WANTED

Swimming or Tumbling

B. burgdorferi
L. interrogans
H. gracilis
C. jejuni

BLAST XIII 2015
TUCSON, AZ
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AWARDS INFORMATION

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.

Robert M. Macnab Memorial Travel Awards

We are pleased to announce the establishment of the first travel awards for the BLAST meeting, remembering our colleague Dr. Robert M. Macnab on the 10th anniversary of his death. 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 XIII are Matthew Baker from Lawrence Lee’s lab at the Victor Chang Cardiac Research Institute, Dinah Tambalo from Chris Yost’s lab at the University of Regina and Yuki Yamanaka from Kaneyoshi Yamamoto’s lab at the Hosei University.

(The Macnab poster and travel awards are 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.

BLAST Board of Directors’ Award for an Outstanding Talk

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 is open to Graduate Students who present a talk at BLAST XIII.

BLAST Founders’ Award for an Outstanding Talk

The BLAST Board of Directors is pleased to announce the establishment the BLAST Founders’ Award in recognition of those who founded BLAST, Joe Falke, Mike Manson, Phil Matsumura and Sandy Parkinson. The BLAST Founders’ Award is open to Postdoctoral Fellows who present a talk at BLAST XIII.
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<th>TIME</th>
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<tr>
<td><strong>Sunday, January 18, 2015</strong></td>
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<tr>
<td>4:00 pm</td>
<td>Poster room available for poster setup</td>
<td>Gala Rooms A &amp; B</td>
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<tr>
<td>4:00 pm – 7:00 pm</td>
<td>Meeting Registration</td>
<td>Outer Vistas</td>
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<td><strong>Monday, January 19, 2015</strong></td>
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<tr>
<td>7:30 am – 8:30 am</td>
<td>Breakfast</td>
<td>Private Dining Room</td>
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<tr>
<td>8:45 am – 9:00 am</td>
<td>Welcome &amp; Announcements</td>
<td>Salon B &amp; C</td>
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<tr>
<td>9:00 am – 12:00 pm</td>
<td>Meeting Session – “Flagella”</td>
<td>Salon B &amp; C</td>
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<tr>
<td>10:15 am – 10:30 am</td>
<td>Coffee Break</td>
<td>Salon A</td>
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<tr>
<td>12:00 pm – 1:30 pm</td>
<td>Lunch</td>
<td>Private Dining Room</td>
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<td>1:30 pm – 4:00 pm</td>
<td>Meeting Session – “Flagella Motor”</td>
<td>Salon B &amp; C</td>
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<td>2:45 pm – 3:00 pm</td>
<td>Coffee Break</td>
<td>Salon A</td>
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<td>4:30 pm – 6:00 pm</td>
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<td>Poster Session</td>
<td>Gala Rooms A &amp; B</td>
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<td><em>Poster Groups A and B presented from 7:30 PM – 8:30 PM</em></td>
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<tr>
<td>8:45 am – 12:00 pm</td>
<td>Meeting Session – “Chemoreceptors and Signaling”</td>
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<tr>
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<td>Lunch</td>
<td>Private Dining Room</td>
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<tr>
<td>1:30 pm – 4:00 pm</td>
<td>Meeting Session – “Chemotaxis”</td>
<td>Salon B &amp; C</td>
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<tr>
<td>2:45 pm – 3:00 pm</td>
<td>Coffee Break</td>
<td>Salon A</td>
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<tr>
<td>6:00 pm – 7:30 pm</td>
<td>Dinner</td>
<td>Private Dining Room</td>
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<tr>
<td>7:30 pm – 10:00 pm</td>
<td>Poster Session</td>
<td>Gala Rooms A &amp; B</td>
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<td><em>Poster Groups A and C presented from 7:30 PM – 8:30 PM</em></td>
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<td><strong>Wednesday, January 21, 2015</strong></td>
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<tr>
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<td>Breakfast</td>
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<td>8:15 am – 11:30 am</td>
<td>Meeting Session – “Motors and Motility”</td>
<td>Salon B &amp; C</td>
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<td>Lunch</td>
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<tr>
<td>8:45 am – 12:00 pm</td>
<td>Meeting Session – “c-di-GMP Signaling/Collective”</td>
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<td>Coffee Break</td>
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<tr>
<td>12:00 pm – 1:30 pm</td>
<td>Lunch</td>
<td>Private Dining Room</td>
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<tr>
<td>1:30 pm – 4:00 pm</td>
<td>Meeting Session – “Two-Component &amp; Other Signaling”</td>
<td>Salon B &amp; C</td>
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<tr>
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<td>Coffee Break</td>
<td>Salon A</td>
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<tr>
<td>4:00 pm – 5:00 pm</td>
<td>Town Hall Meeting for students &amp; postdocs</td>
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<tr>
<td>7:30 pm – 8:15 pm</td>
<td>Business Meeting for all attendees</td>
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<td>Awards Presentation</td>
<td>Salon B &amp; C</td>
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<td>Reception</td>
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<tr>
<td>7:00 am – 8:30 am</td>
<td>Breakfast</td>
<td>Private Dining Room</td>
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# BLAST XIII PROGRAM

## January 19, 2015

### Flagella

**Chair – Seiji Kojima**

#### Monday Morning (8:45 am – 12:00 pm)

<table>
<thead>
<tr>
<th>PRESENTER</th>
<th>TITLE</th>
<th>ABSTRACT PAGE NO.</th>
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<tbody>
<tr>
<td>Philip Aldridge</td>
<td>Growth rate control of flagellar abundance differs in <em>E. coli</em> and <em>Salmonella enterica</em> sv. Typhimurium</td>
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<tr>
<td>Santosh Koirala</td>
<td>A nutrient-tunable bistable switch control motility in <em>Salmonella enterica</em> serovar Typhimurium</td>
<td>3</td>
</tr>
<tr>
<td>Mary Stewart</td>
<td>Mutually repressing repressor functions and multi-layered heterogeneity regulate the bistable <em>Salmonella FlIC</em> census</td>
<td>4</td>
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<tr>
<td>Guillaume Paradis</td>
<td>Can bacterial filaments regrow?</td>
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**COFFEE BREAK**

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<thead>
<tr>
<th>PRESENTER</th>
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<th>ABSTRACT PAGE NO.</th>
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</thead>
<tbody>
<tr>
<td>Tao Lin</td>
<td>Mutations in <em>B. burgdorferi</em> flagellar export profoundly affect spirochete flagellar assembly, morphology, motility, structure, cell division, and mouse infectivity</td>
<td>6</td>
</tr>
<tr>
<td>Marc Erhardt</td>
<td>ATPase-independent Type-III protein secretion in <em>Salmonella enterica</em></td>
<td>7</td>
</tr>
<tr>
<td>Matthew Baker</td>
<td>Molecular emergence: the mechanism for the self-organization of FlIG in the bacterial flagellar motor</td>
<td>8</td>
</tr>
<tr>
<td>Anna Roujeinikova</td>
<td>Structure of <em>H. pylori</em> pseudaminic acid biosynthesis N-acetytransferase PseH (flagella glycosylation), and its implications for the novel substrate specificity</td>
<td>9</td>
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</table>

## January 19, 2015

### Flagella Motor

**Chair – Judy Armitage**

#### Monday Afternoon (1:30 pm – 4:00 pm)

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<tr>
<th>PRESENTER</th>
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<th>ABSTRACT PAGE NO.</th>
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</thead>
<tbody>
<tr>
<td>Yasuhiro Onou</td>
<td>Asymmetric role of conserved charged-residues in rotor-stator interface of the flagellar motor</td>
<td>10</td>
</tr>
<tr>
<td>Xiaowei Zhao</td>
<td><em>In situ</em> structures of the switch complex provide novel insights into CheY-P binding and switching</td>
<td>11</td>
</tr>
<tr>
<td>Michael Morse</td>
<td>Flagellar motor switching rates in <em>C. crescentus</em> follow first passage time distribution</td>
<td>12</td>
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<tbody>
<tr>
<td>Junhua Yuan</td>
<td>Switching dynamics of the bacterial flagellar motor near zero load</td>
<td>13</td>
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<tr>
<td>Afanzar Oshri</td>
<td>Switch mechanism of the flagellar motor deduced from phosphorylation-independent signaling</td>
<td>14</td>
</tr>
<tr>
<td>Huawei Zhang</td>
<td>Molecular interaction of FlIM and spermidine synthase in <em>Helicobacter pylori</em></td>
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</tbody>
</table>
### January 20, 2015
#### Tuesday Morning (8:45 am – 12:00 pm)
Chair – Mike Manson

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<tr>
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<tbody>
<tr>
<td>Peter Ames</td>
<td>Transmembrane signaling roles for Tsr control cable residues</td>
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<tr>
<td>Nicholas Bartelli</td>
<td>Structure and conformational signaling in the cytoplasmic domain of bacterial chemoreceptors characterized by EPR spectroscopy</td>
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<tr>
<td>Dipanjan Samanta</td>
<td>Bacterial chemoreceptor dynamics as revealed by pulsed dipolar and continuous wave ESR</td>
<td>18</td>
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<tr>
<td>Hayden Swisher</td>
<td>Effects of core complex incorporation and on-off switching on the dynamics of CheA P1 substrate domain</td>
<td>19</td>
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<td></td>
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<tr>
<td>Emily Sweeney</td>
<td>Structure-function insights from the <em>Helicobacter pylori</em> chemoreceptor TlpA</td>
<td>20</td>
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<tr>
<td>Christopher Cassidy</td>
<td>All atom structure and dynamics of an intact bacterial chemosensory array</td>
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<td>Tino Krell</td>
<td>Mechanism for the specific targeting of methyltransferases to chemoreceptors</td>
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<td>Davi Ortega</td>
<td>Evolution of the chemotaxis system in Archaea</td>
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### January 20, 2015
#### Tuesday Afternoon (1:30 pm – 4:00 pm)
Chair – Tom Shimizu

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<td>Diana Di Paolo</td>
<td>Single molecule imaging of electroporated motility proteins in live <em>E. coli</em></td>
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<tr>
<td>Yann Dufour</td>
<td>Mapping <em>E. coli</em> swimming behavior to chemotaxis protein counts in single cells</td>
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<tr>
<td>Katya Taute</td>
<td>3D tracking for the people: high-throughput label-free imaging of bacterial motility on a standard phase contrast microscope</td>
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<tr>
<td>Rémy Colin</td>
<td>Fast, high-throughput measurement of collective bacterial chemotaxis</td>
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</tr>
<tr>
<td>Michael Manson</td>
<td>Chemotaxis to norepinephrine metabolites</td>
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<tr>
<td>Monica Gerth</td>
<td>Exploring the functional diversity of chemoreceptors from <em>Pseudomonas syringae pv. actinidiae</em></td>
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<tr>
<td>Mark Wooten</td>
<td>Intravital assessment of <em>B. burgdorferi cheY3</em> mutant in mouse skin tissues reveals spirochetal properties for dissemination and persistence in Lyme disease</td>
<td>30</td>
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</tbody>
</table>
### January 21, 2015
#### Motors & Motility
##### Wednesday Morning (8:30 am – 11:30 pm)

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<tr>
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<tr>
<td>Steven Esquivel</td>
<td>Biochemical, genetic, and microfluidic characterization of the bacterial flagella system</td>
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<td>Susanne Brenzinger</td>
<td>Upgrade of flagellar motility in <em>Shewanella oneidensis</em> MR-1</td>
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<tr>
<td>Yuhai Tu</td>
<td>How efficient are bacterial flagellar motors?</td>
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<tr>
<td>Ben Webb</td>
<td>Combining the simplicity of the modified agarose capillary assay with automated data acquisition and quantification</td>
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<tr>
<td>Beiyan Nan</td>
<td>The actin homologue MreB and gliding motors show interdependent movements in <em>Myxococcus xanthus</em></td>
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<tr>
<td>Yongtao Zhu</td>
<td>Flavobacterium gliding motility: where is the motor?</td>
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<td>Abhishek Shrivastava</td>
<td>A rotary motor drives <em>Flavobacterium</em> gliding</td>
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<tr>
<td>Sonia Bardy</td>
<td>Role of PilJ periplasmic domain in signal transduction in <em>Pseudomonas aeruginosa</em></td>
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### January 22, 2015
#### c-di-GMP Signaling/Collective Behaviors
##### Thursday Morning (9:00 am – 12:00 pm)

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<th>PRESENTER</th>
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<tbody>
<tr>
<td>Urs Jenal</td>
<td>A novel CheY family regulates motility in <em>C. crescentus</em></td>
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<tr>
<td>Christopher Jones</td>
<td>Role of c-di-GMP and MshA pili in the motile to sessile transition in <em>Vibrio cholerae</em></td>
<td>40</td>
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<tr>
<td>Amy Baker</td>
<td>Mechanisms of c-di-GMP-mediated swarming motility repression in <em>Pseudomonas aeruginosa</em></td>
<td>41</td>
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<tr>
<td>Hyo Kyung Kim</td>
<td>A new role for a diguanylate cyclase: YfiN controls cell division in response to envelope stress</td>
<td>42</td>
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<tr>
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<tbody>
<tr>
<td>Roy Welch</td>
<td>Quantifying <em>M. xanthus</em> developmental dynamics</td>
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<td>Yilin Wu</td>
<td>Macroscopic self-organization in a bacterial colony</td>
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<tr>
<td>Ismael Duchesne</td>
<td>The motility of bacteria in a liquid crystal</td>
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<tr>
<td>Emilia Mauriella</td>
<td>A cytoplasmic receptor segregates along with the nucleoid in <em>Myxococcus xanthus</em></td>
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### January 22, 2015
#### Two-Component & Other Signaling
##### Thursday Afternoon (1:30 pm – 4:00 pm)

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<tr>
<td>Stuti Desai</td>
<td>A unique role for SPI-2 response regulator SsrB: Regulation of the <em>Salmonella</em> carrier state in the absence of the SsrA kinase</td>
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<td>McKenzie Lehman</td>
<td>Identification of amino acids important for the <em>Staphylococcus aureus</em> two-component system LytSR and implications for biofilm formation</td>
<td>48</td>
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<td>John Kirby</td>
<td>Jousting between <em>Myxococcus xanthus</em> and <em>Bacillus subtilis</em>: secondary metabolites, predation, and sporulation</td>
<td>49</td>
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<tr>
<td>Jonathan Willett</td>
<td>An essential phosphorelay signal transduction system regulating the intracellular survival of <em>Brucella</em></td>
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<td>Tarek Msadek</td>
<td>Peptide antibiotic resistance and intramembrane signaling in <em>Staphylococcus aureus</em></td>
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<tr>
<td>John Helmann</td>
<td>Signal transduction by Zinc from atomic to the cellular level</td>
<td>52</td>
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<tr>
<td>1</td>
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<td>2</td>
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# BLAST XIII POSTER PRESENTATION SCHEDULE

**Monday, January 19, 2015:**
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SPEAKER ABSTRACTS
GROWTH RATE CONTROL OF FLAGELLAR ABUNDANCE DIFFERS IN *ESCHERICHIA COLI* AND *SALMONELLA ENTERICA* SV. TYPHIMURIUM

Phillip D. Aldridge¹, Martin Sim¹, Santosh Koirala², Paul Hoskisson⁴, Colin Gillespie³, Christopher V. Rao³

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BACKGROUND: The bacterial flagellum is a complex organelle requiring the coupling of gene expression to the assembly pathway. In *Escherichia coli* and *Salmonella enterica* serovar Typhimurium the flhDC operon encodes the essential FlhD₄C₂ flagellar-specific master transcriptional regulator. The activity of FlhD₄C₂ is influenced by many flagellar-specific and global regulatory stimuli. FlhD₄C₂ activity responds to cellular and environmental factors through regulators including OmpR, RcsB and CRP. Flagellar-specific signals known to influence FlhD₄C₂ activity are controlled by the action of FliT and FliZ. flhDC expression is also sensitive to cell growth.

