BLAST XIV MEETING
ROYAL SONESTA HOTEL
NEW ORLEANS, LOUISIANA
JANUARY 15-20, 2017

Meeting Chairperson
Dr. Alan Wolfe – Loyola University, Chicago, Maywood, IL

Meeting Vice-Chairperson
Dr. Birgit Scharf – Virginia Tech University, Blacksburg, VA

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Dr. Birgit Scharf – Virginia Tech University, Blacksburg, VA
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Dr. Nyles Charon – West Virginia University, Morgantown, WV
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Dr. Mark Johnson - Loma Linda University, Loma Linda, CA
Dr. Michael Miller - West Virginia University, Morgantown, WV
Dr. Birgit Pruess – North Dakota State University, Fargo, ND
Dr. Thomas Shimizu – AMOLF Institute, Amsterdam, Netherlands
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Dr. Roy Welch – Syracuse University, Syracuse, NY

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Dr. Tino Krell (Chairperson) – Estacion Experimental del Zaidin, Granada, Spain
Dr. Simon Rainville – Laval University, Quebec, Canada

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Dr. Joe Falke – University of Colorado, Boulder, CO
Dr. Rasika Harshey – The University of Texas at Austin, Austin, TX
Dr. Urs Jenal – University of Basel, Basel Switzerland
Dr. Karen Ottemann – University of California, Santa Cruz, CA

Conference Coordinators
Ms. Peggy O’Neill – Molecular Biology Consortium, Alsip, IL
Ms. Sarah Van Heusen – University of North Carolina, Chapel Hill, NC
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AWARDS

Robert M. Macnab Award for an Outstanding Poster Presentation by a Postdoctoral Scientist

This award was established at BLAST VIII (2005) and is named in memory of the late Robert M. Macnab, Ph.D., who was an integral member of the Bacterial Locomotion and Signal Transduction Community. Dr. Macnab spent his 30 year career studying the assembly, structure and function of the bacterial flagellum. Bob actively participated in the BLAST meetings and served on the Program and Review Committees for BLAST IV. At the time of his death in 2003, Bob was a professor in the Department of Molecular Biophysics and Biochemistry at Yale University. The Macnab poster award is sponsored by generous donations from Mrs. May K. Macnab.

Robert J. Kadner Award for an Outstanding Poster Presentation by a Graduate Student

This award was established at BLAST IX (2007) and is named in memory of the late Robert J. Kadner, Ph.D., who was an integral member of the Bacterial Locomotion and Signal Transduction Community. Dr. Kadner spent his career studying microbial physiology of E. coli transport systems. Bob actively participated in the BLAST meetings and served as Chair of the Review Committee for BLAST V, Vice-Chair of BLAST VII and Meeting Chair of BLAST VIII. At the time of his death in 2005, Bob was the Norman J. Knorr Professor of Basic Sciences in the Department of Microbiology at the University of Virginia, School of Medicine. This award is sponsored by BLAST.

Nucleic Acids Research Award for an Outstanding Poster Presentation by a Young Investigator

This award was established at BLAST XI (2011) and is presented to a young investigator whose research is in on regulation of transcription. The award is sponsored by Nucleic Acids Research (NAR), an Oxford University Press Journal, which publishes the results of leading edge research into physical, chemical, biochemical and biological aspects of nucleic acids and proteins involved in nucleic acid metabolism and/or interactions (http://nar.oxfordjournals.org).

Microbiology Award for an Outstanding Poster Presentation by a Graduate Student

We are pleased to announce the new Microbiology Award for the second best poster by a graduate student. Microbiology Senior Editor Tarek Msadek will present the winner with a cash prize, one-year complimentary Microbiology Society membership and certificate. The award is sponsored by Microbiology, a journal published by the Microbiology Society. Microbiology publishes topical, high-quality reviews and research papers on all aspects of the field. The journal combines editorial expertise from around the world with exceptional breadth of coverage, providing access to research in a single accessible source – find out more here.
AWARDS

BLAST Board of Directors' Award for an Outstanding Talk by a Graduate Student

This award was established at BLAST XI (2011) by Phil Matsumura, Founding Chair of the BLAST Board of Directors. *The BLAST Board of Directors' Award was initiated by a generous donation from Dr. Phil Matsumura and is now sponsored by BLAST.*

BLAST Founders' Award for an Outstanding Talk by a Postdoctoral Scientist

This award was established at BLAST XIII (2015) by the BLAST Board of Directors in recognition of those who founded BLAST: Joe Falke, Mike Manson, Phil Matsumura, and Sandy Parkinson. *This award is sponsored by BLAST.*

Robert M. Macnab Memorial Travel Awards

The Macnab travel awards were created to remember our colleague Dr. Robert M. Macnab on the 10th anniversary of his death, at BLAST XII (2013). The intent of the awards is to help young scientists from outside the country that hosts BLAST to attend the meeting. The recipients chosen by the Board of Directors for BLAST XIV are graduate students Arely Marcos from George Dreyfus' lab at the Universidad Nacional Autónoma de México and Nitin Kamble from Graham Stafford's lab at the University of Sheffield. *The Macnab travel awards are sponsored a generous donation from Mrs. May K. Macnab.*
# MEETING SCHEDULE

**Sunday, January 15, 2017**

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>4:00 PM</td>
<td>Poster room available for poster setup</td>
<td>Evangeline Suite</td>
</tr>
<tr>
<td>4:00 PM to 7:00 PM</td>
<td>Meeting Registration</td>
<td>South Ballroom</td>
</tr>
<tr>
<td>7:00 PM to 8:30 PM</td>
<td>Dinner</td>
<td>Fleur de Lis Room</td>
</tr>
<tr>
<td>8:30 PM to 10:00 PM</td>
<td>Poster Sneak Preview</td>
<td>Evangeline Suite</td>
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**Monday, January 16, 2017**

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Location</th>
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</thead>
<tbody>
<tr>
<td>7:30 AM to 8:30 AM</td>
<td>Breakfast</td>
<td>Fleur de Lis Room</td>
</tr>
<tr>
<td>8:45 AM to 9:00 AM</td>
<td>Welcome &amp; Announcements</td>
<td>South Ballroom</td>
</tr>
<tr>
<td>9:00 AM to 12:00 PM</td>
<td>Meeting Session – “Biofilms”</td>
<td>South Ballroom</td>
</tr>
<tr>
<td>10:15 AM to 10:30 AM</td>
<td>Coffee Break</td>
<td>Foyer</td>
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<tr>
<td>10:30 AM to 11:00 AM</td>
<td><strong>Keynote Speaker</strong></td>
<td>South Ballroom</td>
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<tr>
<td>10:30 AM to 11:00 AM</td>
<td>George O'Toole, Ph.D.</td>
<td>South Ballroom</td>
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<tr>
<td>12:00 PM to 1:30 PM</td>
<td>Lunch</td>
<td>Fleur de Lis Room</td>
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<tr>
<td>1:30 PM to 4:30 PM</td>
<td>Session – “Flagellar Structure”</td>
<td>South Ballroom</td>
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<tr>
<td>3:00 PM to 3:15 PM</td>
<td>Coffee Break</td>
<td>Foyer</td>
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<tr>
<td>4:30 PM to 6:00 PM</td>
<td>Student Mixer</td>
<td>Fleur de Lis Room</td>
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<tr>
<td>6:00 PM to 7:30 PM</td>
<td>Dinner</td>
<td>Fleur de Lis Room</td>
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<tr>
<td>7:30 PM to 10:00 PM</td>
<td>Poster Session</td>
<td>Evangeline Suite</td>
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<td></td>
<td><strong>Poster Groups A &amp; B</strong></td>
<td>Evangeline Suite</td>
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<td></td>
<td>from 7:30 PM – 8:45 PM</td>
<td>Evangeline Suite</td>
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<tr>
<td></td>
<td><strong>Poster Groups C &amp; D</strong></td>
<td>Evangeline Suite</td>
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<tr>
<td></td>
<td>from 8:45 PM - 10:00 PM</td>
<td>Evangeline Suite</td>
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# MEETING SCHEDULE

## Tuesday, January 17, 2017

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>7:30 AM</td>
<td>Breakfast</td>
<td>Fleur de Lis Room</td>
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<tr>
<td>8:45 AM</td>
<td>Session – “Signal Transduction”</td>
<td>South Ballroom</td>
</tr>
<tr>
<td>10:15 AM</td>
<td>Coffee Break</td>
<td>Foyer</td>
</tr>
<tr>
<td>12:00 PM</td>
<td>Lunch</td>
<td>Fleur de Lis Room</td>
</tr>
<tr>
<td>1:30 PM</td>
<td>Session – “Chemotaxis”</td>
<td>South Ballroom</td>
</tr>
<tr>
<td>3:00 PM</td>
<td>Coffee Break</td>
<td>Foyer</td>
</tr>
<tr>
<td>6:00 PM</td>
<td>Dinner</td>
<td>Fleur de Lis Room</td>
</tr>
<tr>
<td>7:30 PM</td>
<td>Poster Session</td>
<td>Evangeline Suite</td>
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<tr>
<td></td>
<td>Poster Groups A &amp; C</td>
<td>from 7:30 PM – 8:45 PM</td>
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<tr>
<td></td>
<td>Poster Groups B &amp; D</td>
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## Wednesday, January 18, 2017

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<tr>
<td>7:00 AM</td>
<td>Breakfast</td>
<td>Fleur de Lis Room</td>
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<tr>
<td>8:15 AM</td>
<td>Session – “Signal Transduction”</td>
<td>South Ballroom</td>
</tr>
<tr>
<td>10:00 AM</td>
<td>Coffee Break</td>
<td>Foyer</td>
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<tr>
<td>11:30 AM</td>
<td>Lunch</td>
<td>Fleur de Lis Room</td>
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<tr>
<td>12:45 PM</td>
<td>Tours Depart</td>
<td>Hotel Lobby</td>
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# MEETING SCHEDULE

**Thursday, January 19, 2017**

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<tr>
<td>7:30 AM</td>
<td>Breakfast</td>
<td>Fleur de Lis Room</td>
</tr>
<tr>
<td>8:45 AM</td>
<td>Session – “Chemoreception”</td>
<td>South Ballroom</td>
</tr>
<tr>
<td>10:15 AM</td>
<td>Coffee Break</td>
<td>Foyer</td>
</tr>
<tr>
<td>12:00 PM</td>
<td>Lunch</td>
<td>Fleur de Lis Room</td>
</tr>
<tr>
<td>1:30 PM</td>
<td>Session – “Locomotion”</td>
<td>South Ballroom</td>
</tr>
<tr>
<td>3:00 PM</td>
<td>Coffee Break</td>
<td>Foyer</td>
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<tr>
<td>4:00 PM</td>
<td>Town Hall Meeting for Students &amp; Postdocs</td>
<td>South Ballroom</td>
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<tr>
<td>6:00 PM</td>
<td>Dinner</td>
<td>Fleur de Lis Room</td>
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<tr>
<td>7:30 PM</td>
<td><strong>Keynote Speaker</strong></td>
<td>South Ballroom</td>
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<tr>
<td>8:15 PM</td>
<td>Business Meeting for all Attendees</td>
<td>South Ballroom</td>
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<tr>
<td>8:45 PM</td>
<td>Awards Presentations</td>
<td>South Ballroom</td>
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<tr>
<td>9:00 PM</td>
<td>Reception</td>
<td>Fleur de Lis Room</td>
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**Friday, January 20, 2017**

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<tr>
<td>7:30 AM</td>
<td>Breakfast</td>
<td>Fleur de Lis Room</td>
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# SPEAKER PROGRAM

**Monday Morning**  
9:00 am – 12:00 pm  
Biofilms  
Chair – George O’Toole

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<tr>
<th>PRESENTER</th>
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<th>PAGE #</th>
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<tbody>
<tr>
<td>Nicola Farthing</td>
<td>The Fluid Dynamics of Nascent Biofilms</td>
<td>2</td>
</tr>
<tr>
<td>Tianyi Zhou</td>
<td>The Regulatory Roles of Exopolysaccharides (EPS) in Bacterial Motility and Biofilm Formation</td>
<td>3</td>
</tr>
<tr>
<td>Melene Thompson</td>
<td>mirA is a Novel Motility Inhibitor That Influences the Motile-To-Sessile Switch in <em>Agrobacterium tumefaciens</em></td>
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**COFFEE BREAK**

<table>
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<tr>
<th>KEYNOTE SPEAKER</th>
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<tbody>
<tr>
<td>George O’Toole</td>
<td>To Build a Biofilm</td>
<td>5</td>
</tr>
<tr>
<td>Richard Meek</td>
<td>Investigating the Structure and Function of Diguanylate Cyclases in <em>Bdellovibrio bacteriovorus</em></td>
<td>6</td>
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<tr>
<td>Michael Galperin</td>
<td>Cyclic di-GMP-binding Proteins and Signaling Mechanisms</td>
<td>7</td>
</tr>
<tr>
<td>Monica Gerth</td>
<td>Engineering Biofilm-Blocking Enzymes</td>
<td>8</td>
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</tbody>
</table>
# SPEAKER PROGRAM

**Monday Afternoon**  
Flagellar Structure  
Chair – Kelly Hughes  
**1:30 pm – 4:30 pm**

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<tr>
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<tbody>
<tr>
<td>Morgan Beeby</td>
<td>Evolution of Higher Torque in Bacterial Flagellar Motors</td>
<td>9</td>
</tr>
<tr>
<td>Shiwei Zhu</td>
<td><em>In Situ</em> Structural Analysis of Vibrio Flagellar Motor Reveals Novel Stator-Rotor Interactions</td>
<td>10</td>
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<tr>
<td>Seiji Kojima</td>
<td>Structure of the MotB Fragment That Activates Flagellar Stator Complex</td>
<td>11</td>
</tr>
<tr>
<td>Marc Erhardt</td>
<td>Bacterial Flagella Grow Through an Injection-Diffusion Mechanism</td>
<td>12</td>
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<td><strong>COFFEE BREAK</strong></td>
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<tr>
<td>Milinda James</td>
<td>Compounds That Alter Cysteine Can Either Inhibit or Stimulate <em>in vitro</em> Cross-Linking of the Spirochete Flagellar Hook Protein FlgE</td>
<td>13</td>
</tr>
<tr>
<td>Michael Lynch</td>
<td>Structural and Mechanistic Insights into the Lysinoalanine Cross-Linking Reaction of the <em>Treponema denticola</em> Hook Protein FlgE</td>
<td>14</td>
</tr>
<tr>
<td>Eli Cohen</td>
<td>Outer Membrane-Dependent Termination of Distal Rod Assembly in <em>Salmonella enterica</em> spp. Typhimurium</td>
<td>15</td>
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<tr>
<td>Ismael Duchesne</td>
<td>Observation of a Locked Hook Rotation in Flagellated Bacteria</td>
<td>16</td>
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# SPEAKER PROGRAM

## Tuesday Morning 8:45 am – 12:00 pm

**Signal Transduction**  
Chair – Dan Kearns

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<tbody>
<tr>
<td>Bernardo Mello</td>
<td>How do Adaptation and Gain Depend on Sequential Methylation?</td>
<td>17</td>
</tr>
<tr>
<td>Anja Paulick</td>
<td>Mechanism of Bidirectional Thermotaxis in <em>Escherichia coli</em></td>
<td>18</td>
</tr>
<tr>
<td>Sara Kilmury</td>
<td>Type IV Pilins Regulate Their Own Expression Via Direct Intramembrane Interactions with the Sensor Kinase PilS</td>
<td>19</td>
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<tr>
<td>Barbara Kazmierczak</td>
<td>Modulation of Flagellar Rotation in Surface-Attached Bacteria: A Circuit for Rapid Surface-Sensing</td>
<td>20</td>
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<tr>
<td></td>
<td><strong>COFFEE BREAK</strong></td>
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<tr>
<td>Christine Diethmaier</td>
<td>The Flagellar Filament Regulates K-State Development in <em>Bacillus subtilis</em>, Perhaps Through Rotational Load</td>
<td>21</td>
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<tr>
<td>Roy Welch</td>
<td><em>Myxococcus xanthus</em> Development as Motility Regulated Phase Separation</td>
<td>22</td>
</tr>
<tr>
<td>Anna Hughes</td>
<td>Overlapping Degron and Active Site in SwrA, The Swarming Master Regulator</td>
<td>23</td>
</tr>
<tr>
<td>Birgit M. Prüß</td>
<td>Spontaneous Mutations in the flhD Operon Generate Motility Heterogeneity in <em>Escherichia coli</em> Biofilm</td>
<td>24</td>
</tr>
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# SPEAKER PROGRAM

## Tuesday Afternoon  
1:30 pm – 4:00 pm

Chemotaxis  
Chair – Sandy Parkinson

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<tr>
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<tbody>
<tr>
<td>Johannes Keegstra</td>
<td>Variability in <em>E.coli</em> Chemotaxis Measured by Single-Cell FRET</td>
<td>25</td>
</tr>
<tr>
<td>Thierry Emonet</td>
<td>Non-genetic Phenotypic Variability and its Effect on Population Performance</td>
<td>26</td>
</tr>
<tr>
<td>Leanid Laganenka</td>
<td>The Interspecies Quorum-Sensing Signal AI-2 Mediates Autoaggregation of <em>Escherichia coli</em></td>
<td>27</td>
</tr>
<tr>
<td>Tino Krell</td>
<td>Two Different Mechanisms, Based on Direct and Indirect Signal Recognition, Mediate <em>Pseudomonas aeruginosa</em> Chemotaxis to the Major Virulence Signal Inorganic Phosphate</td>
<td>28</td>
</tr>
<tr>
<td>Zhou Huang</td>
<td>Chemotaxis Towards Aromatic Compounds by Direct Sensing and TCA Intermediates in <em>Comamonas testosteroni</em></td>
<td>29</td>
</tr>
<tr>
<td>Kieran Collins</td>
<td>TlpD Mediates a Repellent Response to Oxidative Stress and Regulates Antral Colonization in the Host Gastric Epithelium</td>
<td>30</td>
</tr>
<tr>
<td>Jolene Garber</td>
<td>The Role of CJ0485 in L-Fucose Metabolism and Chemotaxis in <em>Campylobacter jejuni</em></td>
<td>31</td>
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# SPEAKER PROGRAM

## Wednesday Morning
8:15 am – 11:30 am

**Signal Transduction**

**Chair** – Alan Wolfe

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<tr>
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<tbody>
<tr>
<td>Payman Tohidifar</td>
<td>DNA Sensing in <em>Bacillus subtilis</em></td>
<td>32</td>
</tr>
<tr>
<td>Tarek Msadek</td>
<td>SpdC, A Novel Virulence Factor, Controls Histidine Kinase Activity in <em>Staphylococcus aureus</em></td>
<td>33</td>
</tr>
<tr>
<td>Brian Stevenson</td>
<td>Bacterial Replication Rate Controls Production of <em>Borrelia burgdorferi</em> Virulence Factors</td>
<td>34</td>
</tr>
<tr>
<td>Evan Hilt</td>
<td>Investigation Into a Cell-Density Dependent Pathway in <em>Aerococcus urinae</em></td>
<td>35</td>
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<tr>
<td>Ekaterina Filippova</td>
<td>Structure of the Two-Component Response Regulator RcsB from <em>Escherichia coli</em> in Complex with DNA Reveals Mechanism of Phosphorylation-Independent Activation of Transcription</td>
<td>36</td>
</tr>
<tr>
<td>Oshri Afanzar</td>
<td>Hidden Dynamics of CheY at the Switch of the Bacterial Flagellar Motor</td>
<td>37</td>
</tr>
<tr>
<td>Tao Lin</td>
<td><em>Borrelia burgdorferi</em> Flagella Export Apparatus and Virulence: Insight into Type III Secretion System</td>
<td>38</td>
</tr>
<tr>
<td>Davi Ortega</td>
<td>Exploring Alternative Roles of Chemotaxis Pathway in Bacteria.</td>
<td>39</td>
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## SPEAKER PROGRAM

### Thursday Morning 8:45 am – 12:00 pm

**Chemoreception**  
Chair – Jerry Hazelbauer

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<tr>
<td>Marharyta Petukh</td>
<td>Inter-Dimer Interactions Throughout the Chemoreceptor Signaling Domain</td>
<td>40</td>
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<tr>
<td>German Pinas</td>
<td>A Molecular Mechanism for Kinase Control in Chemoreceptor Signaling Complexes</td>
<td>41</td>
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<tr>
<td>Wenlin Pan</td>
<td>Chemotaxis Signaling Complexes Act by Altering the Rate Constant of Kinase Autophosphorylation</td>
<td>42</td>
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<tr>
<td>Ady Vaknin</td>
<td>Networked Chemoreceptors Benefit Bacterial Chemotaxis Performance</td>
<td>43</td>
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### COFFEE BREAK

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<tr>
<td>Jessica Gullett</td>
<td>Distinct Domains Confer CheA with Unique Functions in Chemotaxis and Cell Length at Division in <em>Azospirillum basilense</em> SP7</td>
<td>44</td>
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<tr>
<td>Mark Wooten</td>
<td><em>Borrelia burgdorferi</em> CheY1, CheY2, And CheY3 Possess Distinct Chemotaxis and/or Virulence Functions During the Natural Enzootic Cycle in Tick and Mouse Reservoirs</td>
<td>45</td>
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# SPEAKER PROGRAM

## Thursday Afternoon  
**Locomotion**  
Chair – Karen Ottemann  
1:30 pm – 4:00 pm

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Speaker Abstracts
THE FLUID DYNAMICS OF NASCENT BIOFILMS

Nicola Farthing, Martin Bees, Laurence Wilson  
The University of York, Heslington, York, YO10 5DD, UK.

Background  
Biofilms are ubiquitous in nature, and are the focus of many studies with emphasis often on developing methods to inhibit their growth. A common aim of these studies is to minimize biomass on a given surface by targeting mature biofilms with biochemical or physio-chemical interventions. We aim to investigate the effect of interventions at an earlier stage.

It is well known that motility affects the cells’ approach to surfaces, and that individual cells ‘seed’ biofilms. This regime is associated with some particularly interesting fluid dynamics: a free-swimming cell causes a relatively small disturbance in the surrounding fluid, whereas a surface-attached cell with rotating flagella can generate a significant and relatively long-ranged flow field. We investigate the effect of these surface-associated fields on the recruitment of cells to nascent biofilms.

Methods  
This is a combined experimental and theoretical study. Digital inline holographic microscopy is used to determine the three-dimensional flow field around Pseudomonas aeruginosa cells as they become stuck within a flow cell. By introducing tracer particles into our system, we can follow both the swimming cells and the flow profile within the surrounding fluid. We have compared experimental results of the flow around single and multiple cells to those determined theoretically. Singularity solutions of Stokes’ flow and the method of images were employed to model the boundary interactions that are critical to surface-associated flow phenomena.

Results  
Preliminary experimental data show that flow velocity decreases with the square of distance from a surface-attached cell in this geometry. Moreover, we find that the flow fields around the bacteria have a significant component normal to the wall - that is, attached bacteria pull fluid in from a plane parallel to the wall and pump it out into the bulk. Using a simplified theoretical model, preliminary results are producing the same quantitative behaviour to within a reasonable accuracy.

Conclusion  
We have demonstrated that the flow field around a surface-attached cell is significantly different to that around a freely-swimming cell. A simplified mathematical model of the long-range flow field captures this behaviour, and offers opportunities for future modelling efforts aimed at uncovering quorum sensing interactions between assemblies of cells in a nascent biofilm.

A comparison of experimental and theoretical data: a) shows visualisation of experimental holographic data. The 3D position and instantaneous velocity of tracer beads around a stuck P. aeruginosa cell are used to determine the flow field due to the cell. b) shows the flow field due to a modelled stuck cell.
THE REGULATORY ROLES OF EXOPOLYSACCHARIDES (EPS) IN BACTERIAL MOTILITY AND BIOFILM FORMATION

Tianyi Zhou and Beiyuan Nan
Department of Biology, Texas A&M University, College Station, TX 77843, USA.

Background
The biofilm-forming bacterium Myxococcus xanthus moves on surfaces as structured swarms utilizing type IV pili-dependent social (S-) motility. In contrast to isolated cells, individual cells within swarms rarely reverse their moving direction. The regulatory mechanisms that inhibit cellular reversals and promote the formation of swarms are not well understood.

Methods
We used genetic and microscopic approaches to investigate the function of exopolysaccharides (EPS).

Results
Here we show that EPS, the major extracellular components of M. xanthus swarms, inhibit the reversal of individual cells in a concentration-dependent manner. Thus, individual wild type cells reverse less frequently in swarms due to high local EPS concentrations. In contrast, cells defective in EPS production hyper-reverse their moving direction and show severe defects in S-motility. Surprisingly, S-motility and wild type reversal frequency are restored in double mutants that are defective in both EPS production and the Frz chemosensory system, indicating that EPS regulates cellular reversals in parallel to the Frz pathway. Using fluorescence microscopy, we found that the Ras-like GTPase MglA and its cognate GTPase activating protein, MglB, both inverted polar localizations at high frequencies in EPS cells.

Conclusion
We clarify that besides functioning as the structural scaffold in biofilms, EPS is a self-produced signal that coordinates the group motion of the social bacterium M. xanthus.
**MirA IS A NOVEL MOTILITY INHIBITOR THAT INFLUENCES THE MOTILE-TO-SESSILE SWITCH IN AGROBACTERIUM TUMEFACIENS**

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**Background**  
A critical stage in the development of many bacterial infections is the motile-to-sessile transition that drives initial physical interactions with the host. In the facultative plant pathogen *Agrobacterium tumefaciens*, one of the proteins that governs this transition is the periplasmic regulator ExoR. ExoR controls the ChvG-ChvI two component system, a global regulatory pathway known to provide a response to acidic conditions such as in the host environment. When activated, the ExoR-ChvG-ChvI pathway abolishes motility gene expression. In *A. tumefaciens*, motility is also regulated by the solo two-component-type response regulator Rem, a protein which lacks the canonical phospho-accepting aspartate residue, but is absolutely required for expression of most motility and chemotaxis genes. The ExoR-ChvG-ChvI likely regulates motility through indirect interactions with Rem. In this study, we sought to identify a genetic link between ExoR-ChvG-ChvI and Rem.

**Methods**  
We isolated motile suppressor mutants of the activated ExoR-ChvG-ChvI pathway and the identified mutation sites via whole genome sequencing. The point mutations were recreated in a naïve background and were characterized for flagellar motility and reporter activity.

**Results**  
We found that ChvI-mediated motility inhibition could be suppressed by secondary point mutations in a previously uncharacterized region of the *A. tumefaciens* genome with sequence conservation limited to the *Rhizobiales*. The suppressor mutations clustered within an undefined open reading frame which we have named the motility inhibitor via Rem, or *mirA*. Initial characterization of *mirA* indicates that it encodes a small protein (76 aa) which lacks any amino acid motifs indicative of its structure or function. Mutation of the predicted *mirA* start codon imparts the same null phenotype as the original point mutations. Preliminary studies indicate that *mirA* transcription is activated by the ChvG-ChvI two component system. Initial work indicates that, while *mirA* does not appear to affect transcription of *rem*, a plasmid-borne version of *mirA* broadly inhibits motility.

