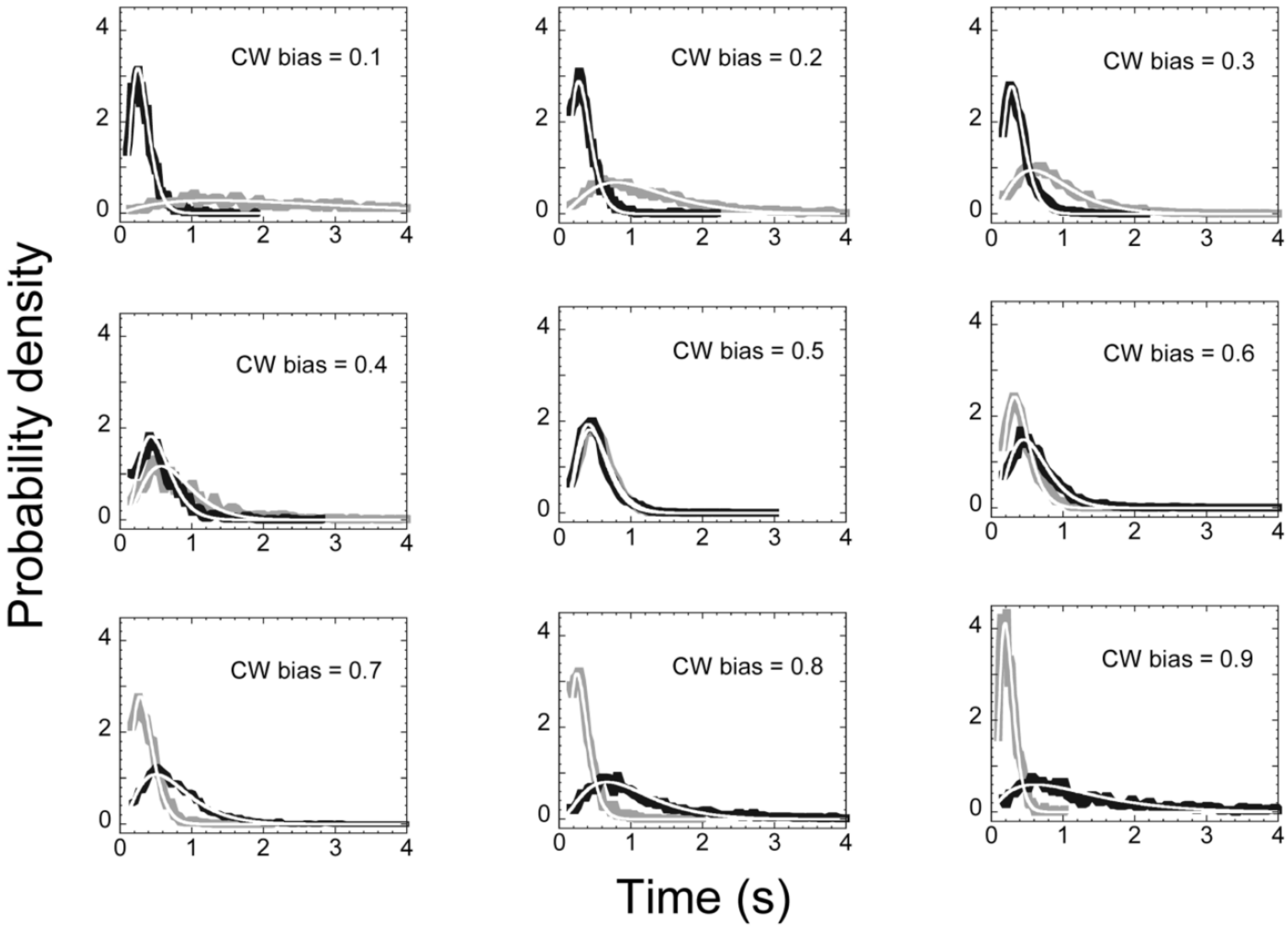


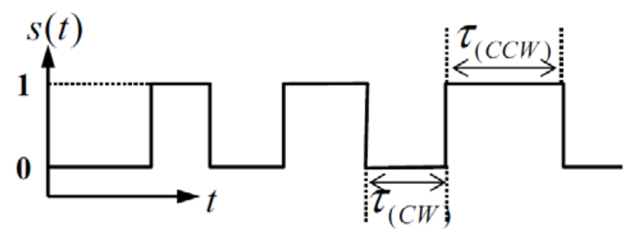


$$H_{DB} = -\sum_i (E_L + E_A s_i)\left(\sigma_i - \frac{1}{2}\right) - E_J \sum_{<ij>} \frac{s_i s_j + 1}{2}$$

Duke, T.A.J. et al. J. Mol. Biol. ***308****, 541-553 (2001)*

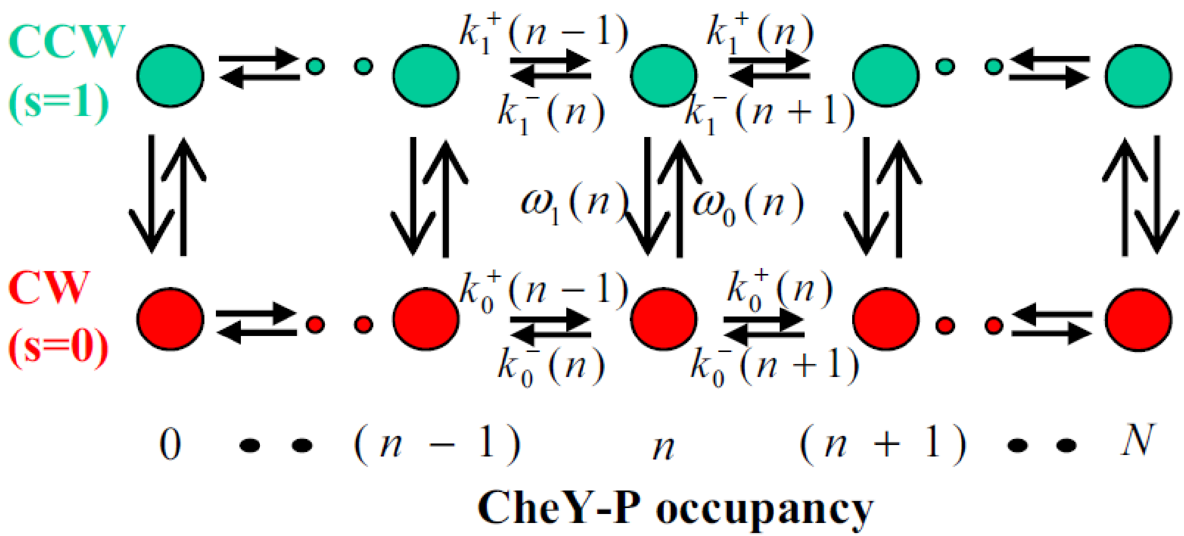
Interval distributions





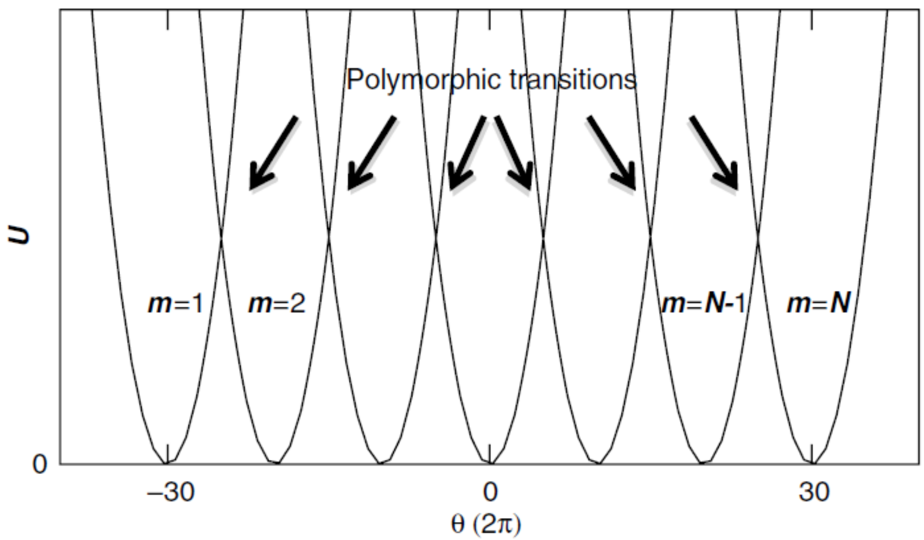
Korobkova et al. Phys. Rev. Lett. ***96****, 058105 (2006)*

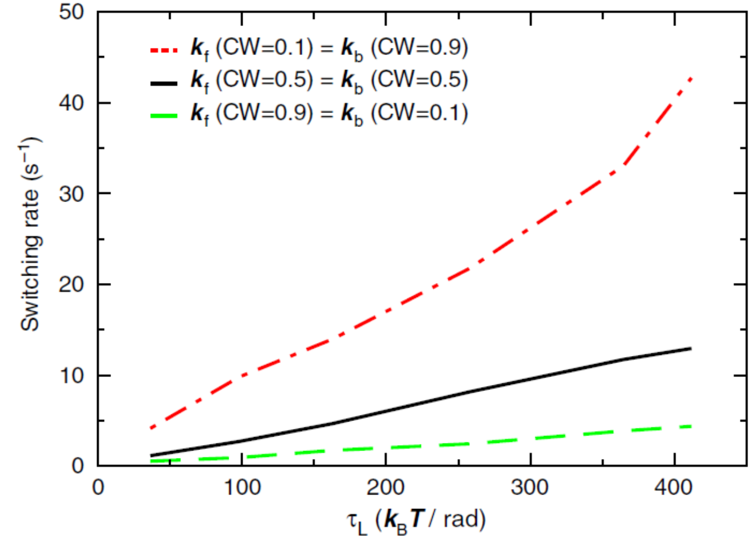
Non-equilibrium effects

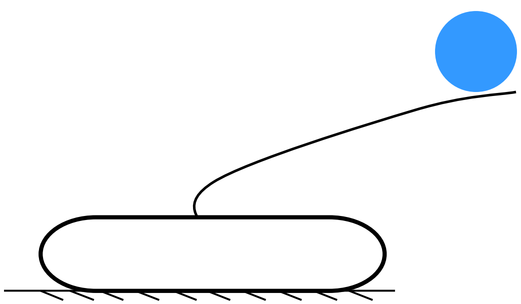


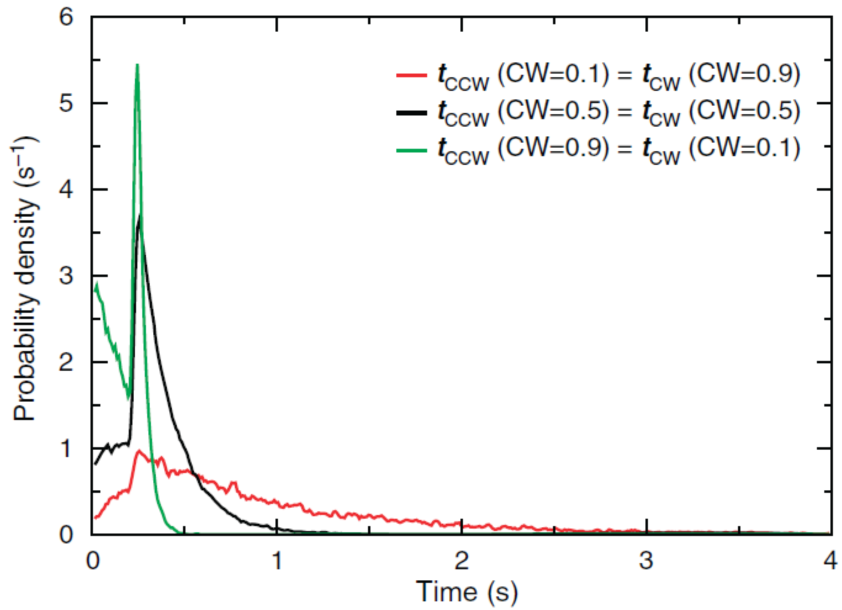
*Yuhai Tu, PNAS **105**, 11737-11741 (2008)*