METHOD: Here, we investigated the impact of growth rate on flagellar formation in *E. coli* and *S. Typhimurium* using steady-state chemostat cultures. Our measure was flagellar abundance using a functional FliM-GFP fusion protein.

RESULTS: We will show that flagellar abundance correlates with growth rate, where faster growing cells produce more flagella in both species. We will present data showing that the impact of transcriptional, post-transcriptional and flagellar-specific regulation of flhDC with respect to the growth rate response of the flagellar system leads to a marked difference in flagellar abundance when the species are compared.

CONCLUSION: Our data suggests that even though both flagellar systems have a high degree of genetic similarity the way *E. coli* and *S. Typhimurium* assimilate signals during flagellar regulation may reflect the lifestyle of these two bacterial species.
A NUTRIENT-TUNABLE BISTABLE SWITCH CONTROLS MOTILITY IN SALMONELLA ENTERICA SEROVAR TYPHIMURIUM

Santosh Koirala¹, Philip D. Aldridge², and Christopher V. Rao¹
1. University of Illinois at Urbana-Champaign, Urbana, Illinois, United States, 61801
2. Newcastle University, Framlington Place, Newcastle upon Tyne, United Kingdom, NE2 4HH

Background: Motility is a common strategy used by diverse organisms to adapt to changes in their environment. For instance, when proximal sources of nutrients are depleted, one simple strategy is to seek out new ones. This process has been extensively studied in the enteric bacteria Escherichia coli and Salmonella enterica serovar Typhimurium. Both employ flagella to move. Both swim towards nutrients and away from toxins by altering the rotational bias of their flagella. Both regulate the expression of their flagellar genes in response to nutrient availability, independently of chemotaxis. Interestingly, this response is quite different despite otherwise close similarities between the two motility systems. In particular, nutrients repress flagellar gene expression in E. coli whereas they enhance it in Salmonella.

Methods: All strains are derivatives of S. enterica serovar Typhimurium 14028. All experiments were performed in Vogel-Bonner Minimal E medium supplemented with glucose and various amount of yeast extract at 37°C. Cell tracking was done with a Zeiss standard microscope fitted with B&W video camera. The movie was then analyzed using custom Matlab software. The gene expression measurement was performed using transcriptional fusion to fluorescent proteins. The samples were analyzed using BD LSRII flow cytometer.

Results: In this work, we investigated phase variation within the Salmonella flagellar regulon. We have shown that the response to nutrients is bistable and two distinct phenotypes, motile and sessile, coexist in an isogenic cell population. We have further shown that the response exhibits hysteresis which results from a competitive interaction of two regulatory proteins, FliZ and YdiV. Additionally, we found that a positive feedback loop involving alternate sigma factor FliA is required for bistability which amplifies the expression of FliZ by enhancing its expression from class 3 component of P_fliAZ promoter, but two other feedback loops involving FlgM and FliT are not.

Conclusion: These results reveal a new facet of motility in S. enterica serovar Typhimurium and demonstrate how these bacteria employ phenotypic diversity as general mechanism for adapting to changes in their environment.

Lab: Christopher Rao
MUTUALLY REPRESSING REPRESSOR FUNCTIONS AND MULTI-LAYERED HETEROGENEITY REGULATE THE BISTABLE SALMONELLA FLIC CENSUS

Mary K. Stewart¹ and Brad T. Cookson¹,²
Departments of Microbiology¹ and Laboratory Medicine², University of Washington, Seattle, 98195, USA

Background: A switch-like and heterogeneous response at a promoter can generate a bistable distribution, or census, of gene expression for a population of cells. We have shown that bistable expression of the flagellin monomer FliC depends upon the EAL-like protein YdiV. YdiV functions as a rheostat within and outside the host, tuning the fraction of Salmonella populations expressing flagellar genes. YdiV-dependent flagellar gene repression allows Salmonella to evade caspase-1 mediated host defenses and enhances systemic colonization. YdiV and the class II flagellar protein FliZ are mutually repressing repressors, a genetic feature demonstrated to contribute to bistability in other systems. FliZ 1) transcriptionally represses ydiV and 2) increases the level of the flagellar class I protein FlhC; independence of these activities had not previously been tested. We investigated the role of the mututally repressing repressor circuit formed by YdiV and FliZ in the bistable expression of fliC.

Methods: Flow cytometry with GFP reporter fusions was used to take the single cell census of fliC expression in WT and mutant Salmonella strains. β-galactosidase assays and western blots were used to measure ydiV and FlhC expression at the population level.

Results: YdiV partitions cells into the fliC-OFF subpopulation, while FliZ partitions cells into the fliC-HIGH subpopulation at late timepoints during growth. Both YdiV-dependent and – independent effects of FliZ shape flagellar gene expression. Bistability of ΔfliZ populations and ydiV-independent FliZ control of flagellar gene expression demonstrate that the YdiV-FliZ mutually repressing repressor circuit is not required for bistability under our conditions. YdiV and FliZ play important individual roles, and bistability collapses into a predominant intermediate population in the absence of both regulators. Metered expression of YdiV and FliZ identifies conditions where FliZ maximizes the fliC-ON population and where control of fliC expression by FliZ is constrained.

Conclusions: Maintenance of a fliC-OFF subpopulation by YdiV is sufficient to shape a bistable gene expression census without FliZ-mediated positive feedback. Determinants of the fliC population distribution change with growth phase, and multiple layers of heterogeneity shape the census of fliC expression.

Lab: Brad Cookson
CAN BACTERIAL FILAMENTS REGROW?

Guillaume Paradis¹, Marc Erhardt², and Simon Rainville¹
¹Department of Physics, Engineering Physics and Optics and Centre of Optics, Photonics and Lasers, Laval University, Québec, Québec, Canada
²Helmholtz Center for Infection research, 38124 Braunschweig, Germany

Background: Bacterial flagellar filaments extend many body lengths (10-15µm) outside of the cell and are constructed of as many as 20000-30000 protein subunits (called flagellin). Each filament grows at its distal end by self-assembly of flagellin subunits that have to be transported in an unfolded conformation through the narrow channel of the filament. At the very end of the filament, an essential “cap” structure made of the FliD protein promotes flagellin self-assembly and polymerization. This work attempts to answer the simple question: can the filament regrow if it is cut?

Methods: Using femtosecond laser ablation, we cut individual bacterial filaments and observed whether they could regrow. Bacterial filaments were first labeled with an orange fluorophore, cut with the laser (see figures), and then re-labeled with a green fluorophore after a 2h regrowth period (in TB at 37°C). The experiments were performed with the EM800 strain (Salmonella Enterica strain with only one filament per cell because of a fliO deletion), which allows us to make sure that we revisit (after the regrowth period) the exact individual filaments that were cut by the laser. The same experiment was also done with a strain where the cap protein FliD could be overexpressed.

Results: Overall, more than 60 filaments were cut and we did not observe any regrowth. Interestingly, this conclusion differs from the results reported a few years ago by the Berg Lab who reported that mechanically broken (sheared) E. coli filaments do grow back [Turner and al, J. Bact, 2012]. Using a similar approach (sequential labeling of filaments with different colors) we also investigated the rate at which bacterial filaments grow as a function of their initial length. Statistics from 456 filaments lead us to the conclusion that the growth rate decreases with length (from ~4 µm/h at 1µm down to ~1µm/h at 10 µm). These observations again contrast with the constant growth rate (2.3 µm/3h) reported by the Berg Lab in the paper cited above.

Conclusion: Those two separate results appear to disagree with the chain mechanism model proposed to describe flagellum growth [Evans and al., Nature, 2013]. The data of ongoing experiments on these interesting questions will be presented.
MUTATIONS IN THE *B. BURGDORFERI* FLAGELLAR EXPORT APPARATUS GENES PROFOUNDLY AFFECT SPIROCHETE FLAGELLAR ASSEMBLY, MORPHOLOGY, MOTILITY, STRUCTURE, CELL DIVISION, AND MOUSE INFECTIVITY

Tao Lin, Lihui Gao, Xiaowei Zhao, Jun Liu, and Steven J. Norris
Department of Pathology and Laboratory Medicine, Medical School, University of Texas Health Science Center at Houston, Houston, Texas, USA

**Background:** The Lyme disease spirochete *Borrelia 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. FliH and FliI are two cytoplasmic proteins that play important roles in the type III secretion system- mediated export and assembly of flagellar structural proteins. However, the structures and functions of FliH and FliI genes have not been reported for *B. burgdorferi*.

**Methods:** In this study, we inactivated the *fliH* and *fliI* genes in an infectious strain of *B. burgdorferi* by signature tagged transposon mutagenesis system. The *fliH* and *fliI* transposon mutants were utilized 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 verified prior signature tagged mutagenesis analysis indicating that *fliH* and *fliI* mutants are noninfectious in the mammalian host, although they can colonize *I. scapularis* ticks. The *fliH* and *fliI* mutants exhibited rod-shaped or string-like morphology, greatly reduced ‘twitching’ motility, and division defects (resulting in elongated, string-like and/or rod-shaped organisms). Mutants in *fliH* and *fliI* were incapable of translational motion in 1% methyl cellulose or soft agar. Inactivation of either *fliH* or *fliI* resulted in the loss of the FliH/FliI complex from otherwise intact flagellar motors, as determined by cryo-electron tomography. Flagellar assemblies were still present in the mutant cells, albeit in lower numbers and with truncated flagella. Genetic complementation of *fliH* and *fliI* mutants *in trans* restored their wild-type morphology, motility, swarming ability, and flagellar motor structure; however, infectivity was not recovered in these complemented mutants. Disruption of *fliH* or *fliI* also resulted in the altered abundance of certain proteins in the protein profiles of these mutants.

**Conclusion:** Based on these results, disruption of either *fliH* or *fliI* in *B. burgdorferi* results in a severe defect in flagellar structure and function, cell division, and infectivity, but does not completely block the export and assembly of flagellar hook and filament proteins.
ATPase-INDEPENDENT TYPE-III PROTEIN SECRETION IN SALMONELLA ENTERICA

Marc Erhardt¹, Max E. Mertens², Florian D. Fabiani¹, and Kelly T. Hughes²
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²Department of Biology, University of Utah, 257 South 1400 East, Salt Lake City, UT, 84112, USA.

Background: Type-III protein secretion (T3S) is utilized by bacteria to secrete building blocks of the bacterial flagellum. T3S is also used by gram-negative pathogens to secrete virulence effectors from the cytoplasm into host cells, and the structural subunits that make up the injectisome complex. The flagellar T3S apparatus utilizes both the energy of the proton motive force and ATP hydrolysis to energize substrate unfolding and translocation.

Results: We report formation of functional, full-length flagella in the absence of FliHIJ type-III ATPase activity by mutations that increased the proton motive force and flagellar substrate levels. We additionally show that increased proton motive force bypassed the requirement of the Salmonella pathogenicity island 1 (Spi1) virulence-associated type-III ATPase for secretion.

Conclusion: Our data support a role for T3S ATPases in enhancing secretion efficiency under limited secretion substrate concentrations and reveal the dispensability of ATPase activity in the type-III protein export process.

Lab: Marc Erhardt
MOLECULAR EMERGENCE: THE MECHANISM FOR THE SELF-ORGANISATION OF FliG IN THE BACTERIAL FLAGELLAR MOTOR

Matthew A. B. Baker¹, Robert M G Hynson¹, Lorraine Galuemas¹, Nasim Shah Mohammadi¹, Anthony Rey¹, Anthony Duff², Cy Jeffries³, Nicolas Delalez⁴, Yusuke Morimoto⁵, Daniela Stock¹, Judith Armitage⁴, Keiichi Namba⁵, Richard Berry⁶, Lawrence Lee¹
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³EMBL Hamburg, DESY, Notkestraße 85, 22603 Hamburg, Germany
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⁵Graduate School of Frontier Biosciences, Osaka University, 1-3 Yamadaoka, Suita, Osaka 565-0871 Japan
⁶Department of Physics, University of Oxford, South Parks Rd, Oxford, OX13QU UK

Background: Complex natural systems at all scales emerge from many components capable of nothing more than a simple interaction with neighbours. On a molecular scale, the basis for the self-organisation of sophisticated, multi-protein machines is unknown. Epitomising this is in the synthesis of the bacterial flagellar motor, a self-assembling, adaptable and tunable rotary motor where one of the first proteins to self-assemble is a rotor protein known as FliG. Several crystal structures have revealed its structure in different conformations with no consensus on its stable state in solution.

Methods: We use small-angle X-ray scattering to determine the solution structure of size-exclusion purified monomer. We confirm the relevance of monomeric solution structure using evolutionary covariance and biochemical crosslinking to investigate in vivo assembly.

Results: We show that FliG predominantly exists in the cytoplasm in a compact conformation with self-binding ARM motifs from the middle (ARMm) and C-terminal (ARMc) domains, and with a strained helixMC connecting the two bound domains. We confirm through targeted disulfide crosslinking that in vivo the FliG ring structure consists of intermolecular interactions between ARM motifs on adjacent proteins, and that key residues pairs involved in ARMm-ARMc binding covary evolutionarily across all FliG sequences.

