Paper Presentation: Physics of Living Matter
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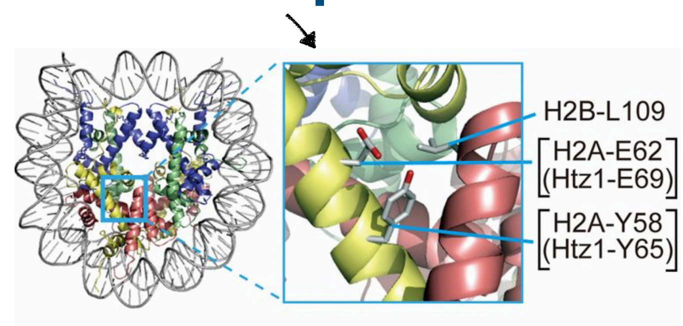
Torque-dependent remodeling of the bacterial flagellar motor

Navish Wadhwa, Rob Phillips, and Howard C. Berg, 2019

Vinay Kumar, 17th May 2022

Hello everyone. I’ll be talking about this paper by Wadhwa et al. on the torque dependant remodeling of the bacterial flagellar motor.

MultiSubUnit Protein Complexes



(Nakabayashi et al 2013)

Functions:

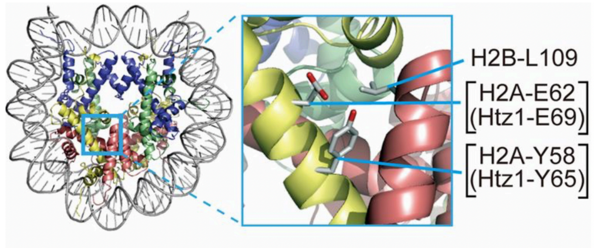
- Protein Synthesis
- ATP Production
- DNA Replication
- Intracellular Transport

The bacterial flagellar motor is an example of a multisubunit protein complex. MultiSubUnit Protein complexes are macromolecules formed of self assembling units, and according to the authors, are found everywhere in Biology. I've listed some of the functions they perform on the screen.

MultiSubUnit Protein Complexes

- Structure : assumed to be static (until degraded)
- Function : independent of method of assembly

True??



(Nakabayashi et al 2013)

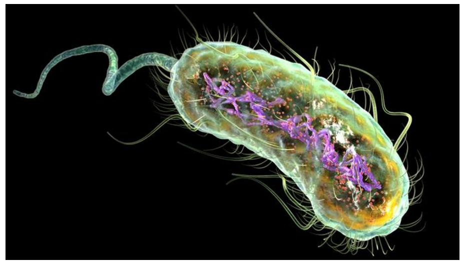
For a long time, these were assumed to have a static structure and a function independent of MO of assembly. This paper and the experiment described therein tests the validity of these assumptions by looking at Bacterial Flagellar motors.

Bacterial Flagellar Motor

E. coli

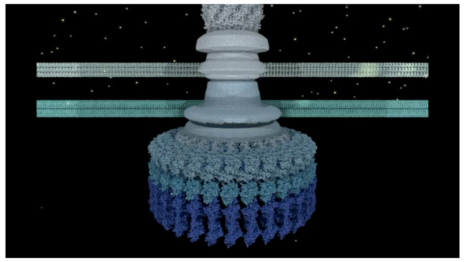
- Rotor
  - rotating part
  - rotates helical filaments for motion
- Stator
  - stationary, torque generating part
  - bind to peptidoglycan layer as and when required

E. Coli



(TechnologyNetworks)

Motor Structure



(YouTube)

They look at the motor responsible for motion in E coli. The motor is assembled at the cell wall. It has two main parts - the rotor which rotates and the stator which is responsible for torque generation. The video on the bottom right shows the structure. There exists a pool of stator units from which individual units bind and unbind to the peptidoglycan layer and drive the motor. More bound stators means more torque. Clearly the assembly is not static and changes with requirement. The obvious way to study this remodeling is to study the stoichiometry of stator proteins, which is what we'll do now.

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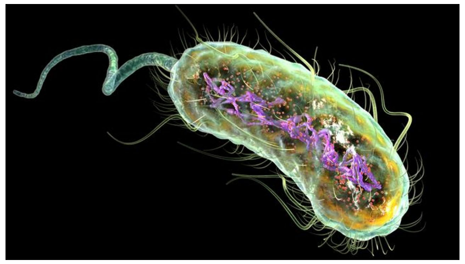

(TechnologyNetworks)

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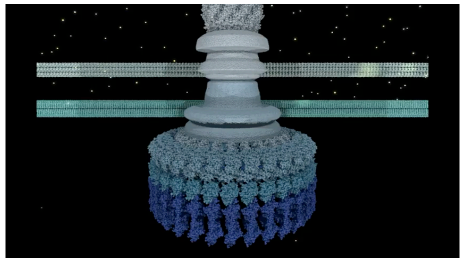