Filament polymorphic transitions





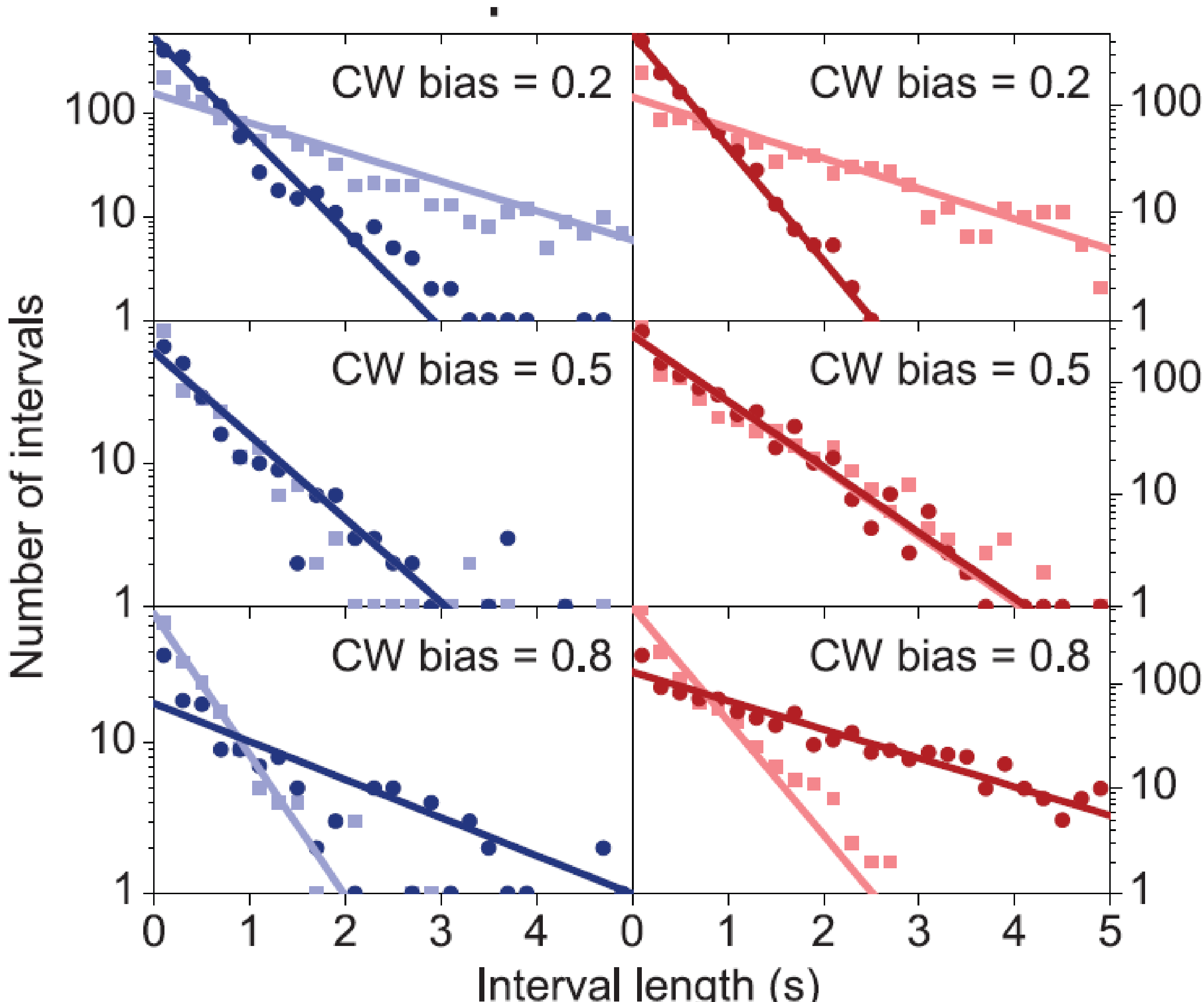




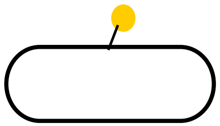
Van Albada et al. Mol. Syst. Biol. ***5****, 316 (2009)*

Interval distributions at medium load

0.5 μm bead on filament

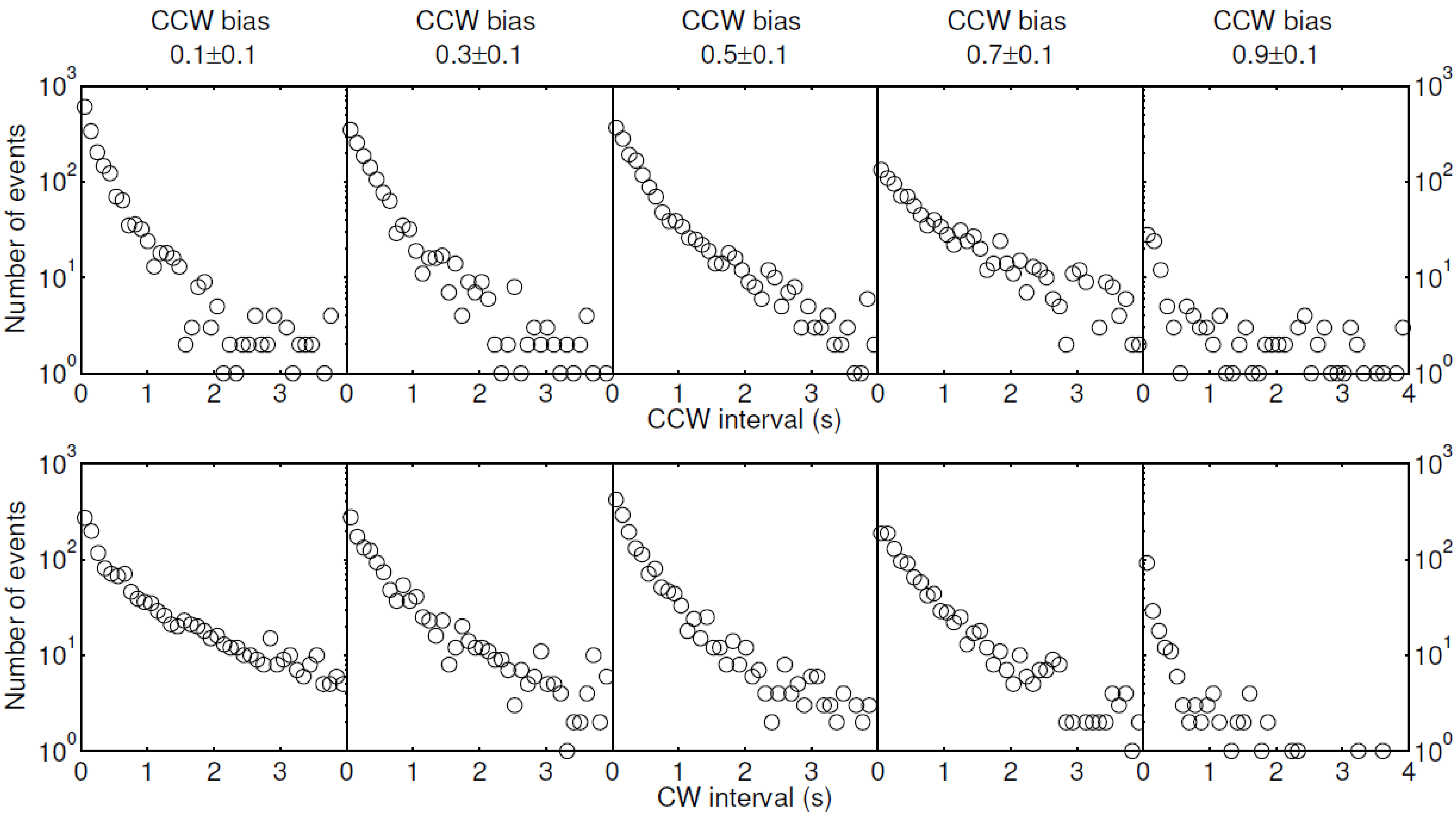


F. Bai *et al*. Science (2010)



Interval distributions near zero load

100 nm gold on hook

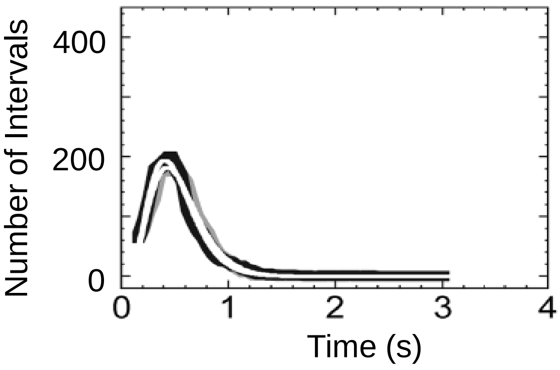


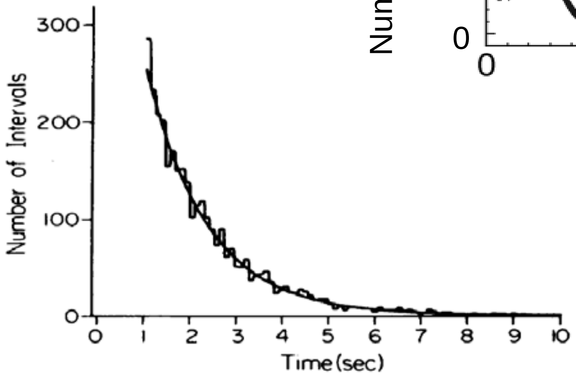
Wang *et al*. PNAS (2014)

Summary of previous results

（驻留时间统计分布）

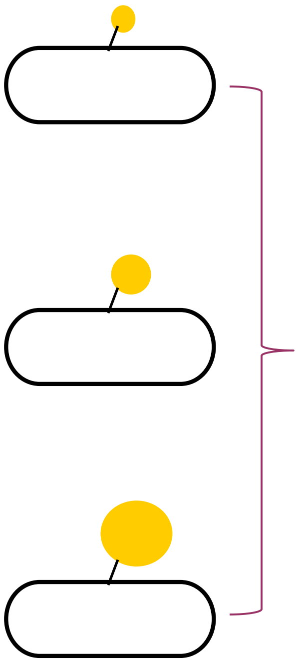
以前工作	结论
J. Bacteriol. 154(1983)	指数
PNAS 95(1998)	指数
Phys. Rev. Lett. 96(2006)	Γ函数
Mol. Syst. Biol. 5(2009)	Γ函数
Science 327(2010)	指数
PNAS 111(2014)	近指数





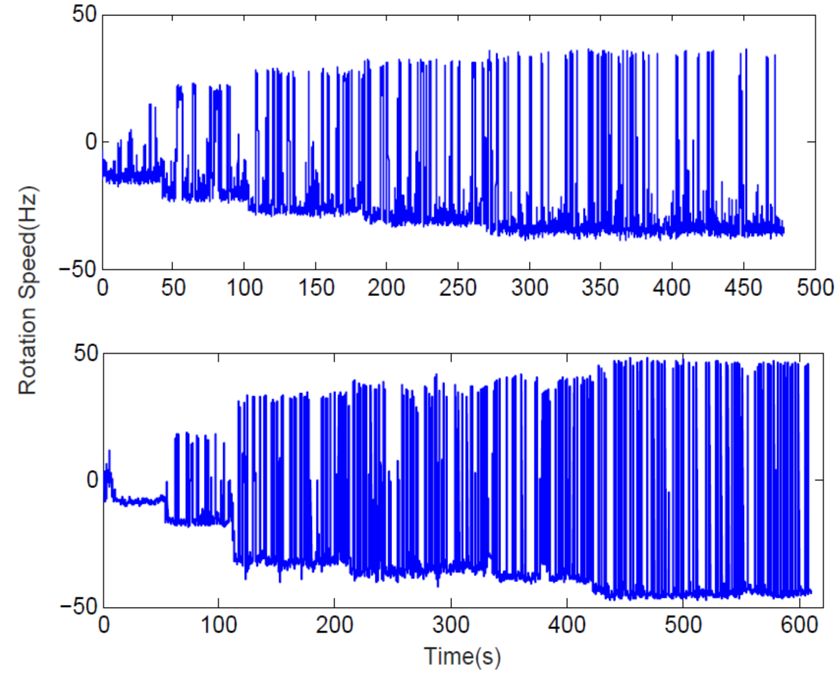
⇒ 原因:无法精确控制马达实验条件

H^+电化学势(pmf)、马达负载(load)、马达定子数(# of stators)