Conclusion: We propose a simple structural and free energy model for assembly that coordinates the self-organisation of FliG in the rotor and extends to a general model for macromolecule assembly.

Lab: Lawrence Lee
STRUCTURE OF H. PYLORI PSEUDAMINIC ACID BIOSYNTHESIS N-ACETYLTRANSFERASE PSEH AND ITS IMPLICATIONS FOR THE MOLECULAR BASIS OF THE NOVEL SUBSTRATE SPECIFICITY

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Background: Persistent Helicobacter pylori infection requires functional flagella that are heavily glycosylated with pseudaminic acid (Pse). Pse biosynthesis protein H (PseH) catalyzes the third step in its pathway. It belongs to the GCN5-related N-acetyltransferase (GNAT) superfamily.

Methods: The crystal structure of the PseH complex with cofactor acetyl-CoA has been determined at 2.3 Å resolution. Based on structural homology with MccE and WecD, the Michaelis complex has been modeled.

Results: This is the first crystal structure of the GNAT superfamily member with specificity to UDP-4-amino-4,6-dideoxy-β-L-AltNAc. Interestingly, PseH is more similar to the GNAT enzymes that utilize amino acid sulfamoyl adenosine or protein as a substrate than a different GNAT-superfamily bacterial nucleotide-sugar N-acetyltransferase of the known structure, WecD. Analysis of the complex of PseH with acetyl-CoA revealed the location of the cofactor-binding site between the splayed strands β4 and β5. Analysis of the model for the Michaelis complex suggests that the nucleotide- and 4-amino-4,6-dideoxy-β- L-AltNAc-binding pockets form extensive interactions with the substrate and are thus the most significant determinants of substrate specificity. A hydrophobic pocket accommodating the 6'-methyl group of the altrose dictates preference to the methyl over the hydroxyl group and thus contributes to substrate specificity of PseH.

Conclusion: The first crystal structure of the GNAT superfamily member with specificity to UDP-4-amino-4,6-dideoxy-β-L-AltNAc provides a molecular basis for understanding the third enzymatic step in the biosynthesis of pseudaminic acid in bacteria. The structure of PseH, together with the conservation of the active-site general acid among GNAT superfamily transferases, are consistent with a common catalytic mechanism for this enzyme that involves direct acetyl transfer from AcCoA without an acetylated enzyme intermediate. Our analysis pinpoints key structural features that contribute to specificity of this enzyme.

Lab: Anna Roujeinikova
THE ASYMMETRIC ROLE OF CONSERVED-CHARGED-RESIDUES IN ROTOR-STATOR INTERACTION IN FLAGELLAR MOTOR

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Background: Most bacteria can swim towards favorable conditions by rotating their flagella bidirectionally. It has been known that the conserved-charged residues in the rotor and stator have an important role in the rotor-stator interaction. Previous studies, however, mainly focused on the role of these residues in rotation in the forward direction (counter-clockwise rotation, CCW). Here, we verified that these conserved-charged residues are also important for rotation in the reverse direction (clockwise rotation, CW).

Methods: Chimeric rotor and stator were expressed in *Escherichia coli*. Using tethered-cell assay, we observed the rotation of this motor and controlled its rotational directions by changing the solution in the flow chamber from a repellent to an attractant. We measured rotational speeds in both directions of each cell and analyzed CCW-CW relationship. Comparing this relationship of wild-type with that of the charge-neutralizing mutations in the rotor or the stator, we examined the rotational-direction-dependent role of these charged-residues.

Results: In wild-type cells, rotational speeds in both directions were almost the same, as demonstrated previously. This indicates our experimental system is working well. Similarly, in case of the charge-neutralizing mutations in the stator, rotational speeds in both directions were almost the same. However, in the case of some mutations in the rotor, there was a significant difference in the rotational speed in the two directions.

Conclusion: Conserved-charged residues in the stator contribute equally to rotations in both directions. Some conserved-charged residues in the rotor are more critical for rotational-direction dependence than that in the stator.

Lab: Michio Homma
IN SITU STRUCTURES OF THE SWITCH COMPLEX PROVIDE NOVEL INSIGHTS INTO CheY-P BINDING AND FLAGELLAR SWITCHING

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Background: The Lyme disease spirochete, Borrelia burgdorferi, is highly motile and invasive pathogen. The spirochete motility, which is driven by periplasmic flagella, is unique as the entire bacterium is involved in two distinct motility phenotypes: “run” and “flex”. A sophisticated signaling system allows the spirochete to sense chemical stimuli and ultimately regulates the direction of flagellar rotation and the spirochete motility (Figure). Recent studies indicate that the non-chemotactic mutants ΔcheA₂ or ΔcheY³ constantly run, while the ΔcheX mutant constantly flexes, suggesting that cheA₂, cheY³, and cheX play critical roles in B. burgdorferi motility. However the underlying mechanism remains elusive.

Methods: We use cryo-electron tomography (Cryo-ET), targeted mutagenesis, and GFP fusion labeling to examine the in situ flagellar motors of B. burgdorferi.

Results: Here, we first characterize the subunit organization of the switch complex in the spirochetal motor by systemically analyzing deletion mutants of FliG1, FliG2, FliM, and FliN. Furthermore, we determine two distinctive motor conformations directly associated with two swimming behaviors in ΔcheX and ΔcheY³ mutants, respectively. Employing a GFP-fusion of a phosphorylated form of the signaling protein CheY (CheY-P), our studies provide direct structural evidence that CheY-P interacts with the switch complex protein FliM, triggering a large conformational change in the switch complex, which we propose is essential for flagellar switching during motility.

Conclusion: The in situ flagellar motor of B. burgdorferi reveals dramatic structural difference in the switch complex, when the cells are locked in running and flexing motility modes. Based on the structure evidence, we propose a CheY-P binding induced switch complex conformational change and flagellar motor switching mechanism.

Lab: Jun Liu
FLAGELLAR MOTOR ROTATION SWITCHING RATES IN CAULOBACTER CRESCENTUS FOLLOW FIRST PASSAGE TIME DISTRIBUTION

Michael Morse, Guanglai Li, and Jay X. Tang
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Background: The bacterial flagellar motor rotates a helical flagella filament, propelling cells through their fluid environment. Most uni-flagellated bacteria switch the direction of motor rotation between clockwise (CW) and counterclockwise (CCW) to alternate between forward and backward swimming. The intervals between motor switches determine a cell's swimming trajectory and altering these times can lead to directed motility. In particular, the level of signaling protein CheY-P regulates chemotactic response by affecting the intervals of the CW motor rotation. We aim to measure the motor switching behavior of uni-flagellated Caulobacter crescentus and use a model of dynamic CheY-P binding to explain the resulting distribution of switching intervals.

Methods: Experiments were performed using the mutant C. crescentus strain, CB15 Delta Pilin, which does not grow pili used by the wildtype cells to adhere to surfaces. Two types of measurements were performed. First, we imaged cells with their flagella stuck to a glass slide at 60X magnification while the cell body visibly rotated due to the rotation of the flagellar motor. Second, cells swimming freely in a microfluidic chamber were imaged at low 10X magnification using dark field so that their trajectories could be followed over long distances and time. In both cases, the times at which motor switching occurred were recorded based on the motion of the cell body and used to construct distributions of CW and CCW rotation intervals (Figure 1).

Results: Both types of measurements return similar distributions. For both CCW and CW rotation, there is a clear peak time interval around one second. The frequency of switches drops to zero as time goes to zero and there is an exponential decay at longer times. The distribution of CCW rotation is more sharply peaked and CW rotation has a larger tail at long times, resulting in a longer average interval. We find the data can be well fit using a curve generated by first passage time theory for a biased random walker, implying a stochastic process that governs the observed behavior.

Conclusion and Model: The clear presence of a peak switching time indicates motor direction is not controlled by constant switching rate, as governed by familiar Poisson statistics. Additionally, the agreement between the two experiments indicates that the switching rate of the Caulobacter motor is not load dependent. The first passage time behavior of switching can be explained by modeling the dynamic binding of CheY-P to FliM subunits as a biased random walk. Over time, the number of subunits bound will fluctuate as a function of the binding and unbinding rates of an individual subunit. The C-ring’s total degree of CheY-P binding makes CW or CCW rotation energetically favorable and allows for motor switching. The simplest model suggests that the motor switches rotation direction whenever the number of CheY-P bound FliM subunits reaches a high or low threshold number.

Lab: Jay Tang
SWITCHING DYNAMICS OF THE BACTERIAL FLAGELLAR MOTOR NEAR ZERO LOAD

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Background: Switching dynamics of the motor has been observed by tethering single filaments to a glass slide and observing rotation of the cell body, or by attaching micron-sized beads to short or long filament stubs, and watching the beads wobble. A recent model noted that conformational transitions of filaments can contribute to the observed dynamics. To remove this complication and to observe the motor dynamics directly, we utilized the nanogold method developed to study motor behavior near zero load, in which 60- to 200-nm-diameter gold spheres are attached to hooks of cells lacking flagellar filaments.

Methods: To eliminate possible complications due to conformational transitions of these filaments and to look at the output of motors more directly, we monitored motor rotation by attaching nanogold spheres to hooks of cells lacking filaments. We measured the CCW/CW interval distributions for an E. coli strain with all CheY are phosphorylated and its expression can be adjusted by using various amount of the inducer IPTG, and a strain with active CheY mutant cheY¹³⁰K¹⁰⁶W.

Results: We observed exponentially-distributed counterclockwise (CCW) and clockwise (CW) intervals and Lorentzian power spectra of the switching time series, consistent with models that treat motor switching as a two-state Poisson process.

Conclusion: Motor switching near zero load appears to be an equilibrium two-state Poisson process.

Lab: Junhua Yuan
SWITCH MECHANISM OF THE FLAGELLAR MOTOR DEDUCED FROM PHOSPHORYLATION-INDEPENDENT SIGNALING

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Background: In chemotaxis of *Escherichia coli*, binding of the response regulator CheY to the N-terminus of the switch protein FliM (FliM_N) is phosphorylation-dependent and the outcome is clockwise rotation. Here we demonstrate the occurrence of a phosphorylation-independent switching mechanism and reveal, in part, its way of function.

Methods: We constructed mutants with truncated FliM_N and examined their chemotactic responsiveness. Using motors containing YFP-labeled truncated FliM_N isolated from cells at different CheY expression levels we quantified by TIRF microscopy their FliM levels. We produced *in silico* simulation of the switch with different levels of FliM.

Results: We found that FliM_N is not essential for clockwise rotation and chemotactic responsiveness and that CheY acetylation greatly enhances these functions. We further found that, in the absence of FliM_N, clockwise rotation is correlated with CheY level and inversely correlated with the level of exchangeable FliM units within the motor (see figure).

Conclusion: CheY reduces the number of exchangeable FliM units in the motor in a phosphorylation-independent manner. The outcome is enhancement of the motor’s responsiveness on account of its sensitivity, with a consequent enhanced clockwise rotation.

Enhancement of the motor’s responsiveness on account of its sensitivity. (A) Illustration of the CheY effect on the exchange of FliM in the motor, in a model where conformational spread is initiated from exchangeable FliM units. Each color shows conformational spread initiated from an exchangeable FliM unit. (B) Simulation demonstrating that low levels of exchangeable FliM in the motor can enhance the responsiveness of the switch on account of its sensitivity. The responsiveness is the relative fraction of the C-ring being in clockwise state in a given activation probability (i.e., a given probability of CheY binding to exchangeable FliM unit) for 1, 6 or 12 exchangeable FliM units.

Lab: Eisenbach Michael
MOLECULAR INTERACTION OF FLIM AND SPERMINDE SYNTHASE IN HELICOBACTER PYLORI

Huawei ZHANG, Shannon Wing Ngor Au
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Background: Chemotaxis is regarded as the main signaling pathway in bacterial motility, in which phosphorylated CheY binds to switch protein FliM, resulting in rotational switching of flagellum. Additionally, several protein factors have been reported to associate with motor function by binding to the switch complex, such as YcgR in S. enteritidis, EpsE in B. subtilis, H-NS and fumarate reductase in E. coli. It appears that bacteria exploit different strategy to modulate the motor activity. In this study, we aim to identify and characterize the interacting partners of FliM in H. pylori.

Methods: GST pull-down in conjunction with mass spectrometry were employed to identify FliM interacting partners. Their direct biophysical interaction was further confirmed by size exclusion with purified recombinant proteins. The crystal structure of the binary complex was solved by X-ray crystallography.

Results: We have identified spermidine synthase (SpeE) as an interacting partner of FliM in H. pylori. Stoichiometric ratio of SpeE to FliM interaction is 2:1. A 2.8 Å crystal structure of FliM-SpeE complex reveals their interacting surface. Results from pull- down assay further show that the binding of SpeE can reduce the FliG-FliM interaction, suggesting that SpeE may play a role in flagellar assembly and rotational switching.

Conclusion: SpeE is an interacting partner of FliM in H. pylori. SpeE may involve in the switching of flagellar rotation. Biological significance of their interaction still need to be elucidated.

Lab: Shannon Au
TRANSMEMBRANE SIGNALING ROLES OF TSR CONTROL CABLE RESIDUES

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Background: The *E. coli* Tsr protein is a homodimeric transmembrane receptor that mediates chemotaxis to serine. The Tsr molecule contains a periplasmic ligand binding domain and a cytoplasmic kinase control domain that regulates autophosphorylation activity of the histidine kinase CheA. Tsr signaling complexes have two output states, kinase-off, which allows forward swimming, and kinase-on, which causes random directional changes. Serine binding initiates an asymmetric conformational change in the Tsr molecule that produces a small (~2Å) inward movement of one transmembrane helix (TM2) in the four-helix TM bundle. Piston displacements of TM2 in turn modulate the conformation or packing stability of the four-helix HAMP bundle to shift Tsr to the kinase-off output state. A five-residue “control cable” segment connects TM2 to the AS1 helix of HAMP and plays a key role in transmembrane signal transmission in Tsr (figure).

Methods: To explore the transmembrane signaling role of the Tsr control cable we constructed a variety of Tsr mutants with structural changes in TM2 or the control cable, including single amino acid replacements, one-residue additions or deletions, and synthetic control cables and TM2 helices with simplified primary structures. We characterized the signaling properties of the mutant receptors with an *in vivo* kinase assay based on Förster resonance energy transfer (FRET) between YFP-tagged CheY, whose phosphorylation state reflects CheA activity, and CFP-tagged CheZ, which interacts with and dephosphorylates phospho-CheY. This FRET assay measures the amount of CheA kinase activity in mutant receptor signaling complexes and the sensitivity and cooperativity of their responses to serine.