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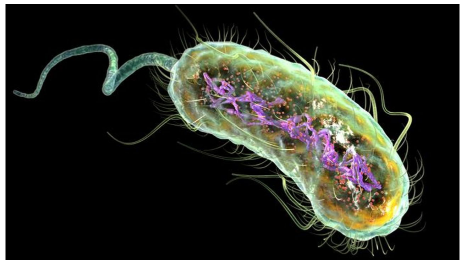

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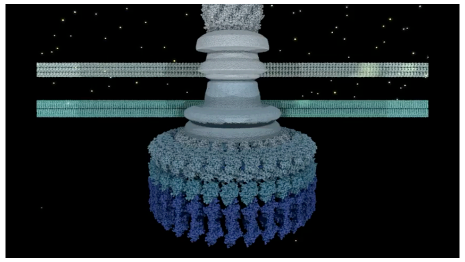
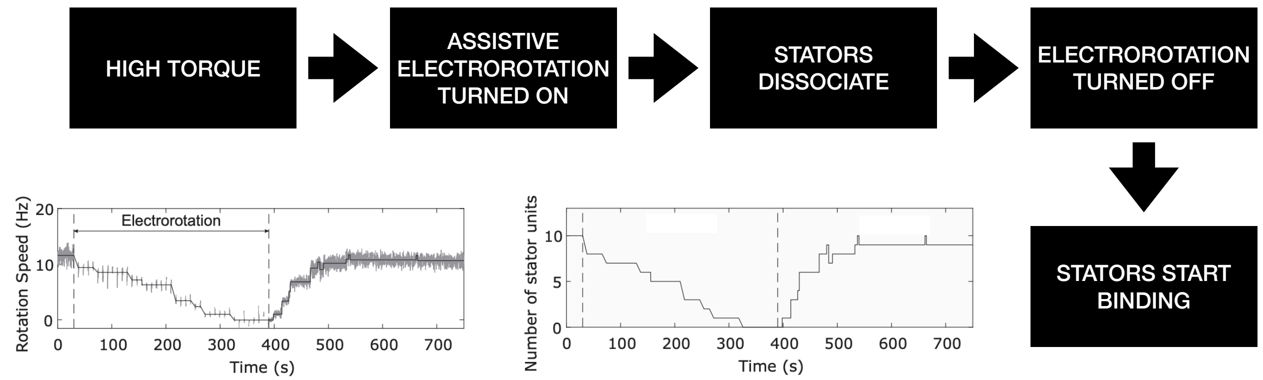


(YouTube)

Study the Stoichiometry of Stator Proteins

The Experiment

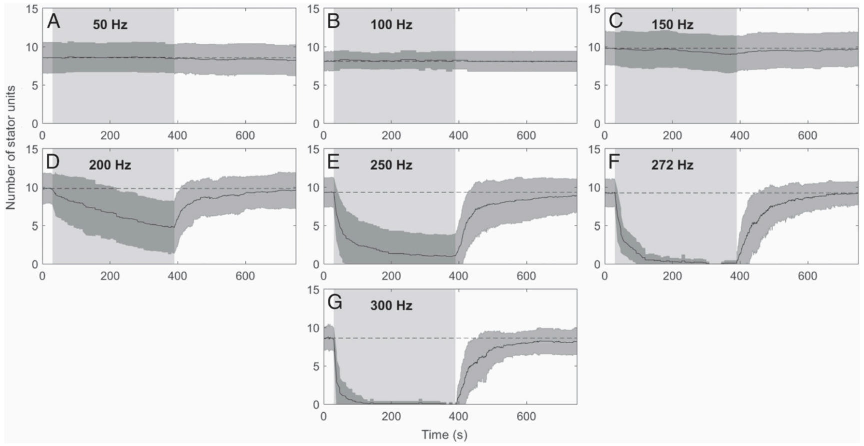
- Bacteria lacking CheY regulator were used, so the motors only rotate CCW.
- Tethered to sapphire.
- Electrorotation used for providing external torque.



These guys used bacteria which lacked this particular regulator and so cannot rotate clockwise. The experiment looks at how the stators dissociate when an assistive external torque is provided and subsequently associate when the external torque is removed. Bacteria are tethered to a sapphire sheet. We start with zero external torque and so the number of stator units is high initially. When electrorotation is turned on, the motor speed decreases and so does the number of stator units because less number of units can provide necessary torque. Further, when electrorotation is turned off, stator units start binding again to provide the required torque.

The Experiment

- This was done for many cells at many different speeds of electrorotation.
- Results:



The speed written on each curve is the speed of electrorotation. We see that below a certain speed, the knee speed, which is around 150 Hz in this case, no stator units were released. This can be seen in figures A to C. From D to F, we see that the number of stator units released depends on the speed of electrorotation, as does the rate of recovery post removal of the electrorotation torque. Figure F corresponds to the zero torque speed - speed at which the cells don’t produce any torque - 272 Hz. It can be seen that the number of stator units at the end of the electrorotation period drops to zero for this speed. The final plot shows that at speeds higher than the zero torque speed, stator units are released very rapidly.