**Conclusions**  
Thus far, our studies indicate that the regulation of the motile-to-sessile switch by the ExoR-ChvG-ChvI system in *A. tumefaciens* may be directed by *mirA* (see figure). Our initial work indicates that the small peptide *mirA* is a novel motility inhibitor in the *Rhizobiales.*
TO BUILD A BIOFILM

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Background
Work in the O’Toole lab focuses on the study of surface-attached microbial communities known as biofilms. These surface attached communities can be found in medical, industrial and natural settings. In fact, life in a biofilm probably represents the predominate mode of growth for microbes in most environments. Biofilm microbes are typically surrounded by an extracellular matrix, which provides structure and protection to the community. Biofilm-grown bacteria are notorious for their tolerance to a range of antimicrobial agents including clinically relevant antibiotics.

Methods
We use a combination of genetic, molecular, biochemical and imaging techniques.

Results
Our studies indicate that Pseudomonas aeruginosa, an important opportunistic pathogen, can detect surface contact via a pathway requiring Type IV pili (TFP) and a membrane-bound signaling complex that generates the second messenger cAMP. Moreover, our recent findings using cell tracking of entire communities at single-cell resolution combined with a cAMP reporter support the model that multi-generation signaling via this cAMP-dependent pathway is required for this microbe to commit to initiating biofilm formation. That is, cAMP levels accumulate over multiple generations in response to TFP engagement with a surface. Our data also indicate that irreversible attachment, the first committed step in biofilm formation, requires this cAMP-mediated signaling to modulate TFP and flagellar function interactively and systematically, thereby promoting stable surface attachment and downstream biofilm development.

Conclusions
P. aeruginosa, and likely other microbes, have developed means to sense surface contact.

![Early Events in Biofilm Formation](image-url)
INVESTIGATING THE STRUCTURE AND FUNCTION OF DIGUANYLATE CYCLASES IN *BDELOVIBRIO BACTERIOVORUS*

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**Background**  
*Bdellovibrio bacteriovorus* is a predatory bacterium which feeds on a number of Gram-negative bacteria including both human and plant pathogens. Like most bacteria, *Bdellovibrio* coordinates a number of cellular processes via the cyclic nucleotide messenger, c-di-GMP. To dissect this signalling network in *Bdellovibrio* we have investigated both the structure and function of novel diguanylate cyclases (DGCs) within *Bdellovibrio*.

**Methods**  
X-ray crystallography has provided high-resolution structural data. This method was complemented with numerous biochemical and biophysical techniques to build a picture of the mechanisms that underlie DGC function.

**Results**  
Diffraction data obtained to 1.8Å for one of the candidates (Bd0742; required for invasion) has suggested a novel means of DGC regulation involving a forkhead-associated domain. Additional structures (truncation constructs) validate our working hypothesis that an asymmetric forkhead interaction holds the catalytic domains in a non-productive state.

**Conclusion**  
Simulation by a phosphopeptide, is likely to drive large-scale conformations within the forkhead-DGC, and is potentially responsible for activating DGC activity.
CYCLIC DI-GMP-BINDING PROTEINS AND SIGNALING MECHANISMS

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Background
Cyclic di-GMP is a nearly universal bacterial second messenger that is produced in representatives of all major bacterial lineages, but not in archaea or multicellular eukaryotes. C-di-GMP synthetases and hydrolases (containing GGDEF, EAL, and HD-GYP domains) are readily identified in bacterial genome sequences using standard bioinformatic tools (see http://www.ncbi.nlm.nih.gov/Complete_Genomes/c-di-GMP.html). In contrast, identification of c-di-GMP receptors remains a difficult task, owing to their diversity and the limited number of the c-di-GMP-interacting residues.

Methods
We have examined the sequences and structures of experimentally characterized c-di-GMP receptors reported so far and analyzed their properties.

Results
Several c-di-GMP receptors with dramatically distinct c-di-GMP-binding modes have been structurally characterized, including (i) PilZ domain-containing proteins; (ii) I-sites of GGDEF domains; (iii) enzymatically inactive EAL domains; (iv) STING protein; (v) BldD-type transcriptional regulators; (vi) VspT-type transcriptional regulators; (vii) FleQ-like ATPases, and the recently described (viii) MshEN domain proteins and (ix) CckA-like histidine kinases. There is also a variety of proteins that appear to be capable of c-di-GMP binding but whose structures have not yet been solved. For these proteins, the mechanisms of c-di-GMP-binding remain to be characterized. In most cases observed so far, c-di-GMP did not cause any significant conformational changes in its receptors. Rather, c-di-GMP binding typically occurred at the interface of two different domains, either affecting their mutual orientation or promoting their interaction in the first place. Thus, c-di-GMP binding appears to facilitate dimerization of transcriptional regulators, which dramatically increases their binding to their respective DNA targets.

Conclusion
The diversity of c-di-GMP receptors underlies the diversity of c-di-GMP-dependent signaling mechanisms, some of which have been characterized in the past several years. C-di-GMP has been shown to regulate a variety of cellular processes, acting through a wide variety of receptors. Further understanding of the regulatory roles of c-di-GMP will require detailed characterization of additional c-di-GMP receptors and signaling modules.
ENGINEERING BIOFILM-BLOCKING ENZYMES

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Background
For many clinically relevant bacterial pathogens (e.g. Pseudomonas aeruginosa) biofilm formation is regulated by a process called quorum sensing. Quorum sensing (QS) is a way for bacteria to communicate via chemical signals. Bacteria use quorum sensing to assess their local population densities; once they have reached a critical mass, they activate their arsenal of virulence genes, establish infection, and/or begin forming biofilms.

Our goal is to engineer highly active and specific 'quorum quenching' enzymes that irreversibly degrade the signalling molecules using in quorum sensing. Our main focus is N-acyl-L-homoserine lactones (AHLs), as they are the most common type of QS molecules used by Gram-negative pathogens.

Methods
Iterative rounds of site-saturation mutagenesis are used to identify amino acid substitutions that confer improved activity and/or specificity. Libraries of variants are expressed and purified in 96-well plates, and then screened for enzyme activity in vitro. Promising variants are then tested against P. aeruginosa biofilms, using a variety of techniques including crystal violet assays, scanning electron microscopy and confocal microscopy.

Results
We have successfully engineered two enzymes with improved ability to degrade AHL signalling molecules. Biofilms grown in the presence of our 'first-generation' engineered enzymes have significantly reduced matrix formation (i.e. reduced extracellular polymeric substances (EPS)). These enzymes also reduce EPS levels when added to established biofilms.

Conclusion
Enzyme-based quorum quenching is a promising approach for the prevention and/or disruptions of biofilms.

Figure 1. Iterative rounds of mutation and screening are used to identify enzymes with improved activity. (i) Enzyme modeling; (ii) iterative site-saturation mutagenesis; (iii) in vitro enzyme activity assays; and (iv) biofilm assays (such as scanning electron microscopy, shown here).
EVOLUTION OF HIGHER TORQUE IN BACTERIAL FLAGELLAR MOTORS

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Background
Although it is known that diverse bacterial flagellar motors produce different torques, the mechanism underlying torque variation is unknown.

Methods
To understand this difference better, we combined genetic analyses with electron cryo-tomography subtomogram averaging to determine in situ structures of flagellar motors that produce different torques, from Campylobacter and Vibrio species.

Results
For the first time, our results unambiguously locate the torque-generating stator complexes and show that diverse high-torque motors use variants of an ancestrally related family of structures to scaffold incorporation of additional stator complexes at wider radii from the axial driveshaft than in the model enteric motor. We identify the protein components of these additional scaffold structures and elucidate their sequential assembly, demonstrating that they are required for stator-complex incorporation. These proteins are widespread, suggesting that different bacteria have tailored torques to specific environments by scaffolding alternative stator placement and number.

Conclusion
Our results quantitatively account for different torques, complete the assignment of the locations of the major flagellar components, and provide crucial constraints for understanding mechanisms of torque generation and the evolution of multiprotein complexes. I will conclude by discussing possible pathways to evolve this diversity in the flagellar motors.
IN SITU STRUCTURAL ANALYSIS OF VIBRIO FLAGELLAR MOTOR USING VARIOUS MUTANTS

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Background: Vibrio species are gram-negative, rod-shaped bacteria that can swim to natural inhabitants in all types of aqueous environments including marine, freshwater and estuary. The fast swimming motility of Vibrio can be attributed to a rotating unipolar bacterial flagellum that is driven by a flagellar motor located beneath the cell envelope. It has been established that the flagellar rotation is driven through an interaction between the rotating unit (rotor) and the membrane bound torque-generating unit (stator), which is powered by the sodium ion gradient. Our previous works pointed out that a membrane FliL, behaving like stator being dynamic assembly and disassembly from the rotor, is involved in supporting torque generation of flagellar motor. A recent report claimed that the density locating beneath the T-ring and top of C-ring belongs to the stator. We hypothesize that both stator and FliL might locate there not just only the stator.

Methods: To pinpoint a clear and well comprehensive flagellar motor image including rotor, stator and FliL. Here, we used Vibrio alginolyticus, well-known as the fastest swimmer in the inaugural Microbial Olympics and as a paradigm for understanding unipolar flagellum system by using extensive biochemical, genetic and X-ray crystallography studies. Next, we performed cryo-electron tomography (cryo-ET) to visualize over thousands of V. alginolyticus cells, which have been genetically modified to generate multiple polar flagella for pursuing high-throughput cryo-ET in situ structural studies. Subtomogram average and classification are performed to analyze over 10,000 flagellar motors from wild type and five specific flagellar mutants.

Results: We determined the intact V. alginolyticus flagellar motor structure with unprecedented details and reveal novel interactions between the rotor and the stator. 1) The density beneath the T-ring and top of C-ring is attributed to both stator and FliL. Stator locates outside of the C-ring not on the top of the C-ring as another paper reported. 2) Vibrio species-specific T-ring structure recruits and stabilizes 13 stator complexes together with 13 FliLs around the rotor. 3) We also observed striking structural difference in pomAB and fliL, respectively, suggesting that FliL also plays important role in mediating the rotor-stator interaction. 4) Strikingly, we found a novel ring structure functioning like a belt to bind the sheathed flagellum, thus named it as sheath ring.

Conclusions: The bacterial flagellar motor (BFM) structure we solved to unprecedented detail reveals novel interactions among the rotor, stator and FliL. Thus, we proposed that the BFM should be composed of the FliL supporters in addition to the rotor and stator. The BFM has also evolved to generate species-unique structures to adapt to the environment as we found with the sheath ring in Vibrio. This novel feature explains that how outer membrane curves almost 90 degree to form flagellar sheath. We speculate that the sheath ring like structure might exist in all sheathed flagellated species such as Helicobacter pylori. Overall, our structural results provide exciting and comprehensive insights into the understanding of BFM especially on the highly conserved interactions among flagellar stator, rotor and FliL.

Figure A, Slice through the tomogram of Vibrio alginolyticus with multiple sheathed flagella at the cell pole; Figure B, The examples of flagellar Hook Basal Body (HBB); Figure C, The subtomogram averages of wild-type flagellar HBB. OM and IM indicates outer and inner membranes. PG indicates peptidoglycan layer. Scale bars, 200 nm (A), 50 nm (B), 20 nm (C).

STRUCTURE OF THE MOTB FRAGMENT CARRYING A POINT MUTATION THAT ACTIVATES FLAGRLLAR STATOR COMPLEX

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Background
Stator units of the flagellar motor become active only when they are anchored around a rotor via the periplasmic region of the stator B subunit. Previously we determined the crystal structure of this region from Salmonella MotB (MotBc) and proposed a large conformational change during the stator incorporation into the motor. The mutation L119P in helix α1 of MotBc caused increased proton conduction and incorporation into the motor, suggesting that this mutant stator mimics the active conformation. To investigate this active conformation, we solved the crystal structure of MotBc with the L119P mutation, and analyzed its solution structure by NMR.

Methods
We introduced the L119P mutation in the MotBc fragment, and determined its crystal structure. To investigate the disordered N-terminal region of MotBc-L119P, we labeled this region with 15NH-Lys and analyzed its solution structure by NMR. The peptidoglycan (PG)-binding ability of MotBc was assessed by the co-sedimentation with the purified PG sacculus.

Results
The crystal structure showed that the helix α1 of MotBc was disordered by the L119P mutation. NMR analysis showed the significant structural disruption localized on the N-terminal half of α1 of L119P mutant, supporting the conformational changes revealed by the crystal structure. Not wild-type MotBc but its L119P mutant was co-sedimented with PG, suggesting that the mutation changes a MotBc conformation to be competent with PG binding.

Conclusion
This study links the functional and structural changes of stator units by the mutation L119P in MotB, and supports our model for assembly-coupled stator activation, in which the conformational change in the helix α1 of MotBc is required for both stator activation and anchoring at the PG layer.
BACTERIAL FLAGELLA GROW THROUGH AN INJECTION-DIFFUSION MECHANISM

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Background
Many bacteria move by rotation of a helical organelle, the flagellum. The external flagellar filament is several times longer than a bacterial cell body and is made out of up to 20,000 flagellin subunits. A type III export apparatus (T3SS) located at the base of the flagellum utilizes the proton motive force (pmf) as the primary energy source to translocate axial components of the flagellum across the inner membrane. Exported substrates travel through a narrow 2 nm channel within the structure and self-assemble at the tip of the growing flagellum. The T3SS-dependent export of flagellar building blocks is a remarkable fast process and more than 1500 amino acids per second are transported during filament growth. A fundamental problem concerns the molecular mechanism of how the long, external filament grows at a rapid rate in the absence of any cellular energy sources.

Methods
We determined the growth rate of single flagella using in situ labeling and real-time immunostaining of growing flagellar filaments. The growth rate data was combined with mathematical modeling to generate a biophysical model of flagellum growth.

Results
We present a molecular mechanism to explain the growth of flagellar filaments based on simple biophysical parameters. We provide experimental evidence to demonstrate that growth of flagella follows a saturated diffusion mechanism and decreases with length. We determined the growth rate of single flagella using in situ labeling and real-time immunostaining of growing flagellar filaments. The growth rate data revealed a negative correlation between the rate of filament polymerization and the length of the flagellum. Addition of uncoupling agent that disrupted the pmf prevented filament elongation. Growth was resumed after removal of uncoupler, indicating a major contribution of the pmf in driving flagellin export. Competitive export of flagellin mutant proteins deficient in head-to-tail chain linkage did not impair the flagellum growth rate. While inter-subunit interactions between flagellin monomers might be important during substrate docking, these results suggest that the pulling force of chain of flagellin molecules does not contribute substantially to the filament elongation dynamics.

Conclusion
In summary, we propose a flagellum growth model based on simple biophysical parameters where the filament growth rate is driven by both diffusion and pmf-dependent injection of subunits.
COMPOUNDS THAT ALTER CYSTEINE CAN EITHER INHIBIT OR STIMULATE IN VITRO CROSS-LINKING OF THE SPIROCHETE FLAGELLAR HOOK PROTEIN FlgE

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Background
Many bacteria swim with the aid of a rotary motor coupled to a flagellar filament by a flexible, curved universal joint, or “hook.” In spirochetes, the flagella are held within a tight periplasmic space that presumably necessitates that the hooks be capable of dealing with additional mechanical stress. In Treponema denticola (periodontal disease), Treponema pallidum (syphilis) and Borrelia burgdorferi (Lyme disease), the bacteria deal with this stress by forming a hook with a unique covalent cross-link. In T. denticola and B. burgdorferi, the cross-link has been identified as a lysinoalanine interpeptide bond. This bond forms as a result of cysteine being converted to a dehydroalanine intermediate on one subunit reacting with a specific lysine of another subunit. These bonds link the FlgE subunits into a high molecular weight complex (HMWC) that is stable to treatments known to disrupt non-covalent bonds.

Our present work focuses on: 1) screening of compounds that could stimulate or inhibit cross-linking in T. denticola in vitro and 2) whether Treponema pallidum rFlgE forms a HMWC in vitro and if it is a lysinoalanine cross-link similar to that of T. denticola. We also asked if this T. pallidum crosslinking is stimulated and inhibited by specific compounds.

Methods
Maximal cross-linking of purified T. denticola and T. pallidum rFlgE was obtained at 4°C, 40 mM Tris pH 8.5 with 0.9% NaCl and 1.0 to 1.5 M (NH₄)₂SO₄. Mass spectrometry (MS) analysis of the HMWC was carried out by trypsin treatment followed by MS/MS.

Results
We found that β-mercaptoethanol (BME) inhibits cross-linking of T. denticola rFlgE. MS analysis indicated that BME binds to form a dehydroalanine adduct derived from C178.

Cross-linking of T. denticola rFlgE was stimulated by 5,5'-dithiobis 2-nitrobenzoic acid (DTNB) and N-ethyl maleimide (NEM). Compounds that slightly reduced cross-linking include ethanolamine, tris 2 carboxyethyl phosphate and sodium azide.

We found that T. pallidum rFlgE formed HMWC’s in vitro in a manner similar to T. denticola rFlgE as seen in SDS-PAGE. In addition, DTNB was found to stimulate cross-linking (see accompanying figure). The cross-linking was time dependent and mediated by a lysinoalanine interpeptide bond.

Conclusion
T. pallidum rFlgE forms a HMWC in vitro with lysinoalanine being the cross-link. We propose that DTNB and NEM stimulate cross-linking of rFlgE by augmenting conversion of C178 in T. denticola or T. pallidum FlgE to dehydroalanine.
STRUCTURAL AND MECHANISTIC INSIGNS INTO THE LYSINOALANINE CROSS-LINKING REACTION OF THE *TREPONEMA DENTICOLA* HOOK PROTEIN FlgE

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**Background**

Flagellated bacteria propel themselves via the rotation of flagellar filaments that connect to membrane-embedded motors. These filaments rotate in either a clockwise or counterclockwise direction, allowing the cell to tumble or swim smoothly. The Spirochete phylum has a unique flagellar location, as the flagella of these bacteria remain enclosed within the periplasm, rather than extend into the extracellular space. Spirochetes are of increasing interest due to the pathogenic nature of several of its members such as: *Borrelia burgdorferi* (Lyme disease), *Treponema denticola* (periodontal disease), and *Treponema pallidum* (syphilis). Recently, it has been demonstrated that the flagellar hook protein of *T. denticola* and *T. pallidum*, FlgE, undergoes a self-catalyzed lysinoalanine (LAL) cross-linking reaction that polymerizes the FlgE subunits. In *T. denticola*, this cross-linking has been shown critical for motility. The cross-link forms through a reaction that involves the conversion of Cys178 to dehydroalanine¹², followed by Michael addition of Lys165. Herein, we present our ongoing efforts to ascertain the catalytic mechanism of LAL cross-linking.

**Methods**

To study FlgE crosslinking from *T. denticola* (TdFlgE), we have utilized X-ray crystallography, site-directed mutagenesis, colorimetric assays, and mass spectrometry in order to begin to elucidate the mechanism of LAL cross-linking.

**Results**

We have crystallized the active cysteine-containing D2 domain of TdFlgE, and identified key residues that play a role in promoting efficient cross-linking. We have also characterized products and intermediates in the cross-linking reaction, which can be accelerated by Cys178 disulfide formation or alkylation. From this data we propose a preliminary mechanism for LAL formation.

**Conclusion**

We have made initial steps toward characterizing the unique self-catalyzed LAL cross-linking mechanism in *T. denticola* hook protein FlgE. The chemistry, along with the resulting post-translational modification are largely unprecedented.

OUTER MEMBRANE-DEPENDENT TERMINATION OF DISTAL ROD ASSEMBLY IN SALMONELLA ENTERICA SPP. TYPHIMURIUM

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Background
The rod substructure of the bacterial flagellum acts as a driveshaft that couples torque produced by the inner membrane-anchored rotor-stator complex to the extracellular hook and filament. In gram-negative bacteria, the rod can be divided into proximal rod and distal rod sections of ~11 nm each. The proximal rod, composed of four distinct protein subunit types, spans the distance between the inner membrane and the cell wall peptidoglycan. The distal rod is composed of dozens of copies of a single protein subunit (FlgG) and polymerizes on top of the proximal rod until it has reached the inner leaflet of the outer membrane. The regulated termination of distal rod polymerization at the outer membrane is important for the proper assembly of the hook and filament. Mutations in flgG that allow the distal rod to polymerize beyond the WT length (a.k.a. flgG* mutations) impair motility, result in hook and filament assembly in the periplasm and cause growth and morphological abnormalities. The isolation of distal rod length-control mutants, coupled with the observation that FlgG and the FlgE hook protein share ~40% amino acid identity implied that a mechanism for distal rod length control must exist. However, unlike the hook, no molecular ruler exists to terminate distal rod assembly once the proper length has been reached.

Methods
By screening for flgG* motile revertants, a set of suppressor mutations were identified in lppA, the major outer membrane lipoprotein in Salmonella spp. (a.k.a. Braun’s lipoprotein). The C-terminus of LppA anchors in the outer membrane while the N-terminus covalently binds to the cell wall peptidoglycan, thereby tethering the outer membrane to the cell body. The isolation of lppA null alleles that relieved both the motility defect of flgG* mutants suggested that the regulator of distal rod length could be the outer membrane. To test this model, length variants of LppA were constructed to determine if increasing the cell wall to outer membrane distance would result in a concomitant increase in distal rod length.

Results
By incrementally increasing the length of LppA, we observed a proportional increase in the length of the rod (Fig 1). Additionally, we observed an increase or decrease in the cell wall-to-outer membrane spacing upon addition or subtraction of residues from LppA.

Conclusion
Our results support a model for distal rod length control whereby distal rod polymerization terminates upon contact with the outer membrane. We will also provide evidence that the function of LppA is to act as an outer membrane tether under tension as opposed to a support column, i.e. the osmotic pressure in the periplasmic space mirrors that of the cytoplasm as opposed to that of the external environment.
OBSERVATION OF A LOCKED HOOK ROTATION IN FLAGELLATED BACTERIA

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Background

E. coli and Salmonella, like many other flagellated bacteria move efficiently in liquid environments by doing a biased random walk. This trajectory is composed of straight runs interrupted by quick reorientations (tumbles). Their capacity to change their orientation allows them to avoid obstacles and even reverse their trajectory when swimming in restricted or anisotropic environments. These bacteria have one or few flagella composed at their base of a rotary motor anchored in the membrane (see Fig. A). The motor can rotate either clockwise (CW) or counterclockwise (CCW). It is well established that the tumble events are associated with a switch in the direction of rotation of at least one flagellar motor, which leads to a reorientation of the corresponding filament. But the mechanism behind these tumbles is not fully described. The rotation of the motor is transmitted to a "semirigid" filament by a flexible universal joint called the hook, which allows the spatial orientation of the filament to be independent of the rotation of the motor (see Fig. B). This would imply that any displacement of the filament axis must the result of external forces, like the thrust of the filament, hydrodynamic forces, etc. However, our direct observations of the filaments of swimming bacteria in agar and in liquid crystals suggest that the hook can “lock” for short periods so that the motor’s rotation then drives the filament around.

Methods

In this work, we studied reorientation and reversal events by observing fluorescently-labeled filaments of Salmonella and E. coli strains. Swimming bacteria were observed in soft agar, which is a fluid-filled porous medium. To linearize the orientation and displacement of the filaments (to keep them in focus), we also tracked bacteria in a biocompatible liquid crystal (disodium cromolyn glycated (DSCG)). This medium is an anisotropic solution where the trajectory and orientation of the bacteria are confined to the direction of the alignment of the liquid crystal molecules.

Results

In both media (agar and DSCG), we noted that reorientation and reversal events (tumbles) are always initiated when the motor switched from a CCW to a CW direction. In addition, we observed that the filaments do not rotate while they reorient during these events (and the cell body is also stopped). This suggests two things: 1) the rotation of the motor is what drives the movements of the filaments (not just their rotation) and 2) the hook must be momentarily rigid to transmit the motor’s force to the filament (as illustrated on Fig. C).

Conclusion

To our knowledge, this is the first evidence of a new mode of rotation of the hook: the locked hook rotation. Even though a complete explanation of how the hook can lock is still missing, we believe this is an important phenomenon that greatly enhances the motility of flagellated bacteria by enabling reorientation and reversal events (even in water). This work also demonstrates how liquid crystals can be used to study the physical properties of bacteria.
HOW DO ADAPTATION AND GAIN DEPEND ON SEQUENTIAL METHYLATION?

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![Image: The sequential and the non-sequential methylation states.]

**Background**
Adaptation is one of the main properties of bacterial chemotaxis. It is achieved by the addition and the removal of methyl groups at specific sites of transmembrane receptors. Mathematical models of adaptation require that these sites are sequentially methylated. It is not clear, however, if partial adaptation is theoretically possible with non-sequential methylation.

**Methods**
We propose measures of adaptation error and gain in the signal to noise ratio. Monte-Carlo simulation is performed to evaluate these measures on several mutants, which we defined as a given set of parameters controlling the model dynamics. The fittest mutants are select by evaluating their response gain and adaptation error. We analyze the fittest mutants to understand the role played by sequential methylation and by the simulation parameters.

**Results**
Partial adaptation can be obtained on non-sequential methylation at the cost of reduced response gain. In sequential and in the non-sequential methylation, a trade-off was found between adaptation precision and response gain. However, sequential methylation results in a much higher gain for a given adaptation error. In the sequential methylation, the gain in the response increases with the number of methylation sites. On the other hand, the number of methylation sites does not significantly improve the gain of the non-sequential fittest mutants.

**Conclusion**
Although sequential methylation is not essential to achieve a given level of adaptation precision, it leads to a much stronger gain in the signal to noise ratio on the receptor activity. Increasing the number of methylation sites in the receptor improves the fitness of sequential methylation mutants but has virtually no effect on non-sequential ones.
MECHANISM OF BIDIRECTIONAL THERMOTAXIS IN *ESCHERICHIA COLI*

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**Background**

Migration towards or away from chemical stimuli and temperature is inherent to many organisms. In *Escherichia coli* both of these stimuli are sensed by the same pathway but the mechanism has remained unclear.

**Methods**

Here, we use an *in vivo* assay of the pathway activity and microfluidics to investigate the response to dynamically changing temperature. Additionally, quantification of chemoreceptor methylation along with mathematical modeling helped us to understand the mechanism of bidirectional thermotaxis.

**Results/Conclusion**

Un-stimulated, *E. coli* exhibits a thermophilic response, the magnitude of which decreases with temperature. Adaptation to chemoattractants reduces or inverts this response to cryophilic in a dose-dependent manner. Interestingly, stimuli sensed by only one of the two major chemoreceptors leads to a bidirectional thermotactic response, with cells being thermophilic at lower but cryophilic at higher temperatures. We show that this inversion is due to the interplay between thermosensing and chemoreceptor methylation. Finally, for serine the preferred temperature of accumulation corresponds to the optimal growth temperature, demonstrating the importance of thermotaxis to cellular environmental response and proliferation.
TYPE IV PILINS REGULATE THEIR OWN EXPRESSION VIA DIRECT INTRAMEMBRANE INTERACTIONS WITH THE SENSOR KINASE PILS

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Background
Type IV pili (T4P) are important virulence factors for many pathogens, including Pseudomonas aeruginosa, as they facilitate cell surface attachment, formation of antibiotic resistant biofilms and twitching motility. Transcription of the major pilin gene – pilA – is controlled by the PilS- PilR two-component regulatory system in response to previously unknown signals. The absence of a canonical periplasmic sensing domain in the sensor kinase PilS suggested that it may detect intramembrane signals such as PilA itself. In this work, we show that direct interactions between PilA and PilS in the inner membrane reduce pilA transcription when PilA protein levels are high.