Results: Tsr with an all-alanine control cable (AAAAA) exhibited extremely on-shifted output, suggesting that a stable control cable helix promotes kinase activity. In contrast, Tsr-GGGGG responded to serine stimuli, but with slow kinetics and only partial control of CheA activity, suggesting that a control cable with little helical character destabilizes both native signaling states. The aberrant signaling properties of these synthetic control cables were largely corrected by restoring the native I214 residue, indicating that this position plays a key role in promoting piston-induced signal-state transitions. The signaling properties of Tsr mutants with all possible single amino acid replacements at G213 or I214 indicated that these two residues might serve as a piston-sensitive structural swivel to transmit (on-state) or alleviate (off-state) HAMP-destabilizing structural inputs.

Conclusion: Our study suggests that the Tsr control cable may transmit stimulus inputs to the HAMP domain through helix-coil transitions. The talk will present a possible mechanism for how TM2 piston displacements might modulate such structural transitions in the control cable.

Lab: John Parkinson
STRUCTURE AND CONFORMATIONAL SIGNALING IN THE CYTOPLASMIC DOMAIN OF BACTERIAL CHEMORECEPTORS CHARACTERIZED BY EPR SPECTROSCOPY

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Background: Transmembrane bacterial chemoreceptors couple ligand recognition in the periplasmic domain and adaptational modification in the cytoplasmic domain to receptor conformation and allosteric control of a receptor-associated histidine kinase. At present there is an incomplete understanding of chemoreceptor cytoplasmic domain structure, conformational dynamics, and how those features relate to signal transduction.

Methods: We employed site-directed spin labeling and electron paramagnetic resonance (EPR) spectroscopy to probe features of structure and conformational signaling of the cytoplasmic domain of the *Escherichia coli* aspartate chemoreceptor Tar. By attaching spin labels to cysteines at surface positions along the length of the extended, helical coiled-coil cytoplasmic domain, we probed features of helical backbone dynamics on the nanosecond timescale. Via this technique we assessed the relative extent of region-specific helical stability along the length of the Tar cytoplasmic domain as a consequence of detergent solubilization, lipid association, ligand recognition, and adaptational modification.

Results: We found that detergent solubilization perturbed much of the Tar cytoplasmic domain, that certain regions of bilayer-inserted, functional receptors remain highly dynamic, and that adaptational modification altered helical backbone stability in the modification region. Furthermore, we found that the effects of ligand recognition were distinct from the effects of adaptational modification.

Conclusion: Bacterial chemoreceptors demonstrate varying helical stability along the length of their cytoplasmic domain structure and helical stability is modulated by adaptational modification. We propose these are common features of bacterial chemoreceptors.

Lab: Gerald Hazelbauer
BACTERIAL CHEMORECEPTOR DYNAMICS AS REVEALED BY PULSED DIPOLAR AND CONTINUOUS WAVE ESR

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Background: Recent work implicates dynamical changes in the activity states of modular proteins, such as G-protein coupled receptors and receptor tyrosine kinases. This may also be true for conformational changes of bacterial chemoreceptors induced by ligand binding and covalent modification (1, 2). Chemoreceptor structural transitions are well described in periplasmic and transmembrane domains, but less so in the cytoplasmic domain, which modulates the activity of the CheA histidine kinase. We have developed model systems wherein cytoplasmic modules of the E. coli aspartate receptor Tar are coupled to HAMP domains of defined structure. Two such variants produce opposite chemotaxis responses when expressed in cells. Here, we measure dynamics throughout the two functionally distinct variants and show how these properties impact receptor activity.

Methods: Site-directed spin labeling in conjunction with pulsed dipolar and continuous wave ESR is used to evaluate protein dynamics in receptors of varying activity and modification states. These features are correlated with the ability of the receptors to bind and activate CheA in vitro and produce chemotaxis responses in vivo.

Results: H1-Tar, and H1-2-Tar, the two soluble variants, differentially activate CheA in both cellular and in vitro phosphotransfer assays, while maintaining similar interaction with proteins in the signaling complex. The activity state also responds to adaptation as expected. This study discloses polarized dynamical coupling between the HAMP and protein interaction region (PIR): stable HAMP forces the PIR to be dynamic, while dynamic HAMP allows for a stable PIR. Adaptational modification alters the dynamics of the modules to compensate for activation state. Charge-neutralizing adaptational modifications convert the dynamics in the “off” state towards that in the “on” state, whereas negatively charged modifications have converse effects.

Conclusion: We find that the receptor cytoplasmic domain behaves as one large dynamically coupled system in which activation signals cause destabilization of membrane proximal regions, but stabilization of the most distal protein interaction tip. Inhibitory signals or adaptation of the receptor through chemical modification produces the opposite changes in conformational properties. This reciprocal coupling of stability provides a versatile mechanism for sending conformational signals throughout large modular proteins.


Lab: Brian Crane
EFFECTS OF CORE COMPLEX INCORPORATION AND ON-OFF SWITCHING ON THE DYNAMICS OF CheA P1 SUBSTRATE DOMAIN

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Background: It has been established that the core complexes of the chemosensing array switch between kinase on and off signaling states in the absence and presence of attractant, respectively. The mechanism of kinase on-off switching is not fully understood. Two hypotheses focus on the substrate (P1) domain of CheA, which is connected to the CheA core by a flexible linker and must bind to the CheA catalytic domain to be phosphorylated. Hypothesis 1 proposes that the P1 domain is mobile in the on-state and is free to collide randomly with the catalytic domain, whereas in the off state P1 is tightly bound to an inhibitory site (1,2). Hypothesis 2 proposes that the P1 domain is primarily bound to the catalytic site in the on-state, and is tightly bound to a separate inhibitory site in the off-state. To resolve these hypotheses we are using disulfide trapping techniques to investigate the mobility of the CheA P1 domain in the two different signaling states.

Methods: Membrane-bound core complexes are reconstituted using standard methods to incorporate a di-Cys CheA mutant into the core units. The di-Cys mutant possesses one Cys in the P1 domain and a second Cys in a different CheA domain, enabling detection of inter-domain collisions within the core unit by disulfide formation as previously described for free CheA (2). Disulfide-trapped collisions are quantitated by SDS-PAGE analysis as previously described (2).

Results: The initial data reveal that the extensive P1 dynamics observed for free CheA are dramatically reduced when the CheA is incorporated into core complexes. (Fig. 1). My talk will also present ongoing experiments examining the effect of attractant on P1 dynamics in core complexes.

Conclusions: The initial findings reveal a dramatic decrease of P1 dynamics when CheA incorporates into core units. These findings will be discussed, together with ongoing studies of the effect of receptor-mediated on-off switching on the P1 dynamics.

References:  

Lab: Joseph Falke
STRUCTURE/FUNCTION INSIGHTS FROM THE *Helicobacter pylori* CHEMORECEPTOR TLPA

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**Background:** *Helicobacter pylori* is a human pathogen that colonizes the stomach and can cause ulcers and cancer. We are interested in understanding the chemical cues *H. pylori* senses in order to navigate within its unique environment and are conducting structural studies of the chemoreceptors responsible for sensing them. Previously, we presented the structure of the periplasmic portion of the acid-sensing chemoreceptor TlpB, the monomer of which contains a single PAS domain that tightly binds a molecule of urea. Urea binding is directly implicated in the mechanism for sensing ambient pH, which is important for bacterial localization to the stomach lining. Here we present the 2.0 Angstrom crystal structure of the periplasmic region of TlpA, previously suggested to sense arginine and sodium bicarbonate.

**Methods:** X-ray crystallography, absorbance spectra

**Results:** The periplasmic portion of TlpA contains tandem PAS domains and in overall structure is very similar to the LuxQ receptor of *Vibrio harveyi*. The membrane distal PAS domain, implicated in ligand binding, is very similar to the heme-binding PAS domain of redox-sensitive *Escherichia coli* DOSH, which crystal structures demonstrate to bind a molecule of heme. We present spectroscopic evidence for heme binding to TlpA, suggesting a novel role for TlpA as a heme binding chemoreceptor. Heme may act as a chemoattractant to direct movement toward lesions in the stomach lining, or as a co-factor to sense redox changes, iron, or gas levels (O2, NO, CO).

**Conclusion:** We have determined crystal structures of two *H. pylori* chemoreceptors, both of which we have demonstrated contain ligand-binding PAS domains. Based on sequence homology searches conducted by ourselves and others, we predict that ligand-binding PAS domains will be a predominant structural characteristic of bacterial chemotaxis receptors.

**Lab:** S. James Remington
ALL-ATOM STRUCTURE AND DYNAMICS OF AN INTACT BACTERIAL CHEMOSENSORY ARRAY

C. Keith Cassidy\textsuperscript{a}, Benjamin Himes\textsuperscript{b}, Frances Joan D. Alvarez\textsuperscript{b}, Juan R. Perilla\textsuperscript{a}, Jun Ma\textsuperscript{b}, Gongpu Zhao\textsuperscript{b}, Peijun Zhang\textsuperscript{b}, and Klaus Schulten\textsuperscript{a}

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Background. The chemotactic response in bacteria relies on the formation of large, highly ordered complexes of sensory proteins, known as chemosensory arrays, which mediate the transduction and regulation of signals that ultimately control cellular motility. Although, a coarse representation of the array's extended organization has recently been revealed, an outstanding problem concerns the detailed description of the molecular events occurring within the array during signaling. Progress in this area necessitates a high-resolution understanding of the intact chemosensory array structure.

Methods. Combining large-scale, all-atom molecular dynamics (MD) simulations with electron tomography data derived utilizing a novel \textit{in vitro} reconstitution technique, we have constructed and refined an atomic model of the chemosensory array's core structure.

Results. MD simulations of the 1.2 million-atom array unit cell have revealed the molecular details of the interaction interfaces between the receptor, CheA, and CheW proteins, as well as a distinctive conformational change in CheA. Mutagenesis and chemical cross-linking studies have further confirmed the important roles of specific residues at these critical interaction interfaces.

Conclusions. Joining experimental and computational methodologies, we derive a refined picture of the core structure of the chemosensory array and provide molecular insight into key singling events therein.

Figure – All-atom modelling of core structure from \textit{T. maritima} chemosensory array. (Left) Top view (top) and side view (bottom) of 1.2 million-atom unit cell model. (Right) Top view of 11 million-atom extended lattice model obtained from equilibrated unit cell model via tiling.

\textit{Lab: Klaus Schulten}
MECHANISM FOR THE SPECIFIC TARGETING OF METHYLTRANSFERASES TO CHEMORECEPTORS

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Background: Studies with enterobacteria that contain a single CheR and CheB show that, apart from binding at the methylation site, some chemoreceptors recognize CheR and CheB at additional sites at C-terminal pentapeptides in the chemoreceptor. The relevance for these dual binding is not fully understood. We investigate here the interaction of C-terminal pentapeptides from chemoreceptors of the human pathogen Pseudomonas aeruginosa with each of the 4 CheR and CheB paralogues.

Methods: Microcalorimetric binding studies of the 4 purified CheR and CheB with 3 pentapeptides of P. aeruginosa receptors, in vitro methylation assays, sequence analysis, site-directed mutagenesis of CheR2 and the McpB chemoreceptor.

Results: Binding studies show that CheR2 is the only methyltransferase that binds to the GWEEF pentapeptide of the McpB chemoreceptor. The cheR2 and mcpB genes are vicinal in the genome and form part of the gene cluster that encodes the che2 chemotaxis pathway. CheR2 was the only parologue that methylated McpB, and deletion of the pentapeptide abolished both the CheR2-McpB interaction and methylation of McpB. We found that bacterial CheRs form two protein families when clustered according to sequence, those that bind pentapeptide-containing chemoreceptors and those that do not. We also identify a sequence/structural feature that distinguishes pentapeptide dependent CheR from pentapeptide independent proteins. These data have been published recently (1), whereas studies on CheB-pentapeptide interactions are ongoing and will be presented.

Conclusion: The CheR-pentapeptide interaction enabled the specific targeting of one CheR methyltransferase to one chemoreceptor. Because many bacteria contain pentapeptide-containing chemoreceptors and several signaling protein paralogues, we predict that the mechanism described may contribute to the specific assembly of signaling proteins to form distinct, parallel pathways that mediate different responses.


Lab: Tino Krell
THE EVOLUTION OF THE CHEMOTAXIS SYSTEM IN ARCHAEA

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Background: The chemotaxis pathway is the control system of the motility apparatus in prokaryotes. Over the course of evolution, two-components system presumably evolved in complexity to give birth to an ancient version of the modern chemotaxis system. Because two-component systems are thought to have first appeared in an ancient lineage of Bacteria, chemotaxis is thought to have arisen in Bacteria and later been transferred to Euryarchaeota. However, recently sequenced genomes of the deeply branched Thaumarchaeota also contain chemotaxis and motility genes, suggesting the hypothesis that the last archaeal common ancestor (LACA) may have been chemotactic.

Methods: We use phylogenetics and bioinformatics of the chemotaxis genes in archaeal genomes and selected bacterial genomes with closely related chemotaxis systems.

Results: Here we show evidence to support the idea of lateral gene transfer (LGT) of the chemotaxis system from Bacteria to the common ancestor of Euryarchaeota, despite the presence of chemotaxis genes in Thaumarchaeota. Our phylogenetic analysis shows that Thaumarchaeota received their system from an ancient lineage of Euryarchaeota before the branching of Methanococci but after the branching of Thermococci. In addition, some archaeal organisms contain secondary chemotaxis systems, which were received from Bacteria in a much more recent LGT.

Conclusion: Taken together, these results summarize billions of years of evolution of chemotaxis in Archaea. The current view of a massive exchange of genes between Bacteria and Archaea during the evolution of prokaryotes contrasts dramatically with the subsequent evolutionary history of the chemotaxis system in Archaea. After being received from Bacteria, more specifically from a lineage related to Firmicutes, Thermotogales and Synergistetes, the main chemotaxis system appears to have evolved vertically within the Euryarchaeota phylum, and transferred to Thaumarchaeota.