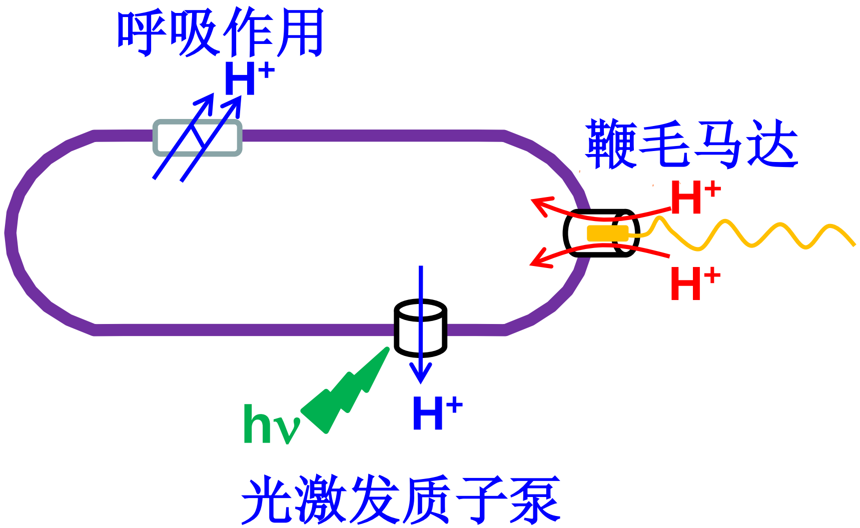


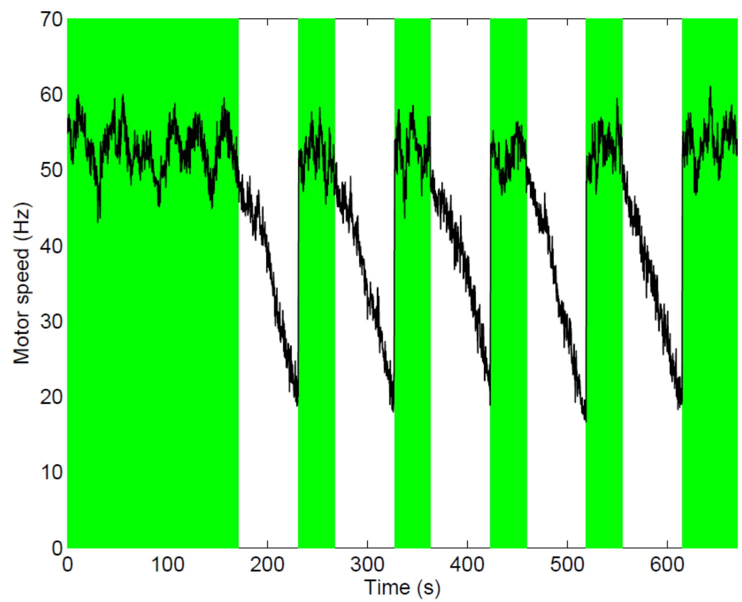
Different loads

Different # of stators



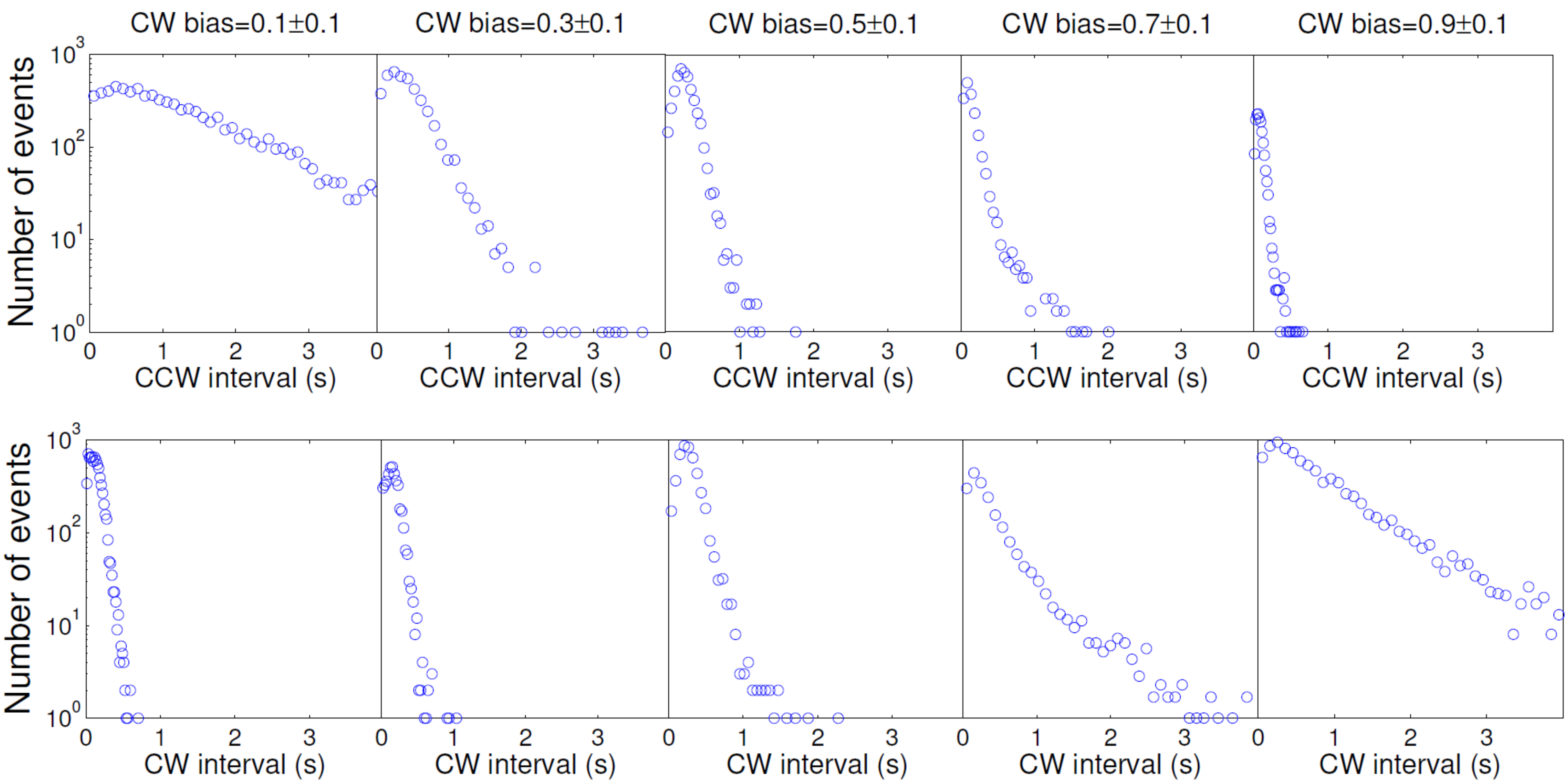
Different pmf





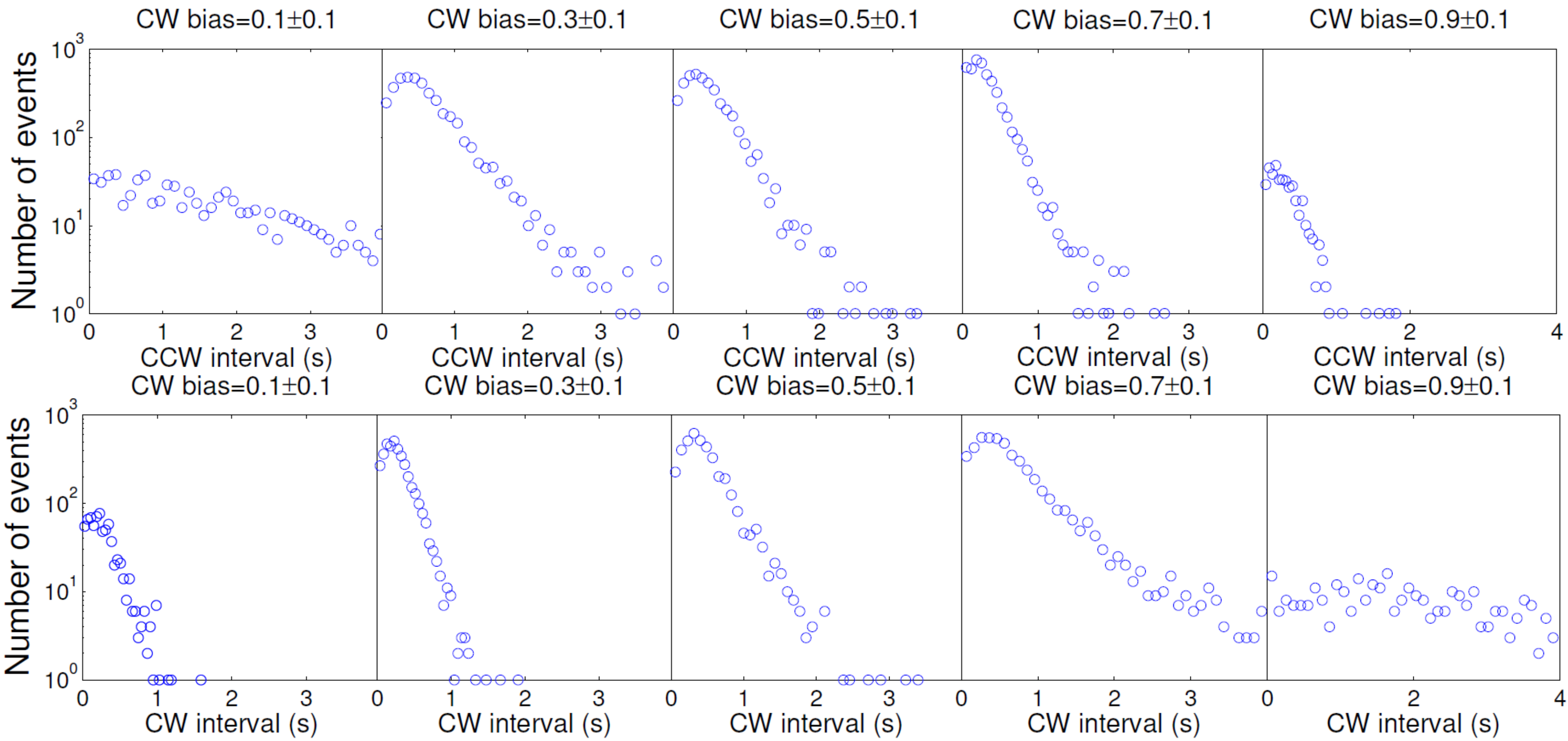
Interval distributions at high load

1 μm bead on filament



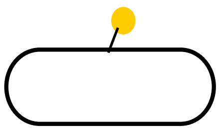
Interval distributions at medium load

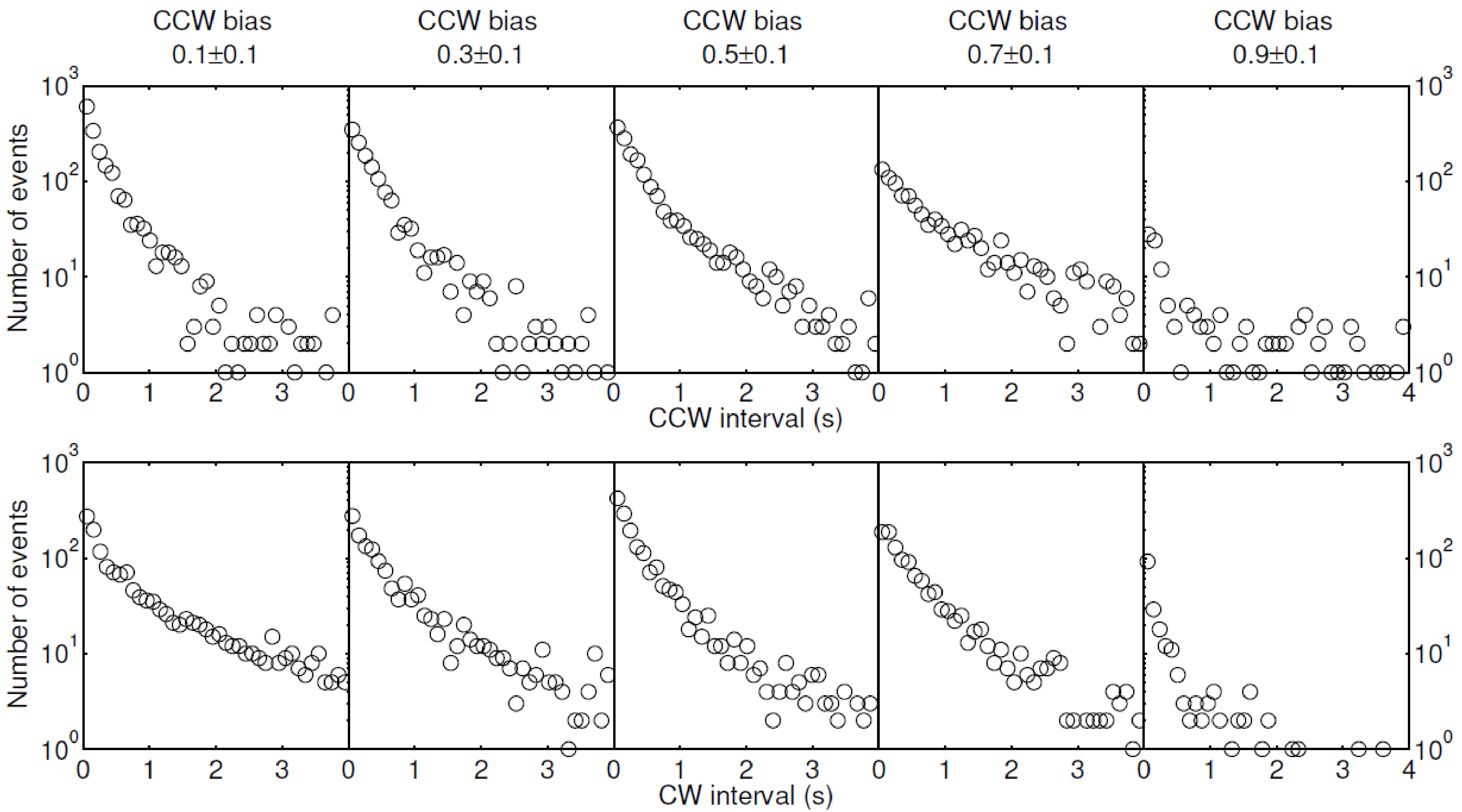
0.5 μm bead on hook



Interval distributions near zero load

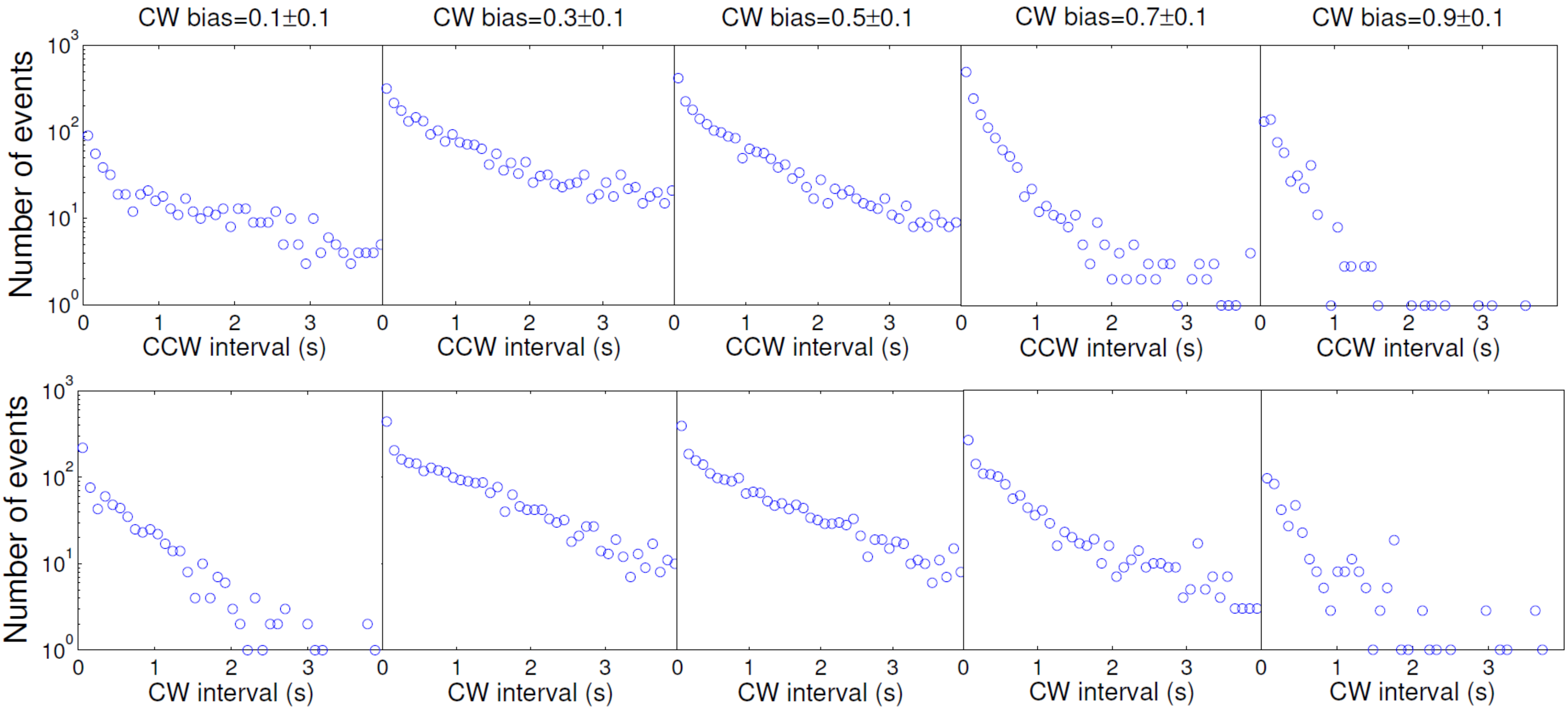
100 nm gold on hook



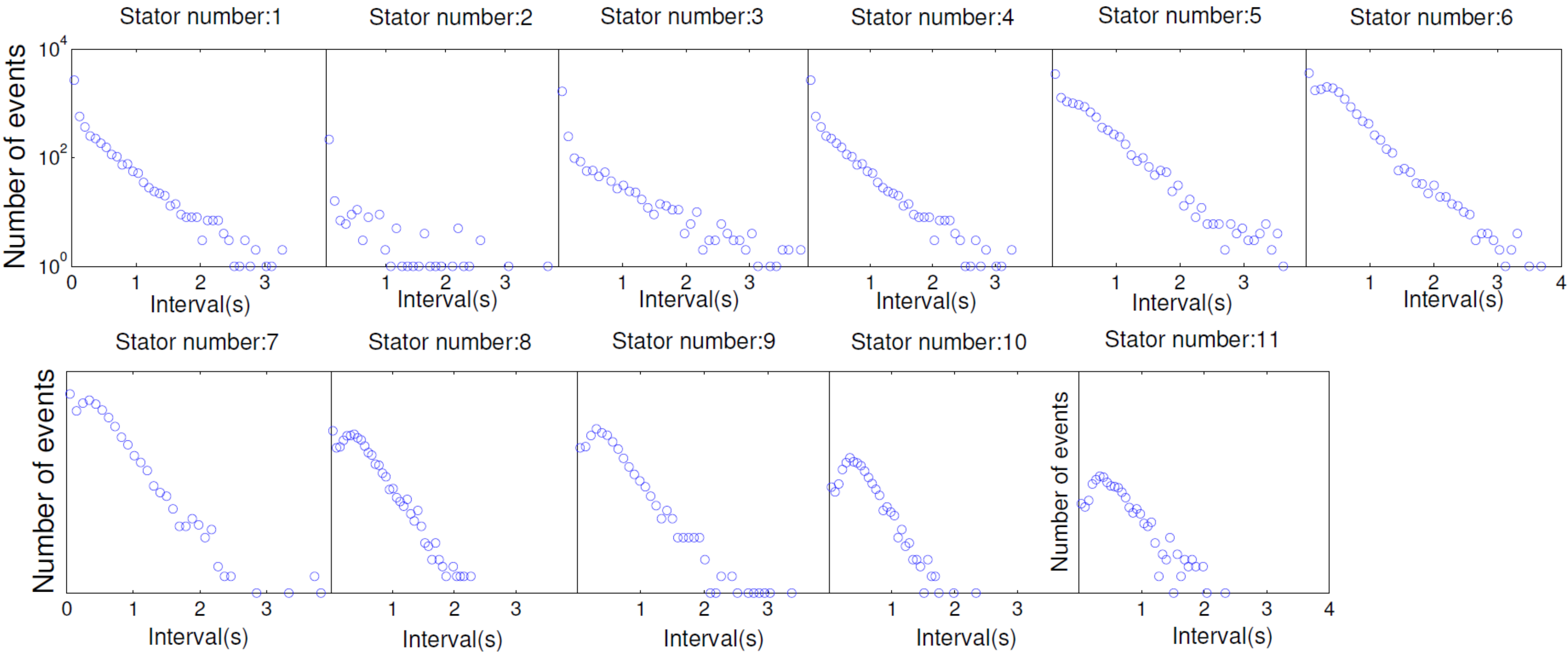


Interval distributions at medium load

@lower PMF



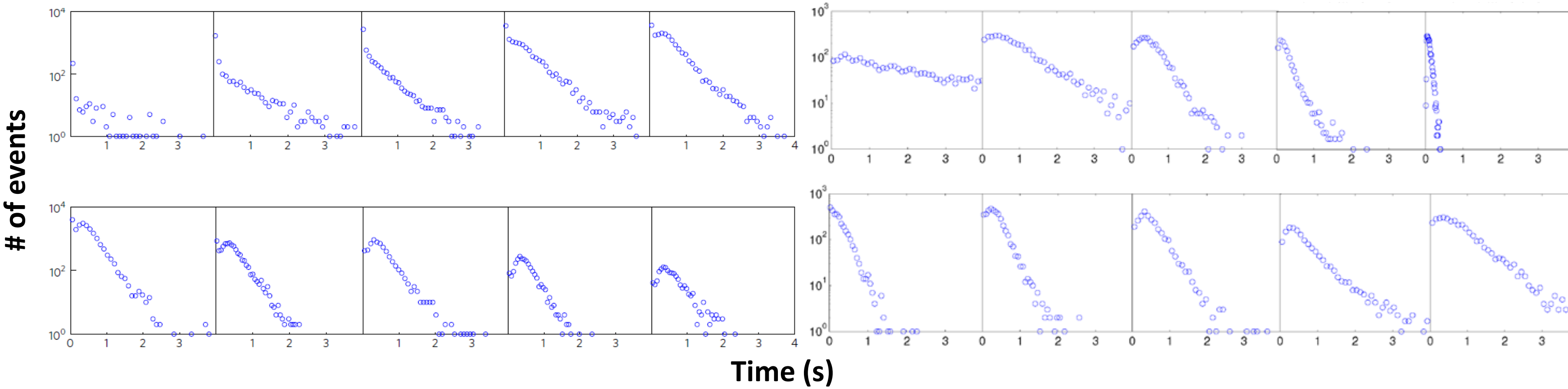
Interval distributions at high load @different # of stators

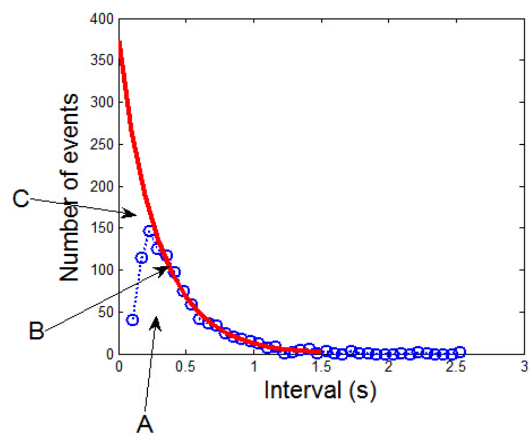


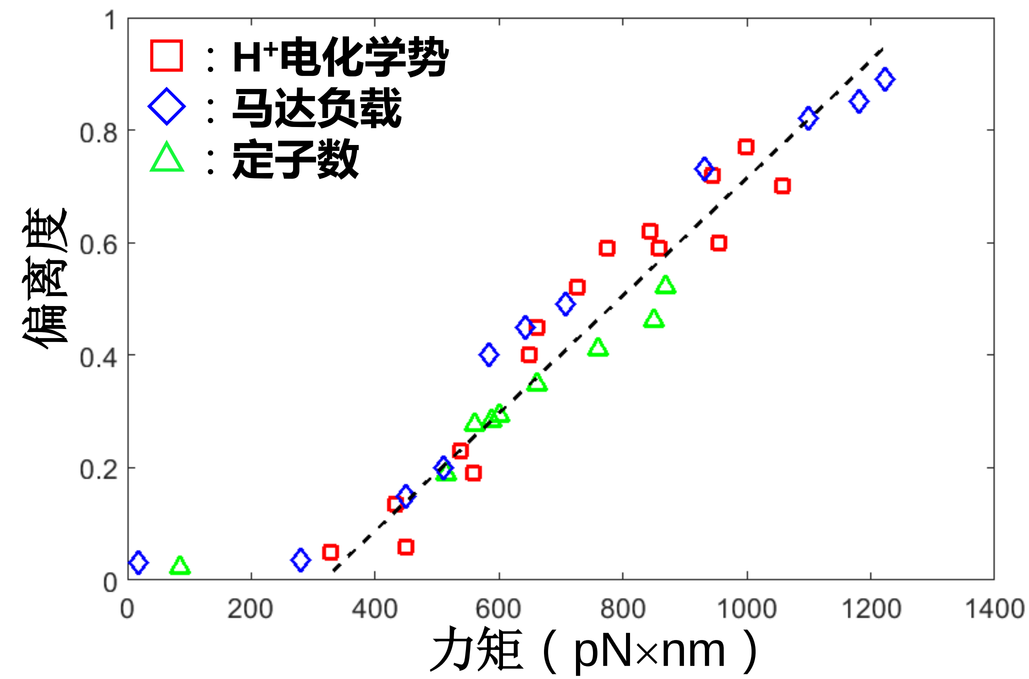