Methods
A bacterial two-hybrid assay was used to test for protein-protein interactions between PilS and PilA or structurally related pilins. Amino acid substitutions in the conserved N-terminus of PilA and in the TM segments of PilS were generated using site directed mutagenesis and tested for their effect on interactions. Western blotting and a lux-pilA luminescent reporter assay were used to identify pilins that repress chromosomal pilA transcription upon overexpression.

Results
Overexpression of pilin proteins with diverse and/or truncated C-termini decreased native pilA transcription, suggesting that the highly conserved N-terminus of PilA is a regulatory signal for PilS. Point mutations in PilA or PilS that disrupted their interaction also prevented autoregulation of pilA transcription, though a subset of mutants retained the ability to interact with PilS without decreasing pilA transcription. Therefore, interaction between the pilin and sensor is necessary but not sufficient for pilA autoregulation. We also showed that PilS likely has intrinsic phosphatase activity but also that this activity was required for the autoregulation of pilA transcription.

Conclusion
Under conditions where pilins are abundant, the pilin-PilS interaction promotes PilS-mediated inactivation of the regulator, PilR and thus downregulation of further pilA transcription. This work reveals a clever bacterial inventory control strategy in which the major subunit of an important P. aeruginosa virulence factor controls its own expression. Furthermore, this work may provide insight into potential autoregulatory functions in other two component systems.
MODULATION OF FLAGELLAR ROTATION IN SURFACE-ATTACHED BACTERIA: A CIRCUIT FOR RAPID SURFACE-SENSING

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Background
Attachment is a necessary first step in bacterial commitment to surface-associated behaviors, e.g. colonization, biofilm formation, and host-directed virulence. The Gram-negative pathogen *Pseudomonas aeruginosa* initially attaches to surfaces via its single polar flagellum. Although some bacteria quickly detach, others become irreversibly attached and express surface-associated structures, such as Type 4 pili, and behaviors, including twitching motility and biofilm initiation. *P. aeruginosa* that lack the GTPase FlhF assemble a randomly placed flagellum that is motile; however, we observed that these mutant bacteria show defects in biofilm formation comparable to those seen for non-motile, aflagellate bacteria. This phenotype was associated with altered behavior of ΔflhF bacteria within 2 min of surface-binding, and decreased progression to a non-rotating, surface-attached phenotype. Events required for this transition to surface-attachment were investigated.

Methods
Videomicroscopy and single cell tracking were used to analyze bacterial motility. Forward and reverse genetic screens were employed to identify gene products required for modulation of flagellar motility at a surface.

Results
We observed that FlhF interacts with FimV, a polar organizer, and that ΔfimV bacteria show identical defects as ΔflhF cells, in modulating flagellar rotation after surface binding, despite assembling a polar flagellum. As *P. aeruginosa* expresses two distinct motor-stators that have been implicated in playing distinct roles in swarming motility through viscous media, we constructed and analyzed ΔmotAB and ΔmotCD mutants for flagellar behavior in liquid and at a surface. Each motor supported swimming, though bacteria demonstrated significantly different swimming speeds and chemotactic behaviors depending on the motor they expressed. Analysis of tethered bacteria suggested that only one motor, MotCD, supported flagellar rotation in tethered cells. Like ΔfimV and ΔflhF mutants, the ΔmotAB mutant failed to modulate flagellar behavior upon surface tethering, resulting in persistent rotation and significantly diminished attachment.

Conclusion
*P. aeruginosa* behavior after flagellar-mediated surface binding appears to provide another example in which specific motor-stators are required to alter bacterial behavior when the flagellum responds to a change in load. Models for how FlhF and FimV might affect this process will be presented.
THE FLAGELLAR FILAMENT REGULATES K-STATE DEVELOPMENT IN BACILLUS SUBTILIS, PERHAPS THROUGH ROTATIONAL LOAD

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Background
The flagellar structure of B. subtilis consists of three architectural domains: the basal body including the motor to drive rotation, the hook and the filament. Cairns et al., (2013) and Chan et al., (2014) have shown that deletion of the motor gene motB results in elevated levels of the response regulator DegU-P. Here we show that specific mutants affecting the filament have the same phenotype as ΔmotB on DegU-P dependent gene expression and we discuss the implications of this finding.

Methods
We used luciferase assays to determine DegU-P dependent gene expression. A maleimide based labeling technique was used to visualize flagella and to monitor rotation of specific motility mutants in tethering experiments.

Results
We found that a deletion of the flagellin gene hag as well as the point mutation hagA233V that encodes straight filaments, both increase the level of phosphorylated DegU. Furthermore, all three motility mutants (motB, hag and hagA233V) are less transformable, due to a decreased expression of comK, a transcription factor for the K-state, which confers genetic transformability and persistence. Our data indicate that high DegU-P in the motility mutants represses the basal transcription rate of comK and therefore lowers the frequency of transitions to the bistably expressed K-state. Tethering experiments have shown that straight flagella and hooks in Δhag cells continue to rotate.

Conclusion
Flagella send a signal to suppress the level of DegU-P and therefore favor transitions to the K-state (see Fig.). This signal may be a perturbation in proton flux, rotation per se or viscous drag on the filaments. Because the hooks and straight flagella continue to rotate, rotation and proton flux may not be the signals. Instead, these findings argue that the signal might be viscous drag, which may be sensed by basal bodies to suppress the levels of DegU-P in response to environmental conditions. Perhaps basal body remodeling, as shown in E. coli (Lele et al., 2013) mediates this regulation.

![Model for regulation of K-State development by motility](image)

Model for regulation of K-State development by motility. The rotation of flagella sends a signal to suppress the level of phosphorylated DegU. DegU activates the K-State, while high levels of DegU-P caused by a loss of the signal in nonfunctional flagella mutants inhibit comK expression and lower the probability of K-state development.
MYXOCOCCUS XANTHUS DEVELOPMENT AS MOTILITY REGULATED PHASE SEPARATION

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Background

Myxococcus xanthus forms a biofilm called a swarm that exhibits a variety of multicellular processes. For example, in response to starvation, an \textit{M. xanthus} swarm containing millions of cells undergoes a process of self-organization called development. During development, swarm cells aggregate to form macroscopic dome shaped fruiting bodies within a period of 24h. Development can occur over a range of initial cell densities, but the process appears very different at different densities. Below a threshold density of approximately $2.5 \times 10^{7}$ cell/mL no aggregates form. As cell density increases above $5 \times 10^{8}$ cell/mL, aggregates form as pin-holes that grow with time; these aggregates are spaced at random throughout the swarm, and require up to 40h to mature into fruiting bodies. At a very high cell density above $5 \times 10^{9}$ cell/mL, aggregates form quickly (3-4h) and synchronously, as a series of elongated and interconnected ridges that condense into dome shaped mature fruiting bodies.

Methods

These density-dependent differences in the process of \textit{M. xanthus} development closely resemble phase separation in a passive system. Because of this observation, we have based our current model for \textit{M. xanthus} development on an Active Brownian Particle (ABP) model of phase separation. In such an ABP model, lower density systems may undergo random and local nucleation, while higher density systems may undergo a spontaneous spinodal decomposition phase transition.

Results

We have tested this model using time lapse microcinematography of \textit{M. xanthus} development at several initial cell densities, and tracked individual cells within them. We found that swarm cells change their movements during aggregation, increasing their speed and decreasing the frequency of their reversals; the impact of these changes is an increase in the overall distance cells travel. We then examined the movements of a FrzE mutant strain, which has hypo-reversing cells, together with Nigerecin, which reduces cell movement.

Conclusion

By controlling reversal frequency and speed through these genetic and chemical means, we were able to push \textit{M. xanthus} either into or out of developmental “phase boundaries.” These data were in good agreement with our ABP model.

Figure 1: Brightfield images of M. xanthus development (40X). Numbers indicate hours after starvation onset. Black spots in the 24 hr. timeframe are fruiting bodies, each of which is $\sim 0.1$ mm in diameter.
OVERLAPPING DEGRON AND ACTIVE SITE IN SWRA, THE SWARMING MASTER REGULATOR

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Background

Bacillus subtilis is a motile bacterium capable of swimming in liquid and swarming atop a surface. Both forms of motility are powered by the same flagellar system, however swarming motility requires approximately two fold more flagella than swimming motility. When inoculated on surface, the transcriptional activator SwrA accumulates within the cell and results in an increase in both flagellar gene transcription and flagellar density. Intracellular SwrA levels are low in swim cells due to proteolytic turnover by a AAA+ protease, LonA, and its putative adapter SmiA. How LonA/SmiA recognizes SwrA remains unknown.

Methods

To genetically determine residues required for SwrA proteolysis, a complementation construct in which swrA, under the control of its native PswrA promoter, was mutagenized by low fidelity PCR and screened for hyper swarming colonies. Hyperswarming mutants were sequenced to identify the mutation, and mutant SwrA stability was tested both in vivo and in vitro.

Results

We identified two mutations in the C-terminal region of SwrA that increase stability of SwrA by inhibiting proteolysis by LonA/SmiA.

Conclusion

Here we show that the C-terminal region of SwrA is important for turnover by LonA. We hypothesize that the C-terminal domain of SwrA contains a specific degron sequence that is recognized by LonA/SmiA and targets SwrA for proteolysis.
SPONTANEOUS MUTATIONS IN THE \textit{FLHD} OPERON GENERATE MOTILITY HETEROGENEITY IN \textit{ESCHERICHIA COLI} BIOFILM

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Background
Heterogeneity and niche adaptation in bacterial biofilm involve changes to the genetic makeup of the bacteria and gene expression control. We hypothesized that i) spontaneous mutations in the \textit{flhD} operon can either increase or decrease motility and that ii) the resulting motility heterogeneity in the biofilm might lead to a long-term increase in biofilm biomass.

Methods
We allowed the highly motile \textit{E. coli} K-12 strain MC1000 to form seven- and fourteen-day old biofilm. Isolated colonies were tested for motility and phenotypic complementation with plasmid pXL27. Portions of the \textit{flhD} operons were investigated with PCR and sequence analysis.

Results
We recovered reduced motility isolates from biofilm at a substantially greater frequency (5.4\%) than from planktonic bacteria (0.1\%). Biofilms formed exclusively by MC1000 degraded after 2 weeks. In contrast, biofilms initiated with a 1:1 ratio of MC1000 and its isogenic \textit{flhD::kn} mutant remained intact at 4 weeks and the two strains remained in equilibrium for two weeks. These data imply that an ‘optimal’ biofilm may contain a mixture of motile and non-motile bacteria.

Twenty-eight of the non-motile MC1000 isolates contained an IS1 element in proximity to the translational start of FlhD or within the open reading frames for FlhD or FlhC (blue arrows in Figure). Two isolates had an IS2 (orange arrow) and one isolate had an IS5 (green arrow) in the open reading frame for FlhD.

An additional three isolates contained deletions that included the RNA polymerase binding site (horizontal yellow box right upstream of the transcriptional start), five isolates contained point mutations (X) and small deletions (horizontal purple box) in the open reading frame for FlhC. The locations of all these mutations are consistent with the lack of motility and further downstream within the \textit{flhD} operon than previously published IS elements that increased motility. We believe that the location of the mutation within the \textit{flhD} operon determines whether the effect on motility is positive or negative.

To test the second part of our hypothesis where motility heterogeneity in a biofilm may lead to a long-term increase in biofilm biomass, we quantified biofilm biomass by MC1000, MC1000 \textit{flhD::kn}, and mixtures of the two strains at ratios of 1:1, 10:1, and 1:10. After three weeks, biofilm of the mixed cultures contained up to five times more biomass than biofilm of each of the individual strains.

Conclusion
Mutations in the \textit{flhD} operon can exert positive or negative effects on motility, depending on the site of the mutation. We believe that this is a mechanism to generate motility heterogeneity in \textit{E. coli} biofilm, which may help to maintain biofilm biomass over extended periods of time.
VARIABILITY IN *E. coli* CHEMOTAXIS MEASURED BY SINGLE-CELL FRET

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Background
Isogenic populations of *E. coli* bacteria demonstrate substantial variability in their motile behavior [1,2]. Theory [3,4,5] suggests that this variability is likely attributable to stochastic biochemical processes in the signaling network controlling bacterial chemotaxis. Experimentally, however, direct measurements of those signaling processes in single cells has been challenging, and existing results on single-cell signaling variability have been limited to indirect inferences based on measurements of motor switching.

Method
We report the first direct measurements of variability in the *E. coli* chemotaxis network by *in vivo* FRET in single cells. FRET between CheY and CheZ at the population level has been used extensively in the past to study chemotactic signaling dynamics [6]. Pioneering experiments [7] had demonstrated the feasibility of single-cell FRET, but extracting parameters from single-cell data remained an open problem. Results
We improved single-cell FRET to enable observation of significant cell-to-cell variability in key signaling parameters, such as ligand sensitivity, adaptation time, and steady-state network output. We find surprisingly large cell-to-cell variability in receptor sensitivity in adaptation-deficient cells. Experiments with CheB mutants in adapting cells revealed that phosphorylation feedback through CheB-P reduces cell-to-cell variability in the steady-state signaling output.

Single-cell FRET also enables measurement of temporal fluctuations in signaling. We observe slow, large-amplitude fluctuations of kinase signaling in CheR+CheB+ cells, but not in ΔCheRΔCheB cells. The ~10s timescale of these fluctuations agree with previous flagellar motor noise experiments [2], but the very large amplitude of these fluctuations (~40% of the mean) are much greater than earlier estimates.

Conclusion
The observed cell-to-cell differences in signaling parameters most likely reflect copy-number variability of chemotaxis proteins due to stochastic gene expression. The slow fluctuations within individual cells are, to our knowledge, the first experimental observation of temporal variability in a protein signaling network, and provide the most direct evidence to date that CheR/B-mediated methylation/demethylation underlies behavioral variability. Our results establish single-cell FRET as a powerful tool for addressing a variety of questions regarding stochastic biochemical processes *in vivo*.

Reference
NON-GENETIC PHENOTYPIC VARIABILITY AND ITS EFFECT ON POPULATION PERFORMANCE

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Background
Biological functions are typically performed by groups of cells that express predominantly the same genes, yet display a continuum of phenotypes. While it is known how one genotype can generate such non-genetic diversity, it remains unclear how different phenotypes contribute to the performance of biological function.

Methods
To address this issue, we developed a microfluidic device to simultaneously measure the phenotype and chemotactic performance of tens of thousands of individual, freely-swimming Escherichia coli as they climbed a gradient of attractant.

Results
We discovered that spatial structure spontaneously emerged from initially well-mixed wild type populations due to non-genetic diversity. By manipulating the expression of a key chemotaxis protein, we established a causal relationship between protein expression, non-genetic diversity, and performance that was theoretically predicted. This approach generated a complete phenotype-to-performance map. Significantly, we found that this relationship is convex at low values of tumble bias, leading to disproportionate performance of populations containing low tumble bias cells. We show that this convex relationship results in "Jensen's inequality" (Jensen, 1906): for populations containing low tumble bias cells, population performance can become greater than the performance of the mean population phenotype.

Conclusion
These results demonstrate how the shape of a phenotypic distribution can have as large of an effect on performance as changing the mean phenotype, suggesting that evolution could act on both during the process of adaptation.

Figure: A bacterial "race" in a microfluidic device revealed non-genetic diversity in behavior and performance of clonal E. coli cells. The "shape" of behavioral diversity affected population performance as much as the mean behavior, supporting the hypothesis that this shape is evolvable.

References
THE INTERSPECIES QUORUM-SENSING SIGNAL AI-2 MEDIATES AUTOAGGREGATION OF
ESCHERICHIA COLI

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**Background:** Many bacteria can communicate with each other, coordinating and synchronizing their behavior by means of production and sensing of extracellular signal molecules called autoinducers. A universal autoinducer AI-2 is the only established quorum-sensing molecule produced by *E. coli*, though its physiological role remains elusive. Here, we investigated the role of AI-2 in autoaggregative behavior of *E. coli*.

**Methods:** All strains were derived from *E. coli* W3110 (RpoS"). Autoaggregation of *E. coli* cells was accessed in µ-slide channels using phase contrast microscopy. Oxidative stress resistance tests were performed using 0.5% H₂O₂ treatment. Intra- and extracellular levels of AI-2 were measured using flow cytometry of plasmid-based Plsr-yfp reporter. Biofilm formation was quantified with crystal violet staining or visualized with the help of confocal laser scanning microscopy.

**Results:** We show that motility and chemotaxis towards self-produced AI-2 mediates collective behavior – autoaggregation – of *E. coli*. In contrast to previous studies, our results thus show that autoaggregation and motility are not mutually exclusive behaviors, but both functional flagellar apparatus and chemotaxis are required for efficient autoaggregation at physiological cell densities. We further demonstrate that such AI-2 dependent autoaggregation benefits bacteria by enhancing their stress resistance and promotes biofilm formation.

**Conclusions:** Our study provides the first evidence that chemotaxis towards a self-secreted attractant mediates autoaggregation of bacteria. Importantly, motility and chemotaxis are required for autoaggregation regardless of whether cell-cell interactions under particular growth conditions are mediated by the major *E. coli* adhesin antigen 43 (exponential growth at 37°C) or by curli fibers (early stationary phase at 30°C). AI-2 mediated autoaggregation not only promotes local AI-2 signalling, but it also provides physical protection against oxidative stress and contributes to the development of surface-attached biofilms. The universal nature of AI-2 production by bacteria lets us presume that chemotaxis to AI-2 is involved in aggregation, or even co-aggregation, of other species.

TWO DIFFERENT MECHANISMS, BASED ON DIRECT AND INDIRECT SIGNAL RECOGNITION, MEDIATE PSEUDOMONAS AERUGINOSA CHEMOTAXIS TO THE MAJOR VIRULENCE SIGNAL INORGANIC PHOSPHATE

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Background
Inorganic phosphate (Pi) is a central signaling molecule that modulates virulence in many pathogens. In Pseudomonas aeruginosa, low Pi concentrations induce transcriptional alterations that increase virulence. Previous studies have shown that the chemoreceptors CtpL and CtpH mediate chemotaxis to low and high Pi concentrations, respectively. Both receptors belong to different families; CtpH has a 4-helix bundle ligand binding domain (LBD), whereas CtpL is predicted to possess a helical bimodular LBD.

Methods
Isothermal Titration Calorimetry (ITC), Differential Scanning Calorimetry, Analytical Ultracentrifugation, pull-down experiments, protein identification, quantitative capillary chemotaxis assays of wt, mutant and complemented strains.

Results
ITC studies showed that CtpH-LBD but not CtpL-LBD recognizes Pi directly. Pull-down experiments of immobilized CtpL-LBD and a protein extract of P. aeruginosa demonstrated that the periplasmic Pi binding protein PstS recognizes specifically CtpL-LBD. PstS is part of the Pi transporter and has thus a double function in transport and chemotaxis. It binds Pi with an ultra-high affinity of 7 nM. Deletion of the psts gene abolished chemotaxis to low Pi concentrations and wt like taxis was observed after complementation. Data have been reported in (1) and results will be presented on our current work on Pi chemotaxis in the non-pathogenic P. putida KT2440.

Conclusion
Data permit to establish a model for the linked Pi transport and chemotaxis as shown in the figure. The expression of psts is tightly controlled by Pi which permits the coordination of Pi taxis and transport. The existence of two receptors with different sensitivities to the same ligand, permit to expand the concentration range of Pi taxis. Indirect binding mechanisms have been reported for sugar and dipeptide chemotaxis in E. coli. The demonstration that such mechanisms also exist in a different bacterial order, to a different ligand and a different receptor type suggests that such mechanisms are widespread in nature.

CHEMOTAXIS TOWARDS AROMATIC COMPOUNDS BY DIRECT SENSING AND TCA INTERMEDIATES IN *COMAMONAS TESTOSTERONI*

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**Background**
Many bacteria show chemotactic responses towards aromatic compounds. Bacterial chemotaxis is either metabolism-dependent or/and -independent. Energy-taxis chemoreceptors such as Aer and Aer2 trigger chemotaxis towards aromatics via metabolism-dependent. Several chemoreceptors such as McpT, NahY, NbaY, PcaY and CtpL were reported to be involved in chemotaxis toward aromatics. However, the true ligands of these chemoreceptors remained unclear. So far, chemoreceptors that trigger chemotaxis toward aromatic compounds via direct binding have not reported. *Comamonas testosteroni* CNB-1 has robust ability to degrade and sense a range of aromatic compounds, and its chemoreceptors and chemotaxis signaling pathway were studied.

**Methods**
Deletion and complementation of each MCP in wide-type and MCP-null strains. Isothermal Titration Calorimetry (ITC) for measuring the binding affinity of ligand binding domain. Intact transmembrane chemoreceptor were purified and assembled into Nanodiscs, and kinase control activities were assayed. Finally, ligand binding domain was crystallized.

**Results**
MCP2901, one of aromatic chemoreceptor, triggered chemotaxis towards multiple metabolizable aromatics. ITC assays identified citrate, gentisate and its several isomers as ligands, they directly bound to the ligand binding domain of MCP2901. Nanodiscs and capillary assay also proved that MCP2901 mediated direct sensing of aromatic compounds. MCP2201, another aromatic chemoreceptor in *C. testosteroni*, triggered chemotaxis towards aromatic compounds via sensing metabolic intermediates.

**Conclusions**
MCP2901 binds to both citrate and gentisate, and triggers both metabolism-dependent and –independent chemotaxis. We proposed a new metabolism-dependent chemotaxis strategy for aromatic compounds, i.e., chemoreceptors could be activated by TCA intermediates of aromatic compounds. MCP 2901 represents the first biochemically identified chemoreceptor that directly bind to aromatic compounds and generate chemotaxis signal.
TLPD MEDIATES A REPELLENT RESPONSE TO OXIDATIVE STRESS AND REGULATES ANTRAL COLONIZATION IN THE HOST GASTRIC EPITHELIUM

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Background
*Helicobacter pylori* relies on the cytoplasmic chemoreceptor TlpD during colonization of the host gastric epithelium although it remains unclear what signals the chemoreceptor responds to in the host. TlpD mediates a repellent response during exposures that may be linked by reactive oxygen species (ROS). The nature of these chemotactic responses, and their roles during early infection of the host were examined to understand the contribution of TlpD to host colonization.

Methods
Chemotactic responses were captured by video microscopy and the frequency of direction changes over a three second window was compared between strains and conditions. Colonization of *H. pylori* was assessed by enumerating and comparing colony-forming units from stomach tissue from the antrum and corpus assessed colonization at two weeks post-infection.

Results
TlpD repellent responses to hydrogen peroxide are sensitive to removal of ROS by catalase addition or dithiothreitol. TlpD also appears to alter *H. pylori* distribution in the stomach as a result of host-generated ROS.

Conclusion
TlpD repellent responses to hydrogen peroxide appear sensitive to the elimination of ROS via catalase or the addition of reductant. It remains unclear however if the chemoreceptor senses oxidative stress by oxidation or perhaps through a ligand whose concentration changes during oxidative stress. TlpD also appears to alter *H. pylori* colonization in the stomach due to host-generated ROS, as hosts deficient in their production were more readily colonized than wild type litter mates in specific regions of the stomach.

mG27 strains were incubated for 15 min in the presence of either 10 μg/ml metronidazole or 10.5 μM paraquat, swimming behavior was recorded, and direction changes were tracked over a 3-s window.
THE ROLE OF CJ0485 IN L-FUCOSE METABOLISM AND CHEMOTAXIS IN *CAMPYLOBACTER JEJUNI*

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**Background**

Strains of the pathogen *Campylobacter jejuni* with the *fuc* locus are capable of fucose metabolism and show a competitive colonization advantage in a piglet disease model (1). There is a link between possession of the *fuc* locus and biofilm formation and chemotaxis (Figure 1). In particular, the putative dehydrogenase Cj0485 has a key role in these phenotypes (2).

**Methods**

There is interest in further characterizing Cj0485 to better understand its role in chemotaxis. Cj0485 was overexpressed and purified for x-ray crystallography to determine its structure and also used in enzymatic assays to characterize its activity. Cj0485 was also used in far western blots to identify potential binding partners.

**Results**

Cj0485 has a structure resembling the dehydrogenase of *Burkholderia multivorans*, and converts L-fucose with NAD+ into L-fuconolactone and NADH at a rate of 8050 /M.s. Comparison of 32 aldoses revealed that Cj0485 can also reduce D-arabinose at a rate of 296/M.s. Growth of *C. jejuni* wild type and *fuc* mutants in minimal media together with D-arabinose confirmed that the organism can indeed use this carbon source and that the enzymes used for fucose catabolism are at least in part involved in the breakdown of D-arabinose. Far western blot experiments with *C. jejuni* cell fractions before and after fucose treatment are currently being optimized.

**Conclusion**

Cj0485 is a dehydrogenase capable of reducing both L-fucose and D-arabinose to lactone intermediates. Although the Cj0485 structure most closely resembles the dehydrogenase of *B. multivorans*, it is currently not clear why the latter is also capable of converting L-galactose and L-xylene, which are not substrates for Cj0485. In addition to playing a role in fucose metabolism, Cj0485 is also involved in sensing this nutrient since mutation of the enzyme inhibits fucose chemotaxis. Also, functional transfer of the fucose locus into *C. jejuni* B1-176 enabled the strain to swim toward this chemotactant. We are currently investigating the mechanism responsible for this phenotype.

DNA SENSING IN *BACILLUS SUBTILIS*

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**Background**
We recently found that the bacterium *Bacillus subtilis* performs chemotaxis to DNA. While DNA can serve as a nutrient for *B. subtilis*, our data suggest that the chemotaxis response is not to the DNA itself but rather to the information encoded within the DNA. Our evidence comes from experiments showing that *B. subtilis* prefers the DNA of more closely related species than the DNA of more distantly related ones. These results suggest that *B. subtilis* responds to particular DNA sequences that are enriched within the genomes of closely related bacteria.

**Methods**
We employed the capillary assay to measure chemotaxis to DNA from different organisms. We then used SELEX-seq to identify the specific sequences of DNA that *B. subtilis* responds to. The binding properties of these sequences were then evaluated using isothermal titration calorimetry (ITC) and the *in vitro* receptor-kinase assay.

**Results**
Chemotaxis to DNA is dose-dependent. It occurs to both chromosomal and synthetic DNA. Among the organisms tested, *Bacilli* are the preferred sources of DNA. McpC is the sole chemoreceptor for DNA. Using SELEX-seq, we identified a number of chemotactic DNA motifs. DNA sequences enriched in these motifs elicit a strong chemotactic response. The abundance of these motifs partially explains the organismal preference of DNA chemotaxis.