Kinetics

- A master equation can be written for the remodeling process:

$$\frac{\mathrm{d}p(n,t)}{\mathrm{d}t} = (n+1)k_{-}p(n+1,t) + (N-n+1)k_{+}p(n-1,t) - nk_{-}p(n,t) - (N-n)k_{+}p(n,t)$$

- The rate of change of the average number of stator units can be found to be:

$$\frac{\mathrm{d}\langle n\rangle}{\mathrm{d}t} = k_{+}(N-\langle n\rangle) - k_{-}\langle n\rangle$$

- Setting this rate of change to zero gives the steady state value of the number of stators per sites available or the average occupancy as:

$$r = \frac{\langle n\rangle_{ss}}{N} = \frac{1}{1+\frac{k_{-}}{k_{+}}}$$

Kinetics

- Also, the solution to the rate equation can be given as:

$$\langle n \rangle = \langle n \rangle_{ss} + (n_0 - \langle n \rangle_{ss}) e^{-(k_+ + k_-)t}$$

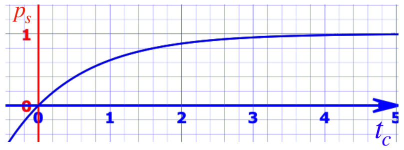
- which gives the time constant of this process as:

$$\tau = \frac{1}{k_+ + k_-}$$

- We will now see how to model the dependence of k_+ and k_- on the speed of the motor.

Speed Dependent ON Rate

- Binding of stator onto motor - 2 steps:
  - Contact of a free stator unit with motor - diffusion sets rate - k_0
  - Probability of Success in assembling - p_s



- Effective rate, $k_+ = k_0 p_s$
- p_s depends on the Contact time, t_c, which depends inversely on speed, F.
- $\implies p_s = 1 - e^{-\kappa t_c} \implies k_+ = k_0 \left(1 - e^{-\kappa/F}\right)$

The binding of the stator can be modelled as a two step process. 1. A freely diffusing stator coming in contact with the motor. The rate of this step, k_0 is set by diffusion. Every contact may not lead to assembly and so there is a success probability p_s. The effective rate then is the product of these two values. Now p_s depends on the motor speed. This is because the higher the speed, the less is the contact time t_c between the stator and the. Motor. Higher t_c means higher p_s. This dependance is assumed to be like that of the charge on a charging capacitor as a function of time as it follows the same asymptotics. So we get the following form for p_s and subsequently we get the expression for k+ as shown.

Torque Dependent Occupancy

- Assume assembly is in quasi-equilibrium - use equilibrium stat-mech.
- Binding causes free energy to drop by μ to $\epsilon_b - \mu$.
- Torque causes free energy to further drop by $\epsilon_T = \epsilon_T(T)$ to $\epsilon_b - \mu - \epsilon_T$
- Steady-state occupancy:

$$r = \frac{\langle n \rangle_{ss}}{N} = \frac{1}{1 + \exp\left[-\beta\left(\epsilon_b - \mu - \epsilon_T\right)\right]}$$

$$\frac{1}{1 + \exp\left[-\beta\left(\epsilon_b - \mu - \epsilon_T\right)\right]} = \frac{1}{1+\frac{k_-}{k_+}}$$

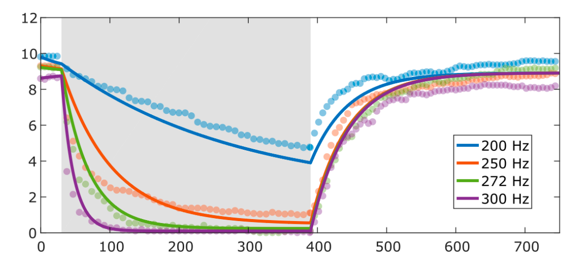
We assume that the assembly is in quasi-equilibrium since the measurement timescale is much smaller than the remodeling timescale. The process of binding of a stator causes a drop in the free energy by an amount mu. Presence of an external torque is hypothesised to decrease the free energy further by an amount Epsa_T. So the average steady state occupancy is given by r = read. Which we had previously in terms of k+ and k-. This r can be measured in experiment and the expression can be inverted to get how epsa_T varies with F. Solving for k- we get

Torque Dependent Remodeling

$$\frac{k_-}{k_+} = \exp\left[-\beta\left(\epsilon_b - \mu - \epsilon_T\right)\right]$$

$k_+ = k_0\left(1 - e^{-\kappa/F}\right)$ and $k_- = k_0 e^{-\beta\left(\epsilon_b - \mu - \epsilon_T\right)}\left(1 - e^{-\kappa/F}\right)$

$$\tau = \frac{1}{k_0\left[1 + e^{-\beta\left(\epsilon_b - \mu - \epsilon_T\right)}\right]\left(1 - e^{-\kappa/F}\right)}$$



We had k+ and we get k- as ____. So now we have both k+ and k- as functions of the speed of the motor and the external torque provided. We can now find the time constant for the kinetic equation as tau =.

So finally we see that the reassembly of the bacterial motor depends on the speed and hence the external torque. This is what we wanted to find. The plot on the right shows the experimental verification of the same.

Thank You