Dependence on torque

Experimental conditions		Motor torque	CW/CCW interval distribution shape
Different **loads** (high pmf, high stator number)	Near zero load with over expressed stator proteins	low	Exponential
	Intermediate to high loads (0.5, 0.75, 1.0 μm beads)	high	Non-exponential
Different **pmfs** (high load, high stator number)	Low pmf	low	Exponential
	High pmf	high	Non-exponential
Different **number of stators** (high load, high pmf)	Small number	low	Exponential
	Large number	high	Non-exponential

Dependence on torque



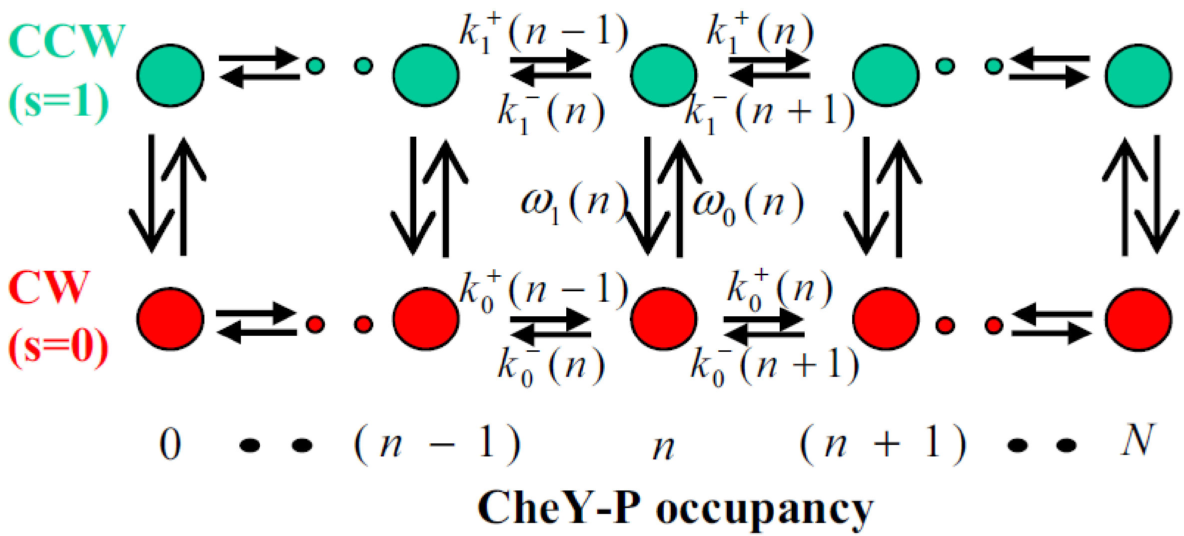




统一成：

与指数函数的偏离度 ∝ 力矩

Non-equilibrium effects



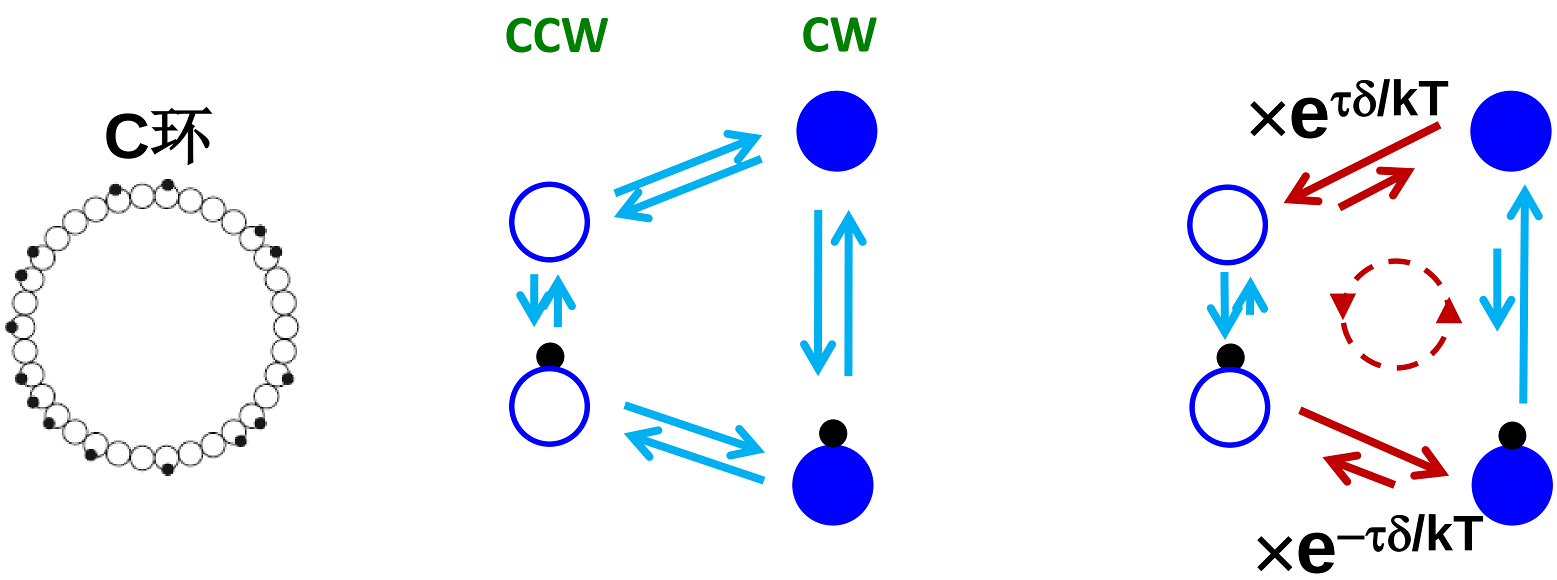
Any equilibrium model:

$$(-1)^m \frac{d^m P_s(\tau)}{d\tau^m} > 0, \quad \forall \, \tau > 0, \quad m = 1, 2, 3, \ldots,$$