**Conclusion**
*B. subtilis* performs chemotaxis to DNA. Furthermore, it appears to prefer the DNA of closely related species. While the physiological role of DNA chemotaxis is unknown, its selectivity suggests that it may be involved in horizontal gene transfer or kin selection.
SPDC, A NOVEL VIRULENCE FACTOR, CONTROLS HISTIDINE KINASE ACTIVITY IN STAPHYLOCOCCUS AUREUS

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Background
The WalKR two-component system (TCS) is essential for viability of Staphylococcus aureus, playing a central role in controlling cell wall metabolism. We produced a constitutively active form of the WalR response regulator in S. aureus and showed that transcription of several genes was increased in the presence of the walR⁶ allele. Among these, spdC encodes a membrane-anchored protein with 8 predicted transmembrane segments and an Abi domain (CAAX protease self-immunity). We sought to define the role of SpdC in the WalKR signal transduction pathway.

Methods
Transcriptome analysis, RNASeq, antibiotic resistance assays, qRT-PCR, GFP fusions, fluorescence microscopy, chimeric protein expression, bacterial adenylate cyclase two-hybrid assays, biofilm formation, virulence assays

Results
A ΔspdC mutant was constructed in S. aureus and used in an RNAseq analysis. Among the genes negatively-regulated by SpdC, 24 belong to the WalKR regulon. Using Bacterial Two-Hybrid experiments and fluorescence microscopy, we showed that SpdC interacts with the WalK histidine kinase and is co-localized at the division septum. Furthermore, the ΔspdC strain was sensitive to certain cell wall antibiotics and strongly impaired in biofilm formation as well as in virulence in a murine sepsis model. Many of the genes identified in the RNAseq analysis belong to regulons controlled by other two-component systems and we have shown that SpdC interacts specifically with several of the S. aureus histidine kinases and that this interaction requires their transmembrane domains.

Conclusion
The WalKR TCS positively controls synthesis of SpdC, which in turn negatively regulates the activity of the WalKR TCS by interacting with the WalK histidine kinase, effectively constituting a negative feedback loop. This control system seems to affect multiple TCSs in S. aureus.

The SpdC membrane-bound protein forms a negative feedback control loop for the WalKR two-component system in S. aureus
BACTERIAL REPLICATION RATE CONTROLS PRODUCTION OF BORRELIA BURGDORFERI VIRULENCE FACTORS

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Background
*Borrelia burgdorferi*, the agent of Lyme disease, is maintained in nature through cycles of alternating infection of vertebrate hosts and tick vectors. Colonization of those two dramatically different animals, and efficient transmission between vector and host, requires that the bacterium express distinct repertoires of surface proteins and other factors at each step. Key proteins involved with mammalian infection include the OspC and Erp outer surface proteins. Neither protein is produced during tick colonization, but both are induced upon initiation of tick feeding and are expressed at high levels during transmission. OspC is essential for mammalian infection, although its function remains unknown. Erp proteins bind mammalian proteins and other ligands, such as complement Factor H, plasmin, laminin, and glycosaminoglycans.

Methods
Combinations of western blotting, quantitative RT-PCR, and immunofluorescence microscopy demonstrated that *B. burgdorferi* controls production of Erps and OspC in vivo and in vitro. Transcriptional reporters between *erp* 5’ DNA and *gfp* identified sequences necessary for regulation of transcription (*erpO*). DNA affinity chromatography identified three novel proteins that bind to specific sites within *erpO*. In vitro analyses with recombinant proteins, and in vivo studies of mutant bacteria, revealed how each of the three DNA-binding proteins control *erp* transcription, and RNA-Seq investigated the global impacts of those regulators. Similar techniques are being employed to characterize the mechanisms by which the regulators are regulated.

Results
Production of OspC and Erp proteins is directly proportional to the rate of bacterial division. We hypothesize that the rapid burst of *B. burgdorferi* replication during tick feeding serves to signal the need for the bacteria to adapt to infection of the vertebrate host. Transcription of *erp* genes is directly controlled by three DNA-binding proteins: EbfC, BpuR, and BpaB. Studies from other labs found that transcription of *ospC* is controlled by an alternative sigma factor, RpoS, and our RNA-Seq data indicate involvement of at least one as-yet unidentified intermediate regulatory factor: In addition, BpuR, EbfC, BpaB, and RpoS control production of numerous other *B. burgdorferi* genes. Expression levels of all four regulatory proteins are significantly affected by the rate of bacterial replication. Investigations into the mechanisms by which *B. burgdorferi* controls transcription of *ebfC* and *bpuR* revealed that the DnaA protein binds adjacent to both promoters. DnaA is the master regulator of bacterial chromosomal replication.

Conclusions
We hypothesize that DnaA coordinates the transcription of *bpuR*, *ebfC*, and other regulators, with the rate of chromosomal replication. Ongoing studies are refining that hypothesis, and examining the mechanism(s) underlying replication-dependent regulation of RpoS.
INVESTIGATION INTO A CELL-DENSITY DEPENDENT PATHWAY IN *AEROCCOCUS URINAEE*

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**Background**
Analysis of the Female Urinary Microbiota has identified organisms correlated with Overactive Bladder Syndrome (OAB). One of those organisms is *Aerococcus urinae*, an understudied, Gram-positive, α-hemolytic, emerging uropathogen. To reach our goal of understanding the pathogenesis of *A. urinae* in the context of OAB, we have chosen to examine specific phenotypes using genetic approaches. During growth on media supplemented with Congo Red, clinical isolates of *A. urinae* produce black colonies, a phenotype that in *Staphylococcus aureus* is correlated with biofilm formation. We observed that colonies developed rapidly from red to black during a period from approximately 40 to 48 hours, starting from areas with high cell density and spreading to areas of low cell density. We are currently pursuing the hypothesis that development of the black colony phenotype depends on quorum sensing; our initial results suggest that a soluble factor is involved.

**Methods**
Our lab has a collection of over 5000 clinical urinary isolates and there are currently 153 *A. urinae* isolates. We screened these clinical isolates looking for the presence or absence of the black colony phenotype. In addition, we utilized an autoinducer cross assay developed by the Bassler lab to test if the black colony phenotype is dependent upon quorum sensing (Figure 1).

**Results**
The screen of clinical *A. urinae* isolates for black colony phenotype on media supplemented with Congo Red yielded both black and red isolates. Using the autoinducer cross assay, we observed that some constitutively red isolates developed black colony color in the presence of constitutively black isolates.

**Conclusion**
These results suggest the involvement of a soluble factor, potentially as part of an autoinducer-type pathway in *A. urinae*. To identify the gene(s) responsible for this phenotype, we used UV and chemical mutagenesis protocols to isolate mutant strains that fail to produce black colonies. Ultimately, we will use whole-genome sequencing to identify the genetic loci that control black colony formation. The investigation of the factors controlling this pathway will advance our understanding of the pathogenesis of *A. urinae* in the context of OAB.

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**Autoinducer Cross Assay**

![Autoinducer Cross Assay](image)

*Figure 1 Autoinducer Cross Assay. To test if the black colony phenotype of *A. urinae* is dependent upon quorum sensing, red isolates of *A. urinae* are cultured in the presence and absence of black isolates in a cross formation.*
STRUCTURE OF THE TWO-COMPONENT RESPONSE REGULATOR RCSB FROM *ESCHERICHIA COLI* IN COMPLEX WITH DNA REVEALS MECHANISM OF PHOSPHORYLATION-INDEPENDENT ACTIVATION OF TRANSCRIPTION

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Background
RcsB is a universal two-component response regulator of Rcs phosphorelay signal transduction system that activates transcription from a variety of genes essential for virulence and survival of enteric bacterial pathogens. RcsB can regulate transcription alone or together with auxiliary regulators to stimulate transcription in either phosphorylation-dependent or -independent modes. Despite increasing knowledge of RcsB’s importance, the molecular and cellular mechanisms by which phosphorylation and dephosphorylation drive RcsB’s ability to form homodimers or heterodimers with its auxiliary partners to facilitate DNA binding are not well understood. Here, we present the first crystal structure of non-phosphorylated RcsB from *Escherichia coli* in complex with DNA. The structure reveals important details of the phosphorylation-independent mechanism of DNA binding by RcsB and formation of the RcsB homo and heterodimers.

Methods
X-ray crystallography, electrophoretic mobility shift assay, surface plasmon resonance, size exclusion chromatography with multi-angle light scattering

Results
In the present study, we focus on the structural details of phosphorylation-independent RcsB binding to DNA. We present the crystal structure of non-phosphorylated RcsB from *E. coli* in complex with the consensus DNA-binding sequence of the *flhDC* operon determined at resolution 3.4 Å. The RcsB displays a unique asymmetric dimer accompanied by the formation of hydrophobic interactions within its dimerization interface. The structure suggests that this asymmetry likely provides conformational flexibility and allows RcsB to regulate a large number of genes by forming homodimers or heterodimers with its auxiliary partners.

Conclusions
Our data confirm that RcsB in its non-phosphorylated state can bind to DNA and activate transcription.

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HIDDEN DYNAMICS OF CHEY AT THE SWITCH OF THE BACTERIAL FLAGELLAR MOTOR

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Background
The flagellar motor of E. coli responds to the binding of phosphorylated CheY (CheY~P), the product of repellent signaling, by switching its direction of rotation. CheY~P strongly binds to the N'-terminus of the switch protein FliM (FliM_N) with subsequent binding to other, low-affinity, sites. In the absence of the chemotactic machinery, switching can also be caused by CheY acetylation. The aim of this study was to resolve two related major questions. One is the apparent discrepancy between the non-cooperative binding of CheY~P to the motor and the cooperative switching activity of the motor. The second is the relevance of acetylated CheY to chemotactic signaling.

Methods
We employed a FliM_N-deleted strain to quantify the stimuli-driven responses of flagellar rotation and the contribution of phosphorylation and acetylation to switching produced by binding of CheY to the low-affinity sites. The binding was measured by FRET microscopy. By internalizing single phosphorylated fluorescently labeled CheY(I95V) molecules within living cells with wild-type motors, we measured to contribution of acetylation to the dwell time of CheY at the motor.

Results
We found that, in the absence of FliM_N, stimuli-driven responses are slowed down, the contribution of phosphorylation and acetylation to switching is synergistic, and the binding of CheY to the low-affinity sites in the motor is cooperative. We also found that single molecules of phosphorylated CheY dwell significantly more time at the motor when acetylation is promoted.

Conclusions
We suggest that acetylated CheY binds to the low-affinity sites with a resultant modulation of the motor to favor CheY~P binding due to the mechanism of cooperativity. Thus, acetylated CheY increases the sensitivity of the motor to phosphorylation-dependent signaling.
**BORRELLIA BURGDORFERI FLAGELLA EXPORT APPARATUS AND VIRULENCE: INSIGHT INTO TYPE III SECRETION SYSTEM**

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**Background**
Previous results indicated that almost all motility and chemotaxis proteins are required for the pathogenesis of the Lyme disease spirochete *Borrelia burgdorferi*. *B. burgdorferi* migrates to distant sites in the tick vectors and mammalian hosts through robust motility and chemotaxis activities. Type III Secretion System (T3SS) is essential for the morphology, motility, cell division, structure, infectivity, and life cycle of this spirochete. FliJ is a cytoplasmic chaperone protein that plays important roles in the type III secretion system-mediated export and assembly of flagellar structural proteins. However, the structure and function of FliJ gene have not been reported for *B. burgdorferi*.

**Methods**
In this study, the fliJ gene was inactivated in an infectious strain of *B. burgdorferi*. The functional roles of fliJ protein were investigated to dissect the mechanism of *Borrelia* type III secretion system and their relationship to virulence.

**Results**
Inoculation of C3H/HeN mice via either needle injection or use of infected *Ixodes scapularis* ticks indicated that fliJ mutant is noninfectious in the mammalian host, but it is not required by survival in *I. scapularis* ticks. The fliJ mutant showed rod-shaped or string-like morphology, greatly reduced motility, and division defects (resulting in elongated, string-like and/or rod-shaped organisms). Mutant in fliJ was incapable of translational motion in 1% methyl cellulose.

**Conclusion**
The results indicated that *B. burgdorferi* FliJ plays important roles in flagellar structure, function, cell division, and infectivity.
EXPLORING ALTERNATIVE ROLES OF CHEMOTAXIS PATHWAY IN BACTERIA

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Background
Most motile Bacteria and Archaea respond to chemical cues in the environment through a behavior called chemotaxis. Chemotaxis is best understood in E. coli where a set of 11 core proteins sense the chemical cues in the environment and control flagellar motility accordingly. This observation set the paradigm that proteins from the chemotaxis pathway solely participate in the regulatory pathway of motility. However, a large fraction of organisms has more than one chemotaxis system encoded in their genomes. While typically one of these systems has been shown to control canonical chemotaxis behavior, the function of the additional systems is still unclear. The lack of a clear, discernable phenotype for these additional sets of chemotaxis proteins might indicate that these proteins control motility in environmental conditions that cannot be reproduced under laboratory conditions. Alternatively, these systems could be involved in other cellular response pathways not related to the control of the cells’ motility.

Methods
We use comparative genomics, gene neighborhood analysis, phylogenetics, genetics and electron cryo-tomography to gain insight into structure and function of these less well understood chemotaxis systems.

Results
We analyzed the co-evolution of one of these chemotaxis systems of unknown function in the proteobacteria and structurally characterized them using homology modeling and electron cryo-tomography in four species: Pseudomonas aeruginosa, Vibrio cholerae, Shewanella oneidensis and Methyloicrobium alcaliphilum. We validate the identity of these chemoreceptor arrays with tomography of a series of knockout mutants in P. aeruginosa and V. cholerae. Finally, we compare multiple characteristics of the organisms to gain insights into the functional role of this system in the cell.

Conclusion
Taken together, these results pave the path to a multi-organism approach to determine the biological function of this chemotaxis system.

Figure: Additional chemotaxis gene clusters are conserved in many proteobacteria suggesting an important alternative biological role or control of motility under special conditions.
INTER-DIMER INTERACTIONS THROUGHOUT THE CHEMORECEPTOR SIGNALING DOMAIN

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Background
Chemoreceptors are key components of the bacterial sensory system that modulates flagellar motility. They detect changes in the environment and transmit information to CheA histidine kinase, which ultimately controls flagellar motors. The prototypical Tsr chemoreceptor in *E. coli* is a homodimer containing two principle functional modules: (i) a periplasmic ligand-binding domain and (ii) a cytoplasmic signaling domain comprising an antiparallel, four-helix coiled-coil bundle. Receptor dimers are arranged into a trimer-of-dimers, which is a minimal physical unit essential for modulating the CheA activity. Recent advances in cryoelectron tomography revealed that trimers-of-dimers are arranged into highly ordered hexagon arrays at the cell pole; however, the mechanism underlying the trimer-of-dimer and higher order array formation remains unclear. While some studies claim that the dimers keep contact only at the hairpin tip of the signaling domain, other suggest that oligomerization occurs through the full length of the cytoplasmic part of the receptor.

Methods
Using Cray XK7 “Titan” supercomputer, we performed all-atom, microsecond-range molecular dynamics simulation of the Tsr trimer-of-dimers crystal structure. The evaluation of the plausible organization state of the Tsr chemoreceptor trimer complex was made based on a) the frequency of specific bonds formation (hydrogen bonds, salt bridges, and hydrophobic interactions) between homodimers, b) their distribution throughout the length of the MCP signaling domain, and c) the conservation of residues participating in the specific contacts. Evolutionarily conserved positions in the Tsr signaling domain were revealed by comparative sequence analysis of Tsr homologs. The preference of the system to stay in a “zipped” state was assessed in terms of different components of binding free energy.

Results
*In silico* analysis shows that the oligomerization between homodimers is initiated by hydrophobic interactions at the hairpin tip of the chemoreceptor. The “zipping” between dimers is facilitated by the electrostatics. The compact, fully “zipped” structure is further stabilized by salt bridges. Residues involved in establishing specific contacts are evolutionarily conserved.

Conclusion
Based on the structural and evolutionary analysis we show that Tsr dimers can interact throughout the full length of the signaling domain.
A MOLECULAR MECHANISM FOR KINASE CONTROL IN CHEMORECEPTOR SIGNALING COMPLEXES

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Background
In core chemotaxis signaling units two receptor trimers of dimers, assisted by two CheW monomers, control the autophosphorylation activity of the homodimeric histidine kinase CheA. Unlike most histidine autokinases, the CheA phosphorylation site and ATP-binding pocket reside in separate domains (P1 and P4, respectively). The arrangements and motions of CheA domains in the kinase-on and kinase-off core complex signaling states are not yet known. A recent all-atom molecular dynamics simulation by Cassidy et al. (elife 4:e08419, 2015) revealed two alternative conformations, which we designate “closed” and “open”, for the CheA-P4 domains in core complexes. Our experiments explored the possibility that these two CheA conformations correspond to different signaling states of E. coli chemoreceptor core complexes.

Methods
We created amino acid replacement mutants at CheA residues proposed to stabilize the open conformation and examined their signaling consequences with in vivo FRET-based kinase assays. Cysteine-directed in vivo crosslinking tests and phenotypic suppression analyses were carried out to identify functional interactions among the conformation-controlling residues.

Results
Amino acid replacements at CheA residues participating in the “open” P4 conformation impaired chemotaxis and reduced or abolished CheA kinase activity. Compensatory amino acid replacements at other residues proposed to stabilize the “open” conformation suppressed these functional defects. Crosslinking between cysteine reporters of P4 “open” state was greatly reduced in mutants displaying no kinase activity.

Conclusion
The “open” conformation is likely to be the arrangement adopted by the CheA-P4 domain in its kinase active state. We hypothesize that this configuration exposes P4 docking sites that facilitate productive interactions between P4 and P1, thereby promoting P1 phosphorylation. How chemoreceptor trimers of dimers modulate the CheA open-closed switch in core signaling complexes remains an open question.
CHEMOTAXIS SIGNALING COMPLEXES ACT BY ALTERING THE RATE CONSTANT OF KINASE AUTOPHOSPHORYLATION

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Background
Autophosphorylating histidine kinase CheA is central to signaling in bacterial chemotaxis. This kinase donates its phosphoryl group to two response regulators, one controlling flagellar rotation and thus motility, the other crucial for sensory adaptation. As measured by phosphorylation of the motility-controlling response regulator, incorporation into signaling complexes activates the kinase ~1,000-fold and places it under control of chemoreceptors. By the same assay, receptors modulate kinase activity ~100-fold as a function of receptor ligand occupancy and adaptational modification. These activity changes are the essence of chemotactic signaling. Yet, the enzymatic properties altered by kinase incorporation into signaling complexes, by chemoreceptor ligand binding or by receptor adaptational modification have not been identified.

Methods
We performed steady-state kinetic analysis of autophosphorylation by kinase alone; in isolated, Nanodisc-based core signaling complexes; and in small arrays of core complexes assembled on receptors in native membranes. Autophosphorylation in signaling complexes was measured as a function of ligand occupancy and adaptational modification.

Results
We found that activation by incorporation into signaling complexes reflected almost exclusively increases in the apparent catalytic rate constant ($k_{cat}$) as did modulation by ligand occupancy and adaptational modification. Changes in the autophosphorylation $k_{cat}$ accounted for most of the ~1000-fold kinase activation in signaling complexes observed by measuring coupled CheY phosphorylation, the ~100-fold inhibition by ligand occupancy or modulation by adaptational modification.

Conclusion
The data suggest that kinase activation and inhibition result from alterations of the kinase active site or an equilibrium between active and inactive states of the kinase.
NETWORKED CHEMORECEPTORS BENEFIT BACTERIAL CHEMOTAXIS PERFORMANCE

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Background
Chemoreceptor arrays are found in many motile bacteria. In E. coli these extended arrays are composed of networked signaling complexes, and promote cooperative stimulus control of their associated signaling kinases. Although our understanding of bacterial chemotaxis is quite detailed, the signaling and behavioral advantages of networked receptor arrays had not been directly studied in cells.

Methods
We used structural lesions at the interface between core signaling complexes to create an E. coli strain with functional but dispersed signaling complexes. This strain allowed us to directly study how networking of signaling complexes affects chemotactic signaling and gradient-tracking performance.

Results
We directly demonstrate that networking of receptor complexes provide wild type cells with about 10-fold heightened sensitivity to attractant while maintaining a wide dynamic range over which receptor modifications can modulate their response sensitivity. We further show that adaptation of receptors in dispersed signaling complexes is slower than it is in extended arrays, which could be compensated by overexpression of the adaptation enzymes. Finally, we found that networking of receptor complexes is generally advantageous for chemotaxis under various conditions, and especially critical for chemotaxis towards a non-metabolizable attractant source. The latter, mimics natural conditions in which bacteria are too sparse to significantly alter the attractant distribution.

Conclusion
Networking of signaling complexes heighten sensitivity, increase dynamic range, and expedite adaptation, advantages that allow bacteria to migrate effectively and robustly toward widely varying attractant sources.
DISTINCT DOMAINS CONFER CHEA WITH UNIQUE FUNCTIONS IN CHEMOTAXIS AND CELL LENGTH AT DIVISION IN AZOSPIRILLUM BRASILENSE SP7

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Background
A central regulator of chemotaxis is the histidine kinase, CheA. This cytoplasmic protein interacts with membrane-bound receptors, which assemble into large polar arrays, to propagate the signal. In the alphaproteobacterium, Azospirillum brasilense, Che1 controls transient increases in swimming speed during chemotaxis, but it also affects cell length at division. However, the underlying molecular mechanisms for Che1-dependent control of multiple cellular behaviors are not known. Here, we identify specific domains of the CheA1 histidine kinase implicated in modulating each one of these functions.

Methods
Site-directed and deletion mutagenesis combined with behavioral assays confirmed the role of each domain in chemotaxis and/or modulating cell length. Subcellular fractionation and Western blot analyses revealed the presence of two isoforms of CheA1 in A. brasilense Sp7, and fluorescence microscopy confirmed that these two isoforms were positioned at different locations within the cell.

Results
We show that CheA1 is produced in two isoforms: a membrane-anchored isoform produced as a fusion with a conserved seven-transmembrane domain of unknown function (TMX) at the N-terminus, and as a soluble isoform similar to prototypical CheA. Furthermore, TMX is dispensable in chemotaxis and, instead, mediates changes in cell length.

Conclusion
The data provide a mechanism for the role of Che1 in controlling multiple unrelated cellular behaviors via acquisition of a new domain in CheA1 and production of distinct functional isoforms.
**BORRELIA BURGDORFERI** CheY1, CheY2, AND CheY3 POSSESS DISTINCT CHEMOTAXIS AND/OR VIRULENCE FUNCTIONS DURING THE NATURAL ENZOOTIC CYCLE IN TICK AND MOUSE RESERVOIRS

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**Background**

*Borrelia burgdorferi* (Bb) is a vector-borne spirochetal bacterium that persists within an enzootic cycle between *Ixodes* ticks and certain vertebrate hosts. Each bacterium possesses between 7-11 endoflagella and motor apparatuses at each of their poles, and the direction of their flagellar rotation at each end directs whether they move forward or reverse, with flexing movements to reorient the microbe. Thus, they have evolved a sophisticated motility and chemotaxis system, consisting of over 5% of their genome, which is essential for their persistence within and transmission between the dense tissues of susceptible reservoir hosts. Our previous work has determined that motility is absolutely required for infectivity, however the chemotaxis apparatus is less well studied. The genomic arrangement of the chemotaxis system is somewhat similar to *E. coli*, but is much more complex, with multiple homologs of several chemotaxis genes. The CheY protein is the response regulator in the chemotaxis signal transduction system of bacteria, where it interacts with the flagellar switch proteins to control motor rotation. We hypothesize that each of the three *cheY* genes (i.e. *cheY1*, *cheY2*, and *cheY3*) possessed by Bb are essential for at least one of the transmission events within or between vertebrate and tick hosts during the natural infection cycle. In this study, we intend to delineate the virulence properties for each of the three Bb CheY proteins during the mouse-tick-mouse infection cycle.

**Methods**

We have created individual *cheY1*, *cheY2*, and *cheY3* mutants in an infectious B31 Bb strain, some of which also generate fluorescence. These strains were used in various *in vitro* chemotaxis/motility assays, and also tested for their ability to complete the mouse-tick-mouse infection cycle, including intravital microscopy to directly observe bacteria within intact mouse ear skin.

**Results**

Deletion of *cheY3* demonstrated that this protein acts much like a classic response regulator, with Δ*cheY3* lacking the ability to reverse direction or complete chemotaxis via capillary tube assays. The Δ*cheY3* demonstrated similar defects within mouse tissues, and was defective in its ability to persist in fed ticks or to be transmitted to or persist in mice. The Δ*cheY2* strain displayed no defects in motility or chemotaxis *in vitro*, and was able to persist within tick hosts similar to WT. However, they could not be transmitted from ticks to mice, nor could they persist or disseminate to target tissues in mice. Interestingly, the Δ*cheY1* strain demonstrated no motility or chemotaxis defects *in vitro* or in mice and tick hosts, including co-infection studies with WT Bb in mice.

**Conclusions**

Overall, our studies indicate that the three CheY proteins have distinct, non-overlapping functions related to the ability of Bb to infect tick and vertebrate hosts. The specific functions of CheY1 and CheY2 in chemotaxis or other signaling functions are still under study.
ISOLATION, CHARACTERIZATION AND STRUCTURE OF AN ARCHAEAL FLAGELLUM

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Background
The Archaea like Bacteria employ flagella to accomplish motility and taxis although we know little about the structure or functioning of their appendages. To address this we examined the obligate anaerobic mesophilic methanogen, Methanospirillum hungatei strain JF1, a rod shaped cell type isolated from a municipal waste digester in Urbana Illinois in the mid 1970’s. This methanogen has the special ability to partner with many syntrophic bacteria and support a biochemical-based symbiosis.

Methods
A combination of microbiological, mass spectroscopy and cryo electron microscopy (cryoEM) techniques were employed to identify and analyze the M. hungatei strain JF1 flagellum yielding the protein composition, dimensions and atomic level structure.

Results
The M. hungatei flagellum was shown to be distinct in protein composition and structure from the bacterial counterpart. Here, we report an atomic description of the filament (also termed an archaellum) at 3.4 Å resolution. Each flagellum contains ~61,500 archaellin subunits in an average 10,000 nm length and forms a curved helix of 10 nm in diameter. The tadpole-shaped archaellin monomer has two domains, a beta barrel domain and a long, mildly kinked alpha helix tail. Our structure reveals multiple posttranslational modifications including six O-linked glycan additions plus an unusual N-linked modification. The extensive interactions among neighboring archaellin subunits explain how the long but thin flagellum maintains structural integrity required for motility-driving rotation.

Conclusions
These extensive inter-subunit interactions and the absence of a central pore in the archaeal flagellum make it distinct from both the bacterial flagella and type IV pili.
OPTIMALITY AND INDIVIDUALITY IN RUN-TUMBLE MOTILITY

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Background
E. coli's run-tumble motility strategy (Fig. a) has been hypothesized to represent a compromise between two conflicting chemotactic performance requirements [1,2] (Fig. b). The recently discovered substantial individuality in E. coli run-tumble motility [3] however raises the question whether and how such a balance between traits can be maintained in the face of pervasive variability between individuals.

Methods
We characterize E. coli run-tumble motility by performing statistical analyses (Fig. a) of a large 3D trajectory dataset, obtained using a recently developed high-throughput 3D tracking technique [3]. We then compare our findings against theoretical predictions for the chemotactic drift velocity, vd, and localization precision, a ([2], Fig. b).