Figure: Main evolutionary events of the chemotaxis system in Archaea mapped onto the prokaryotic tree of life. Presence or absence of the system is marked by dark or white nodes. Arrows represent LGT of the main chemotaxis system (solid) or secondary systems (dotted and dashed). Bars represent...
SINGLE-MOLECULE IMAGING OF ELECTROPORATED MOTILITY PROTEINS IN LIVE E. COLI

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Background: The flagellar motor of Escherichia coli is powered by a transmembrane flux of H⁺ and the chemical energy is converted into work through a ring of stator units pushing on a central rotor. Bacterial chemotaxis is the biasing of movement towards regions that contain higher concentrations of beneficial, or lower concentrations of toxic, chemicals and is one of the most well-understood sensory pathways. Upon phosphorylation, the response regulator protein CheY transduces changes of environmental chemical concentrations detected by specific transmembrane chemoreceptors to the flagellar motors: it binds to the N-terminus of the FliM proteins in the C-ring part of the motor (also known as the switch complex) inducing a cascade of conformational changes that modulate the direction of rotation (see Figure).

Methods: We combine a novel technique for protein internalization in live bacteria based on electroporation¹ and single-molecule imaging using a custom-built microscope. Our aim is to exploit electroporation of fluorescent dye-labelled chemotaxis and motor proteins in electrocompetent E. coli cells to perform an in-depth investigation of the interactions between the latter and the motor complex in vivo, aided by the long-lasting fluorescence from organic dyes.

Results: We have developed protocols to purify motility and chemotaxis proteins, label them with organic dyes and insert them into live E. coli cells by electroporation. We have characterized such system in depth quantifying cellular uptake, dye-protein conjugates’ photobleaching lifetimes and cellular growth and motility recovery. In the first experiments exploiting this new capability, video fluorescence microscopy shows single molecules of one of these proteins (CheY) diffusing within cells, binding to the sensory cluster at the cell pole and to individual flagellar motors where they are known to cause switches of direction.

Conclusion: Our work will allow fluorescent protein fusion tag-free imaging and tracking of a single CheY protein performing its function in real time, travelling between the polar cluster and the motor complex, and measurement of the relevant binding constants and dwell times at each end of its journey. Depending on results obtained for CheY, other proteins relevant to the functioning of the bacterial flagellar motor will be considered for future work.

Figure (taken from [2])


Lab: Richard Berry
MAPPING E. COLI SWIMMING BEHAVIOR TO CHEMOTAXIS PROTEIN COUNTS IN SINGLE CELLS

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Background: Clonal populations of E. coli cells display a broad diversity of swimming behavior from cell-to-cell even when cultured in homogeneous conditions. Theoretical analyses suggest that these differences arise from cell-to-cell variability in protein numbers and that phenotypic diversity can be beneficial to clonal bacterial populations as a mean to overcome performance tradeoffs or to hedge their bets under uncertain conditions (Dufour Y, Fu X, Hernandez-Nunez L, Emonet T, PLoS Comp Bio 10(6): e1003694 (2014); Frankel NW, Pontius W, Dufour Y, Long J, Hernandez-Nunez L, Emonet T, eLife 10.7554/eLife.03526 (2014)).

Method: To understand how protein counts determine the swimming behavior of individual cells, we developed an experimental protocol that combines cell tracking with epifluorescence microscopy. After recording the trajectories of free swimming cells over a wide field of view, the cells are immobilized in a hydrogel to count fluorescently tagged proteins in each tracked cell using high magnification.

Results: From cell trajectories, we characterized the shapes of the distributions for swimming speed, tumble bias, and diffusion coefficient within the wild type E. coli population. By matching cell trajectories to calibrated fluorescence measurements, we determined the quantitative relationship between counts of two chemotaxis proteins, CheR and CheB, and individual cell swimming behavior.

Conclusion: We found that the tumble bias of individual cells is partially determined by the ratio of CheR over CheB proteins counts. The remaining variability is likely caused by fluctuations in the numbers of the remaining proteins of the chemotaxis system. This new experimental method enables the careful dissection of the relationships between protein counts and cell behavior for bacterial chemotaxis and other biological systems at the single-cell level.

Figure 1. Mapping swimming behavior to chemotaxis protein counts in single cells. A. Cell trajectory tracking and tumble detection. B. Fluorescence acquisition in three channels from single-cell trapped in a hydrogel. C. Cell tumble biases mapped on tagged CheR and CheB counts.

Lab: Yann Dufour
3D TRACKING FOR THE PEOPLE: HIGH-THROUGHPUT LABEL-FREE IMAGING OF BACTERIAL MOTILITY ON A STANDARD PHASE CONTRAST MICROSCOPE

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Background: Unbiased characterization of bacterial motility requires the ability to follow individual cells in three spatial dimensions. The pioneering work of Berg (Nature 1972) established the run-and-tumble motility strategy of *E. coli* using a custom-built 3D tracker, but there is now a renewed interest in 3D tracking to better understand alternative motility strategies (e.g. run-reverse-flick of *V. alginolyticus*, or run-and-pause of *R. sphaeroides*). Berg’s physical tracking method, however, requires sophisticated engineering that is inaccessible to most laboratories, and is also limited to tracking one cell at a time. Other subsequently proposed tracking techniques are limited either in temporal resolution and range of applicability (Wu, Appl. Environ. Microbiol. 2006), spatial range (Edwards, J. Micro-Bio Robot. 2014) or technical ease of implementation and use (Molaei Phys. Rev. Lett. 2014; Vater, PLOS ONE 2014).

Methods: We demonstrate a label-free 3D bacterial tracking technique that extracts position information in the direction along the optical axis (z) from the diffraction patterns observed out of focus in phase contrast microscopy.

Results: Dozens of bacteria can be followed simultaneously at micron-scale spatial resolution and video rate temporal resolution over a z range of ~200 µm and lateral range of ~300 µm x 350 µm. We demonstrate the application of this technique to a range of enteric, marine, and soil bacteria where we confirm previously observed motility patterns. In addition, we employ the new technique to reveal that behavioural individuality, rather than stochasticity, underlies the broad population distribution observed for a key motility parameter (the flick angle) of the marine bacterium *Vibrio alginolyticus*.

Conclusions: With its minimal technical requirements, broad applicability and high throughput, our technique presents a simple and powerful new tool for the characterization of bacterial motility patterns.

**Figure 1**: A compilation of example trajectories obtained by 3D tracking of a selection of bacterial species. Trajectory starting points are marked by black dots.

Lab: Tom Shimizu
FAST, HIGH-THROUGHPUT MEASUREMENT OF COLLECTIVE BACTERIAL CHEMOTAXIS

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Background: The population-level measurements of the chemotactic behavior of microorganisms have been limited so far to semi-quantitative (agar plate assay) or indirect, poorly time-resolved (cell density profile responses) techniques. Elaborating on recent developments in Fourier image analysis, we designed a new method (φDM) which allows the fast accurate measurement of collective drifts using video-microscopy. As a proof of principle, we investigated the chemotaxis of E coli populations.

Methods: φDM was tested using computer simulations and then applied to experiment. Populations of planktonic bacteria are subjected to steady gradients of attractants, created using home-made millifluidic devices. The motion of thousands of cells is recorded using low-magnification video-microscopy. A subsequent computer analysis of the temporal evolution of the Fourier components of the image intensities enables to measure the collective drift velocity of the population of cells, without actually tracking them.

Results: The technique is found to measure collective drifts with a precision of 50 nm/s and a temporal resolution set by the camera (down to 2 ms in our case). The response to gradients of methyalaspartate is found to display a regime of absolute gradient sensing at low background concentrations of attractants, followed by a relative gradient sensing at higher concentration. A model for the collective chemotactic velocity was derived based on the classical single cell response models, and found to be in excellent agreement with our data.

Conclusion: φDM is a robust technique for measuring chemotactic drifts, with unprecedented precision and excellent time resolution, making it fit to investigate weak responses and time varying stimuli. This population-level assay has a clear interpretation: only the chemotactic efficiency is probed, independently of other factors like swimming efficiency or growth rate. The effect of cell density on the chemotactic efficiency is now under investigation.

Lab: Victor Sourjik
CHEMOTAXIS TO NOREPINEPHRINE METABOLITES

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Chemotaxis to host molecules in the gut is proposed to play an important role in pathogenesis. However, little is understood about the mechanism of such chemotaxis responses in bacteria. We addressed this issue by investigating chemotaxis to metabolites derived from the catecholamine norepinephrine (NE), which is reported to induce virulence in pathogenic bacteria. K-12 strains of Escherichia coli exposed to NE produce enzymes that can convert NE into 3,4-dihydroxymandelic acid (DHMA; see Figure). The quorum-sensing kinase QseC, previously shown to regulate virulence and motility in enterohaemorrhagic E. coli (EHEC), is essential for induction of the enzymes required for conversion of NE into DHMA. Here, we present new information to explain the unusual dose response of DHMA, which is an attractant at low concentrations but fails to evoke an attractant response at high concentrations. Our recent results also show that DHMA induces virulence in enterohemorrhagic E. coli (EHEC), although a BLAST search indicates that EHEC lacks an obvious TynA homolog. NE is ubiquitous in the gastrointestinal (GI) tract. The fact that DHMA is sensed at nM concentrations by the serine chemoreceptor (Tsr), possessed by many enteric bacteria, suggests that chemotaxis to DHMA produced by resident commensal E. coli may direct motile pathogens to their preferred sites of colonization on the intestinal epithelium and induce the production of virulence factors required for effective pathogenesis.

**Pathway for conversion of NE to DHMA**

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<table>
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<tr>
<th>Norepinephrine</th>
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<td>Aldehyde Dehydrogenase (FeaB)</td>
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<td>DHMA</td>
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**Model for the role of NE and DHMA as inducers of virulence**

*Pathogens*

*Intestinal lumen*

*Mucous layer*

*DHMA gradient*

*Commensals*

*Epithelium*

*Lab: Michael Manson*
EXPLORING THE FUNCTIONAL DIVERSITY OF CHEMORECEPTORS FROM PSEUDOMONAS SYRINGAE PV. ACTINIDIAE

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Background: Pseudomonas syringae pv. actinidiae (Psa) is a pathogen of kiwifruit, causing severe crop losses in New Zealand and throughout the world. Psa invades plant tissues through openings and lesions on the plant surface, resulting in wilting, cankers and plant death. It is known that chemotaxis is critical for many pathogenic bacteria to colonise and invade a host. For example, other plant-associated microbes are known to be specifically attracted to host plant exudates such as amino acids, organic acids and sugars. Genome sequencing of a virulent strain of Psa isolated in New Zealand (strain NZ-V13) revealed that it has 43 predicted chemoreceptors, none of which have been previously studied. To shed light on how Psa senses and responds to environmental signals (including those from its host), we have begun to characterise the ligand specificities of its chemoreceptors.

Methods: We have developed a high-throughput, fluorescence thermal shift (FTS) assay for identifying the signal molecules that are recognised by a given chemoreceptor ligand binding region (LBR). Each LBR was expressed recombinantly in Escherichia coli, purified, and screened using FTS assays against a commercially available ligand collection. Ligands identified via high-throughput screening were confirmed by isothermal titration calorimetry, and qualitative capillary assays were used to assess chemotactic response in vivo.

Results: To date, we have screened 5 chemoreceptor LBDs from Psa against ~400 potential ligands. We have identified three amino acid chemoreceptors, which are homologs of the P. aeruginosa PAO1 amino acid chemoreceptors PctA, PctB and PctC. In each case, the binding profiles of the amino acid chemoreceptors from Psa were distinct from their P. aeruginosa PAO1 homologs. Notably, Psa PctA-LBR only bound the acidic amino acids (aspartate and glutamate), while P. aeruginosa PctA-LBR binds all of the L-proteinogenic amino acids except for these two. A combination of homology modeling, site-directed mutagenesis and functional screening identified a single amino acid residue in the Psa PctA-LBR (A146) that is important for determining its narrow specificity. We have also identified two chemoreceptors which mediate chemotaxis towards nitrates and nitrites.

Conclusion: By using a new high-throughput screen, we are characterising the complete chemosensory repertoire of Psa. This screening approach can also be used to identify chemoeffectors in other bacteria with similarly large numbers of chemoreceptors.

Figure 1. Functional characterization of Psa chemoreceptors (A) SDS-PAGE gel of six purified Psa LBRs. (B) Detection of chemoreceptor-ligand binding via thermal shift assays. (C) Qualitative capillary assays to assess the chemotactic response of Psa to identified ligands.
INTRAVITAL ASSESSMENT OF A BORRELIA BURGDORFERI cheY3 MUTANT IN MOUSE SKIN TISSUES REVEALS CRITICAL SPIROCHETAL PROPERTIES FOR MOTILITY, DISSEMINATION AND PERSISTANCE IN THE DEVELOPMENT OF LYME DISEASE

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Background: Borrelia burgdorferi (Bb) is a spirochetal bacterium that utilizes endoflagellar motility to efficiently disseminate through dense host tissues, and eventually persist to cause Lyme disease. Unfortunately, the importance of Bb motility/chemotaxis in dissemination and immune evasion cannot be correctly assessed in vitro, since the complexity of host tissues cannot be accurately replicated. Our goal is to determine the importance of the chemotaxis-associated cheY3 gene on Bb motility/dissemination patterns within intact mouse tissues using our novel intravital microscopy techniques, and to correlate these activities with persistence in host tissues.

Methods: Inbred mouse lines, as well as transgenic lines that possess different fluorescent immune cell lineages, were infected intradermally with fluorescent wild-type (WT) and a cheY3-deficient (∆cheY3) Bb strain. Both bacterial and immune cell motility and dissemination patterns were assessed using multiphoton fluorescent confocal microscopy to directly observe these patterns by direct visualization within intact skin tissue and in real-time. Parallel studies were performed using quantitative real-time PCR to confirm any differences observed by microscopy.

Results: Initial experiments determined that WT Bb remain at the skin inoculation site for ~48h before disseminating through skin tissues, primarily through dermal layers associated with collagen. Langerhans cells, macrophage/dendritic cells, and neutrophils appear to recognize these bacteria and some are able to phagocyte/kill these pathogens. However, Bb are able to achieve speeds that are ≥40x faster than any immune cell within skin tissues. Bb eventually disseminate throughout host tissues, where they can persist for >2 years. Interestingly, the bacteria maintain a steady population during this time and continually move in a forward-backward pattern. A ∆cheY3 Bb strain possessed similar cellular morphology, growth rates, and velocities as WT in vitro, though ∆cheY3 did move slower in murine skin tissue than WT Bb. Notably, GFP-∆cheY3 were unable to reverse direction both in vitro and in vivo, whereas WT display primarily a sequential forward-backward motility pattern. Within skin, ∆cheY3 appear to be cleared by 72-96h post- infection, and more global qPCR analyses indicate that ∆cheY3 was unable to disseminate to more distant sites.