*Yuhai Tu, PNAS **105**, 11737-11741 (2008)*

Non-equilibrium Ising model

与力矩相关的非指数分布 ⇒ 非平衡过程，正比于力矩



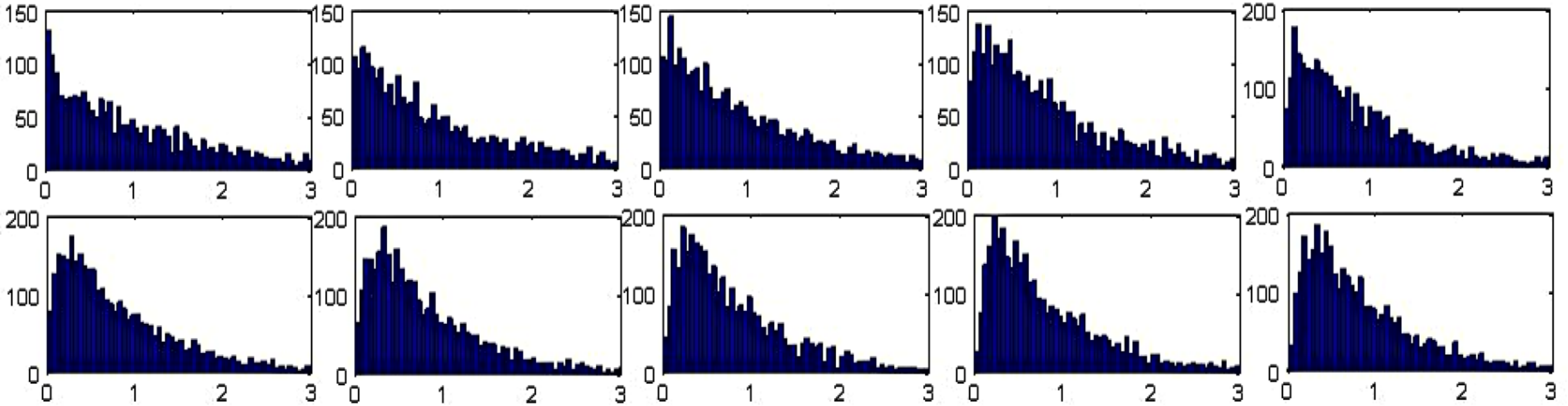
细致平衡　　　　非平衡

- 将力矩与转向调控联系起来 ⇒ 马达统一模型
- 小系统非平衡热力学的范例

Wang,...,Zhang*,Yuan* *Nature Physics* 13,710 (2017)

Interval distributions @ 1- 10 stators

# of stators: 1 → 5



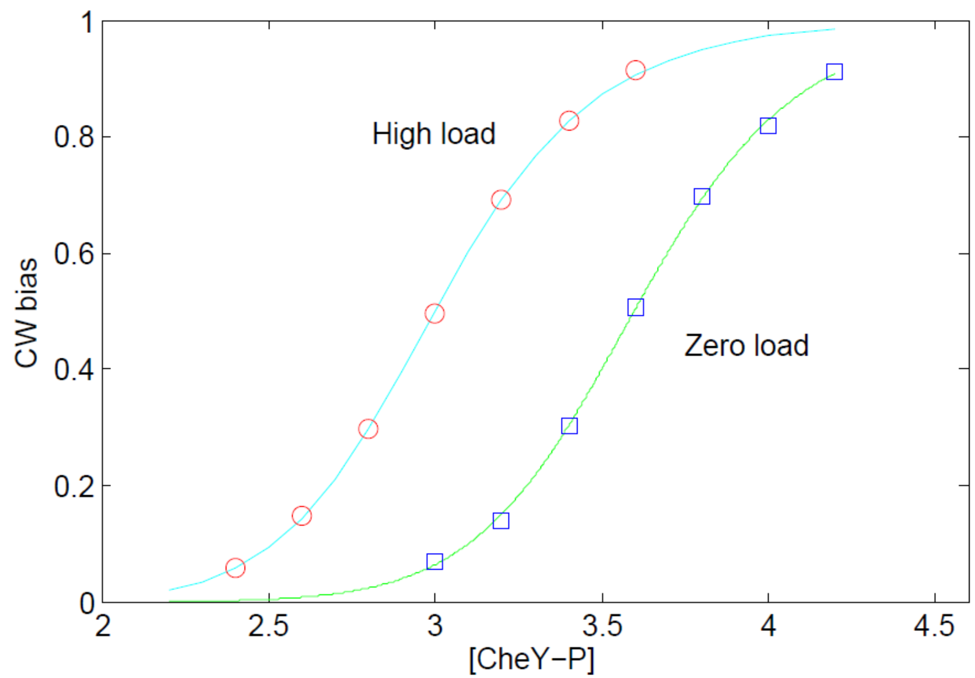
# of stators: 6 → 10

Sensitivity increased for high load

- 非平衡能量输入提高了马达的灵敏度：

$$\dot{W} = \sum_i (J_i^+ - J_i^-) \ln \frac{J_i^+}{J_i^-}$$

%1 马达能量 ⇨



Summary

- Previous controversies are resolved.
- Nonequilibrium effect in motor switching.

王芳彬, 何瑞, 史慧, 汪仁杰, 张榕京

Wang,...,Zhang*,Yuan* *Nature Physics* 13,710 (2017)

科学问题

信号 ⟹ 马达

① 信号如何可靠地传递到马达?
（输入输出如何鲁棒耦合）

② 马达转向如何调控?

转向改变

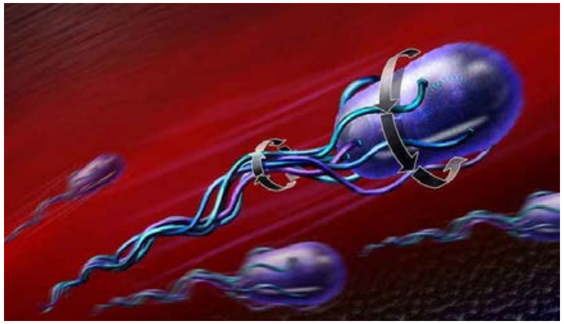
③ 马达力矩如何产生?

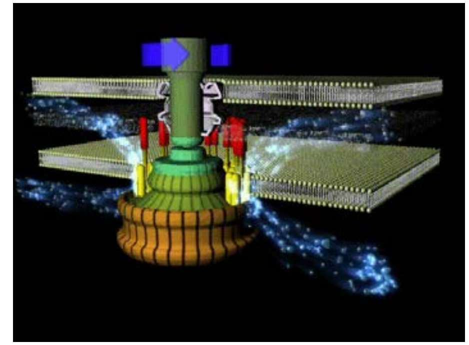
力矩

鞭毛马达力矩产生的动力学

力矩产生单元（定子）占空比

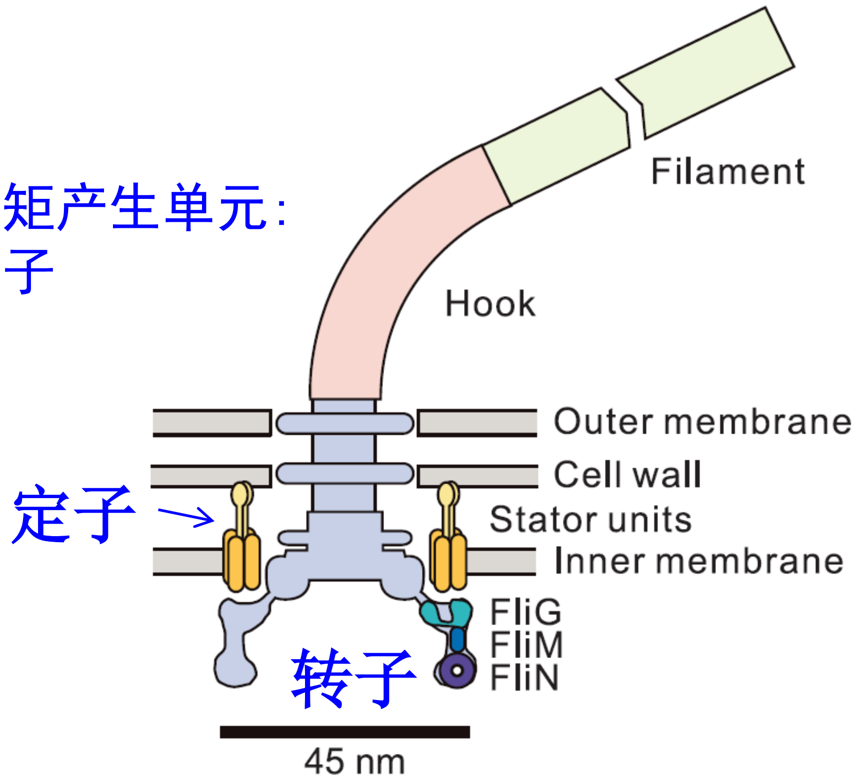
研究背景：大肠杆菌鞭毛马达



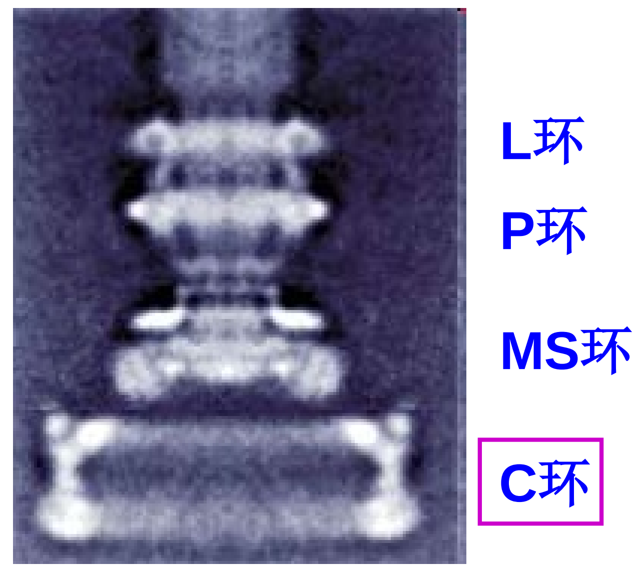


(视频制作：Ishijima)

力矩产生单元：
定子

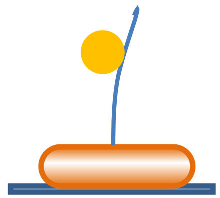


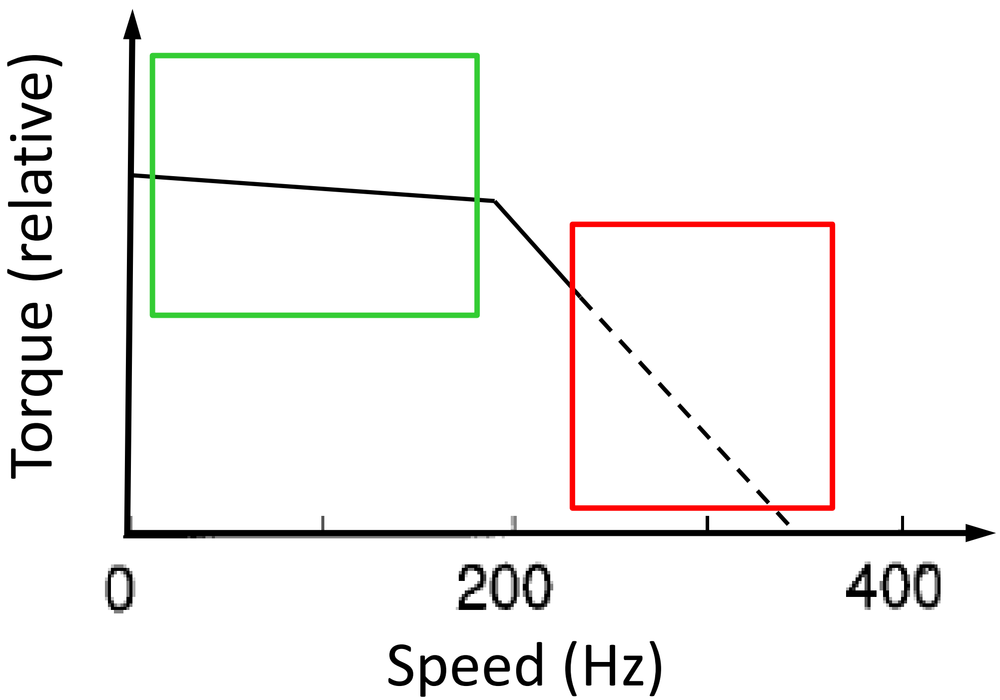
Bai *et al.* ***Science*** 327:685(2010)

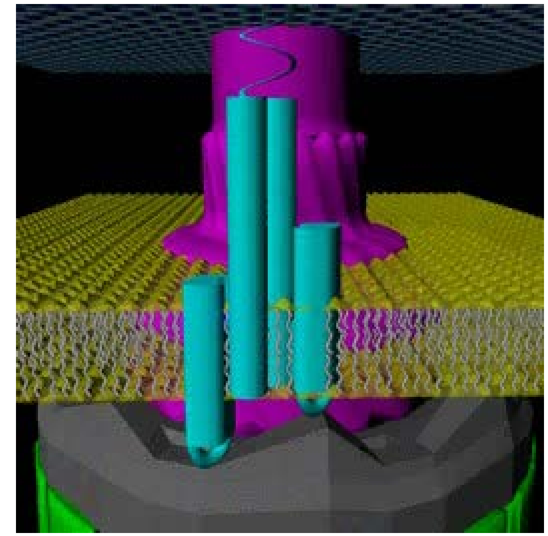


(电镜照片： D. DeRosier)