Results
We find that the directional persistence, \(<\cos\Theta>\), decreases and the effective rotational diffusion coefficient, DR, increases with decreasing individual run speed, vr, impacting predicted chemotactic drift velocity, vd, and localization precision, a. For each run speed range separately, however, the measured \(<\cos\Theta>\) and DR yield a compromise between these two performance requirements.

Conclusions
We identify the concerted variation of directional persistence, \(<\cos\Theta>\), and effective rotational diffusion coefficient, DR, as an effective mechanism for resolving the conflicting requirements of high drift velocity, vd, and high localization precision, a, in the face of vr-dependent individuality.

NOVEL PSEUDOTAXIS MOTILITY MUTATIONS AND DIFFUSION OF BACTERIAL CELLS IN POROUS MEDIA

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Background
The chemotaxis signal transduction network regulates the biased random walk of many bacteria in favorable directions and away from harmful ones through modulating the frequency of directional reorientations. In mutants of diverse bacteria lacking the chemotaxis response, migration in classic motility agar, which constitutes a fluid-filled porous medium, is compromised: Straight-swimming cells unable to tumble become trapped within the agar matrix. Spontaneous motility mutations that restore spreading, albeit not under control of the chemotaxis network, were previously observed in the enteric bacterium *Escherichia coli*, notably in a classic work by Wolfe and Berg [Wolfe A. J. and H.C. Berg. 1989. Proc. Natl. Acad. Sci. USA 86:6973-6977]. Recent work in other bacterial species has isolated and quantified different classes of non-chemotacting mutants exhibiting the same spreading phenotype [Mohari B., et al. 2015. mBio 6:e00005-15].

Methods
We present a theoretical description of bacterial diffusion in a porous medium – the natural habitat for many cell types – which elucidates how diverse modifications of the motility apparatus resulting in a nonzero tumbling frequency allows for unjamming of otherwise straight-swimming cells at internal boundaries and leads to net migration. These theoretical results, combined with a simple model of bacterial diffusion and growth in agar, are compared with our experimental measurements of swim ring expansion as a function of time, demonstrating good quantitative agreement.

Results
Our results suggest that the details of the cellular tumbling process may be adapted to enable bacteria to propagate efficiently through complex environments.

Conclusions
For engineered, self-propelled microswimmers that navigate via alternating straight runs and changes in direction, these results suggest an optimal reorientation strategy for efficient migration in a porous environment with a given micro-architecture.
FLAGELLAR INSTABILITY-DRIVEN ESCAPE MECHANISM FOR TRAPPED BACTERIA

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Background
Many bacteria are motile by means of a single flagellum at the cell pole which has a rather rigid helical shape allowing forward and backward swimming in liquid media. However, many natural bacterial habitats consist of structured environments, such as sediments, water-saturated soils, or tissues, where bacteria run the risk of getting stuck.

Methods
Using high-speed microscopy, we monitored the swimming behavior of the mono-polarly flagellated species Shewanella putrefaciens with fluorescently labeled flagellar filaments. To mimic the conditions of its natural habitats we observed the cells at an agarose-glass interface featuring both wide liquid filled channels and constricted areas.

Results
We found that when cells get stuck and regular backing out does not release the cell, they use a yet undescribed movement: the flagellar filament wraps around the cell body in a spiral-like fashion, enabling the cells to escape by a screw-like backward motion. Microscopy and modeling suggest that this propagation mode is triggered by an elastic instability at the base of the filament upon switching to clockwise motor rotation and when higher torque is applied. The switch is reversible, so cells can return to regular swimming mode by switching motor direction back to counterclockwise rotation.

Conclusion
An instability of the flagellar structure can be employed for a novel rescue and movement mechanism and is likely found in numerous environmental and pathogenic bacteria.

Figure: Illustration of trapping, screw formation and escape of a swimming cell in a constricted environment based on high-speed fluorescence microscopy. The cell enters from the left, swims until it gets stuck at the upper right and tries to get out by swimming backward. Then the flagellar instability sets in, the flagellum wraps around the cell body (see inset) and the bacterium escapes in a screw-like motion toward the lower half of the frame.

For microscopy data of a similar screw formation event visit: www.goo.gl/ChP9Bn or scan this QR code:
THE SCREW-LIKE MOVEMENT OF A GLIDING BACTERIUM IS POWERED BY SPIRAL MOTION OF CELL-SURFACE ADHESINS

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Background
Bacteria that swim are driven forward by helical filaments that rotate like propellers. The number and location of filaments vary among different bacteria, yet the core mechanism remains the same. Motile but non-swimming bacteria do not have propellers, yet they achieve self-propulsion over surfaces. Such movement is divided into two categories: (1) twitching and (2) gliding. Twitching involves the extension and retraction of type IV pili, but gliding bacteria do not use type IV pili. Although the mechanism for gliding is barely understood, we know that Flavobacterium johnsoniae, which exhibits some of the fastest gliding of all known bacteria, has a powerful rotary motor and a mobile cell-surface adhesin, SprB. How the motion of SprB might result in gliding is not clearly understood.

Methods
We coated gold nanoparticles with an SprB antibody and tracked them in three-dimensional space in an evanescent field where the nanoparticles appeared brighter when they were closer to the glass surface. We used cephalixin to generate elongated F. johnsoniae cells with irregular shapes. We followed the displacement of asymmetric cells in three dimensions and developed a model to test if the cells moved in a screw-like fashion.

Results
The nanoparticles bound to SprB followed a spiral trajectory on the surface of the cell. We found that cells rolled about their long axes as they moved forward, following a right-handed trajectory. Thus, if SprB were to adhere to the glass surface rather than to a nanoparticle, the cell would move forward along a right-handed trajectory, as observed, but in a direction opposite to that of the nanoparticle.

Conclusion
Our results suggest that for F. johnsoniae, a bacterium that prefers to move over and colonize external surfaces, designing a mobile and adhesive external thread and placing it such that it moves spirally on the surface of the cell appears to be the mechanism for forward motion. F. johnsoniae works as a self-propelled screw, with SprB moving along its external threads.
TYPE IVb PILUS RETRACTION IN CAULOBACTER CRESCENTUS

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Background
Caulobacter crescentus is a gram-negative bacterium that divides asymmetrically to produce a sessile stalked cell and a motile swarmer cell, which expresses a polar flagellum and polar type IVb pili. φCbK is a bacteriophage that initially adsorbs to the C. crescentus flagellum through a head filament and uses flagellar rotation to reach the cell pole. It has been hypothesized that φCbK uses pilus retraction to position itself for attachment at the pilus portals, although there is no known retraction ATPase. In this study, we are using φCbK adsorption to investigate type IVb pilus retraction in C. crescentus.

Methods
To investigate the function of pilus retraction in C. crescentus, we used soft agar motility assays and phage adsorption assays after treatment with anti-PilA antibody or carbonyl cyanide m-chlorophenyl hydrazine (CCCP), a proton motive force inhibitor. φCbK interactions and pilus expression phenotypes were analyzed using cryo-correlative light and electron microscopy (cryo-CLEM) and cryo-electron tomography (cryo-ET).

Results
Treatment with anti-PilA antibody reduces both motility and φCbK adsorption, suggesting that antibody binding blocks pilus retraction. φCbK adsorption is also negatively impacted by the addition of CCCP, indicating that the proton motive force may regulate pilus dynamics, which then impacts phage adsorption that is regulated by the pilus. To separate the role of flagellar of the flagellum and the impact of flagellar rotation in phage adsorption, the assays were performed with motA and tipF mutant strains, which also showed a reduction in phage adsorption after CCCP treatment. Cryo-ET revealed that both treatments lead to an increase in the number of cells with extended pili, demonstrating that pilus retraction has been stalled. Additionally, the φCbK tail fiber can be visualized interacting directly with the extended pilus filament.

Conclusions
This study demonstrates type IVb pilus retraction in C. crescentus and its role in motility and φCbK adsorption. The data support a model of φCbK adsorption in which the phage localizes at the cell pole using flagellar rotation, attaches to the pilus filament through its tail fiber, and then uses retraction of the pilus to reach the pilus portal where it injects the genome.

Figure: Cryo-electron tomography surface rendering of a C. crescentus cell treated with anti-PilA antibody, leading to the display of several pili.
MOTILITY IN ARCHAEA

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Background
All three domains of life have developed fundamentally different motility structures. While bacteria swim using the flagellum, eukaryotes cilia, archaea employ archaella. The archaellum is evolutionary related to type IV pili and therefore its filament proteins, the archaellins, are processed in a similar manner as type IV prepilins. The rotation of the archaellar filament is driven by ATP hydrolysis.

Methods
We study the assembly and function of the archaellum in two model archaea, the halophilic euryarchaeote *Haloferax volcanii* and the thermophilic crenarchaeon *Sulfolobus acidocaldarius*. We use genetic, imaging, biochemical and structural methods to understand how the archaellum is assembled and rotating.

Results
In *H. volcanii* we study the interaction of the archaellum with the chemotaxis system, which was acquired by lateral gene transfer from bacteria and fulfills the same function in archaea as in bacteria, but now has to switch the direction of a totally different motor complex. The structure of CheY indicated the necessary changes that have occurred during the adaption of the che system to the archaellum. The interaction of the subunits of the archaellum motor complex of the archaellum were analyzed in *S. acidocaldarius* showing that FlaI/X and H form the cytoplasmic complex and that ATP binding by FlaH is essential for proper assembly of the archaellum.

Conclusions
The archaellum is a unique motility structure for which we can now present a preliminary model of its assembly pathway and mechanism of rotation.

Jarrell and Albers, TIMS, 2012
Poster Abstracts
HOW TO BE INVISIBLE AS A MICROSCOPIC SWIMMER

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Background
Microscopic organisms live a difficult life in open waters, having to continuously scan large amounts of water for nutrition (and sometimes mates), and hide from predators at the same time. In the absence of vision at these small scales, all interactions are dominated by chemical and hydromechanical cues. Thus, there is an evolutionary pressure to minimize the hydromechanical disturbance generated during locomotion.

Results
We have made experimental observations that breast stroke swimming plankton generate a fluid disturbance that decays faster with distance than what is predicted from the commonly used stresslet model of a self-propelled organism. We rationalize these observations by using a three-Stokeslet model of a breast stroke swimmer.

Conclusions
Our results show that it is possible for a swimmer to dramatically reduce its fluid disturbance by appropriately positioning the propulsive apparatus. A simple rearrangement of flagella suggests that the same result may apply to amphitrichous bacteria such as van Leeuwenhoek’s “little eel” \textit{Spirillum volutans}. A generalization of this concept may be used in understanding the large diversity of shapes and swimming modes found in the microscopic world.
TEMPLATING ASSEMBLY OF C-RING COMPONENTS IN VITRO WITH DNA NANOSTRUCTURES

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Background
During *E. coli* motor construction, the FliF ring templates the sequential self-assembly of FliG, FliM and FliN, collectively the “C-ring”, the site of torque generation and motor switching. The stoichiometries of FliG, FliM and FliN are disputed and a symmetry mismatch between the FliF and FliG rings seen with cryo-EM is unexplained. These questions and others are difficult to resolve with *in vivo* techniques, and purification of intact motors is likely compromised by the dynamic nature of FliM / FliN incorporation. C-ring assembly *in vitro* could overcome these problems. Furthermore, substitution of the FliF ring with a designable template could provide a handle to probe self-assembly from the bottom-up. Among other things, this would allow a test of the domain-swap polymerization model recently proposed for FliG assembly [1]; a possible mechanism for the FliF-FliG symmetry mismatch.

Methods
We have constructed short linear templates from DNA, mimicking fragments of the FliF ring. DNA linkers target FliG to individually addressable template sites via NTA-histag interaction or site-specific covalent conjugation. Stoichiometry of FliG on templates is quantified by native PAGE and single-molecule fluorescence.

Results
We have demonstrated that DNA sequence design can be used to control the stoichiometry and positioning of covalent DNA-FliG conjugates on linear templates.

Conclusion
We can now use the tools we have developed to probe the FliG domain-swap model. With ring-like DNA origami templates and the addition of FliM/N, complete C-ring assembly *in vitro* may be possible.

Reference
THE MASTER REGULATORS OF THE FLA1 AND FLA2 FLAGELLA OF RHODOBACTER SPHAEROIDES CONTROL THE EXPRESSION OF THEIR COGNATE CHEY PROTEINS*

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Chemotaxis is a complex cellular response through which bacteria can bias a random swimming path to move to attractants or swim away from repellents. A wide cytoplasmic protein complex and some associated receptors control this response. In this system, signals are transduced from chemoreceptors to the flagellar motor through a two-component system. The histidine kinase CheA phosphorylates the response regulator CheY, in response to the signaling state of the chemoreceptors. CheY-P interacts with the flagellar switch, changing the flagellar rotation and, as a consequence inducing a change of direction in the swimming path of the cell.

Rhodobacter sphaeroides is an alpha proteobacterium which possesses two functional flagellar systems, Fla1 (acquired by horizontal transfer from an ancestral gamma proteobacterium) and Fla2 (the vertically inherited flagella) as well as multiple copies of the chemotactic genes. The chemotactic genes are arranged in three operons (cheOp1, cheOp2, cheOp3) and an independent locus. The control of each flagellar system is achieved by different sets of chemotactic proteins, for instance, CheY2 and CheY5 (included in cheOp1) control Fla2, while CheY3 (cheOp2), CheY4 (independent locus) and CheY6 (cheOp3) are responsible for the chemotactic control of Fla1. Transcription of fla2 genes is controlled by the master regulator CtrA, while the fla1 genes are controlled by FleQ; however, the regulation of their respective chemotaxis genes has been poorly elucidated so far. We show in this work that, expression of the chemotaxis genes that control fla1 and fla2, is mediated by their cognate flagellar master regulators.

The expression of the chemotactic proteins McpA, CheY2, CheY3, CheY4, CheY5 and CheY6 was evaluated by Western blot. To analyze the transcriptional regulation of these genes, primer extension, RT-PCR, and activity of a reporter gene were performed.

The expression of cheOp1 that encodes for McpA, CheY2 and CheY5, which control the Fla2 flagella is dependent on the master regulator of the fla2 genes (CtrA). On the other hand, expression of cheOp3, which encodes for CheY6, that controls the Fla1 flagellum, is dependent on the master regulator of the fla1 genes (FleQ). Likewise, the \( \sigma^70 \)factor, which is a regulator of some fla1 genes, is involved in the expression of cheY4. The expression of cheOp2 that includes CheY3 is partially dependent on this sigma factor as suggested before.

Conclusion
Our work shows that the expression of the chemotactic proteins in \( R. \) sphaeroides is coordinated with the expression of their cognate flagellar system. \({ }^{*}\)This work was supported by DGAPA-UNAM (IN204614), CONACyT (235996) and Posgrado en Ciencias Bioquímicas, UNAM.
SHEDDING LIGHT ON E. COLI PHOTOTAXIS

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Background
Along with chemicals, blue light modulates E. coli motile behavior – a process known as phototaxis. While chemotaxis in E. coli has been studied extensively, there have been only a handful of studies on its phototactic response [1-3]. The exact mechanism of blue light sensing in E. coli is still unknown, and the biological significance of this phototactic response remains unclear. It has been previously shown that E. coli respond to blue light by increased tumbling [1]. This response requires a functioning electron transport chain and two types of receptors, Tar and Aer [2-3].

Methods
We study phototaxis in E. coli by analyzing trajectories of populations of free-swimming bacteria in 2D before and after light exposure.

Results
In agreement with previous studies we observe that Tar and Aer receptors mediate tumbling response to blue light. Surprisingly, we find that the growth substrate dramatically affects both the amplitude and kinetics of the response, indicating coupling between phototaxis and metabolism (Figure 1). Our results also reveal a previously unknown role of other E. coli receptors in phototaxis. Similar to Tar and Aer, Tsr and Trg receptors cause increased tumbling when exposed to light, while Tap causes increased running.

Conclusions
All five E. coli receptors sense and respond to blue light under our experimental conditions. This observation suggests that blue light may provide a way to quantify poorly understood interactions between chemotactic receptors [4].

Figure 1. Response to blue light in wild-type E. coli strain RP437 shown as tumble bias vs time, dashed line – bacteria grown with succinate, solid line – with glycerol as growth substrates. Light is turned on at a time zero and light exposure is indicated by the shaded area.

References
STRUCTURAL AND MECHANISTIC INSIGHTS INTO THE LYSINOALANINE CROSS-LINKING REACTION OF THE TREPONEMA DENTICOLA HOOK PROTEIN FlgE

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Background
Flagellated bacteria propel themselves via the rotation of flagellar filaments that connect to membrane-embedded motors. These filaments rotate in either a clockwise or counterclockwise direction, allowing the cell to tumble or swim smoothly. The Spirochete phylum has a unique flagellar location, as the flagella of these bacteria remain enclosed within the periplasm, rather than extend into the extracellular space. Spirochetes are of increasing interest due to the pathogenic nature of several of its members such as Borrelia burgdorferi (Lyme disease), Treponema denticola (periodontal disease), and Treponema pallidum (syphilis).

Recently, it has been demonstrated that the flagellar hook protein of T. denticola and T. pallidum, FlgE, undergoes a self-catalyzed lysinoalanine (LAL) cross-linking reaction that polymerizes the FlgE subunits. In T. denticola, this cross-linking has been shown critical for motility. The cross-link forms through a reaction that involves the conversion of Cys178 to dehydroalanine1,2, followed by Michael addition of Lys165. Herein, we present our ongoing efforts to ascertain the catalytic mechanism of LAL cross-linking.

Methods
To study FlgE crosslinking from T. denticola (TdFlgE), we have utilized X-ray crystallography, site-directed mutagenesis, colorimetric assays, and mass spectrometry in order to begin to elucidate the mechanism of LAL cross-linking.

Results
We have crystallized the active cysteine-containing D2 domain of TdFlgE, and identified key residues that play a role in promoting efficient cross-linking. We have also characterized products and intermediates in the cross-linking reaction, which can be accelerated by Cys178 disulfide formation or alkylation. From this data we propose a preliminary mechanism for LAL formation.

Conclusion
We have made initial steps toward characterizing the unique self-catalyzed LAL cross-linking mechanism in T. denticola hook protein FlgE. The chemistry, along with the resulting post-translational modification are largely unprecedented.


FLAVIN COFACTOR OXIDATION STATE REGULATES CheA SIGNAL TRANSDUCTION OF AEROTAXIS RECEPTOR

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Background
Chemotactic bacteria modify their swimming behavior in response to stimulants from their environment that can include toxins, metabolites, oxygen, and light. Transmembrane methyl-accepting chemotaxis proteins (MCPs) bind attractants and repellants in the periplasmic space and activate the CheA / CheY signaling pathway to effect flagellar rotation. In contrast to other receptors the E. coli aerotaxis receptor (Aer) has been shown to detect chemical changes within the cell. It has been proposed that the redox state of the PAS-bound FAD cofactor reflects the activity of the electron transport chain. Changes to the FAD redox state translate into conformational changes in the cytoplasmic domain of the receptor, which then determine Aer signaling events. Our goal is to understand this conformational signaling in molecular detail.

Methods
The Aer receptor was functionally reconstituted into nanodiscs and the FAD redox state altered with the use of oxidizing and reducing agents. Pulse ESR experiments were used to measure the distance between flavin radicals in the PAS domains of an Aer dimer when the receptor is in the reduced kinase-off state (Figure 1).

Results
Aer is shown to regulate CheA activity based on its FAD redox state. Furthermore, pulse ESR data indicate that the PAS and HAMP domains are tightly associated in the kinase off state (Figure 1). Loading ratios of Aer subunits into nanodiscs further suggest that these receptors rely on high-order oligomerization (i.e. trimer-of-receptor-dimers) to engage the CheA kinase.

Conclusion
The mechanism of Aer signal transduction is reliant on the Aer-bound FAD redox state, which is presumably sensitive to the activity of the electron transport chain. These results provide novel mechanistic details for the activity of Aer and offer an approach for elucidating the conformational transitions essential for Aer signaling.

References
MULTIPLE SENSORY KINASES REGULATE GENERAL STRESS RESPONSE IN THE MARINE PHOTOHETEROTROPH, ERYTHROBACTER LITORALIS

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**Background**
The Alpha-proteobacterial general stress response (GSR) system represents a unique fusion between two-component signaling systems and alternative sigma factor regulation. Specifically, sensor histidine kinase(s) regulate the phosphorylation of PhyR, a hybrid protein containing a receiver domain and a sigma factor-like domain. When phosphorylated, PhyR titrates the anti-sigma factor from the general stress alternative sigma factor and thus activates transcription. While the core pathway components are well defined and broadly conserved in the Alphaproteobacteria, the signals and kinases activating the pathway are varied between species and less understood. Here we present the identification of three GSR-regulating kinases in the marine photoheterotroph, *Erythrobacter litoralis*.

**Methods**
We have established *E. litoralis* as a genetic system and defined the GSR regulon using RNA-seq and reverse genetic approaches.

**Results**
We demonstrate that a) the membrane kinase, GsrP, is a negative regulator of GSR-dependent transcription, b) that the soluble sensor kinase, GsrK, is an activator of GSR transcription, and c) a photosensory LOV kinase is a conditional regulator of GSR transcription. The effect of the LOV-kinase on GSR-dependent transcription depends on the light environment. Notably, photosensory LOV kinases serve as GSR inputs in the freshwater oligotroph, *Caulobacter crescentus*, and in the intracellular mammalian pathogen, *Brucella abortus*.

**Conclusion**
This work supports an emerging trend of blue-light sensor kinases as modulators of GSR input signals. Moreover, we establish that multiple kinases contribute to GSR regulation in *E. litoralis*. 

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**Schematic of the molecular control system for the general stress pathway in E. litoralis.**
INTERACTION OF THE FLAGELLAR MURAMIDASE SltF WITH FlgJ IN *Rhodobacter sphaeroides*

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Background
During flagellar rod formation FlgJ is required for assembly. In *Salmonella enterica* this protein consists of two functional domains: the N-terminus acting as a scaffold for rod assembly and the C-terminus acting as a β-N-acetylglicosaminidase that facilitates rod penetration through the peptidoglycan layer, in the photosynthetic bacterium *R. sphaeroides* FlgJ works only as a scaffold for rod assembly. In our laboratory we identified the lytic transglycosylase responsible for breaking the cell wall: SltF, and we are currently establishing the role of its non-conserved C-terminus in flagellar assembly.

Methods
The details of the interaction SltF-FlgJ were explored by dissecting the non-conserved amino acids of SltF. The effects of the different constructs on motility were analyzed using swimming assays; we studied the interaction between SltF and FlgJ on in vitro assays by performing coimmunoprecipitation and far western blot assays. Additionally bioinformatic analyses were performed on lytic transglycosylases sequences found within the flagellar genetic context of bacteria with single domain FlgJ.

Results
Elimination of the non-conserved C-terminus of SltF induces the loss of motility. We identified a region in SltF that is necessary for the interaction with FlgJ. Bioinformatic work shows that flagellar lytic transglycosylases group together in phylogenetic analyses.

Conclusion
Our work shows the existence in SltF of a crucial region for the interaction with FlgJ. We propose the existence of a family of flagellum specific lytic transglycosylases.

Our work shows that the C-terminus of the enzyme responsible for breaking the cell wall (SltF) interacts specifically with rod’s scaffolding protein FlgJ.

*This work was supported by CONACyT (CB235996) and PAPIIT (IN204614).
GENETIC AND BIOCHEMICAL CHARACTERIZATION OF AN OPEN READING FRAME INVOLVED IN FLA2 MEDIATED MOTILITY OF *Rhodobacter sphaeroides*

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**Background**

*Rhodobacter sphaeroides* has two flagellar systems (*fla1* and *fla2*) and both systems encode for flagella used for swimming in liquid media. It has been shown that Fla1 is constitutively expressed under laboratory growth conditions, while Fla2 in our hands can only be expressed when a FleQ mutant that contains a gain-of-function mutation in the histidine kinase CckA. Phylogenetic analyses show that acquisition of *fla1* was due to horizontal transfer from an ancestral γ-proteobacterium. The *fla2* flagellar locus contains various Open Reading Frames (ORFs) of unknown function. One of these ORFs is RSWS8N_12065, which is located in a putative operon along with *fliL2, motA2* and two more ORFs.

**Methods**

We mutated RSWS8N_12065 and evaluated the resulting phenotype in soft agar swimming plates and by transmission electron microscopy. We also determined the location of the protein by means of GFP fusion. Finally, we performed pull down assays with recombinant his-tagged proteins.

**Results**

The interruption of RSWS8N_12065 results in a mot⁻ phenotype. The GFP fused protein was localized near the cell pole. We also carried out interaction assays between 12065 and MotB2 and found that the interaction between these two proteins was positive.

**Conclusion**

The product of ORF 12065 is possibly involved in the interaction with the stator protein MotB2.


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PHENOTYPIC DIVERSITY OF VIBRIO CHOLERAE

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Background
The signaling networks underlying the transition between motile and biofilm lifestyles in V. cholerae have been under intense investigation and it appears that c-di-GMP signaling plays a central role in controlling phenotypic switching. However, isogenic populations often exhibit phenotypic diversity owing to stochastic molecular fluctuations and bi-stability in the regulatory networks. Phenotypic diversity can be beneficial as a bet-hedging or division of labor strategy, but in V. cholerae, the role and regulation of phenotypic diversity in different environments has not yet been characterized.

Method
We used video-microscopy and multiple-particle tracking to characterize single-cell motile behavior. Swimming speed and reversion frequency were calculated from individual trajectories to measure phenotypic distributions. We quantified cell-to-cell variability in the activity of the vpsT promoter (VpsT is a transcription factor controlling biofilm formation) using a fluorescent reporter to examine the origin of phenotypic diversity in the regulatory network. We tested different V. cholerae mutants in different growth conditions to identify factors that are involved in shaping phenotypic distributions.

Results
Single-cell tracking revealed that motile and non-motile cells coexist during the exponential growth phase in all the conditions we tested. However, all cells become motile when the population enters the stationary growth phase. Mutations in the quorum sensing system (QS) had little effects on the distributions of motility phenotypes. On the other hand, the observed bimodality of the vpsT promoter activity in the wild-type strain is lost when the QS is perturbed. A constitutively active (∆luxO) QS upregulates vpsT, whereas a constitutively inactive (∆hapR) QS downregulates its activity.

Conclusion
Exponentially growing cells are phenotypically diverse even in conditions that are known to promote either motility or biofilm formation. Motility phenotype does not appear to have a perfect inverse correlation with the transcriptional activity of genes that promote biofilm formation.

Figure: Characterizing phenotypic diversity in V. cholerae isogenic populations. (A) Single-cell fluorescence expression controlled by the vpsT promoter. (B) Probability distributions of single-cell fluorescence for different QS mutants. (C) Probability distributions of single-cell motile phenotypes for different QS mutants.
FLAGELLAR MOTILITY IN INTESTINAL MUCUS

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Background
Flagellar motility is an important virulence factor for several pathogenic bacteria, such as, *Escherichia coli*, *Vibrio cholerae*, and *Salmonella enterica* Typhimurium. Presumably, these bacteria use motility to penetrate the thick protective mucus layer in the gut to reach epithelial cell or colonize the intestinal crypts. In addition, some bacteria secrete enzymes to degrade mucins and DNA to change the physical properties of the mucus and facilitate translocation. However, little is known about how these bacteria compromise the mucus defense.