Conclusion: Spirochetal motility appears to be crucial for evading the early cellular immune responses and to establish persistent infection. Chemotactic events that allow forward-backward motility appear essential to prevent Bb from getting stuck in host tissues and efficiently cleared by the innate immune defenses, thus allowing the development of Lyme disease.

Lab: Mark Wooten
BIOCHEMICAL, GENETIC, AND MICROFLUIDIC CHARACTERIZATION OF THE BACTERIAL FLAGELLA SYSTEM

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Background: Bacteria utilize propeller devices called flagella to move through liquid environments. These nanomachines provide an enormous survival advantage by allowing bacteria to move towards nutrients, or away from toxins, at speeds of more than 20 body lengths per second. Reflecting its complexity – more than 50 genes involved – assembly of the flagellar system is highly regulated. The respective genes can be divided into three expression-stages consisting of the early, middle, and late phases. During the first phase of assembly, the flagellar master regulatory complex, FlhD\textsubscript{4}C\textsubscript{2}, encoded by \textit{flhD} and \textit{flhC}, directs $\sigma^{\text{70}}$-mediated transcription of middle genes to build the molecular motor (basal body) and a universal joint (hook) which transmits torque generated by the motor to the propeller device called the filament. The FliA protein is a transcription factor that is specific for late gene transcription. Prior to hook basal body (HBB) completion, FlgM binds to FliA as a flagellum specific inhibitor of the class III genes. After the HBB is completed, there is a substrate specificity switch that allows FlgM to be secreted from the cell, releasing FliA to direct late gene transcription required for filament completion. FliS is a chaperone that binds the disordered C-terminal tail of flagellin to protect it from proteolysis in the cytoplasm and to facilitate its export during the filament assembly process. Strikingly, recent studies in \textit{Salmonella enterica} serovar Typhimurium and \textit{Yersinia pseudotuberculosis} show that FliS also interacts with FlgM both \textit{in vitro} and \textit{in vivo}. Why FliS would bind to FlgM and the potential role of the interaction for flagellum assembly is unknown. Based upon our strong preliminary data, which shows there is localized transcription and translation in the biogenesis of flagella, we hypothesize that the interaction between FliS and FlgM acts as the final switch for filament length control (FLC).

Methods: To characterize the interaction of FliS/FlgM, (1) amino acid substitutions in FliS (FliS*) that are unable to interact with FlgM will be isolated via, targeted, PCR mutagenesis. (2) we will use classic biochemical/genomic tools to elucidate the mechanism of FliS mediated filament length control. In addition, we will (3) fabricate a microfluidic device (“Single Cell Microchannels”) (SCMC) to examine the Flagella System in real time and to further examine single cell physiology & molecular processes.

Results: We expect to find FliS amino acid substitutions that abrogate its ability to interact with FlgM. We also expect to see FlgM localized with FliS prior to completion of the filament using time-lapse fluorescence microscopy coupled to the microfluidic platform.

Conclusion: The combined work performed in these studies will allow us to validate whether or not FliS/FlgM acts as a final switch for FLC. Moreover, this mechanism of a FLC switch could be shared across thousands of bacterial species. Figure (left): Prior to completion of the filament, FliS activates the FLC switch by binding to FlgM. This retains FlgM inside the cytoplasm and turns off FliA-mediated class III transcription. FLC is abolished in FliS point mutants that no longer interact with FlgM, resulting in longer filaments.

\textit{Lab: Kelly Hughes}
UPGRADE OF FLAGELLAR MOTILITY IN SHEWANELLA ONEIDENSI S MR-1

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Background: Many well characterized bacteria harbor a single flagellar system powered by one complementary stator set, either the proton-dependent MotAB or the sodium ion-driven PomAB complex. In contrast, Shewanella oneidensis MR-1 (MR-1) possesses only a single polar flagellar system but harbours both stator types. Our group has shown that both PomAB and MotAB are solely sufficient to drive motility in liquid environments and may interact with the rotor in varying configurations depending on sodium ion concentrations, likely forming a hybrid motor. However, cells encoding just the MotAB stator do not spread on soft agar plates and quickly cease to swim in planktonic cultures presumably upon dropping oxygen concentrations.

Methods: The characterization of the stators occurred via a combined approach of FRAP and fluorescence microscopy in concert with physiological characterizations.

Results: Several spontaneous small mutations in the so-called ‘plug- domain’ of MotB (MotB*) were found to bypass the requirement for high oxygen concentrations although the overall speed of the cells decreases (Fig. 1). This ‘plug-domain’ is a small amphipathic alpha-helix close to the transmembrane domain that is thought to prevent ion-leakage in inactive stators. All changes found are either helix breaking mutations or abolish the amphipathic characteristic of this domain. FRAP analysis demonstrated that, rather unexpectedly, the mutated stator is present in the motor at lower numbers and has a significantly higher turnover than wild-type MotAB.

Conclusion: Apparently, MR-1 has evolved a stator that exhibits a more stable motor – stator interaction but is less efficient in promoting a robust motility at low oxygen concentrations. Thus, the suggested hybrid motor of MR-1 may not only respond to changing Na⁺-concentrations but also adjust motility in dependence to oxygen availability.

Lab: Kai Thormann
HOW EFFICIENT ARE BACTERIAL FLAGELLAR MOTORS?

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**Background:** A fundamental question for molecular motors is on their energy efficiencies. Based on the assumptions that the bacterial flagellar motor is tightly coupled and operates close to equilibrium at high load, it is widely believed that efficiency at high load should be close to 1. Nonetheless, recent experiments found that the work done by each torque-generating stator is approximately equal to the energy provided by $37\pm2$ ions (translocation) per revolution. This suggests that the maximum energy efficiency is at most $\frac{37}{52} = 71\%$.

**Methods:** We studied a thermodynamically consistent model for the rotary motor for a wide range of parameters which are physiologically relevant for *E. coli* motors. The properties (torque, speed, efficiency, etc.) of the motor are determined by solving a Fokker-Planck equation with periodic potential and boundary conditions.

**Results:** 1) Torque-speed curve: Our modeling results suggest that a gating mechanism may be present for the ion translocation and is essential for the model to generate the experimentally observed plateau in torque-speed curve. 2) Efficiency: We calculate the motor's energy efficiency defined as the ratio of the mechanical work to the energy cost associated with the stator's forward stepping transitions (powered by ion translocations). We found that there exists a maximum efficiency at an intermediate load, which depends on the ion motive force, the landscape of the stator-rotor interaction potential, as well as chemical transition rates. Our model can explain the lower-than-expectation energy efficiency observed in recent experiments.

**Conclusions:** Our study reveals a fundamental limit to the energy efficiency of bacterial flagellar motors. The optimum efficiency increases with the ion motive force (imf) and only goes to 1 when imf goes to infinity. Our study also shows the conditions in the stator-rotor interaction energy landscape to achieve the optimum energy efficiency and to broaden its range.

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Lab: Yuhai Tu
COMBINING THE SIMPLICITY OF THE MODIFIED AGAROSE CAPILLARY ASSAY WITH AUTOMATED DATA ACQUISITION AND QUANTIFICATION

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Background: The classic Adler capillary assay is the gold standard for assessing the chemotactic ability of a strain. The assay provides a direct quantitative measurement of the response of a population of bacterial cells to a gradient of attractant. However, this method is rather tedious and time-consuming, and reproducible removal of the capillary content can be difficult. The modified agarose capillary assay has eased the assessment of chemotaxis when adding many variables to an experiment. Yet the results are simply presented in a qualitative format showing the chemotactic response at the mouth of the capillary containing the attractant as picture comparisons.

Methods: We developed a method for quantitating the response in the modified agarose capillary assay by measuring pixel intensities of single photographs taken at the mouth of the capillary. In conjunction with a computer-assisted, motorized stage to avoid manual treatment of the capillary, we gained the ability to photograph up to ten chemotaxis chambers over time. We then quantitate the pixel intensities generated by the reflected light caused by the accumulation of cells at the capillary mouth, thus permitting time-course assessments of ten mutant strains in one experimental repetition.

Results: To evaluate the described method, we assessed Sinorhizobium meliloti wild-type, a chemotaxis-negative, and eight single chemoreceptor deletion strains for their abilities to perform chemotaxis to seed exudate components of the host plant, Medicago sativa (alfalfa). The time courses demonstrated that each deletion strain displayed a distinct reduced response to whole seed exudate. Most notably, the mcpU deletion strain exhibited the most diminished response to whole exudate and to a synthetic mixture mimicking the amino acid content in the whole exudate.

Conclusion: The quantified modified agarose capillary assay combines effectiveness and simplicity with automated data acquisition and quantification.

Scheme for quantifying the response observed in the modified agarose capillary assay. A capillary containing attractant is submersed in a suspension of motile bacteria. The area in front of the mouth of the capillary is photographed, revealing an accumulation of cells. The pixel intensity of each subsequent photograph is quantified and plotted over time. Data points are from three independent experiments.
THE ACTIN HOMOLOGUE MreB AND GLIDING MOTORS SHOW INTERDEPENDENT MOVEMENTS IN THE BACTERIUM MYXOCOCCUS XANTHUS

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Department of Molecular and Cell Biology, University of California, Berkeley, USA

Background: MreB is a bacterial actin homologue involved in a wide range of functions including cell wall biosynthesis and chromosome segregation. MreB also plays crucial roles in Myxococcus xanthus gliding motility, which is impaired when MreB polymerization is inhibited. However, it is still unknown how MreB facilitates and adapts to the fast motion of the gliding machinery.

Methods: Here, we tracked the dynamics of single MreB molecules in M. xanthus using single particle tracking photo-activated localization microscopy (sptPALM).

Results: We found that a subpopulation of MreB moved actively in helical trajectories, similar to the behavior of the motor protein AglR, a MotA homologue. The speed of MreB movement was two orders of magnitude higher than that in Bacillus subtilis and E. coli. Unlike these organisms, the fast motion of MreB was not blocked by inhibitors of cell wall biosynthesis in M. xanthus. MreB interacts directly with AglS, a MotB homologue, and MreB movements were lost in mutants that carried truncated AglS.

Conclusion: Our results imply that in M. xanthus, the function of MreB is adapted to provide a scaffold for the gliding motors and that the gliding machinery drives the movement of MreB filaments.

Lab: David Zusman
FLAVOBACTERIUM GLIDING MOTILITY: WHERE IS THE MOTOR?

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Background: Many Bacteroidetes, including Flavobacterium johnsoniae and Cellulophaga algicola, glide over surfaces. Gliding involves the rapid movement of the adhesin SprB along the cell surface. At least 20 proteins are involved in this process. Eight of these function in the type IX secretion system (T9SS) that secretes SprB. Gliding is powered by the proton motive force (PMF) but the components of the gliding ‘motor’ are not known. Only 4 known motility proteins appear to span the cytoplasmic membrane. GldF and GldG are thought to be part of an ATP-binding cassette (ABC) transporter that also includes GldA, whereas GldL and GldM are T9SS components.

Methods: Genetic and comparative genomic analyses coupled with microscopic observations of cell and protein movements are used to attempt to identify the gliding 'motor'.

Results: The GldA-GldF-GldG complex is an unlikely gliding motor since this predicted ABC transporter presumably uses ATP rather than PMF. Comparative genome analyses supported this idea since C. algicola lacked orthologs to gldA, gldF and gldG but exhibited gliding. C. algicola has other ABC transporters that could have replaced the missing Gld transporter. To examine this possibility we isolated 61 C. algicola motility mutants by transposon mutagenesis and identified the disrupted genes. Orthologs to most of the F. johnsoniae motility genes were identified but insertions in ABC transporter genes were not. The T9SS proteins GldL and GldM are thought to secrete SprB across the outer membrane. Are they also the motor that propels SprB along the cell surface? F. johnsoniae gldL and gldM mutants are nonmotile, which may reflect lack of secretion of SprB, lack of motor function, or both. C. algicola gldL and gldM mutants were deficient in secretion but displayed some very limited gliding movements. This suggests either that GldL and GldM are not essential components of the gliding motor or that C. algicola has more than one motility system. To examine possible motor functions of F. johnsoniae GldL the movement of the motility protein SprD, which is not secreted by the T9SS, was examined. SprD-sfGFP moved rapidly in wild type cells and its movement in a gldL mutant is being analyzed. Genome analyses revealed two predicted ExbBD complexes as other candidate 'motors'. The genes encoding these proteins were not identified by analysis of motility mutants but they may have been missed if they are essential for viability or exhibit redundancy. exbB mutants are being isolated and examined to determine if ExbB is involved in gliding.

Conclusion: The nature of the gliding motor remains uncertain. It may be comprised of T9SS proteins or it may consist of other cytoplasmic membrane proteins that harvest the PMF and propel SprB along the cell surface.

Lab: Mark McBride
A ROTARY MOTOR DRIVES FLAVOBACTERIUM GLIDING

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Background: Cells of Flavobacterium johnsoniae, a rod-shaped bacterium, glide over surfaces at speeds of 2-4 µm/s. Gliding is powered by a protonmotive force, but the machinery required for gliding is not known. Proteins associated with F. johnsoniae gliding are not homologous with those involved in flagellar motility. The adhesin SprB forms filaments about 160 nm long by 6 nm in diameter that move longitudinally down the length of a cell, occasionally shifting positions to the right or the left. Interaction of these filaments with a surface produces gliding.

Methods: We sheared Flavobacterium cells to reduce the number and size of SprB filaments and tethered the cells to glass by adding anti-SprB antibody. We calculated the torque generated by the gliding motor. Using a flow cell apparatus, we changed load on the gliding motor by adding the viscous agent Ficoll to tethered cells. We calculated the changes in speed in torque at a high load. We fluorescently tagged GldL, a bacterial Type IX secretion system (T9SS) protein and imaged its dynamics.

Results: Tethered Flavobacterium cells spun about fixed points, rotating at speeds of 1 Hz or more. Most cells rotated counterclockwise, but a few spun clockwise. None were seen to change directions. The torques required to sustain such speeds were large, comparable to those generated by the flagellar rotary motor. We found that a gliding motor runs at constant speed rather than constant torque: the rotation speeds of the tethered cells did not change upon addition of the viscous agent Ficoll. Fluorescently tagged GldL localized near the point of tether, indicating that T9SS localizes with the gliding motor.