鞭毛马达的力矩产生







Duty ratio (占空比D):

High load:
转速 ∝ 定子数 (任何D)

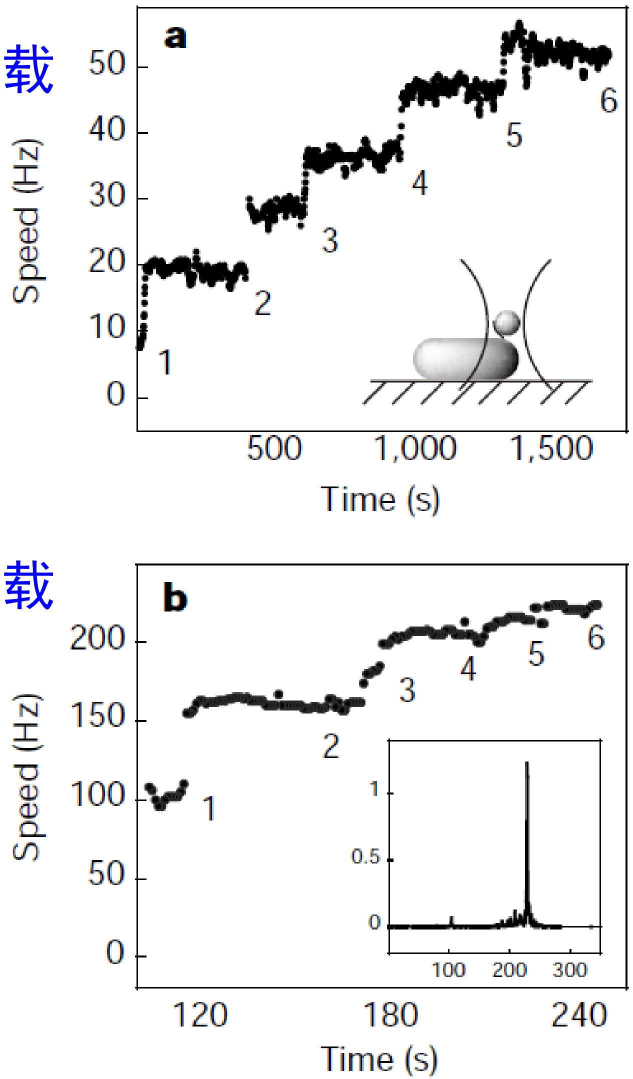
Zero load:
转速 ∝ 定子数 (small D)
转速与定子数无关 (D=1)

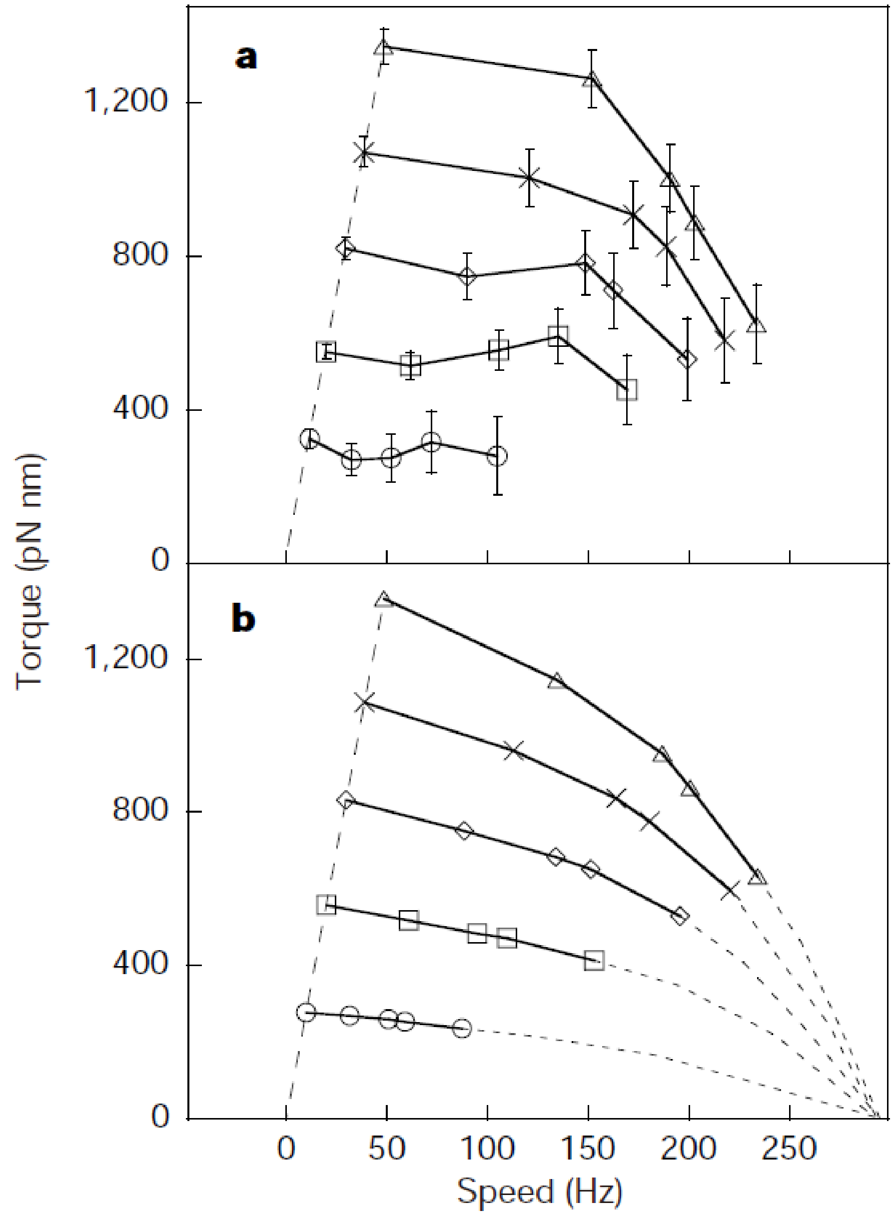
Chen, X. & Berg, H.C. ***Biophys. J.*** *78, 1036-1041 (2000)*

马达复活试验（I）

高负载

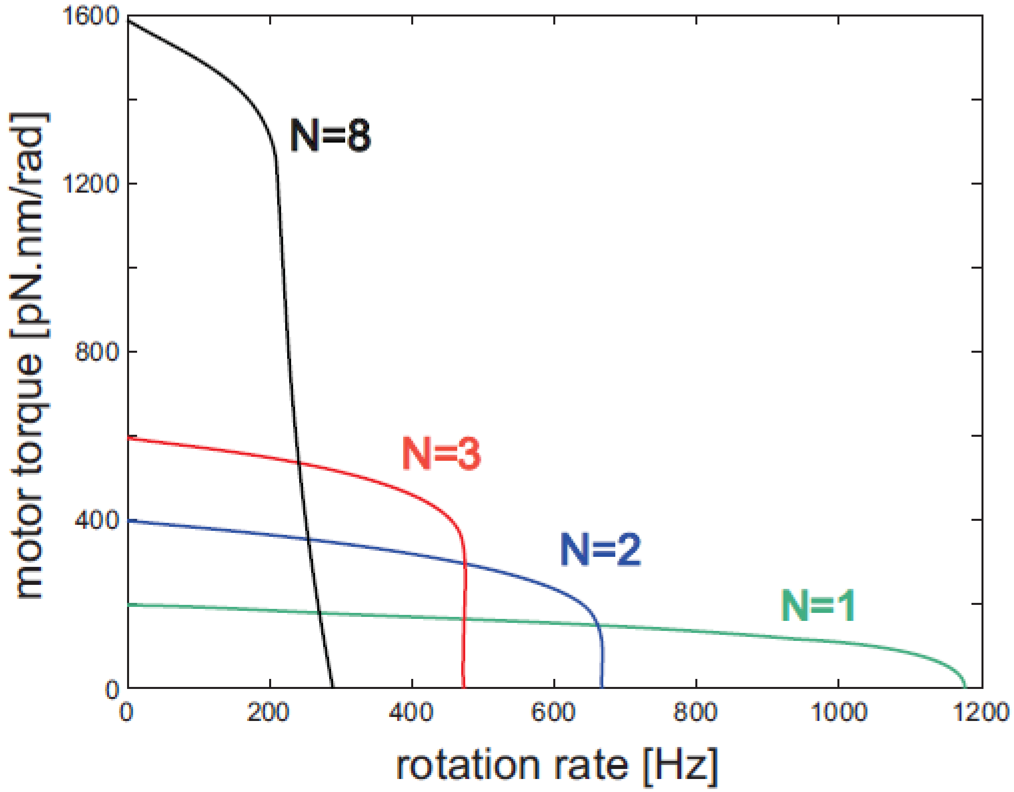
低负载





W.S. Ryu et al. **Nature** 403, 444 (2000)

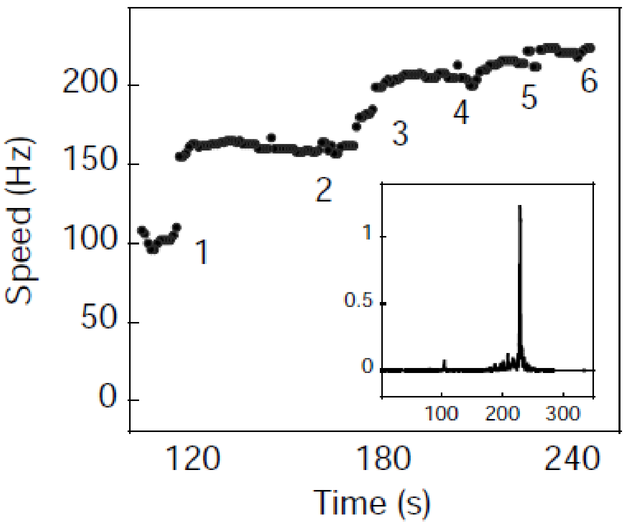
马达理论模型（I）



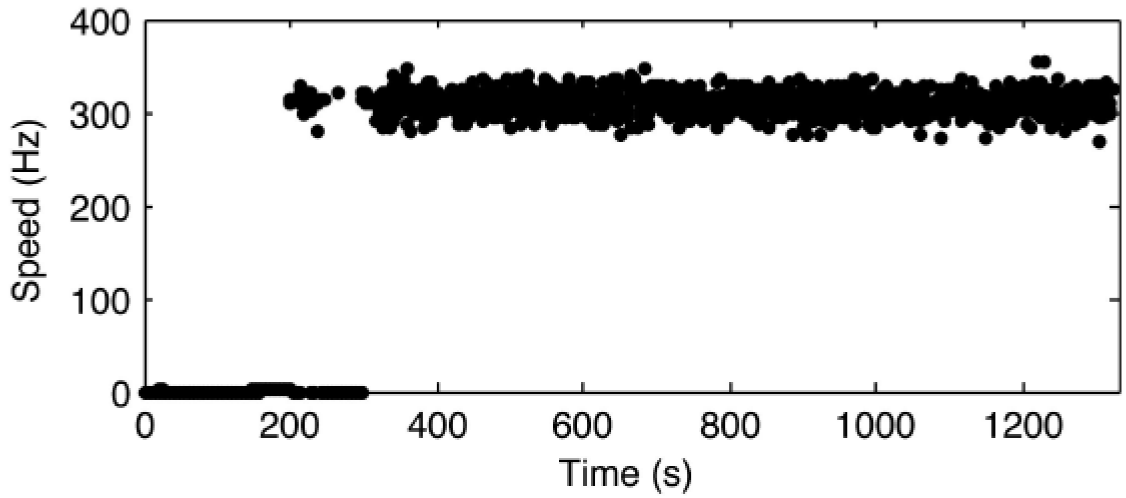
J. Xing et al. ***PNAS*** *103, 1260 (2006)*