Methods
We used video-microscopy and multiple-particle tracking to characterize the behavior of single cells in mucus. Intestinal mucus was extracted from the large intestines of pigs and washed to remove particles and resident organisms while maintaining the physical properties of the mucus gel. We measured viscosity and elasticity of the mucus samples using micro-rheology before introducing bacteria. We measured the distributions of single-cell swimming speed, directional persistence, and diffusion coefficient as single-cell behavioral parameters for populations of different bacterial pathogens.

Results
Motility was inhibited in crude mucus extract for *E. coli* and *S. enterica*. Dilution of mucus with buffered saline to 50% restored motility. This result indicates that these bacteria require additional factors to compromise the mucus defense. *V. cholerae* could swim in crude mucus extract, although, swimming speed and directional persistence was greatly reduced. Analysis of the trajectories revealed that motility is affected by the elastic component of mucus in addition to viscosity indicating that motile cells interact with the mucin matrix while swimming. We hypothesize that the smaller size of *V. cholerae* cells and the higher torque provided by its motor facilitate translocation through mucus.

Future Directions
We will investigate the effect of viscosity, elasticity, and matrix pose size on flagellar motility by preparing synthetic gels. We will investigate which bacterial factors promote motility in mucus, including motor torque, number of flagella, and secreted factors that may alter the local properties of mucus. Overall, we expect that our model will help better understand the role of flagellar motility for enteropathogens.

Figure. Distributions of motile behavior parameters from single-cell trajectories.
PSEUDOMONAS CHEMORECEPTORS: SEQUENCE HOMOLOGY = FUNCTIONAL HOMOLOGY?

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Background
In chemoreceptors, the N-terminal ligand binding domain (LBD) is responsible for sensing environmental stimuli, either directly or via an additional periplasmic binding protein. Recently, our group characterised the LBD of a proline/GABA binding chemoreceptor (named PscC) from Pseudomonas syringae pv. actinidiae. A BLAST search revealed PscC to be highly similar to a chemoreceptor from P. putida (McpC, 73 % amino acid identity), involved in cytosine/nicotinic acid sensing [1,2]. Intriguingly, despite their different sensory repertoires, the six residues predicted to form the binding site in both LBDs are completely conserved. In this work we explore the ligand binding repertoires of these and another homologous LBD from Pseudomonas sp. strain ADP.

Methods
The LBD regions of interest were identified using TOPCONS. Afterwards, the LBDs were cloned and heterologously expressed in Escherichia coli, before being purified using metal affinity chromatography. High throughput, fluorescence thermal shift (FTS) assays were carried out to identify potential ligands using Biolog PM™ plates. Hits from these screens are being further characterised with biophysical techniques (e.g. isothermal titration calorimetry) and chemotaxis assays.

Results
All three proteins were readily expressed and purified. In FTS assays, L-proline was found to stabilize each of the tested LBDs. The LBDs of PscC and its homolog from Pseudomonas sp. strain ADP were also stabilized by multiple other compounds. Preliminary ITC data confirmed the binding of proline to the LBD of PscC with a $K_d$ of $\sim 4.5 \mu M$.

Conclusion
Overall, the comparison between the three homolog LBDs have shown that further studies are required to elucidate the relationship between sequence similarity, structural similarity and binding repertoires.

ADAPTIVE RESISTANCE IN SWARMING BACTERIA: LESSONS FROM THE DEAD

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Background
There are multiple modes of bacterial dispersal in the environment. When faced with the challenge of moving on solid or semi-solid surfaces, many bacteria elicit an en masse flagellum-driven motility called swarming. This type of movement has been implicated in bacterial survival in hostile environments. For example, swarming bacteria can withstand exposure to antibiotics at concentrations that are lethal for bacteria swimming in bulk liquid. This phenomenon is called 'adaptive resistance', because it is maintained only during the swarming state and dissipates once the bacteria are suspended in liquid media i.e. the resistance is non-genetic. Cell density and speed of movement are important factors for this resistance. Neither quorum sensing regulators nor induction of new gene expression pathways appear to be involved, and the mechanism of this resistance is still unknown. It was reported (Butler et al., 2010) that during swarming over an antibiotic surface, a substantial population of dead cells accumulate on agar, indicating a possible contribution of these cells to the observed resistance.

Methods
To test whether dead cells indeed play a role in adaptive resistance, we used the 'border crossing' assay designed by Butler et al., where E. coli cells inoculated on a 'no antibiotic' surface, are allowed to swarm onto an antibiotic surface seeded with dead cells (see figure).

Results
We find that the presence of dead cells, whether killed by antibiotics, by oxidative stress or by physical methods, enhances adaptive resistance against multiple antibiotics. This suggested that cell lysis by any means might release an 'adaptive resistance factor' or ARF. We find that ARF is cytosolic, heat sensitive, and protease sensitive, and therefore likely a protein.

Conclusion
This work suggests the existence of a novel signaling pathway, where cell death is detected by the extracellular presence of a normally intracellular protein, potentiating the adaptive resistance response.

CRYSSELLIZATION TRIALS OF A NANODISC-INSERTED CHEMORECEPTOR

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Background
Transmembrane chemoreceptors are central components of the sensory system that mediates bacterial chemotaxis. Receptors detect attractants and repellents, convey informational signals across the membrane, control the signaling histidine kinase and mediate sensory adaptation. These processes involve receptor conformational changes that are incompletely understood. A major impediment is lack of atomic-resolution structures of intact, functional transmembrane chemoreceptors. Although X-ray crystallography has provided structures of receptor fragments, attempts to crystallize intact transmembrane receptors have not yielded useful crystals, perhaps because detergent-solubilized chemoreceptors are structurally and functionally disrupted. We are addressing this issue by utilizing Nanodisc technology to create water-soluble forms of intact chemoreceptors that are structurally stabilized and functionally active.

Methods
We used E. coli serine receptor Tsr with a six-histidine tag at its carboxyl terminus and a protease site at the beginning of the 36-residue, carboxyl-terminal unstructured arm as well as glutamines at four sites of adaptational modification and thus reduced structural dynamics. After incorporation of affinity-purified Tsr homodimers into Nanodiscs made with native E. coli lipids, the carboxyl-terminal unstructured arm plus histidine tag were removed and one dimer/disc material isolated by size-exclusion chromatography. These Tsr Nanodiscs could be concentrated up to ~30mg Tsr/ml and were stable for weeks at room temperature. We used this material and a crystallization robot to screen thousands of crystallization conditions.

Results
A group of related crystallization conditions consistently yielded small, needle-like crystals. Crystals were examined for X-ray diffraction at synchrotron beamlines. The diffraction patterns were diagnostic of protein and indicative of helical repeating units with large unit cells dimensions. Several diffraction patterns contained spots corresponding to resolution better than 3Å.

Conclusions
We can prepare intact, functional chemoreceptor homodimers inserted in Nanodiscs that are sufficiently well-behaved for crystallization trials and these trials yielded small crystals from which promising diffraction patterns were obtained. The crystals were not suitable for structural analysis, but the ability to obtain them provides a basis for ongoing optimization.
STRUCTURAL CHARACTERIZATION USING SOLUTION NMR, OF THE C-TERMINAL REGION OF ROTOR COMPONENT FLIG FROM VIBRIO ALGINOLYTICUS

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Background
Bacterial flagellar motor consists of rotor and stator. A rotor component FliG has three domains, N terminal, middle and C terminal domains, and the C terminal domain (FliGC) is closely participated in torque generation. In Vibrio, whose motor couples Na⁺ for torque generation in contrast to major H⁺ type one, no structure of FliG has been resolved. To investigate the mechanism for torque generation of the Na⁺-driven motor, we tried getting the structural information on the FliGC.

Methods
FliG of Vibrio is composed of 351 amino acids, and FliGC ranges from amino acid 214 - 351. We constructed and purified the C terminal fragments (amino acid 214 - 351) with or without motility-defective mutations reported previously, and used for NMR analysis. To confirm the results of NMR analysis, we performed molecular dynamics simulation for this domain using crystal structure of Thermotoga maritima (PDB# 1lkv). From these results, we discuss the correlation between structural dynamics and biological function of Vibrio FliGC.

Results
We found that the FliGC fragment (amino acid 253 - 351) gave the better NMR signals. Comparing the results of the wild type FliGC fragment and its mutant, A282T, it was revealed that the conformational exchange in some helices of A282T was slower than that of wild type based on the backbone assignment and relaxation measurement. Moreover, based on the results of molecular dynamics simulation, it was shown that the wild type fragment had the three representative conformations though the A282T fragment had only one.

Conclusion
The structural information from both NMR analysis and molecular dynamics simulation indicates the significant correlation between the structural dynamics of Vibrio FliGC and its biological function in motility.
THE ROLE OF THREONINE RESIDUES IN STATOR OF Na⁺-DRIVEN FLAGELLAR MOTOR

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Background
The stator complex of the bacterial flagellar motor is embedded in the cell membrane and couples ion translocation through itself to interaction with the rotor. In Vibrio alginolyticus, PomA and PomB are the component of the stator complex, form hetero tetramer (PomA₄PomB₂) and use Na⁺ as a coupling ion. A conserved aspartic acid residue (D24 in Vibrio) is identified as a critical residue for the ion-binding site. To understand an ion-biding site more deeply, we focus on threonine residues whose side chain contacts with Na⁺ in Na⁺-coupled transporters. We try demonstrating that these residues are essential for the ion-binding in the Na⁺-driven flagellar motor.

Methods
We introduced mutations in threonine residues in PomA/PomB and examined motility on soft agar plates and in solution, and ion-transport activity by measuring bacterial growth rate. We also examined stability of the complex in these mutants by pull-down assay. Furthermore, we purified the stator complex and examined Na⁺-binding ability by ATR-FTIR spectroscopy.

Results
There are four and one threonine residues that are located in transmembrane alpha helix and are conserved in Na⁺-driven stator complex in PomA (T5, T158, T185 and T186) and PomB (T21), respectively. PomA-T5A and PomB-T21A mutants slightly maintained motility and ion transport activity. However, PomA-T158A, PomA-T185A and PomA-T186A lost motility completely, suggesting that they are critical residues for function. In addition, PomA-T185A could not form a stable complex with PomB but not PomA-T158A and PomA-T186A.

Conclusion
PomA-T158, PomA-T185 and PomA-T186 are essential residues for motor function. Especially, PomA-T185 is involved in stabilization of the complex with PomB. Probably, PomA-T158 and PomA-T186 are associated with the Na⁺ binding and we are testing this possibility by FTIR spectroscopy.
SECRETION-SUBSTRATE TARGETING BY THE FLAGELLAR TYPE THREE SECRETION SYSTEM (T3S)

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Background
Many of the proteins involved in flagellar assembly are external to the cytoplasmic membrane; thus, they require export through the membrane-embedded type three secretion apparatus. In order for this to occur, substrates need to be selectively recognized and targeted by the apparatus. Despite twenty years of effort by a number of labs, little is known about how the secretion signal targets substrates for secretion. It is known that late flagellar proteins require chaperones for secretion and that the secretion signal is disordered and N-terminal. My lab has developed positive selections for and against secretion in Salmonella. Therefore, my question is: how are substrates targeted for T3S?

Methods
I have used these selections in conjunction with PCR and doped oligo mutagenesis to isolate mutants affecting secretion of the anti-sigma 28 factor, FlgM. My selection has two important features: substrate fusion to beta lactamase (bla) and fusion to the pore-forming region of colicin-A. The latter fusion is expressed under the arabinose promoter (Para). When FlgM-bla is secreted into the periplasm, it confers ampicillin resistance. This selects for secretion and is a way of ensuring that the secretion apparatus is functional. When Para-FlgM-ColA is secreted into the periplasm in the presence of arabinose, it forms a pore in the inner membrane, killing the cell. This selects against secretion of substrates. However, I select life to isolate mutant substrates defective for secretion.

Results
The mutants isolated using these methods contain single amino acid changes within the first fifty amino acids of the FlgM protein and display a secretion-defective phenotype. That is, they become resistant to arabinose while remaining resistant to ampicillin. However, when exposed to arabinose and ampicillin at the same time the mutants become sensitive to both.

Conclusion
Resistance to ampicillin and arabinose separately indicates that the secretion apparatus itself is functional, but that FlgM-ColA is not being secreted into the periplasm. Sensitivity to ampicillin and arabinose together suggests that the defect in FlgM-ColA secretion also prevents FlgM-bla secretion. The current understanding of these mutants is that they cause a “jam” within the apparatus after being targeted for secretion, thereby preventing other substrates from being secreted. These mutants will be used to find suppressors to gain new insight on the interactions between flagellar substrates and the secretion apparatus.
cAMP ALTERS CHEMOTACTIC BEHAVIOR OF TETHERED PSEUDOMONAS AERUGINOSA

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Background
Pseudomonas aeruginosa cells tethered to a surface via their polar flagellum quickly stop rotation and become attached via their long axis. This requires the SRP-like GTPase, FlhF, and the HubP-like protein FimV. FimV is known to positively regulate adenylate cyclase activity. We therefore tested whether exogenous cAMP would alter flagellar behavior in tethered bacteria.

Methods
High speed microscopy was used to record movement of tethered bacteria.

Results
Extracellular cAMP can complement P. aeruginosa phenotypes that depend on intracellular cAMP signaling. We therefore tested whether extracellular cAMP would cause ΔflhF or ΔfimV bacteria to stop rotating after surface tethering, and found this to be the case. Analysis of ΔflhF cells taken with a high-speed camera showed that cAMP changed the behavior of the rotor in several ways: The median number of reversals observed in tethered bacteria increased significantly, CCW rotation speed increased significantly, and CW bias decreased. Next, potential targets of cAMP were studied in the tethering assay in presence and absence of cAMP. So far P. aeruginosa has two known proteins that bind cAMP: the transcription regulator Vfr which regulates type IV pili gene expression and the polarly localized cAMP-binding protein CbpA of unknown function. We observed that cAMP still stopped flagellar rotation in bacteria lacking Vfr or type IV pili. This effect of cAMP does not appear to be mediated by CbpA, as the rotation of tethered ΔcbpA bacteria can be stopped by exogenous cAMP as well. We have found that the two motor-stators of P. aeruginosa differ in their ability to power rotation of tethered bacteria: ΔmotCD cells do not rotate after tethering, while ΔmotAB cells rotate but—much like ΔflhF and ΔfimV mutants—fail to stop rotation. Addition of cAMP to ΔflhF motAB cells failed to complement this defect, in contrast to what we observed for ΔflhF and ΔfimV strains. Analysis of flagellar rotation in ΔflhF motAB bacteria before and after exposure to cAMP revealed no change in median reversal frequency or rotation speed, while CW bias increased.

Conclusion
CAMP alters the behavior of tethered P. aeruginosa bacteria and can complement defects observed for tethered ΔflhF or ΔfimV cells. The effects of cAMP may be directly or indirectly mediated via MotAB.
STRUCTURAL PARAMETERS OF THE FLAGELLAR ROD IN *BACILLUS SUBTILIS*

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**Background**  
Bacterial flagella consist of a membrane-bound basal body, and external hook and filament, and an axle-like rod transiting the cell envelope that connects the basal body to the hook. Although rod assembly is a crucial component of flagellar synthesis, mechanisms regulating this step are poorly understood. Previous reports defining the order of rod assembly in Gram-negative bacteria suggest that the rod is metastable and requires four rod proteins to successfully assemble. However, these studies faced difficulties in interpretation, as improperly polymerized subunits secreted into the periplasm are proteolyzed by degradative enzymes.

*Bacillus subtilis* is a Gram-positive, genetically tractable model organism used for many studies of flagellar function in motility, and provides an established and unique toolset to study rod formation. Importantly, they lack a periplasm containing degradative enzymes. The rod composition in *B. subtilis* is unknown. Four genes within the flagellar cluster – *flgB, flgC, flhO*, and *flhP* – encode putative rod proteins, but their function is untested. Here, we utilize *B. subtilis* as a model organism to assess rod order and assembly.

**Methods**  
Cell motility is measured by swarm assay using LB plates containing 0.7% agar, briefly dried, and incubated in a 37°C humid chamber. Swarming radii are measured every 30 minutes. Cell membranes are isolated from cytoplasm via ultracentrifugation of lysed cells. Flagellar complexes were further isolated for EM imaging via ammonium sulfate precipitation and separation on a CsCl gradient.

**Results**  
Swarm assays of *flgB, flgC, flhO*, and *flhP* mutants indicate that the putative rod proteins are required for swarming motility. Immunoblots of cell membranes show that relative to the wild type strain, putative rod mutants do not assemble hooks but do incorporate basal body components. Further, although immunoblots of wild type and *flgE* strains indicate that all four proteins are present in membranes, *flgB* membranes do not contain any putative rod proteins.

Similarly, *flgC* membranes do not incorporate FlhO or FlhP, but do produce a signal when probed with α-FlgB antibody. Only FlgB and FlgC signals are present in the *flhO* strain, and *flhP* membranes contain FlgB, FlgC, and FlhO proteins.

**Conclusion**  
We infer from these data that the *Bacillus subtilis* rod consists of the proteins FlgB, FlgC, FlhO, and FlhP, and that they are assembled – from cell proximal to cell distal – in that order. We hypothesize that the rod is not actually metastable but broken down by degradative enzymes if not fully formed.
**SWIMMING AND GLIDING MOTILITIES OF THE SPIROCHETE LEPTOSPIRA**

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**Background**

*Leptospira* are spirochetes, possessing one periplasmic flagellum (PF) at each end of the cell body. The rotation of PFs transforms the cell end into either spiral (S) shape or hook (H) shape. *Leptospira* not only swim in fluids but also glide over solid surfaces. Recently, we isolated a mutant that remains coiled configuration of right-handed protoplasmic cylinder (PC) but lacks the bended cell end, i.e., S-end and H-end, due to straightened PFs. In this study, we quantified movements of wild-type *Leptospira* and the “unbent” mutant to understand how the three distinct rotations are involved in leptospiral swimming and gliding motilities.

**Methods**

We recorded free-swimming and gliding movements of the WT *Leptospira* and the unbent mutant in various viscous liquids, and measured their speeds (v) and cell-body rotation frequencies (f); the ratio of v to f (v/f) indicates the migration distance by one revolution of the helical cell body. We evaluated how much the bacteria slip while translating by calculating the ratio of v/f to the wavelength of PC.

**Results**

The WT cells swam in a wide range of viscosity. The unbent mutant did not translate in low viscosity, but its swimming speed was increased as viscosity was increased by the addition of methylcellulose or mucin to media. Both WT and the mutant glided on the glass surface; the gliding speeds were faster than the swimming speeds. Both strains showed translation without slippage in extremely high viscosity and on a glass surface.

**Conclusion**

Our data showed that S-end rotation was essential for free-swimming in low viscosity and its contribution got lower with viscosity in viscoelastic fluids. The rotation of PC allowed the cell to glide on the surface without slippage. These results quantitatively prove the theoretical model suggested by Berg et al. (Symp. Soc. Gen. Microbiol., 1978) and the pioneering observation by Goldstein & Charon (PNAS, 1990).
TORQUE-DEPENDENT MOTOR REMODELING AND MECHANOSENSING IN *E. COLI*

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**Background**  
Bi-directional flagellar motors rotate helical filaments in *Escherichia coli* to propel the cell. Each motor comprises of a torque-generating stator complex and a rotor that consists of a molecular directional-switch. We showed previously that the stator-complex is responsible for surface-sensing, an ability that is likely involved in triggering swarming-motility. The stators remodel in response to perturbations in the viscous loads, and we hypothesized that the underlying mechanism is torque-dependent. That is, the amount of torque generated by individual units regulates the affinity between the stator-unit and the rotor. FliL, an inner membrane protein, has been reported to be important in the stabilization of torque-generation, at high viscous loads. As a result, fliL mutants are generally defective in swarming motility in several bacterial species.

**Methods**  
Mechanical load-stimulation experiments were employed to test whether torque regulates stator-binding in strains lacking FliL.

**Results**  
Our results suggest that the loss of FliL has no effect on stator-remodeling at near stall loads. At low loads, direct tests of fliL mutants and wildtype cells confirmed previous results that suggested only minor differences in the swimming speeds.

**Conclusions**  
We will discuss how these results can be interpreted to test the torque-dependency hypothesis for remodeling and motor-mechanosensing.
FILAMENT-SUBSTRATE INTERACTIONS AND THEIR EFFECTS ON SWARMING MOTILITY

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Background
Certain species of motile bacteria exhibit a striking form of two-dimensional group motility associated with solid substrates, called swarming motility. Swarming is sustained by the rotation of flagellar filaments and the thrust generated by each swarming cell depends on a variety of factors, prominent among these are viscous drag, motor torque, substrate properties, and filament shape. We are interested in determining how flagellar shapes and filament-substrate hydrodynamic interactions affect swarming.

Methods
Fluorescent labeling and high-speed imaging were employed to visualize the filament forms in these mutants.

Results
We will discuss our preliminary findings detailing the effect of specific mutations within the flagellum on swarm diameters and running speeds.

Conclusion
It is anticipated that our experiments will enable an estimation of the influence of filament shape on swarming, as well as the resultant thrust developed per cell over changing substrate-properties. These results are likely to provide important insights into the hydrodynamics of swarming at a single-cell level.
ANALYSIS OF A flID MUTANT REVEALS A DUAL POSTTRANSCRIPTIONAL REGULATORY MECHANISM OF BORRELIA BURGDORFERI FLAGELLIN

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Background
The Lyme disease spirochete Borrelia burgdorferi (Bb) lacks the transcriptional cascade control of flagellar protein synthesis common to other bacteria. Instead, it relies on a post-transcriptional mechanism to control its flagellar synthesis; however, its underlying mechanism remains elusive. The hook-associated protein HAP2 (also known as flID) functions as a rotary cap that promotes flagellin assembly to form a long helical filament. Herein, we report that flID (BB_0149) is not only essential for filament formation and spirochete motility but also controls the translation and stability of FlaB, a major flagellin protein that is essential for flagellation and motility of Bb.

Methods
In this study, we used gene inactivation followed by Cryo-electron tomography (Cryo-ET) to study the morphology of the mutant; Furthermore, we utilized genetic and biochemical assays to dissect the mechanism of the regulation of Bb flagellin.

Results
An isogenic mutant of flID is non-motile. Single cell cryo-electron tomography (cryo-ET) analysis reveals that the flID mutant lacks the filaments and the pentagon-shaped cap at the tip of the hook. Interestingly, the level of FlaB in the flID mutant is even lower than those in the hook- (FlgE), rod- (FlgG), and MS-ring- (PliF) deficient mutants, suggesting that deletion of flID significantly impairs the accumulation of FlaB. Protein turnover analysis reveals that FlaB is degraded in the flID mutant but not in the flgE mutant. Genetic and biochemical assays indicate that the turnover is most likely mediated by BB_0104, a protease of Bb. Our recent studies indicate that CsrA, a homolog of carbon storage regulator A, regulates the FlaB synthesis by inhibiting translation initiation of the flaB transcript. Deletion of csrA in the flID mutant partially restores FlaB accumulation.

Conclusion
Taken together, we propose that CsrA and BB_0104 collectively control the synthesis and homeostasis of FlaB and subsequent filament assembly and that deletion of flID leads to the leakage of FlaB monomers into the periplasmic space where it is degraded by BB_0104.
IMAGING THE MOTILITY AND CHEMOTAXIS MACHINERIES IN HELICOBACTER PYLORI BY CRYO-ELECTRON TOMOGRAPHY

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Background
Helicobacter pylori is a highly motile bacterial pathogen that can move readily within the viscous mucosal layer of the stomach. Its unique motility, which is essential for successful gastric colonization and pathogenesis, is driven by chemotactic signaling system and multiple unipolar flagella. However, the motility and chemotaxis machineries in H. pylori have not yet been visualized at high resolution, considerably limiting our understanding of its motility.

Method
We employed cryo-electron tomography to image intact wild type H. pylori cells, with particular focus on flagella and chemoreceptor arrays. We collected ~180 tomograms from the frozen-hydrated bacteria, and analyzed them by subtomogram averaging and classification. The final structures of the chemotaxis signaling complex and the flagellar motor were derived from several hundred subtomograms of hexagonal lattices and flagellar motors, respectively.

Result
H. pylori possesses one of the largest flagellar motors observed by far. It is ~86 nm in diameter and ~81 nm in height (measured from the export apparatus to the outer membrane). Remarkably, a cage-like periplasmic structure with 18-fold symmetry was observed for the first time. It appears that the cage-like structure forms a robust platform for recruiting 18 torque generators. Furthermore, a series of key flagellar assembly intermediates were captured, providing structural evidence that flagellar assembly is tightly coupled with the biogenesis of the membrane sheath. Besides, the overall structure of the chemotaxis array in H. pylori is consistent with the universal architecture of bacterial chemoreceptor arrays (1).

Conclusion
Our cryo-ET studies provide new structural insights into the unique motility of H. pylori. The unipolar flagellum of H. pylori possesses a unique periplasmic cage-structure, which likely provides a stable platform to recruit and stabilize more stators for higher torque generation.

DECIPHER THE INTACT CHEMOTAXIS SIGNALING COMPLEX STRUCTURES IN SITU

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Background
The bacterial chemotaxis receptors form highly ordered arrays, which sense chemical stimuli and transmit this information through a phosphorylation cascade that regulates cell motility. The core signaling complex contains two trimers of homodimeric receptors, two monomers of the couple protein CheW, and one dimer of histidine kinase CheA (Li and Hazelbauer, 2011). The receptor dimer has three functional domains (ligand binding domain, HAMP domain and kinase-control domain), and the CheA is composed of five domains (named as P1 to P5). The structure and function of the core complex and its components have been extensively studied (Parkinson, et al 2015). In particular, cryo-electron tomography (cryo-ET) was used to visualize the chemotaxis arrays, providing structural models of the core complexes and their higher-order assemblies (Briegel et al., 2012; Cassidy et al., 2015; Liu et al., 2012). However, previous cryo-ET studies fails to reveal some key functional domains including the ligand binding domain and HAMP domain of the receptor and the P1 and P2 domains of CheA, likely because of their conformational heterogeneity. In this study, we aim to reveal the intact core complex structures *in situ* as they are required for a better understanding of the signaling and sensory adaptation within the array.

Method
We developed a new cryo-ET pipeline to extract and analyze the receptor arrays in *Salmonella* minicells. Our new procedure allows us to identify over 10 thousands of core complexes from several hundred bacteria cells. Subtomogram classification was used to determine distinct conformations of the core complexes. Atomic models of the intact core complex were built by homology modeling and molecular dynamic flexible fitting.

Result
The overall structure of the core complex *in situ* was resolved at 18 Å resolution. However, the electron densities of ligand binding domain and the HAMP domain are absent in the global average map. By using classification, we found that the ligand binding domain apparently adopts multiple conformations, and thus built the models of the intact core complex based on the class averages. Furthermore, we back plotted the hexagon and the trimers to the minicells to analysis their distribution.