Conclusion: Rotation of the gliding motor leads to lateral movement of cell-surface filaments and, subsequently, to gliding. We now know of three rotary motors powered by protonmotive force, the bacterial flagellar motor, the FO ATP synthase, and the gliding motor.

Figure. Two speculative models of Flavobacterium gliding. (A) A Flavobacterium cell with gliding motors attached to baseplates arranged along a looped track (B) A Flavobacterium cell with gliding motors arranged along a looped track.
THE ROLE OF PILJ PERIPLASMIC DOMAIN IN SIGNAL TRANSDUCTION IN *PSEUDOMONAS AERUGINOSA*

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**Background:** *Pseudomonas aeruginosa* moves across surfaces using twitching motility, which is mediated by type IV pili. The Pil/Chp chemosensory system controls type IV pili mediated twitching motility. Recently, a second function was discovered for the Pil/Chp chemosensory pathway: the regulation of intracellular adenosine 3', 5'-cyclic monophosphate (cAMP) through the post-translational modification of the adenylate cyclase CyaB. A third output related to the Pil/Chp system is the directional twitching towards phosphatidylethanolamine (PE). In this study, we set out to determine if twitching motility, directional twitching to PE, and intracellular cAMP regulation were all modulated by classical signal transduction through the MCP PilJ.

**Methods:** An in-frame deletion of the putative periplasmic domain was created by SOE-PCR. This strain PilJΔ74-273 was tested for cAMP levels via reporter assay, twitching motility levels, surface pilation, and directional twitching towards PE.

**Results and Conclusion:** The deletion of the putative periplasmic domain altered twitching motility, but not cAMP levels, nor directional twitching towards PE. This suggests that only control of twitching is mediated via classical signal transduction.

Directional twitching of wild type, pilJΔ74-273, and ΔpilJ towards PE. Lower panels show leading edge of the colony (10x magnification).

Lab: Sonia Bardy
A NOVEL CheY FAMILY REGULATES MOTILITY IN CAULBACTER CRESCENTUS

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Introduction: To optimize their fitness bacteria use chemotaxis to direct their movement in chemical gradients. Environmental input is coupled to motor behavior via surface receptor mediated phosphorylation cascades that culminate in the activation of CheY and the interaction of CheY~P with the motor switch. Recent findings suggested that flagellar motility can also be modulated by the second messenger c-di-GMP (cdG).

Methods: Using cdG-specific Capture Compound Mass Spectrometry we isolated several members of the CheY family in C. crescentus. Proteins were analyzed biochemically and their role in motility control and regulation by cdG was addressed in vivo.

Results: Biochemical analysis demonstrated that the newly identified CheY proteins specifically bind cdG with high affinity. These studies also identified a conserved Arg-rich domain adjacent to the CheY_{cdG} receiver domain, which is required and sufficient for specific binding of cdG. Activation by cdG binding localizes members of this family to the flagellated pole of C. crescentus cells where they interact with the flagellar switch to control motor function. While the presence of cdG and an intact cdG binding site are important for CheY_{cdG} function, substitutions of residues known to be required for CheY phosphorylation remain functional.

Conclusion: These experiments define a new class of CheY proteins, members of which are not controlled by phosphorylation but are activated by binding to the second messenger cdG. This adds another level of complexity to the intricate control of flagellar motor activity and suggests that cdG converges with phosphorylation mediated control to set motor performance. Future studies are geared towards dissecting the individual contributions of CheY_{cdG} proteins to motor function.

Figure: ITC experiment demonstrating cdG binding to wild-type CheY_{cdG} but not to a mutant carrying an Arg to Ala substitution in its Arg-rich domain. Blue=receiver domain; red=Arg-rich domain; grey=C-terminal domain.
ROLE OF c-di-GMP AND MshA PILI IN THE MOTILE TO SESSILE TRANSITION OF VIBRIO CHOLERAE

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Background. The second messenger cyclic diguanylate (c-di-GMP) is an important signaling molecule for motile to sessile transition. c-di-GMP is synthesized by diguanylate cyclases (DGC) and degraded by phosphodiesterases (PDE). In general, increased concentrations of c-di-GMP result in increased attachment and reduced motility. The pathogen Vibrio cholerae encodes 41 proteins containing a DGC domain and 31 proteins containing a PDE domain, demonstrating the complex regulation involved in this signaling cascade. Though DGCs and PDEs that affect motility and biofilm formation have been identified, the molecular mechanism by which they affect motility remains unknown. We have previously identified two PDEs, CdgJ and RocS, which demonstrate a defect in flagellar motility when mutated. In this study we investigated how the PDE CdgJ affects motility and surface attachment.

Methods. To identify mechanism of CdgJ mediated motility defect, transposon mutagenesis was performed in a cdgJ mutant and the mutants were screened for enhancement of motility to wild type levels. Biofilm phenotypes were analyzed with flow cell biofilm reactors and confocal scanning laser microscopy. Near-surface motility phenotypes were observed with high-speed brightfield microscopy. MshA pilus production was analyzed with transmission electron microscopy. Isothermal calorimetry was utilized to determine the interactions between c-di-GMP and the pilus motor proteins.

Results. Many transposon insertions that suppress the ΔcdgJ motility defect were found in genes encoding the MshA pilus. The effect of c-di-GMP on this pilus was previously unrecognized. High-speed imaging revealed that deletion of the predicted extension ATPase, MshE, resulted in impaired interaction with the surface. In contrast, deletion of the predicted retraction ATPase PilT resulted in transient interactions with the surface. This finding suggests that the MshA motor proteins are essential for the irreversible attachment to abiotic surfaces. We also determined that ΔcdgJ has an enhanced surface attachment phenotype. ATPases have been known to bind c-di-GMP, so we hypothesized that either MshE or PilT binds c-di-GMP to allow the integration of this signal to MshA pilus production or activity. We determined that MshE, but not PilT can bind to c-di-GMP in isothermal calorimetry experiments indicating that c-di-GMP may affect MshA assembly.

Conclusions. Collectively, these data show that the MshE and PilT are the respective extension and retraction ATPases for the MshA pilus in V. cholerae. Surface interaction studies suggest that the dynamic nature of the MshA pilus established by the assembly and disassembly of pilin subunits is essential for transition from the motile to sessile lifestyle. Finally, this study establishes that c-di-GMP affects MshA pilus assembly and function through direct interactions with the MshE ATPase.

Figure 1. Proposed mechanism of c-di-GMP signaling through MshE to affect motility and surface attachment.
MECHANISMS OF c-di-GMP-MEDIATED SWARMING MOTILITY REPRESSSION IN PSEUDOMONAS AERUGINOSA

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Background: The second messenger c-di-GMP is a broadly conserved intracellular signaling molecule that plays an important role in controlling bacterial motility. In the opportunistic pathogen, Pseudomonas aeruginosa, elevated levels of c-di-GMP promote biofilm formation and repress swarming motility. The mechanism by which P. aeruginosa responds to increasing levels of c-di-GMP to repress cell motility is not understood. Ultimately, motility is controlled by rotation of the bacterium’s single polar flagellum, which may be powered by two stator complexes encoded by P. aeruginosa, MotAB and MotCD. Hypothesizing that these distinct stator complexes play unique roles in motility, we examined the involvement of each stator complex in swarming motility. To investigate how c-di-GMP may be impacting flagellar function, we also explored the role of a class of c-di-GMP binding proteins (PilZ domain-containing proteins) in regulating c-di-GMP-mediated swarming repression.

Methods: We performed swarming and swimming assays with motAB, motCD, and PilZ-domain mutants in both low and high c-di-GMP strain backgrounds to observe the impact of these genes on flagellar motility. Fluorescence microscopy of GFP-labeled stator proteins allowed us to analyze how c-di-GMP levels impact stator residency in the flagellar motor. Possible interactions between a c-di-GMP effector protein and flagellar components were analyzed using a bacterial two-hybrid assay.

Results: We show that MotCD is required for swarming motility and MotAB contributes to swarming repression. Elevated c-di-GMP levels cause a decrease in polar localization of the stator protein MotD, suggesting that c-di-GMP decreases motility by driving MotCD complexes out of the flagellar motor. We also show that when c-di-GMP levels are high, cell motility is inhibited by both the c-di-GMP binding protein FlgZ and by Pel polysaccharide accumulation. Bacterial two-hybrid analysis shows that FlgZ interacts with the stator protein MotC, and not with MotA.

Conclusion: Our results show that in P. aeruginosa, levels of c-di-GMP regulate the swapping of unique stator sets MotAB and MotCD to impact flagellar function. The involvement of FlgZ in swarm repression and its ability to bind to MotC suggests that FlgZ influences this stator switching in response to c-di-GMP (Figure 1).

**Figure 1.** One possible model for how FlgZ may be interacting with MotC to facilitate stator swapping in response to increasing c-di-GMP levels. Figure adapted from “Protein dynamics and mechanisms controlling the rotational behaviour of the bacterial flagellar motor” by Brown et al., 2011, Curr Opin Microbiol.
A NEW ROLE FOR A DIGUANYLATE CYCLASE: YfiN CONTROLS CELL DIVISION IN RESPONSE TO ENVELOPE STRESS

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Background: The cyclic dinucleotide c-di-GMP is a signaling molecule that controls cellular processes mainly involved in the transition between motility and sessility. Cellular levels of c-di-GMP are set by two classes of enzymes having opposing activities, diguanylate cyclases (DGCs) and phosphodiesterases (PDEs). Most DGCs and PDEs contain various N-terminal sensory input domains linked to transmembrane helices, suggesting that these proteins respond to environmental signals.

Methods: Fluorescent fusions to YfiN were used to monitor its cellular localization in response to various growth conditions. Interaction of YfiN with cell division proteins was monitored both by localization of fluorescent fusions and by two-hybrid assays. Site-directed mutations were used to dissect the importance of the DGC activity of YfiN in its cell division-arrest function.

Results: We find that in both E. coli and Salmonella, YfiN localizes to the division site as cells approach the stationary phase of growth. The mid-cell localization is dependent on the catalytic GGDEF residues, and occurs through direct interaction with the main cell division protein FtsZ. There is a concomitant lengthening in cell size and a growth defect, indicating that YfiN has a negative regulatory effect on cell division. In E. coli, the dynamic relocation of YfiN to the mid-cell is triggered following exposure to membrane-targeting stress conditions, including high salt concentrations and outer membrane-permeabilizing agents.

Conclusion: We have identified the conserved DGC YfiN as a cell division inhibitor in E. coli and Salmonella. This new function for a DGC highlights the versatility of c-di-GMP signaling in bacteria.
QUANTIFYING M. XANTHUS DEVELOPMENTAL DYNAMICS

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Background: The starvation stress response of the bacterium Myxococcus xanthus represents a minimalist example of multicellular development. 48 hours after being spotted on non-nutritive agar, a swarm of several million cells coordinates its motility and self-organizes into hundreds of dome-shaped aggregates called fruiting bodies. Numerous studies support a functional genomic model in which development in M. xanthus results from the activity of a set of transcriptional regulators that directly coordinates gene expression patterns, thereby indirectly controlling cell motility through both inter- and intra-cellular signal transduction networks. We are working to understand how this molecular-scale model can explain swarm-scale dynamics by performing a quantititative phenotypic characterization.

Methods: All mutant M. xanthus strains were generated using standard insertion-disruption protocols. Development was recorded using time-lapse microcinematography at between 40X-100X, and aggregate quantification was performed using custom image analysis software.

Results In wild-type M. xanthus, approximately half the aggregates that appear and grow will actually shrink and disappear by the time development is completed (see fig 1A). To understand this phenomenon, we modeled the disappearance of aggregates using equations that describe Ostwald ripening of droplets in thin liquid films, and were able to predict aggregate disappearance with an average accuracy of 85%. We then applied a similar type of characterization to the analysis of mutant M. xanthus strains, examining the development of more than 400 single-gene disruption mutants covering four families of homologs. We used both qualitative and quantitative methods to analyze dynamic patterns. We have determined that >50% of these mutant strains can be divided into ten phenotypic categories, some of which can only be clearly differentiated from wild-type after close comparison (see fig 1B).

Conclusion: Data and analysis of wild-type M. xanthus support a relatively simple physical model for the formation of aggregates. By comparing these observations to hundreds of mutant strains, we were able to make three significant observations: developmental phenotypes previously thought to be unique or “extreme” are actually common; some of the phenotypic categories appear to cluster with mutant strains representing one of the homologous families; some phenotypic features are more likely to appear together within categories.

Figure 1: 40X Images from a wild-type swarm (A) over 24 hours of development. Aggregates form within 10 hours and darken within 24. A slightly different pattern is observed for the insertion-disruption mutant strain MXAN_0721 (B). Although aggregates form, they differ in both number and opacity.

Lab: Roy Welch
MACROSCOPIC SELF-ORGANIZATION IN A BACTERIAL COLONY

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Background: When establishing biofilms, many bacterial species are able to move across surfaces in multicellular groups, a process known as swarming. Swarming exhibits rich behavior in multicellular dynamics and cell-environment interactions.

Methods: We aimed to understand the interaction between swarming cells and their microfluidic environment using large scale particle tracking.

Results: We discovered macroscopic self-organization in *Escherichia coli* swarms that spans a distance over 10 millimeters. This centimeter-scale self-organization most likely results from biomechanical coupling between swimming bacteria and their local microfluidic environment.

Conclusion: The self-organization may lead to spatial segregation of different subpopulations and may have profound significance to colony development.

Lab: Yilin Wu
THE MOTILITY OF BACTERIA IN A LIQUID CRYSTAL

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Background: Natural environments for bacteria are often asymmetric and possess an ordered organization at small scale (such as collagen, cell membranes, biofilms, soils enriched in chitin, etc...). The discovery of lyotropic liquid crystals (LLC) soluble in water enables the study of biological phenomena in such anisotropic media whose physical properties are not identical in all directions. At high concentration, the molecules in LLC form columnar aggregates that align with each other (the direction of alignment is called director), to create an anisotropic medium. Below a critical concentration, the aggregates do not align and the solution is isotropic. The behavior of motile bacteria in these environments has recently been shown to be drastically different than in normal isotropic aqueous solutions. Rather than doing their well-known 3D random walk, flagellated bacteria move along one-dimensional tracks (forward and backward) aligned with the director of the LLC medium (see figure above).