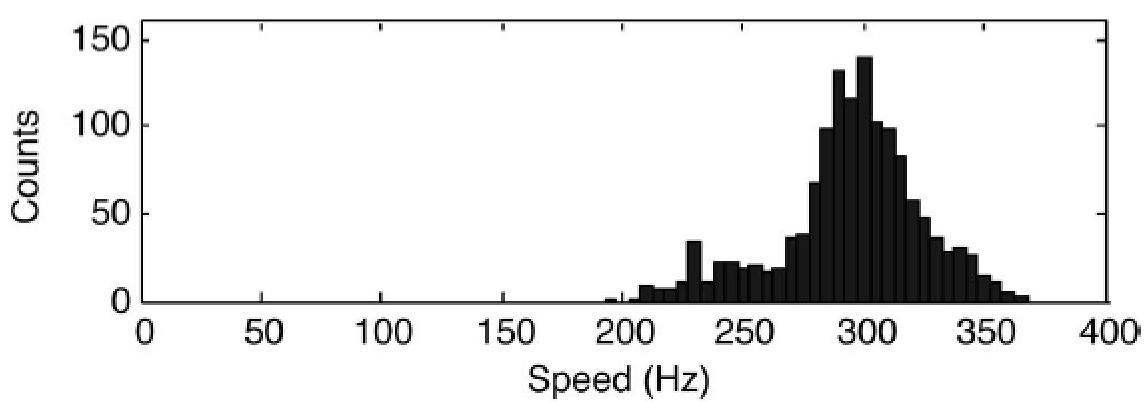
马达复活试验（II）

低负载



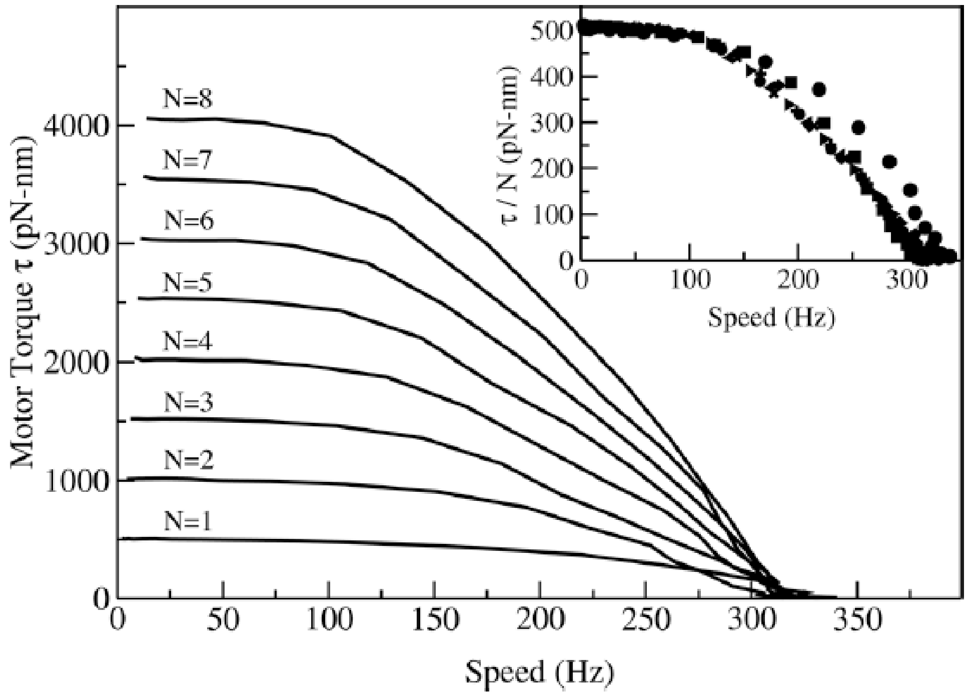
零负载





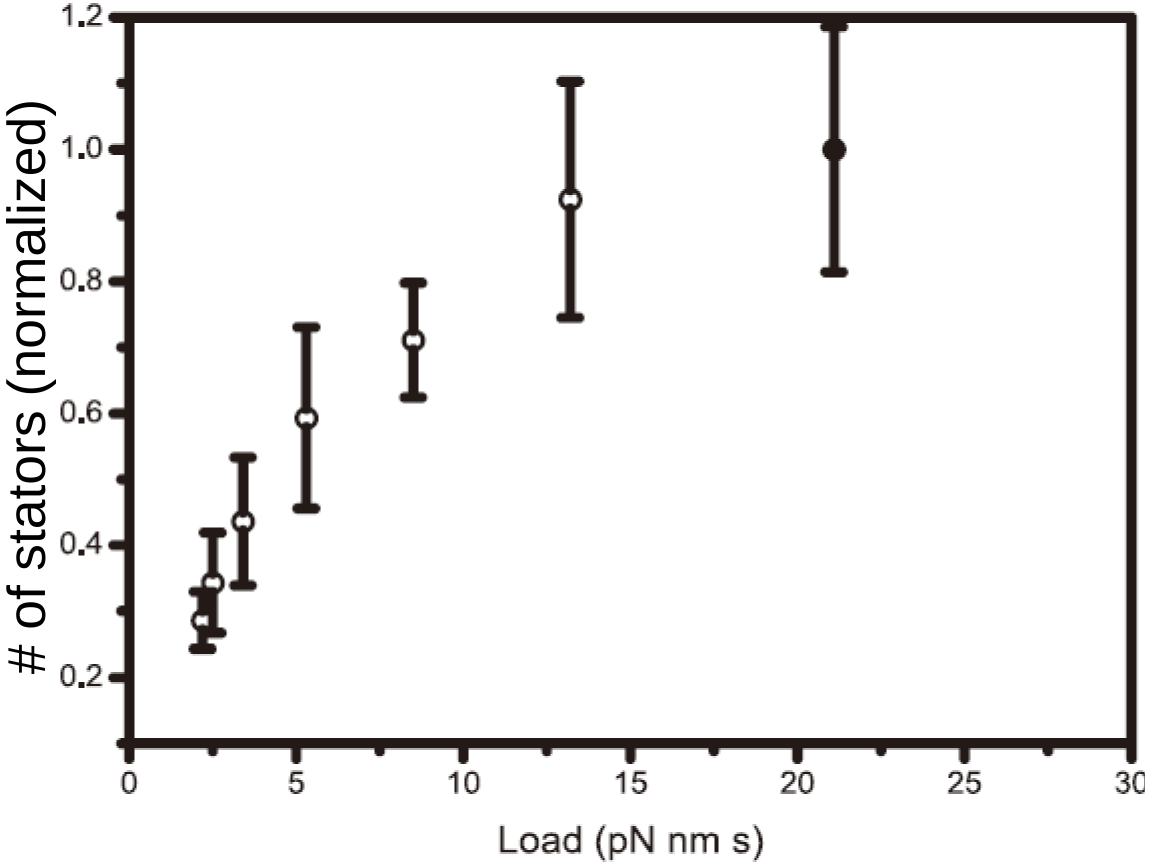
J. Yuan et al. ***PNAS*** *105, 1182 (2008)*

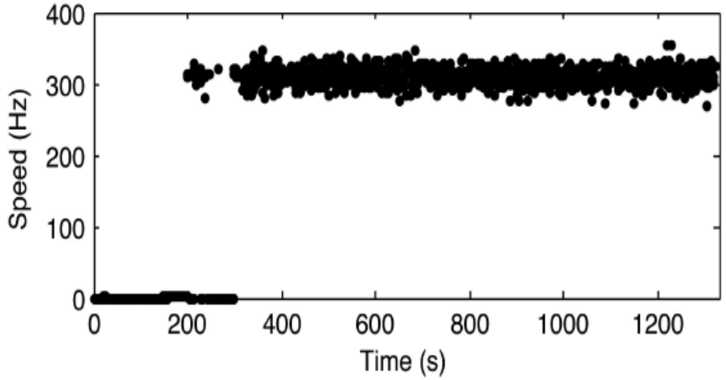
马达理论模型（II）



G. Meacci & Y. Tu ***PNAS*** *106, 3746 (2009)*
F. Bai et al. ***Biophys. J.*** *96, 3154 (2009)*

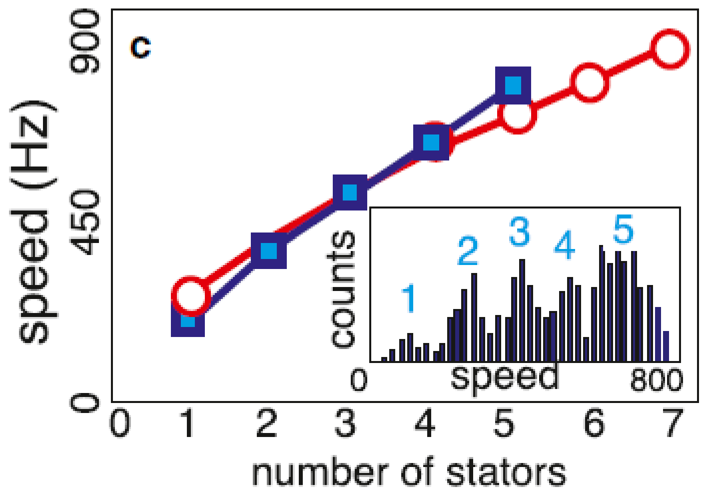
定子数目与负载相关





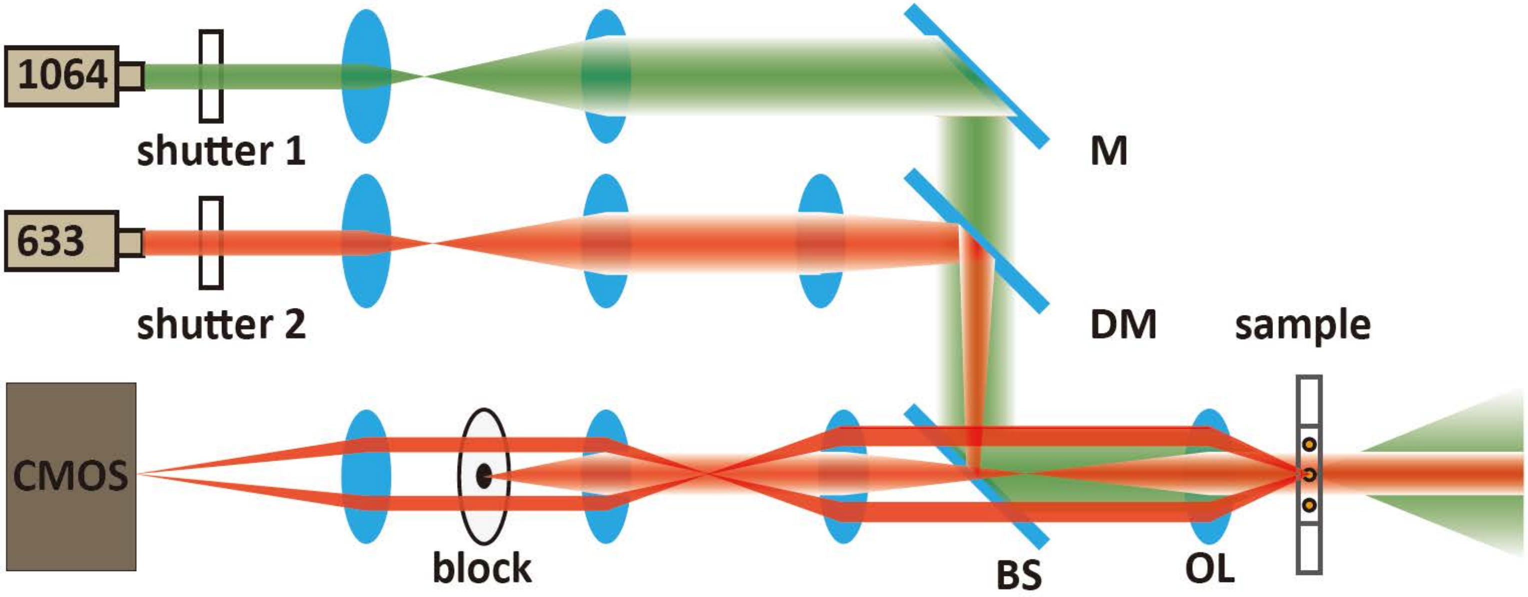
P. Lele et al ***PNAS*** *110, 11839 (2013)*
M.J. Tipping et al. ***mBio*** *4, 00551 (2013)*