Conclusion
We developed methods for structure determination of the core complex in situ. This method allows us to determine the *in situ* structures of the intact core signaling complex for the first time and revealed its intrinsic plasticity.
ROLE OF AI-2 CHEMOTAXIS IN E. coli BIOFILM DEVELOPMENT

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Background
Bacteria use chemotaxis to navigate within their environment by responding to gradients of chemical cues, including nutrients, toxins, pH, temperature and oxygen. Recent studies have demonstrated that E.coli can also sense signaling molecules, including the bacterial interspecies autoinducer-2 (AI-2). These signals are likely to be encountered in the microenvironments inhabited by E. coli and can, therefore, affect niche localization and colonization. In this study, we explore the role of AI-2 chemotaxis in biofilm formation and elucidate the molecular mechanism employed to sense AI-2.

Methods
We quantified biofilm formation using the crystal violet assay and observed the different stages of biofilm development using GFP-tagged cells. We measured the AI-2 chemotaxis response of LsrB and Tsr single-residue variants using capillary assays to validate a docking model of the interaction between AI-2-bound LsrB and Tsr.

Results
Strains lacking either of the key AI-2 receptor components, the binding protein LsrB and the Tsr membrane sensor, are defective in biofilm formation. A wild-type strain rapidly transitions from individual motile cells to surface-associated aggregates under static conditions. Cells deleted for lsrB or tsr delay this transition, suggesting that chemotaxis toward AI-2 enhances biofilm development. Strains lacking either Tsr or LsrB are defective in AI-2 chemotaxis, providing additional support for this hypothesis. Furthermore, detection of an AI-2 gradient does not require induction of the lsrACDBFG operon, and a very small amount of LsrB (~50-70 molecules/cell) suffices to elicit a normal chemotaxis response. Strains expressing proteins with specific single-residue substitutions in Tsr or LsrB were defective for AI-2 chemotaxis. These results support a computer-generated model for the docking of AI-2-bound LsrB to Tsr.

Conclusion
Our results suggest that AI-2 chemotaxis facilitates formation of dense aggregates to enhance biofilm formation. Chemotaxis appears to enhance biofilm development via sensing of AI-2 gradients through the direct interaction of ligand-bound LsrB with Tsr. This interaction must be of high affinity because of the relatively low level of LsrB required for AI-2 chemotaxis. Residues of opposite charge at the interaction surfaces of both proteins may be involved in stabilizing the docked complex.
A MUTANT LACKING FliR, A COMPONENT OF THE FLAGELLAR EXPORT APPARATUS, EXHIBITS DEFECTS IN FLAGELLAR BIOSYNTHESIS AND EXOPOLYSACCHARIDE PRODUCTION THAT ARE OVERCOME BY MODULATING CYCLIC DI-GMP LEVELS.

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Background
*Pantoea* sp. YR343 is a soil microbe that was isolated from the rhizosphere of *Populus deltoides* where it was found to be a robust colonizer of poplar roots. *Pantoea* sp. YR343 possesses plant growth-promoting properties, such as indole-3-acetic acid biosynthesis and phosphate solubilization. This microbe also exhibits both swimming and swarming motility. In a transposon mutagenesis experiment, we isolated 137 mutants that were unresponsive to high levels of c-Di-GMP and over one third of these mutants were affected in the flagellar export apparatus component, FliR. Currently, the role of FliR within the flagellar export apparatus is not well known. We will present ongoing work on characterizing the function of FliR, particularly within the context of c-Di-GMP signaling and root colonization within the rhizosphere.

Methods
In addition to transposon mutagenesis, we have also constructed a Δ*fliR* deletion strain. This mutant has been characterized using various techniques such as genetic tools for gene expression and labeling, motility assays, immunofluorescence, and electron microscopy. We have also examined exopolysaccharide production and root colonization for this mutant.

Results
We constructed a strain of *Pantoea* sp. YR343 lacking the flagellar export apparatus component, FliR, based on identification of this gene through transposon mutagenesis. We have found that the loss of FliR results in a lack of swimming motility due to incomplete formation of flagella; however, this mutant can still swim, but at a slower pace. We have also found that a Δ*fliR* mutant is defective in exopolysaccharide production and root colonization. Interestingly, we found that increasing the levels of c-di-GMP by overexpressing diguanylate cyclases resulted in "wild type" motility and exopolysaccharide production phenotypes.

Conclusions
FliR is a critical component of the flagellar export apparatus, without which there is no fully functional flagella formation. Loss of FliR affects various behaviors in *Pantoea* sp. YR343 including exopolysaccharide production, biofilm formation, and root colonization. One of the more interesting observations includes the finding that increasing the levels of c-di-GMP in a Δ*fliR* mutant partially rescued the motility defect as well as resulting in a decrease in biofilm formation, which is "backwards" to that observed in a wild type strain of *Pantoea* sp. YR343. We are currently investigating the mechanism(s) by which FliR affects flagella biosynthesis and EPS production, and how levels of c-di-GMP can modulate these behaviors.

Figure 1. Fluorescent staining of flagella in wild type *Pantoea* sp. YR343 and a Δ*fliR* mutant.
UNCOMMON DUAL FUNCTION OF THE CYCLIC-DI-GMP BINDING PROTEIN IN BORRELIA BURGDORFERI

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Background and Results
The Lyme disease spirochete Borrelia burgdorferi cycles between disparate environments of the Ixodes scapularis tick and mammalian host. The second messenger cyclic-di-GMP is known to control a variety of cellular processes including, but not limited to, motility, chemotaxis, biofilm formation, gene expression, and virulence. Unlike most other bacteria, B. burgdorferi possesses only one set of c-di-GMP-metabolizing genes and one c-di-GMP-binding PilZ-domain protein designated as PlzA. Cyclic-di-GMP is primarily instrumental in orchestrating the adaptation of B. burgdorferi to the tick environment. Our data indicate that c-di-GMP controls motility/chemotaxis as well as persistence of B. burgdorferi in the hosts. To determine the mechanism of how the second messenger controls these factors, we performed iTRAQ proteomics. Our quantitative proteomics data suggest that expression of several proteins were modulated in ΔplzA. Moreover, we demonstrate that PlzA specifically interacts with the chemotaxis response regulators CheY1 and CheY3 but not CheY2 or the phosphatase CheX.

Conclusion
The interactions described above may be crucial for influencing the flagellar motor rotation and chemotaxis behavior of the spirochete. Because motility and chemotaxis are critical for the spirochete’s persistence, dissemination, and transmission from tick to mammalian host, this shows a new avenue of controlling ‘overall virulence’ by the c-di-GMP of B. burgdorferi. Moreover, we demonstrate that PlzA binds to DNA to control gene expression. PlzA possesses no classical DNA-binding domain, however, we were astounded to discover that this c-di-GMP binding protein, that shares very little homology with other PilZ-proteins, has dual function; protein-protein interaction to control chemotaxis and DNA-binding activity to govern the expression of genes. We are currently investigating the consequences of these interactions in the enzootic life cycle of B. burgdorferi.

A new direction of controlling motility. Cyclic-di-GMP receptor PlzA binds to DNA to control gene expression and CheY proteins to regulate flagellar motor rotation in Borrelia burgdorferi.
CONSEQUENCES OF GLYCOSYLTRANSFERASE DISRUPTION IN *BORRELIA BURGDORFERI* BIOFILM FORMATION

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**Background**
*Borrelia burgdorferi*, the spirochete causing Lyme disease is an ingenious pathogen known for its antibiotic resistant nature and accordingly alters its motile wave-like morphology to cystic, granular or cell wall-deficient forms to counteract eradication from its vertebrate or *Ixodes* tick host. Besides, *B. burgdorferi* forms biofilm *in vitro* as well as *in vivo*, which might enhance its persistence despite antibiotic treatment. Alginate is the major component of the extracellular polymeric substances (EPS) making up the biofilm. Here, we engineered a potential candidate for the biofilm EPS in *B. burgdorferi* that potentiates transfer of sugar moieties to a variety of substrates significant in biological processes including signaling, biofilm formation, adhesion, and cell wall biosynthesis.

**Methods**
A candidate gene that encode for glycosyltransferase in *B. burgdorferi* was inactivated to test its biofilm forming capabilities using *in vitro* crystal violet assays and *in vivo* within tick-mouse models.

**Results**
The genes involved in *B. burgdorferi* biofilm formation are still not classified. Additionally, there is no homology of biofilm-forming genes in *B. burgdorferi* when compared to the biofilm-forming model organisms. We identified a gene termed glycosyltransferase in *B. burgdorferi* which shares only limited homology with *pelF* gene of *Pseudomonas aeruginosa*. The candidate mutant engineered is currently under investigation.

**Conclusion**
In this work, we intend to demonstrate if glycosyltransferase plays a role in biofilm formation in *B. burgdorferi* and could thus be used as a potential target for the development of antimicrobial agents against these complex structures.

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**Lyme patient 1**

**Lyme patient 2**

Two skin tissue sections obtained from two separate Lyme disease patients showing aggregates stained positive with *Borrelia*-specific antibody (Panels A and C) and alginate- specific antibody (Panels B and D). Source: Sapi et al., 2015.
TYPICAL PROTEINS WITH ATYPICAL FUNCTION UNCOVERED IN THE LYME DISEASE SPIROCHETE BORRELIA BURGDORFERI

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Background
*Borrelia burgdorferi*, the causative agent of Lyme disease, possesses a sophisticated and complex chemotaxis system, which includes three response regulators CheY1, CheY2 and CheY3. Each CheY is phosphorylated by the histidine kinase CheA1 or CheA2. Moreover, we found that CheY2 and CheY3, but not CheY1, are involved in virulence (i.e. dissemination within the host, transmission from tick to mouse, and persistence infection of the host). However, we noticed that only CheY3 is involved in motility or chemotaxis despite possessing the “characteristics” of a classical chemotaxis response regulator. A CheY protein typically binds FliM to alter flagellar motor rotation. However, the patterns of how spirochetal CheYs interact with FliM was unknown. Moreover, the stability of each CheY-P, which is also important to depict how the pathway of chemotaxis and motility works in *B. burgdorferi*, was unknown.

Methods & Results
Our affinity blot data suggest that CheY1 and CheY3, but not CheY2, binds specifically to FliM. Furthermore, it appears that the binding affinity of FliM is considerably higher for CheY3 compared to CheY1. *In vitro* phosphorylation assays demonstrated that both CheY1-P and CheY3-P had a similar half-life, which is remarkably longer than that of CheY2-P, and that replacement of *B. burgdorferi* CheY2 Asp59 with glutamate completely abolished the phospho-transferring from CheA.

Conclusion
Based on our data, we propose that *B. burgdorferi* CheY3 plays a role that is similar to what was reported in most other bacterial chemotaxis response regulators. CheY1 appears to be dispensable for motility, chemotaxis, and persistence infection. CheY2 plays an atypical role despite resembling a classical chemotaxis response regulator. We propose that CheY2 serves as a regulator for some *B. burgdorferi* virulence determinant(s) that are required for productive infection within vertebrate, but not tick hosts.
DETECTION OF LOCAL PH DIFFERENCES IN LIVING BACTERIAL CELL BY HIGH-RESOLUTION PH IMAGING

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Background
The flagellar type III protein export apparatus is required for construction of the bacterial flagellum beyond the cellular membranes. The type III protein export apparatus is composed of a transmembrane export gate and a cytoplasmic ATPase complex consisting of FliH, FliI ATPase and FliJ. The export apparatus utilizes ATP and proton motive force as the energy source for efficient and rapid protein export during flagellar assembly, but the energy transduction mechanism remains unclear.

Methods
We have developed an in vivo pH imaging system with high spatial and pH resolutions with a pH indicator probe, pHluorin(M153R)-FliG, to measure local pH differences within living Salmonella cells, especially in the close proximity of the cytoplasmic membrane and the export apparatus.

Results
The local pH near the membrane was ca. 0.2 pH units higher than the bulk cytoplasmic pH. However, the local pH near the export apparatus was ca. 0.1 pH units lower than that near the membrane. This drop of local pH depended on the activities of both transmembrane export components and FliI ATPase.

Conclusion
We suggest that ATP hydrolysis by the ATPase complex and the following rapid protein translocation by the export gate are both linked to efficient proton translocation through the gate.

Local pH around the flagellar export apparatus

\[
\begin{align*}
\text{pH}_{\text{local}} & = 7.43 \\
\text{pH}_{\text{local}} & = 7.55
\end{align*}
\]
TO BUILD A BIOFILM

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Background
Work in the O’Toole lab focuses on the study of surface-attached microbial communities known as biofilms. These surface attached communities can be found in medical, industrial and natural settings. In fact, life in a biofilm probably represents the predominate mode of growth for microbes in most environments. Biofilm microbes are typically surrounded by an extracellular matrix, which provides structure and protection to the community. Biofilm-grown bacteria are notorious for their tolerance to a range of antimicrobial agents including clinically relevant antibiotics.

Methods
We use a combination of genetic, molecular, biochemical and imaging techniques.

Results
Our studies indicate that Pseudomonas aeruginosa, an important opportunistic pathogen, can detect surface contact via a pathway requiring Type IV pili (TFP) and a membrane-bound signaling complex that generates the second messenger cAMP. Moreover, our recent findings using cell tracking of entire communities at single-cell resolution combined with a cAMP reporter support the model that multi-generation signaling via this cAMP-dependent pathway is required for this microbe to commit to initiating biofilm formation. That is, cAMP levels accumulate over multiple generations in response to TFP engagement with a surface. Our data also indicate that irreversible attachment, the first committed step in biofilm formation, requires this cAMP-mediated signaling to modulate TFP and flagellar function interactively and systematically, thereby promoting stable surface attachment and downstream biofilm development.

Conclusions
P. aeruginosa, and likely other microbes, have developed means to sense surface contact.

Early Events in Biofilm Formation

- **Bacteria Contact a Surface**
- **Increase a Signal**
  - $\Rightarrow$ **cAMP**
- **Pili**
DETERMINING THE SENSING PROFILE OF A HELICOBACTER PYLORI CHEMORECEPTOR THAT MODULATES HOST INFLAMMATION

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Background
Helicobacter pylori is a Gram-negative bacterium that infects half of the world’s population, causing gastritis, peptic ulcers, and gastric cancer. To establish chronic infection within the stomach, H. pylori utilizes chemotaxis. This system allows H. pylori to sense environmental signals, guiding its motility towards its preferred niche within the stomach. In addition to its importance for colonization, chemotaxis modulates the host immune response. More specifically, strains lacking the transmembrane chemoreceptors TlpA or TlpB cause hyperinflammation within the stomach. Known ligands are sensed by each chemoreceptor, however the list is likely not exhaustive as chemoreceptors are capable of sensing multiple ligands. To better understand the role of TlpA in vivo, this work aims to identify and characterize additional ligands sensed by TlpA.

Methods
Potential ligands of TlpA are screened via ligand binding arrays and surface plasmon resonance against the periplasmic, sensing portion of these chemoreceptors. Chemotactic responses to hits from these screens are then assessed using supplemented soft agar motility plates, live cell tracking, and a wet-mount microscope slide assay.

Results
Preliminary screening revealed TlpAper binds arginine, a known ligand sensed by TlpA (Cerda et al, 2003; Cerda et al, 2011). We have employed several assays to assess whether arginine is a chemotaxis-active ligand for TlpA. Using supplemented soft agar motility plates and live cell tracking, both swarm diameter and frequency of reversals, respectively, were unchanged upon addition of arginine. Additionally, we were unable to detect a chemotactic response to arginine that was dependent on TlpA in the wet-mount microscopy slide assay.

Conclusion
Ligand binding arrays allow for the identification of potential chemoreceptor ligands in a high throughput fashion. While arginine, a previously identified ligand for TlpA, was a hit from the screen, we have not detected a significant chemotactic response to arginine in vitro. Optimization of in vitro chemotaxis assays is required to improve their sensitivity and detection of potentially subtle chemotactic responses.
SPATIAL AND TEMPORAL SHIFTS IN BACTERIAL BIOGEOGRAPHY AND GLAND OCCUPATION DURING THE DEVELOPMENT OF A CHRONIC INFECTION

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Background
*Helicobacter pylori* chronically infects more than 50% of the human population worldwide, living in the gastrointestinal tract causing gastritis, ulcers and gastric cancer. Chemotaxis has been shown to be essential for correct colonization deep inside gastric glands and for pathogenicity. To understand, how chemotaxis assists bacteria to colonize different sub-regions of the stomach and the glands, we tracked bacterial localization patterns inside different stomach regions over time. Therefore, we developed a quantitative gland isolation method – BLIG (bacterial localization in isolated glands).

Methods
Infections of mice with different *H. pylori* mutant strains expressing fluorescent proteins as GFP or RFP and strains deficient in chemotaxis. Single or Sequential infections of different strain combinations, Colonization and localization studies (plating and microscopy), BLIG

Results
After infections in the murine model system, *H. pylori* populations multiply both inside and outside glands in a manner that requires the bacteria to be motile and chemotactic. *H. pylori* is able to achieve gland densities averaging 25 to 40 bacteria/gland after 2 to 4 weeks of infection. After 2 to 4 weeks of infection, a primary infection leads to colonization resistance for a secondary infection. Nonetheless, about ~50% of the glands remained unoccupied, suggesting there are as-yet unappreciated parameters that prevent gastric gland colonization. During chronic infections, *H. pylori* populations collapsed to nearly exclusive gland localization, to an average of <8 bacteria/gland, and only 10% of glands occupied. We analyzed an *H. pylori* chemotaxis mutant (Che-) to gain mechanistic insight into gland colonization. Che- strains had a severe inability to spread to new glands and did not protect from a secondary infection but nonetheless achieved a chronic gland colonization state numerically similar to that of the wild type.

Conclusions
Our analysis shows that bacteria undergo substantial population dynamics on the route to chronic colonization, that bacterial gland populations are maintained at a low level during chronic infection, and that established gland populations inhibit subsequent colonization.

Figure: BLIG – Bacterial localization inside isolates gastric glands. Stomach isolations and gland isolation to track GFP expressing bacteria inside the stomach glands.
CHEMORECEPTOR SIGNAL CONTROL THROUGH ALTERNATIVE HELIX-PAIRING INTERACTIONS AT THE MH BUNDLE CAP

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Background
Escherichia coli senses attractant chemicals with transmembrane receptors known as methyl-accepting chemotaxis proteins (MCPs). These homodimeric molecules typically contain a periplasmic ligand-binding domain and a cytoplasmic signaling domain that regulates the autokinase activity of CheA in response to ligand occupancy changes. MCP signaling domains contain 4-helix HAMP, methylation, and protein interaction bundles. The HAMP domain is thought to control CheA output signals by modulating the packing stability of the adjacent methylation helix (MH) bundle, but the structural mechanism is poorly understood. The E. coli serine chemoreceptor Tsr contains three highly conserved residues (F515, L508, L501) at the C-termini of the MH2 and MH2’ helices. These MH bundle cap residues are located on a packing face that interacts with the MH1/MH1’ helices adjoining the AS2/AS2’ HAMP output helices and might play an important role in HAMP-mediated signal control.

Methods
Mutant receptors with amino acid replacements at F515, L508, or L501 were constructed by all-codon mutagenesis and characterized with soft agar plates and in vivo FRET kinase assays.

Results
Almost every amino acid replacement at F515, L508, and L501 abrogated serine chemotaxis. Most of those mutant receptors activated CheA, but failed to down-regulate kinase activity in response to serine. The locked-ON behavior of the mutant receptors indicates that the conserved LLF motif enables the receptor to reach the kinase-OFF structural state during HAMP signal transmission. Perhaps the F515/F515’ residues stabilize two-helix packing (MH2/MH2’), reinforced by hydrophobic interactions between the L508/L508’ and L501/L501’ residues.

Conclusions
Previous studies implicated a stably packed four-helix (4H) methylation bundle in production of kinase-ON output. Our results suggest that stable packing of MH2-MH2’, a two-helix (2H) structure, produces kinase-OFF output. This 2H-4H model predicts that serine stimuli promote kinase-OFF output by favoring the 2H structure at the bundle cap, which in turn destabilizes the 4H conformation in the MH bundle. The poly-alanine tract adjacent to L501 might allow the MH bundle to adopt predominantly 4-helix packing arrangements in both output states, as the MH bundle cap transitions between 2H and 4H conformations.
DOES BACILLUS SUBTILIS LIKE TO GET DRUNK?

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Background
Alcohols are common bactericides. In many bacterial species, they are chemorepellents. By contrast, we recently found that alcohols are chemoattractants for Bacillus subtilis.

Methods
We employed the capillary assay to measure chemotaxis to different alcohols. We then used the combination of site-directed mutagenesis and isothermal titration calorimetry (ITC) to identify binding properties of alcohols to chemoreceptors.

Results
Chemotaxis to alcohols is dose-dependent. Only short-chain alcohols (methanol, ethanol, and n-propanol) are chemoattractants. The chemoreceptors for alcohols are HemAT, involved in aerotaxis, and McpB, involved in asparagine chemotaxis. Using site-directed mutagenesis and ITC, we identified the amino acid residues on HemAT that directly interact with alcohols. These residues on HemAT are also involved in oxygen-sensing associated with heme group. These results suggest that HemAT may use a similar mechanism in sensing oxygen and alcohols.

Conclusion
B. subtilis naturally inhabits the soil and can colonize plant roots. In the rhizosphere, B. subtilis can benefit from plant exudates and can promote growth and protect plants from infections. How bacteria are recruited to plant roots is not well understood. In anoxic conditions, plant roots ferment carbohydrates to ethanol. Ethanol secretion by plant roots during fermentative metabolism may mediate the accumulation of B. subtilis at its site of secretion. This hypothesis is also supported by earlier evidence, where ethanol chemotaxis was shown to encourage accumulation of zoospores of Phytophthora cinnamomi at plant roots.
NOVELTY IN SINORHIZOBIUM MELILOTI CHEMOTAXIS: CHET

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Background
*Sino*rhizobium *meliloti* moves through the soil using several peritrichous flagella. It is best known for its symbiotic relationship with legumes, in which it fixes nitrogen in exchange for a carbon source. An early step in forming this symbiosis is chemotaxis towards the host plant. A number of attractants have been discovered for *S. meliloti*, namely amino acids mediated by McpU and betaines mediated by McpX. Previous studies revealed that *S. meliloti*’s chemotaxis signal transduction system differs significantly from *Escherichia coli*. Although phosphotransfer reactions are well understood, the adaptation system is uncharacterized. *cheT* is the last gene of unknown function in *S. meliloti*’s major chemotaxis operon. Here, we are presenting initial results showing involvement of CheT in adaptation.

Methods
A glutaraldehyde crosslinking assay was implemented to screen all *S. meliloti* chemotaxis proteins for interaction with CheT. The putative interaction partner was co-expressed with CheT in *E. coli* from a pETDUET™-1 vector. Interactions were confirmed with affinity and size exclusion chromatography. Finally, a methylation assay was performed to observe the effects of CheT on CheR receptor methylation.

Results
CheR was revealed as an interaction partner of CheT. This result was confirmed by co-purification of CheT and CheR. We then analyzed the effect of CheT on CheR’s methyltransferase activity. We detected a small but significant increase in total methylation in the presence of CheT. Methylation of McpX cause conformational changes which alters McpX’s mobility during SDS-PAGE. We observed differences in this mobility of McpX during SDS-PAGE, in the presence of CheR and CheT, compared to CheR alone.

Conclusions
We have identified a new interaction partner for CheR in the chemotaxis system of *S. meliloti*. Our preliminary results suggest CheT is increasing overall methylation of McpX. However, we speculate that this effect is secondary and that the primary function of CheT is to confer methylation site preference. In future work, we will further investigate the contribution of CheT to methylation site preference in McpX, analyze the effect of McpX ligand binding on CheR and CheT activity, and test the involvement of CheT in methylation of other *S. meliloti* chemoreceptors.
THE ORGANIC ACID SENSING PROFILE OF *SINORHIZOBIUM MELiloti* AS MEDIATED BY MCPV

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**Background**  
Alfalfa is an important forage crop that has very high protein yields due to the symbiotic association with the bacterium *Sinorhizobium meliloti*. Through a series of concerted plant-microbe interactions, the bacterium is ingested into a root organ called a nodule, where it is provided plant photosynthates in order to support nitrogen fixation activity, ultimately yielding ammonium to the plant. We hypothesize that localization of the bacterium to the plant root is the first step in initiating this symbiosis and is mediated by chemotaxis to the source of attractant compounds. Plants secrete organic acids from their roots, and we have identified a chemoreceptor responsible for sensing some of these compounds.

**Methods**  
Behavioral assays were performed with the traditional capillary assay. Motile cells swim into a capillary filled with an attractant and are removed and plated to obtain cell counts per capillary. Binding of compounds from Biolog plate PM1 to a ligand binding domain of a chemoreceptor was screened *in vitro* using Differential Scanning Fluorimetry (DSF). Unfolding of the protein construct was observed with a fluorescent dye, and ligands that heat stabilize the protein are putative ligands. Structural modeling was done using the SWISS Model site and the periplasmic region of a putative acetate sensor from *Anaeromyxobacter dehalogenans* (PDB id 4k08).

**Results**  
In this work, 2-4 carbon acids are shown to be chemoattractants for *S. meliloti*. *In vitro* experiments and structural homology modeling suggest Methyl-accepting Chemotaxis Protein V (McpV) as a likely receptor for small monocarboxylic acids. Modelling to a homologue of McpV revealed the protein with acetate in the binding pocket.

**Conclusion**  
Our data suggest that *S. meliloti* uses McpV to sense small carboxylic acids and swim to their source. We postulate that McpV directly binds organic acids that are 2-3 carbons in length.
MOLECULAR MODELING AND SIMULATION OF *E. coli* SERINE RECEPTOR

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Background
Central to their chemotactic ability, *E. coli* cells possess dedicated chemoreceptors, which couple ambient concentration gradients to the activity of an associated histidine kinase and ultimately cellular swimming pattern. Forming a long series of coiled-coils and helices, chemoreceptors are comprised of several adjacently coupled signaling elements whose structure and dynamics are affected by ligand binding and adaptational modification. Through the subtle balance of these perturbations, sensory information is robustly transmitted across the cell’s inner membrane and over 30 nm to the site of kinase binding. Though the subjects of intense study for several decades, a number of features regarding the detailed molecular mechanism of chemoreceptor signal transduction and regulation are unresolved.

Methods
We have utilized computational modeling and molecular dynamics (MD) simulations, in tandem with existing biochemical and crystallographic data, to construct an atomistic model of the intact, membrane-bound *E. coli* serine receptor (Tsr, Fig. 1). Through the appropriate mutation of critical adaptation sites along the receptor cytoplasmic domain, models representing the kinase-ON and kinase-OFF states are also constructed.

Results
Our Tsr model provides atomistic insight into the structure of several key signaling regions, including the TM four-helix bundle and HAMP domains as well as the elusive five-residue control cable region. In addition our model highlights important protein-lipid interactions and suggests a role for the unstructured N-terminus. A series of long-timescale, MD simulations display regions of increased flexibility and reveal a number of distinct hinges around which chemoreceptors are able to bend. Additional MD simulations of the fully methylated and fully demethylated Tsr receptor suggest that patterns of flexibility and bending are not functions of adaptation state.

Conclusions
An atomic model of the intact, membrane-bound *E. coli* serine receptor is presented. MD simulations are used to provide further insight into the dynamical characteristics of chemoreceptors and investigate the role of adaptational modification.

