Whipping Out Bacteria

A New Era of Flagellum-Targeted Therapeutics

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ABSTRACT

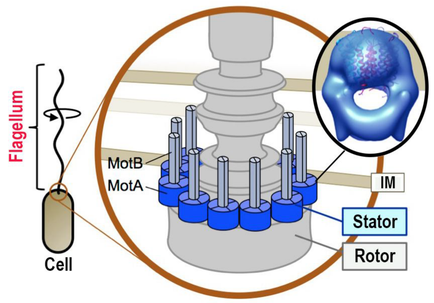
The bacterial flagellum enables bacterial motility and is important in enhancing the virulence or ability of bacteria to cause disease. Bacteria such as E. coli and Salmonella typhi are known to rely on their flagella to attack multicellular organisms, making it an important target when considering potential treatments for infection. This project seeks to determine the structure of flagellar proteins responsible for bacterial motility and proposes a structure-based drug design that utilizes the flagellar stator as a novel drug target for antibiotics.

INTRODUCTION

- Swimming is the most common form of locomotion among bacteria and is propagated by the flagellum.
- The flagellum is important in determining the virulence of some bacteria - *Salmonella typhi* for example
- Most structural analyses of the flagellum focus on the rotor, and not the stator, yet the stator generates the mechanical work needed to power the rotation of the flagellar motor.

Function of the Stator:

- The stator is involved in the passage of protons down an electrochemical gradient across the cell membrane and generates a turning force to spin the rotor. (Terashima et al., 2008)



Source: Michio Homma, https://phys.org/news/2016-09-japanese-team-elucidates-bacterial-flagellar.html (2016)

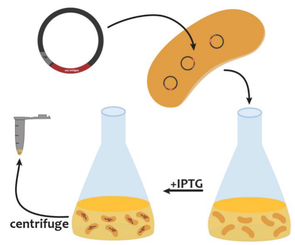
Two Proteins within the Stator: Mot A/B

- MotA and MotB are two stator proteins that change their conformation in response to the proton gradient and interact with FliG, a rotor protein, to generate torque for rotation.
- Mutants of *E. coli* with defective MotA or MotB proteins have been noted to become paralyzed. Gradual expression of MotA and MotB, however, resulted in stepwise increases in rotation speed. (Silverman et al., 1976)
- A low resolution structure of MotA has been determined using electron microscopy, but this is not effective for drug design.

PROCEDURE

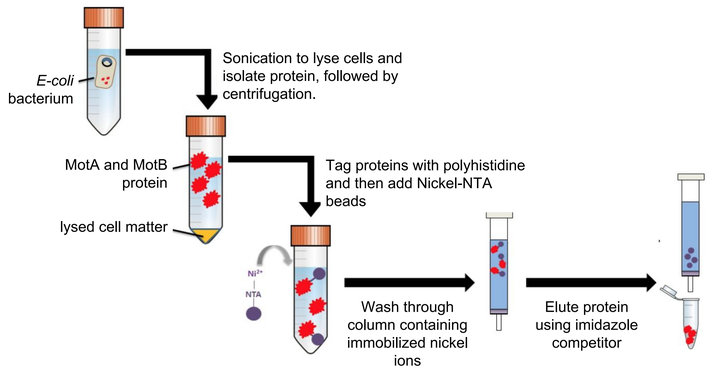
Protein Production

- Isolate genes from Salmonella bacteria, form recombinant DNA, and insert into *E-coli*.
- Culture *E-coli* in IPTG (to allow transcription of the *Lac* operon).
- Centrifuge to isolate bacteria.



Source: http://2015.igem.org/Team:Freiburg/Project/Protein_Purification (2015)

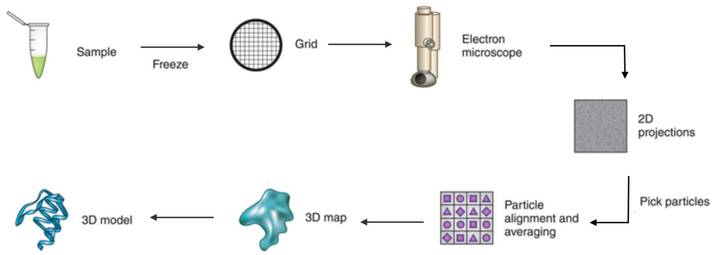
Protein Purification



Adapted From: Raiha T., Wikimedia Commons (2014)

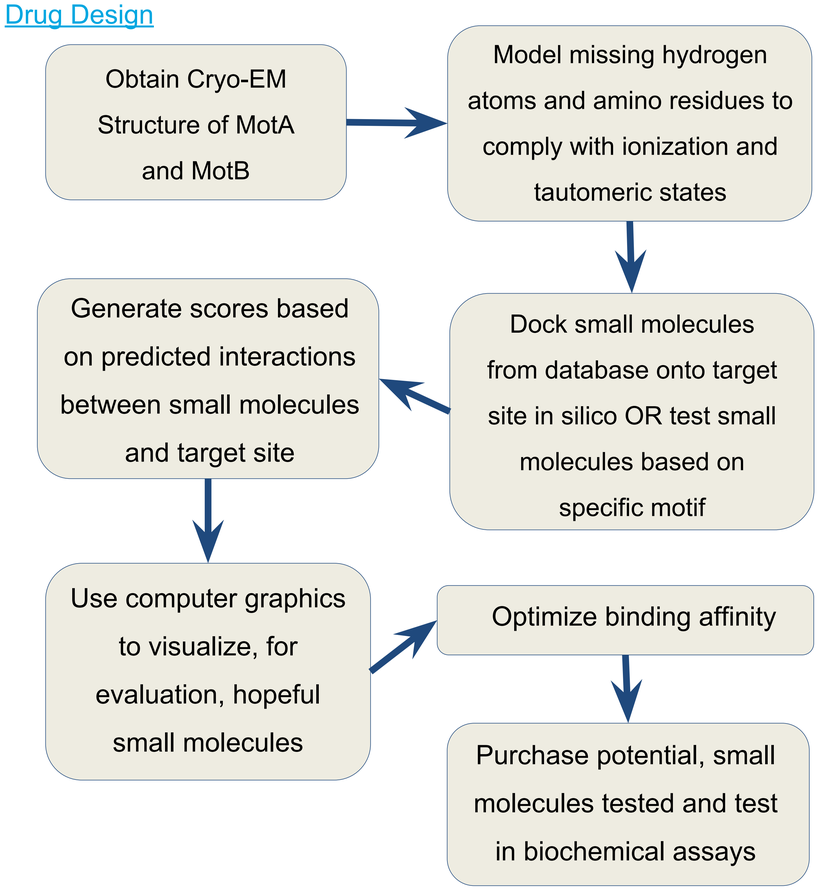
Cryo-Electron Microscopy

- Load 3 microliters of the sample onto a copper grid.
- Plunge grid into liquid ethane to flash freeze protein.
- Load grid on electron microscope.
- Rotation and averaging of images from one orientation.
- Rotation and averaging repeated for different orientations to determine 3D structure of protein.



Source: Doerr Allison, Nature Methods (2015)

Drug Design



SIGNIFICANCE / IMPLICATIONS

- Drugs, specifically targeted to MotA/B proteins, could be used to inhibit proper assembly of the flagellar stator. By reducing the ability of bacteria to generate torque for flagellar rotation, the locomotive ability of pathogenic bacteria can be reduced.
- In an era of increasing antibiotic resistance, such a drug would enable treatment of diseases caused by bacteria that use flagella as virulence factors.

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