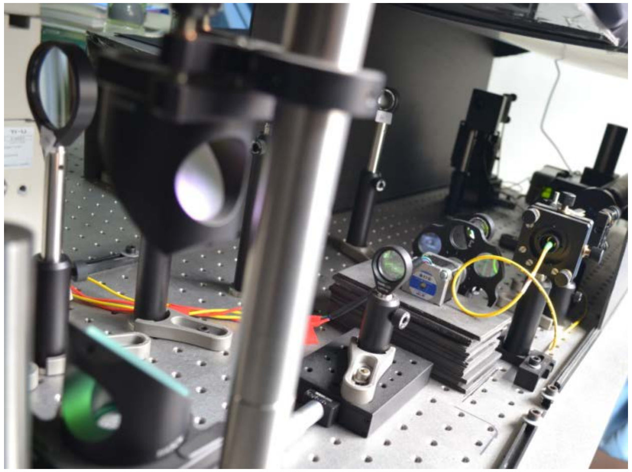
马达实验和模型（III）

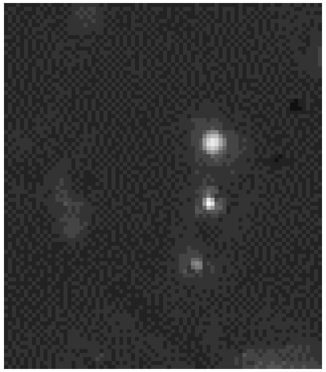


Y. Sowa et al ***PNAS*** *111, 3436 (2014)*
J.A. Nirody et al. ***Biophys. J.*** *111, 557 (2016)*

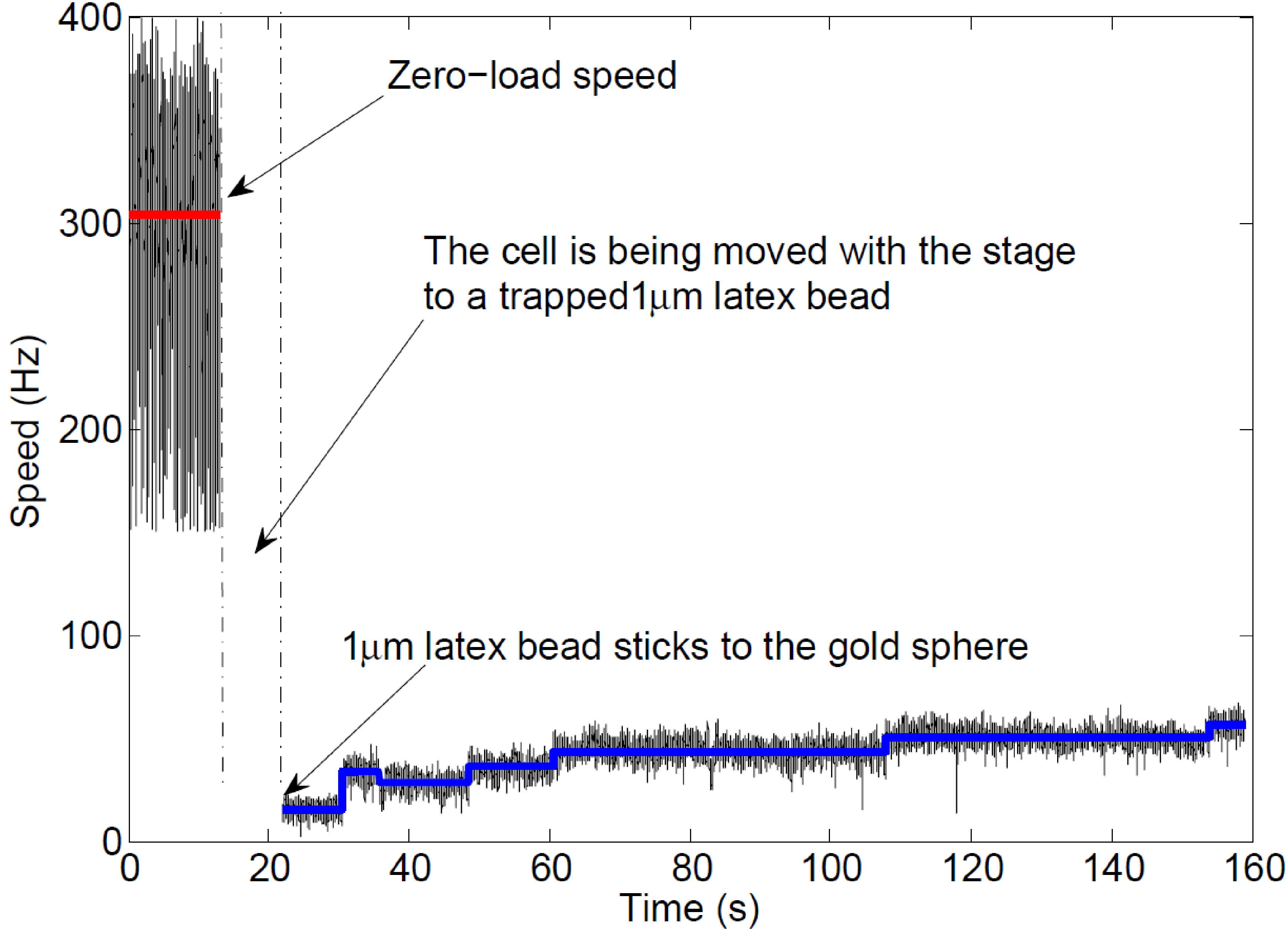
我们的实验设计





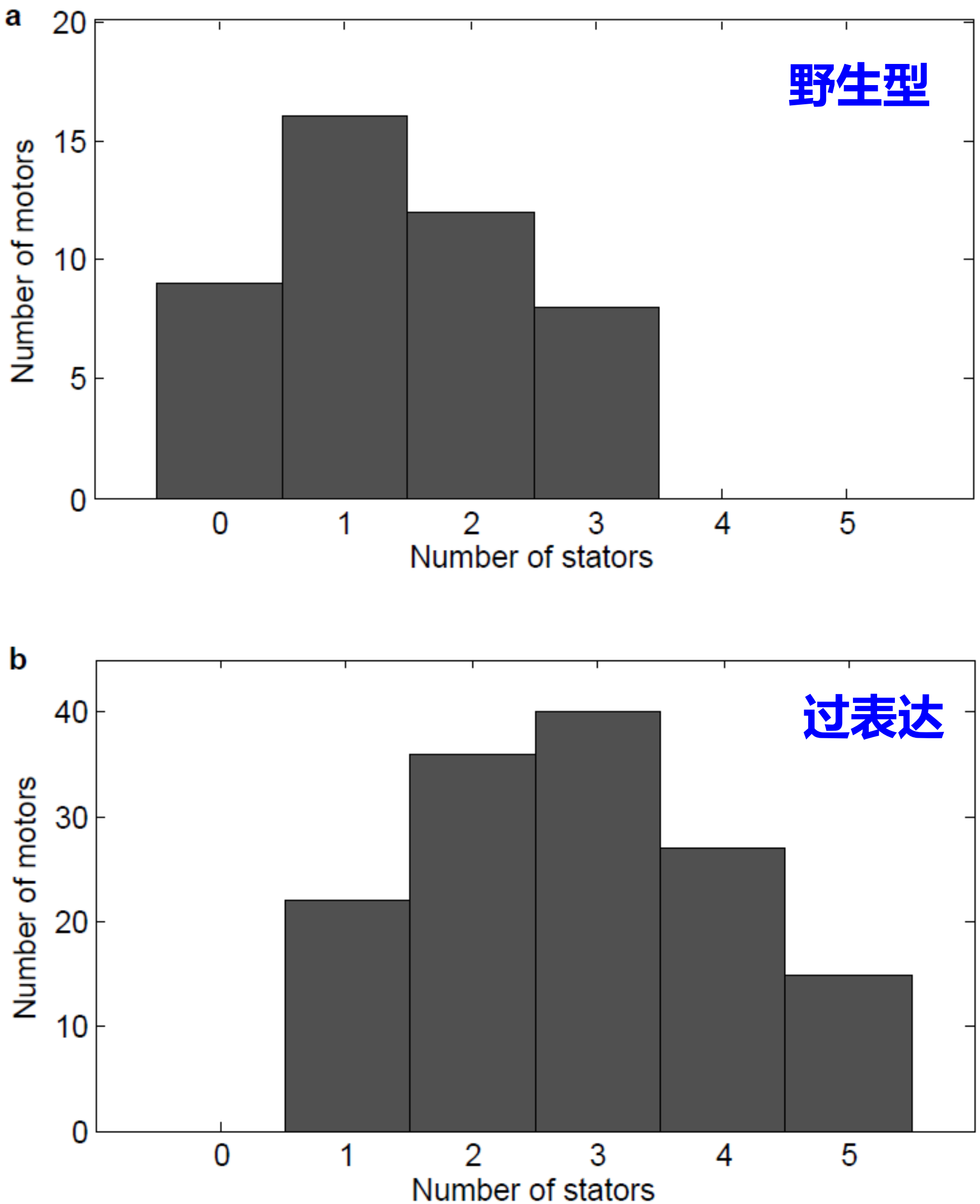


实验设计

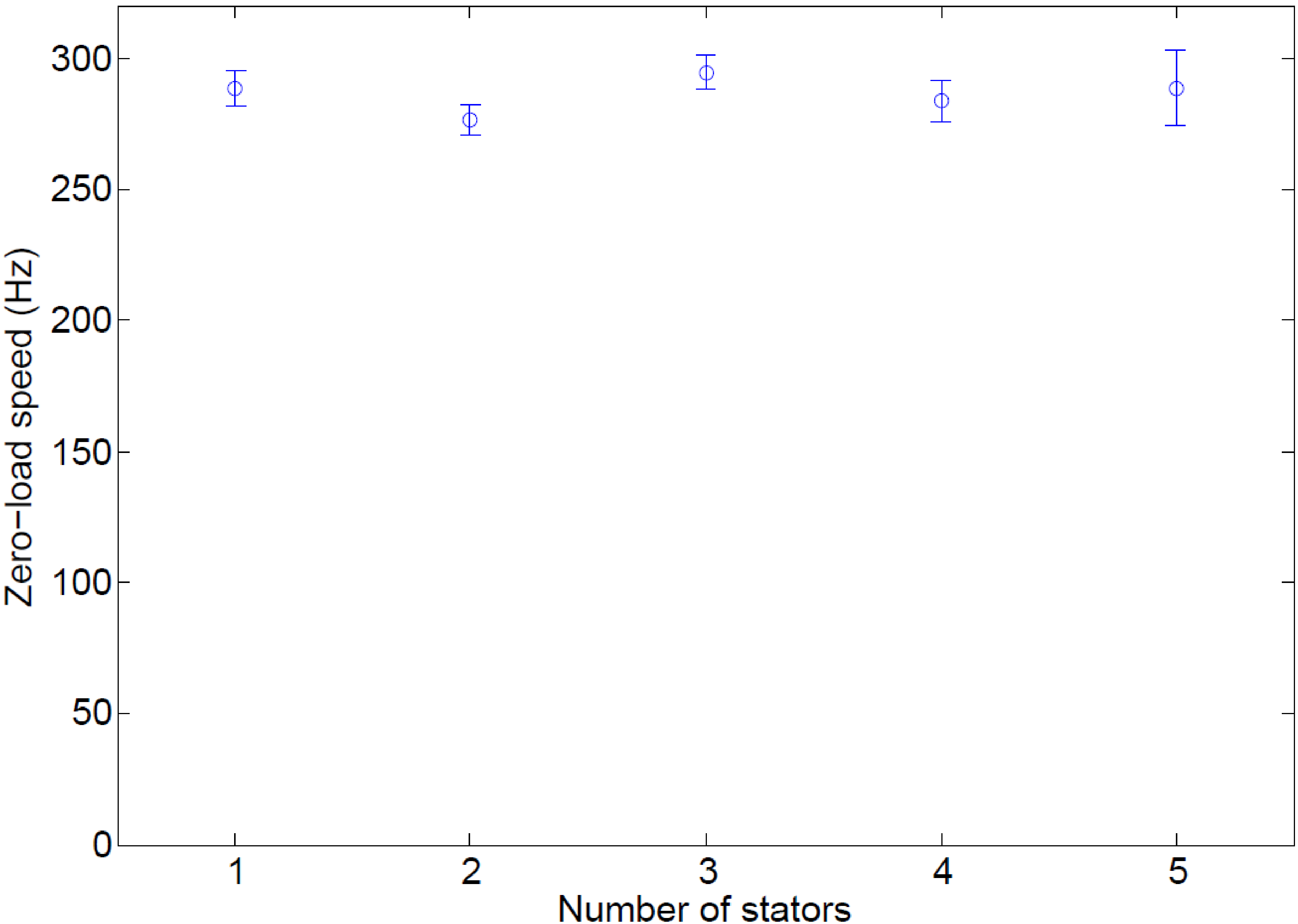


Zero−load speed
The cell is being moved with the stage
to a trapped1μm latex bead
1μm latex bead sticks to the gold sphere
Speed (Hz)
400
300
200
100
0
0
20
40
60
80
100
120
140
160
Time (s)

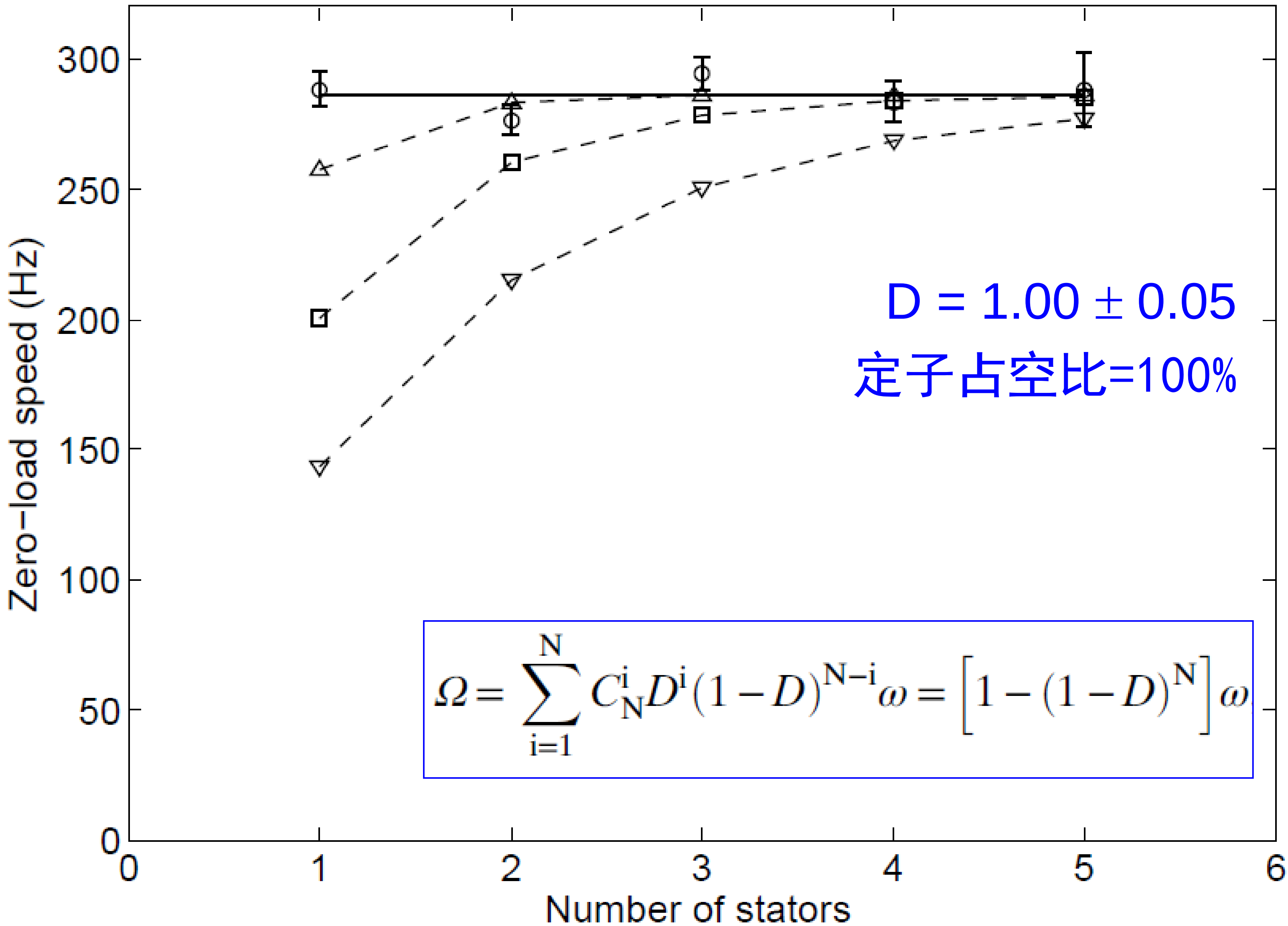
零负载下定子数目统计


a
野生型
Number of motors
Number of stators
0
5
10
15
20
0
1
2
3
4
5
b
过表达
Number of motors
Number of stators
0
10
20
30
40
0
1
2
3
4
5

零负载下速度与定子数目关系



马达定子占空比=1



$$\Omega = \sum_{i=1}^{N} C_N^i D^i (1-D)^{N-i} \omega = \left[1-(1-D)^N\right]\omega$$

总结

- 精确测量零负载下马达定子数目

- 马达零负载速度与定子数目无关 → 定子占空比=1

致谢

王彬、张榕京

国家自然基金委，科技部重点研发计划

Wang, Zhang, Yuan*. ***PNAS*** 114,12478(2017)