*Figure 1:* Atomic model of the *E. coli* Tsr dimer in a lipid bilayer. Key functional areas are labeled to the left. The individual monomers are shown in red and blue. Sites of adaptational modification are shown as red and green spheres.
Clustering of bacterial chemoreceptors is important for functional aspects of signaling such as signal amplification and adaptation\(^1\), but there exist seemingly conflicting reports\(^2-6\) in the literature regarding the stability of these clusters over signaling timescales \textit{in vivo}. We have probed the spatial organization and mobility of \textit{Escherichia coli} chemoreceptor clusters using photoactivation localization microscopy\(^7\) (PALM) and single-particle tracking (SPT). Our results reveal that the cluster-size distribution is altered shortly after ligand stimulation. The change in the observed cluster-size distribution is substantial, yet does not drastically affect either the global pattern of localization (observed previously by diffraction-limited imaging\(^2-5\)), or the nanoscale organization of the ternary complex (resolved previously by cryo-EM\(^6\)). We suspect it is related to the slow ligand-induced changes in chemoreceptor 'packing' that has been observed by fluorescence anisotropy, and shown to affect ligand sensitivity\(^8\). Our results further reveal that CheR/CheB-mediated adaptation restores not only signaling activity but also the spatial organization of receptors upon ligand stimulation.

EFFECTS OF CARBON SOURCE VARIATION AND THE PHOSPHODIESTERASE DIPA ON FLAGELLA-MEDIATED MOTILITY IN PSEUDOMONAS AERUGINOSA

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Background
Pseudomonas aeruginosa exhibits two modes of flagella-mediated motility: swimming in liquid and swarming on surfaces. Studies over the last decade have indicated a phosphodiesterase, DipA, has chemical and physical roles in regulating these motilities, but the extent of the effect varies by study either due to strain or media differences. We have been researching the impact of nutrient cues upon the ability of DipA to influence flagellar motility.

Methods
Swimming and swarming were examined on different growth media. Media tested included FAB/12mM glutamate of glucose, Difco LB Lennox, tryptone, yeast extract, M8/Glucose, M63/Glycerol. Swarm assays were made with 0.1% (wt/vol) Gelzan agar substitute, cured overnight, and dried uncovered 5 hrs. Swarm areas were measured after 24 hrs. Swim assays were made with 0.2% (wt/vol) Noble agar; plates cured overnight. Swim areas were measured after 10 and 24 hrs.

Results
The effect DipA has on flagella-mediated motility varies with the media used. Rich, undefined media and minimal media supplemented with casamino acids supported swimming and swarming in a ΔdipA deficient mutant. In minimal media devoid of casamino acids, DipA was required for both swimming and swarming. Given that richer media supported DipA deficient motility, we also investigated the effect of nutrient level. Doubling components found in common rich media significantly increased both swarming and swimming in both the wild type and a ΔdipA mutant. However, doubling the carbon source concentration in minimal media had no significant effect on flagellar motility in either strain.

Conclusion
DipA is required for flagellar motility in nutrient-scarce environments, but is not required when nutrients are readily available. It does not, however, seem to sense nutrient availability, as a ΔdipA strain responded similarly to the wild type when carbon source was doubled. Doubling rich media led to an increase in flagella-mediated motility, but doubling carbon source in minimal media had no effect. This indicates that only a specific range of nutrients affects flagellar motility.
BACTERIAL CHEMOTAXIS TOWARDS COMPOUNDS IN THE GUT

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Background
Humans share a mutualistic relationship with numerous resident microorganisms, collectively known as the microbiome. Through their long coexistence the microbiome has evolved systems for sensing host-associated signals such as chemicals, nutrients and also hormones. In the last decades compounds present in high concentration in the gastrointestinal (GI) tract have been reported to have an effect in the gut-brain axis. The current study starts to unravel the chemotactic responses of a model commensal bacterium *Escherichia coli* to compounds present in the GI tract namely, catecholamines, thyroid hormones and polyamines, as well as to investigate the effects of these substances on the physiology of *E. coli*.

Methods
To analyse the intracellular response of the chemotaxis pathway of *E. coli* to different stimuli we used previously described fluorescence resonance energy transfer (FRET) assay of the pathway activity. We further studied bacterial behaviour in hormone gradients using microfluidics. Additionally, to determine the physiological importance of the studied compounds we also evaluated the growth rate in presence of these compounds.

Results
FRET experiments showed that the wildtype *E. coli* strain responded to five out of nine analysed chemicals, showing chemotaxis towards melatonin, dopamine, NA (norepinephrine), DHMA (3,4-dihydroxymandelic acid) and spermidine. Interestingly, the response towards NA and dopamine changed sign with the concentration. We also observed that the same compounds affect the growth rate of *E. coli* in a fashion that appears to correlate with their chemotactic preferences.

Conclusion
Here we demonstrated that *E. coli* can detect chemicals found in the GI tract and responds chemotactically accordingly to their physiological importance.
BIO-ENGINEERING OF ESCHERICHIA COLI FLAGELLAR TYPE III SECRETION SYSTEM (fT3SS) FOR IMPROVED SECRETION

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Background
Flagella are the long whip like appendages in motile bacteria that propel them through their environment in search of food and away from danger. They are built in a proximal to distal order with proteins exported by a specialised Type III secretion system or fT3SS that is powered by ATP and Proton motive force (PMF). The flagellum itself can be differentiated into Basal body, hook and filament subunits that are secreted in this order. Proteins secreted up to the formation of hook are called Early substrate protein and those secreted after hook formation are called Late substrates.

Methods
By a combination of molecular cloning and gene synthesis we have created a modular fT3SS secretion plasmid. A range of late and early protein signals with and without UTR regions were cloned in-frame with a range of recombinant proteins and detection tags (FLAG- STREP). In addition using Lambda-Red technology we have created a range of strains that remove regulatory checkpoints and reduce metabolic burden on the flagella gene regulatory hierarchy and tested their ability to secrete proteins via the fT3SS. Secretion was tested using traditional Western blotting techniques on concentrated supernatants while a system was also developed to secrete an esterase enzyme, whose secretion is detected by Enzyme-substrate assay on cleared cell supernatant.

Result
We present data showing that our modular flagellar secretion construct is capable of directing secretion of a range of proteins, one of which potentially allows larger-scale screening of protein secretion since it is an active enzyme. We also show highlight several strain improvements via removal of mot and regulatory genes, and that the 5’ and 3’ FliC-UTRs influence this secretion in the context of late subunit signal and are examining a number of avenues to improve this process in the context of Industrial Biotech.

Conclusion
The fT3SS secretion system, while still low capacity, has a significant capacity for improvement, a possibility enabled by the production of improved strains and modular plasmid systems alongside an efficient screen outlined in this poster. The ultimate aim of the project is to create super secretor flagella mutant strain as a “Mini cell factory” and its corresponding “Super signal” for the maximum protein secretion on Industrial scale.

Figure: Schematic of the synthetic modular secretion construct inserted into an IPTG inducible plasmid
DNAAPPEARS TOSIGNALREPLICATIONRATETOASSOCIATEDEXPRESSION OF AGLOBAL
REGULATORY FACTOR IN BORRELLIA BURGDORFERI

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Background: All organisms must rapidly sense and respond to environmental cues. B. burgdorferi is the
spirochetal pathogen responsible for Lyme disease. This organism occupies an enzootic cycle transmitting
back and forth between an Ixodes tick vector and a vertebrate host. A defining characteristic of these two
niches is their relative nutrient availability and the associated replication rates of B. burgdorferi that
occupy them. The midgut of an unfed tick is a nutrient poor space, where B. burgdorferi do not grow or
divide. Inversely, during tick feeding on blood or infection of a vertebrate host, nutrients abound and B.
burgdorferi replicate at high rates. The Stevenson lab has identified several virulence factors which are
differentially expressed during periods of increased relative growth rates within the enzootic cycle,
and demonstrated their control by several global regulatory factors. A major focus now is how those
regulators are themselves regulated. We sought to characterize the signaling pathway that controls
one of these key factors, BpuR.

Methods: qRT-PCR was used to define the transcriptional profile of bpuR during the enzootic cycle.
Western blots were used to define the expression profiles of bpuR and one of its regulatory targets in
response to altered growth rates in vitro. Promoter fusions and flow cytometry were used to
determine what proportion of this differential regulation was accomplished at the transcriptional level.
Electrophoretic mobility shift assays were utilized to determine if any proteins were bound to the
bpuR upstream DNA and to investigate the binding of potential regulators. DNA-Affinity
chromatography was used to identify which proteins bind the bpuR promoter. RNA-Seq data was
mined to determine the expression profiles of dnaA and bpuR during batch cultivation.

Results: By qRT-PCR and western blot, we found that bpuR expression is correlated with the
alterations of replicative rates both in vitro and in vivo at both the transcript and protein levels. Using
promoter fusions, we found that this regulation is at least partially controlled at the level transcription
from the bpuR promoter. Using whole cytoplasmic gel shifts we found that at least one protein binds
specifically to the bpuR upstream DNA. Using DNA-Affinity chromatography we identified that protein
as DnaA, which is the master regulator of bacterial chromosomal replication. Using gel shifts we
demonstrated that DnaA specifically binds near bpuR's core promoter. Furthermore, by mining our
recent RNA-Seq data, we found that levels of bpuR and dnaA transcript are inversely correlated.

Conclusions: We provide evidence that the sensing of growth rate by B. burgdorferi acts as a signaling cue for the bacteria to adapt and
transmit between the two niches it occupies during its lifecycle. We
sought to understand the mechanism by which this is accomplished
by investigating the regulation of a global regulator of virulence,
bpuR. We found that DnaA binds the core bpuR promoter and is
expressed inversely to bpuR, suggesting a role for DnaA in regulating mammal- and tick-specific proteins in
B. burgdorferi. We hypothesize that DnaA acts as a negative regulator of bpuR expression and that this repression is strongest
during periods of rapid growth, when DnaA levels are elevated.
**B. BURGDORFERI CHEA1 INFLUENCES GROWTH RATE AND ADAPTATION IN RESPONSE TO GLYCEROL, BUT DOES NOT CONTRIBUTE TO CHEMOTAXIS**

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**Background**
Although chemotaxis has been extensively studied in *E. coli*, many other motile bacteria posses multiple homologues to the various chemotactic proteins. In many cases, only one CheA histidine kinase contributes to chemotaxis, while functions have not been ascribed to the other CheA paralogs. *Borrelia burgdorferi* (Bb), the agent of Lyme disease encodes two CheA paralogs, of which only CheA2 has a demonstrated role in chemotaxis. The Stevenson lab has long been interested in how Bb is able to recognize, and appropriately adapt to, the very different environments of ticks and mammals. We have identified a DNA-binding protein, SpoVG, that is highly expressed when Bb is in the tick, and have demonstrated that it is involved in regulating transcription of many genes. Among those are genes for proteins that are involved in glycerol metabolism, which is an essential carbon source for Bb during tick colonization. Bb increases transcription of *spoVG* when glycerol is present, although the signaling mechanism was previously unknown. We hypothesized that the presence of glycerol is signaled through a Bb CheA protein, leading to adaptation to use that carbon source.

**Methods**
Wild type (WT) and ΔcheA1, ΔcheA2, ΔcheA1cheA2 mutants were grown in complete medium, or complete medium plus 4% glycerol. Spirochete morphology and motility was assessed by darkfield microscopy, and growth rate was determined. Cells were harvested on day 3 for RNA extraction and qRT-PCR. Cell pellets were collected for analysis of bacterial mass.

**Results**
WT Bb responded differently to the addition of glycerol than did any of the cheA deletion mutants. The cheA mutants grew more slowly in glycerol than did the WT. Transcription levels of many genes (including *spoVG*) were substantially changed in response to glycerol, while no affects were seen in the cheA mutants. Additionally, ΔcheA2 and ΔcheA1cheA2 mutants and WT formed large cellular aggregates when glycerol was present, whereas the ΔcheA1 mutant did not.

**Conclusions**
The different responses of cheA mutants to glycerol suggest that CheA1, and possibly CheA2, is part of a signaling system that alters cellular processes, including transcription and growth rate. Future work will elucidate whether Bb chemotax to glycerol, and define the pathway(s) that leads from recognition of glycerol to altered cellular processes.
HTC1, A SALICYLATE CHEMORECEPTOR FROM HALOMONAS TITANICA KHS3

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Background

Most chemoreceptors are dimeric transmembrane proteins with a periplasmic domain for ligand binding and a very conserved cytoplasmic domain for kinase control. \textit{Halomonas titanicae} KHS3 was isolated from Mar del Plata harbor (Argentina), and has shown chemotaxis responses towards aromatic hydrocarbons. Two chemosensory clusters and 25 putative chemoreceptors were identified in its genomic sequence.

Methods

Chemotaxis ability of \textit{H. titanicae} KHS3 to different substrates was assessed in soft agar plates. Chemoreceptors were identified in \textit{H. titanicae} genomic sequence based on the annotation obtained from RAST and IMG servers. Both the entire sequence and predicted periplasmic domain of \textit{H. titanicae} chemoreceptor 1 (Htc1) were cloned and expressed in \textit{E. coli} strains. The periplasmic domain was purified and subjected to thermal shift and thermophoresis assays in the presence of different substances. Flagellar control assays were performed in \textit{E. coli} cells expressing Htc1 as the only receptor.

Results

\textit{H. titanicae} KHS3 displays chemotaxis both towards phenanthrene and sodium salicylate in swimming plates. Htc1 was found in a genomic context that suggested a role in aromatic compounds sensing. Thermal shift assays showed that the melting temperature of its purified periplasmic domain was increased by 4 degrees in the presence of 10 mM sodium salicylate, while it was unchanged in the presence of pyruvate, serine or phenanthrene. Thermophoresis assays carried out at different salicylate concentrations resulted in a Kd of 73 µM. \textit{E. coli} cells expressing Htc1 as their only receptor responded to the addition of salicylate (but not phenanthrene) by suppressing CW rotation of their flagella.

Conclusion

Htc1 is a chemoreceptor for sodium salicylate, based on its binding abilities and the flagellar control of \textit{E. coli} cells. Even though Htc1 does not bind phenanthrene, it could be responsible of chemotaxis towards aromatic hydrocarbons, considering that salicylate is an intermediate metabolite of aromatic compounds degradation pathways. Gene replacement experiments to answer this question are in progress.
THE GLYCINE HINGE OF BACTERIAL CHEMORECEPTORS: FUNCTIONAL ANALYSIS OF ITS SIGNALING ROLE IN TSR

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Background
Chemotaxis requires the transmission of information from the environment to the flagellar motors. Chemoreceptors are dimeric transmembrane proteins with a periplasmic domain for ligand binding and a conserved cytoplasmic domain consisting in a long hairpin that forms a four-helix coiled coil bundle. The activity of the CheA kinase, attached to the tip of the cytoplasmic domain, is modulated in response to external signals. The mode of signal propagation along the long chemoreceptor rod is still under study. In this work we focus on the role of three conserved glycine residues, two in the N-terminal and one in the C-terminal helix of the hairpin, that are thought to conform a hinge with signaling function.

Methods
We carried out random-codon mutagenesis at the three glycine hinge positions in Tsr and obtained several non-functional variants. We then characterized the mutants with respect to subcellular localization by a fluorescence reporter protein, trimer-of-dimer formation by crosslinking competition assays, influence on Tar function, control of kinase activity by flagellar rotation assays, and methylation pattern by Western blotting with an anti-Tsr antibody. Furthermore, we generated second-site mutations that restore the chemotaxis proficiency of some of the hinge mutant proteins, and characterized them.

Results
We obtained 14 non-functional replacements, 13 of which retained native receptor interactions and subcellular localization, but were defective in kinase control. All the mutants in G439 (eight) were unable to activate the kinase and showed a hyper-methylated pattern, indicative of an OFF-biased receptor conformation. Only two of them were able to activate the kinase and got demethylated upon stimulus with the repellent glycerol. Second-site mutations lay in the methylation region (increasing hydrophobic interactions) or near the mutated glycine.

Conclusion
The glycine hinge seems to be dispensable for trimer-of-dimer formation but to play a role in ON/OFF switching. The results are consistent with the yin-yang model, in which the glycine hinge separates the signaling hairpin subdomains.
STRUCTURE-FUNCTION STUDIES OF A RESPONSE REGULATOR OF UNKNOWN FUNCTION FROM HYPERVIRULENT CLOSTRIDIUM DIFFICILE R20291

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Background
Clostridium difficile is an anaerobic, spore-forming pathogen and the leading cause of nosocomial infections in the United States, with an estimated 500,000 infections and ~30,000 deaths per year. Strains of C. difficile have gained resistances to multiple antibiotics, including the two common front-line treatments for Clostridium difficile infections (CDI), prominent in clinical settings. Two-component systems (TCSs) are essential signaling pathways in bacteria that are involved in cellular adaptability to environmental stress, virulence and antibiotic resistance. R20291 has 48 such TCSs, plus a number of orphan histidine kinases (HKs) and response regulators (RRs). The function of many of these TCSs remains unknown. We aim to identify biological roles of TCSs using bioinformatics, structural and biochemical assays. Here we present a preliminary X-ray structure and functional assay for RR_1586.

Methods
The hanging drop vapor diffusion method was used to crystallize RR_1586 and the structure was determined using molecular replacement. We are employing a bacterial one-hybrid (B1H) method to identify gene targets regulated by RR_1586. E. coli cells are co-transformed with a plasmid bearing RR_1586 fused to the omega subunit of RNA polymerase (ωRNAP) and a library of plasmids encoding rescue genes under control of randomized promoters. Binding of the RR_1586-ωRNAP fusion to the randomized promoter selectively results in colony formation. We anticipate that comparing sequences of promoters from many colonies will reveal a consensus promoter sequence recognized by RR_1586.

Results
The X-ray structure for RR_1586 was determined at 2.65 Å resolution and revealed a common (α/β)5 N-terminal receiver domain and a winged helix-turn-helix DNA-binding C-terminal effector domain. Initial results from the B1H assay using positive controls show that library coverage is satisfactory. ωRNAP fusions to several full-length mutants or DNA-binding domain constructs of RR_1586 have been tested against this library.

Conclusions
We have determined a preliminary crystal structure of full-length RR_1586. By applying several constructs of RR_1586 to the B1H assay, we can identify the strategy most likely to succeed for the remaining RRs of unknown function in C. difficile R20291.
DECONSTRUCTING 3D HOLOGRAPHIC SWIMMING TRAJECTORIES OF ARCHAEA

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Background
Swimming is an energetically expensive process which many microorganisms adopt to increase encounters with favourable environments. Visualizing these behaviours is challenging with optical microscopy due to the large focal depths and rapid capture rates required. We use digital in-line holographic microscopy and purpose-built tracking codes, to extract the 3D position coordinates of swimming microorganisms from video footage. This high-throughput technique can be applied at high frame-rates to hundreds of cells simultaneously.

Whilst in-depth characterization of the swimming behaviours of some key laboratory strains of bacterial and eukaryotic cells has been established, little is known about the behaviours of archaeal swimming cells. Here we explore the swimming behavior of halophilic archaeal isolated from two sites: The Great Salt Lake (Utah, U.S.A), and Boulby Potash Mine (Cleveland & Redcar, UK).

Methods
Digital Inline Holographic Microscopy (DIHM) uses coherent laser illumination to image outside the focal plane, by creating interference patterns from the light scattered by biological samples. These interference patterns are then captured using high speed video, and can be analysed frame by frame ‘offline’, to produce 3D coordinates of the positions of each cell within a sample. By using this technique, it is possible to simultaneously reconstruct the swimming trajectories of multiple microorganisms from the extracted coordinates. This enables a three dimensional analysis of moving cells at a population scale, facilitating the differentiation of different species within a mixed species population, based upon their swimming ‘style’.

Results
Haloarchaeal cells swim more slowly than most bacterial organisms, with an average straight swimming speed of 1.5-3.5µm/s. Our isolates predominantly reorient via a ‘run and reverse’ technique, with swimming speeds dropping below 1µm/s before each reorientation.

Conclusions
DIHM can be used to track multiple cells simultaneously to obtain trajectories of swimming micro-organisms. These can then be deconstructed to provide insights into previously uncharacterised swimming styles and behaviours.

Figure 1: 3D Plot of Haloarcula sp. swimming trajectories. Colour scale shows swimming speed in µm/s, faster speeds can be observed during straight ‘runs’, with slower speeds highlighting reorientations.
LOSS OF CHEMOTAXIS GENE CHEY2 LEADS TO DECREASED PERSISTENCE OF BORRELLIA BURGDORFERI IN MICE, BUT NOT TICKS, VIA NEEDLE- OR TICK-TRANSMISSION

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Background: Borrelia burgdorferi (Bb) is a spirochetal bacterium that is the causative agent of Lyme disease. It exhibits characteristic endoflagellar motility which allows efficient dissemination through dense host tissues and eventually persistence within multiple tissues. With its 7-11 endoflagella the bacterium can perform forward or reverse movements along with flexing to change direction. Working in conjunction to this motility pathway, Bb possesses an elaborate chemotactic pathway, considerably more complex than the traditional chemotactic pathway. CheY proteins act as the response regulator for prototypic bacterial chemotaxis signal transduction systems, which binds to the flagellar switch protein resulting in reversal of direction of motor rotation. Bb possesses three homologs of the response regulator cheY gene (cheY1, cheY2, and cheY3). Both motility and chemotaxis-related mechanisms appear essential for infection, but little is known regarding specific roles of the genes in these putative pathways. In this study, we investigated the role of the cheY2 gene on persistence and dissemination within tick and mouse tissues using a non-polar mutant in a virulent Bb strain. Our goal is to determine the role/importance of the cheY2 gene for the different stages of the Bb natural enzootic cycle.

Methods
We created a cheY2-deletion (ΔcheY2) mutant on 2 different infectious strains of Bb, namely B31 and K10. Naive mice were intradermally injected in the ear with either wild-type (WT) or ΔcheY2 Bb. Mice were euthanized at indicated time points and various tissues were harvested. Bacterial persistence and dissemination was assessed by quantification of Bb DNA using qPCR. Bb-specific antibody generation was evaluated by ELISA.

Results
ΔcheY2 demonstrated similar growth rates, morphology, motility and chemotactic abilities as WT Bb in vitro. Ticks infected via immersion possessed similar levels of ΔcheY2 and WT Bb up to 7 days post-immersion, regardless of whether the tick was fed or unfed before immersion. Alternatively, mice fed upon by ΔcheY2-infected ticks showed reduced infection rates compared to ticks containing WT Bb. Mice infected by needle-injection possessed significantly lower levels of ΔcheY2 compared to WT Bb at the injection site at all times ≥ Day 4 post-injection, with very few ΔcheY2 disseminating to distant tissues and being cleared by >Day 14 post-infection. ΔcheY2-infected mice generate a Bb-specific antibody response similar to WT infected mice through Day 14 post-infection, but then decreased significantly.

Conclusion
These studies indicate that cheY2 is essential for infection and persistence within mouse tissues, but not for activities within ticks and tissue culture conditions. Future studies will determine the specific properties conferred by cheY2 within murine tissues.
THE ROLE OF MULTIPLE FLAGELLINS IN $\phi$CbK ADSORPTION TO THE CAULOBACTER CRESCENTUS FLAGELLUM

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Background

The Caulobacter crescentus bacteriophage $\phi$CbK initially adsorbs to the bacterial flagellum using a head filament that extends from the phage capsid. Flagellar rotation then brings the phages towards the cell pole where the phage tails can bind host receptors. The $C.\ crescentus$ flagellum is composed of six flagellin proteins encoded by $fljJ$ (29 kDa), $fljK$ (25 kDa), $fljL$ (27 kDa), $fljM$ (25 kDa), $fljN$ (25 kDa), and $fljO$ (25 kDa). There is significant structural redundancy among the flagellins, although different flagellins provide varying levels of motility and filament length. We hypothesized that the flagellins may differentially interact with the $\phi$CbK head filament, providing $C.\ crescentus$ a mechanism to reduce phage predation.

Methods

We used motility assays and phage adsorption assays along with negative stain electron microscopy of intact cells to investigate the functional role of the six $C.\ crescentus$ flagellins in a collection of flagellin deletion strains. Interactions between $\phi$CbK head filaments and $C.\ crescentus$ flagellar filaments were visualized using cryo-electron tomography.

Results

All strains were capable of forming flagellar filaments of various lengths. The $\Delta fljKLMNO$ strain formed extremely truncated filaments, supporting the hypothesis that FljJ forms the hook-proximal portion of the flagellum. Motility of the strains generally correlated with filament length with the presence of FljJ along with either FljK or FljM providing the profiles closest to wild type. $\phi$CbK adsorption increased upon the addition of FljK, particularly in the presence of FljJ and absence of FljL, although this could be compensated by filament length and motility and never exceeded wild type levels of adsorption. $\phi$CbK head filaments were visualized wrapping the flagellar filaments of all strains except $\Delta fljKLMNO$.

Conclusions

The six $C.\ crescentus$ flagellins are all involved in providing motility and flagellar filament length, although they seem to differentially interact with $\phi$CbK. Future studies will examine the adsorption of other $\phi$CbK-like phages to $C.\ crescentus$ flagellin deletion strains. We will also determine high-resolution structures of flagellar filaments to understand the structural implications of incorporating multiple flagellin proteins.
ASSIGNING CHEMORECEPTORS TO MULTIPLE CHEMOSENSORY PATHWAYS IN PSEUDOMONAS AERUGINOSA

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Background
In contrast to Escherichia coli and other model organisms, chemosensory signaling processes in Pseudomonas aeruginosa are much more complex. P. aeruginosa PAO1 has four chemosensory pathways and twenty-six chemoreceptors that feed into these pathways. A few chemoreceptors in P. aeruginosa have been confidently assigned to cognate pathways using a series of experiments by different research groups; however, the majority of chemoreceptors still await their formal assignment to specific pathways. Assigning all chemoreceptors to pathways appears to be a nearly intractable problem, when using experimental approaches alone. Thus, we addressed the problem computationally and used experimental data to interpret and validate computational results.

Methods
Automated pathway; phylogenetic profiling; gene neighborhoods and gene co-expression; multiple sequence alignment and clustering; phylogenetic trees.

Results
Automated pathway assignment and phylogenetic profiling produced ambiguous results. However, comparative approaches targeting specific features of chemoreceptors, namely its highly conserved kinase-interacting domain and methylation sites, enabled accurate assignment of all twenty-six chemoreceptors to four cognate pathways. We identified a conserved motif (LLAxxxxExAR) in the kinase-interacting subdomain of twenty-three chemoreceptors that are all predicted to feed into Che I (F6) pathway. Three remaining chemoreceptors had other uniquely conserved motifs in the same region and are predicted to feed into three other pathways: McpB (ILAxxxVExAR) is specific to Che II (F7) pathway, WspA (LLSxxxIExEK) is specific to Wsp (ACF) pathway, and PilJ (ILAxxxIqxSM) is specific to Chp (Tfp) pathway. In a similar fashion, McpB, Wsp, and PilJ had uniquely conserved methylation sites that distinguished them from all other chemoreceptors.

Conclusion
Using tailored computational genomics approaches that target specific features of chemoreceptors we were able to join multiple, disconnected lines of experimental evidence into a framework that enabled unambiguous assignment of all chemoreceptors in P. aeruginosa to cognate signal transduction pathways.
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