Methods: In this work, we studied the trajectories of over 1800 individual bacteria inside a biocompatible liquid crystal (disodium cromolyn glycated (DSCG)) as a function of its concentration. We carefully measured their swimming speed and the “degree of linearity” of their trajectories in order to better describe and understand their behavior. By tracking the Brownian motion of microbeads, we also measured the viscosity of DSCG solutions which was found to vary by a factor of 2 along or perpendicular to the director. Combining our measures of speed and viscosity, we calculated the propulsive force generated by the bacteria.

Results: In addition, this behavioral study of the motility of E. coli lead us to discover a new “pretransition” zone between the usual isotropic and anisotropic phase (at low and high concentrations of DSCG respectively), during which the bacteria become very sticky. In this zone, the bacteria’s propulsive force as well as the viscosity of the medium suddenly starts to increase. We hypothesized that these two phenomena are caused by the elongation of the rod-shaped DSCG aggregates. This hypothesis is in agreement with previous studies that show an increase of the propulsive force of flagellar bacteria in a solution of rod-shaped polymers.

Conclusion: This study shines light on the motility of flagellated bacteria in realistic environments and it opens new avenues for interesting applications such as the use of motile microorganisms to probe a medium’s physical properties or smart bandage that could guide bacteria out of wounds.

Lab: Simon Rainville
A CYTOPLASMIC RECEPTOR SEGREGATES ALONG WITH THE NUCLEOID IN MYXOCOCCUS XANTHUS

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Background: Directional motility in the gliding bacterium Myxococcus xanthus requires controlled cell reversals mediated by the Frz chemosensory system. FrzCD, a cytoplasmic chemoreceptor, localizes in dynamic cytoplasmic clusters that occupy the central region of the cell length and are excluded from the poles (see Figure below). Such positioning, similar to that of the nucleoid, encouraged us to investigate whether FrzCD was associated to the nucleoid.

Methods: We performed fluorescent microscopy on DAPI stained frzCD-gfp expressing cells to check whether FrzCD colocalized with the nucleoid. We also set up Electrophoretic Mobility Shift Assays (EMSA) of DNA fragments incubated or not with purified 6His:FrzCD to verify whether FrzCD directly interacted with the DNA. Finally, we expressed frzCD-gfp in a parB conditional mutant to test whether FrzCD segregated along with the nucleoid.

Results: First of all, DAPI staining of frzCD-gfp expressing cells showed that FrzCD perfectly colocalized with the nucleoid (see Figure below). Such localization was not cluster-dependent as FrzCD retained the ability to colocalize with the nucleoid even in mutant strains where it appeared diffuse or when it was expressed in heterologous hosts. In vitro gel retardation experiments showed that purified FrzCD strongly and non-specifically interacted with DNA fragments and such interactions depended on a 20 amino acid basic region at the FrzCD N-terminus. Ultimately, we constructed a conditional parB mutant showing impaired chromosome segregation and causing the appearance of anucleated cells. We showed that, in this mutant, FrzCD-GFP was segregated in a chromosome-dependant manner and was, thus, never found in anucleated cells.

Conclusions: This work shows the importance of the nucleoid in the segregation of cytoplasmic macromolecular complexes and the establishment of bacterial cell architecture.

Legend. DAPI staining of frzCD-gfp expressing cells. The figure shows that FrzCD colocalizes with the nucleoid in the central region of the cell length.

Lab: Emilia Mauriello
A UNIQUE ROLE FOR SPI-2 RESPONSE REGULATOR SsrB: REGULATION OF THE SALMONELLA CARRIER STATE IN THE ABSENCE OF THE SsrA KINASE

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Background: Salmonella enterica serovar Typhimurium infects diverse hosts such as humans, cattle, poultry, etc. through contaminated food or water, causing gastroenteritis. The SsrA/B two-component system mediates the activation of virulence genes (on SPI-2), which are essential for surviving the macrophage vacuole. In addition, multi-cellular communities (biofilms) formed by Salmonella on gallstones are important for maintaining its carrier state, allowing it to persist and infect newer hosts. Transcriptional profiling of Salmonella biofilms indicated that ssrA/B was involved in biofilm formation, although the precise mechanism was unknown. We determined that the response regulator SsrB regulated genes outside of SPI-2, one of these was csgD, which encodes the master regulator of Salmonella biofilms.

Methods: Biofilms formed by the wild type, ssrA, ssrB, D56A, kinase mutant and ssrB hns strains were analysed by Crystal Violet staining and fluorescence microscopy. Activation of SPI-2 genes in these strains was measured by beta-galactosidase assay. The expression level of csgD in these strains was analysed by real time RT-PCR and Western Blotting. Atomic Force Microscopy (AFM) analyzed the binding of SsrB and H-NS at the csgD regulatory region.

Results: We discovered that un-phosphorylated SsrB activates the expression of csgD by antagonising the repressor H-NS, thereby regulating biofilm formation. This is in stark contrast to its role as a transcriptional activator of SPI-2 genes, which requires phosphorylation of SsrB by the sensor kinase SsrA.

Conclusion: This study identifies a novel role for an unphosphorylated response regulator. SsrB, located on the SPI-2 pathogenicity island, drives two unique lifestyle decisions in Salmonella by regulating distinct signalling pathways through changes in its phosphorylation state. Thus, SsrB controls the entire gamut of pathogenesis: virulence gene expression and biofilm formation. Supported by Mechanobiology Institute, NUS & VA 5I01BX000372 to LJK.
IDENTIFICATION OF THE AMINO ACIDS IMPORTANT FOR THE STAPHYLOCOCCUS AUREUS TWO-COMPONENT SYSTEM LytSR AND IMPLICATIONS ON BIOFILM FORMATION

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Background: *Staphylococcus aureus* is a gram-positive pathogen that asymptptomatically colonizes approximately 30% of the population in the nares but also causes a variety of diseases ranging from skin and soft tissue infections to more serious maladies such as osteomyelitis, endocarditis, and other biofilm-related infections. The *S. aureus* genome encodes 16 two-component systems (TCSs), including LytSR, which has been found to regulate 267 genes including the lrgAB operon (panel A). Furthermore, LytSR has been reported to have a role in regulating cell death and lysis during biofilm formation. Characterization of the P\textsubscript{lrgAB} activity has revealed that lrgAB expression is limited to biofilm towers and this expression coincides with a large population of dead/lysed cells (panel B). The aim of this study was to characterize the molecular elements important for LytSR signal transduction and examine the effects of signal transduction perturbation on biofilm formation.

Methods: Using point mutations, we identified conserved amino acids that were important for LytSR signal transduction. To explore the effects of these point mutations, a chromosomal P\textsubscript{lrgAB}::lacZ reporter was constructed and β-galactosidase assays were utilized to quantify P\textsubscript{lrgAB} activity after 6 hours of growth in TSB. In addition, in vitro biochemical experiments including autokinase, phosphotransfer, and phosphatase assays were utilized to further characterize the effects of the point mutations on LytSR function. Furthermore, micro-fluidic and static biofilm assays were performed to explore the impact of these perturbations on biofilm development.

Results: Asp53 was shown to be the site of LytR phosphorylation, which enhanced binding to the lrgAB promoter and activation of transcription. We identified His390 as the site of LytS phosphorylation, and Asn394 as a critical amino acid for LytS phosphatase activity. LytS- independent phosphorylation was observed during planktonic growth, but minimally during biofilm growth. In addition, over activation of LytR led to increased P\textsubscript{lrgAB} activity and overall biofilm biomass.

Conclusions: Through the molecular characterization of the amino acids important for LytSR signal transduction, we have identified two separate pathways that regulate lrgAB expression. In the first pathway (arrow 1 in panel A), LytR phosphorylation is dependent on LytS signal transduction whereas the second pathway (arrow 2 in panel A) integrates metabolic cues in the regulation of lrgAB expression by responding to acetyl phosphate pools. This second pathway is only observed in planktonic growth, suggesting that the lrgAB expression we observe in biofilm towers is due to LytS-mediated signal transduction.

Lab: Kenneth Bayles
JOUSTING BETWEEN *MYXOCOCCUS XANTHUS* AND *BACILLUS SUBTILIS*: SECONDARY METABOLITES, PREDATION AND SPORULATION

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**Background:** Predatory *Myxococcus xanthus* and endospore forming *Bacillus subtilis* are common soil-dwelling bacteria that produce a wide range of secondary metabolites, form highly organized biofilms, and sporulate under nutrient-limiting conditions. Both organisms are thought to affect the composition and dynamics of microbial communities within the soil. While laboratory strains of *B. subtilis* are susceptible to predation by *M. xanthus*, the ancestral strain of *B. subtilis*, NCIB3610, is resistant to predation by *M. xanthus*.

**Methods:** Cells from both species, predator and prey, are mixed and plated on agar plates containing starvation medium to promote competition and viewed using a dissecting microscope. Secondary metabolites were purified by HPLC. Transposon mutagenesis was carried out based on standard protocols.

**Results:** We have determined that the *pks* gene cluster, required for production of bacillaene, is the major factor allowing *B. subtilis* NCIB3610 cells to resist predation by *M. xanthus*. In addition, *M. xanthus* is incapable of consuming *B. subtilis* spores even from laboratory strains, indicating the evolutionary fitness of sporulation as a survival strategy. Prolonged predator-prey interactions were found to induce the formation of a new type of *B. subtilis* biofilm, termed megastructures, which are tree-like brachiations as large as 500 µm in diameter, and raised above the agar surface between 150 and 200 µm. The megastructures are filled with viable endospores embedded within a dense matrix, and their formation was found to be genetically distinguishable from colony biofilm formation on MSgg medium. As *B. subtilis* endospores are not susceptible to predation by *M. xanthus*, megastructures appear to provide an alternative mechanism for long-term survival. Lastly, a transposon mutant library was used to screen for defects in predation and revealed that *M. xanthus* produces the secondary metabolite, myxoprincomide, to facilitate predation of *B. subtilis* NCIB3610 cells.

**Conclusion:** Production of secondary metabolites regulates complex interactions within the predator-prey system, which ultimately culminates in complex biofilm formation to facilitate escape into dormancy via sporulation.

*Lab: John Kirby*
AN ESSENTIAL PHOSPHORELAY SIGNAL TRANSDUCTION SYSTEM REGULATING THE INTRACELLULAR SURVIVAL OF BRUCELLA

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**Background:** The zoonotic bacterium, *Brucella abortus*, is an intracellular pathogen that can inhabit a range of mammalian cell types. We have identified and reconstituted a conserved, multi-component *B. abortus* signaling system composed of a sensor histidine kinase (CckA), an essential phosphotransferase (ChpT), and two response regulators (CtrA and CpdR); see figure 1 for model. The genes encoding the CckA-ChpT-CtrA-CpdR regulatory system have been identified in several \(\alpha\)-proteobacteria. However, the function of this system in *B. abortus*, is poorly understood.

**Methods:** In order to define the molecular and structural requirements of signaling through this pathway we utilized a combination of X-ray crystallography, molecular dynamic simulations, biochemistry and genetics.

**Results:** In this study, we describe cellular functions of the *B. abortus* CckA-ChpT-CtrA-CpdR system and define the molecular and structural requirements of signaling through this regulatory pathway. These four proteins comprise an essential phosphorelay that controls multiple features of *B. abortus* biology including cell division, cell morphology and DNA replication. We also determined the 1.6 Å crystal structure of *B. abortus* ChpT and a 2.7 Å structure of ChpT bound to the CtrA receiver domain (ChpT-CtrAREC).

**Conclusion:** Our data provide mechanistic understanding, from the cellular to the atomic scale, of a system that controls division, morphology, and intracellular survival of a globally important zoonotic pathogen. More generally, this study defines structural and dynamical features of two-component phosphorelay systems, which are broadly conserved in bacteria, plants and fungi.

**Lab:** Sean Crosson
PEPTIDE ANTIBIOTIC RESISTANCE AND INTRAMEMBRANE SIGNALING IN STAPHYLOCOCCUS AUREUS

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Background: We previously characterized two peptide antibiotic resistance modules associating two-component systems (TCSs) and ABC transporters in the major human pathogen Staphylococcus aureus: BraS/BraR, essential for bacitracin and nisin resistance; GraS/GraR, controlling cationic antimicrobial peptide resistance (CAMPs). The BraS and GraS histidine kinases belong to the "intramembrane-sensing kinase" subfamily, with a short amino-terminal sensing domain, and two transmembrane helices separated only by a short loop. In both cases we have shown that peptide antibiotic sensing requires the permease of an ABC transporter encoded by genes located immediately downstream from the TCS genes: BraDE for bacitracin and nisin resistance and VraFG for CAMP resistance. We sought to define the respective roles of the permease and the histidine kinase extracellular loop and amino-terminal domains in signal sensing and signaling.

Methods: Site-directed mutagenesis, antibiotic resistance assays, transcriptional lacZ fusions, chimeric protein expression, bacterial adenylate cyclase two-hybrid assays

Results: Contrary to previous reports, alanine replacement of all the charged residues in the GraS extracellular loop had no effect on CAMP resistance or expression of GraSR regulon genes. Replacing all the BraS extracellular loop residues with alanine likewise had no effect on activity of the BraS/BraR bacitracin resistance system. Protein-protein interactions and hybrid protein constructions indicate that the kinases interact specifically with the permeases of their associated ABC transporter through their intramembrane domains.

Conclusion: The BraS and GraS histidine kinases are incapable of direct signal sensing. Peptide antibiotics are instead detected by the permeases of the associated ABC transporters and relayed to the kinases through interactions between their transmembrane domains.

FIGURE: The BraS/BraR BraD/BraE VraD/VraE bacitracin and nisin resistance module in S. aureus

Lab: Tarek Msadek
SIGNAL TRANSDUCTION BY ZINC FROM THE ATOMIC TO THE CELLULAR LEVEL

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**Background:** Zn(II) is an essential element for all cellular life. Zinc homeostasis relies on the appropriate regulation of transporters for Zn(II) import (when zinc is limiting) and Zn(II) export (when zinc is in excess). In addition, bacterial cells frequently alter their proteome under conditions of zinc limitation to mobilize stored zinc and to replace Zn(II)-dependent proteins with non-Zn(II)-requiring alternatives. The Zur (zinc-uptake regulator) protein binds DNA in response to available metal ions. Previous results with *Bacillus subtilis* Zur have revealed that this dimeric repressor contains one structural Zn(II) ion per monomer (not involved in zinc- sensing) and a second regulatory metal binding site. Zn(II) binds to the Zur dimer with negative cooperativity: the first binding event is ~20-fold tighter than the second (Ma et al., 2011, Nucl. Acids Res. 39:9130). As a result, the regulatory protein transitions from the apo-protein containing only the structural Zn(II) ions (Zur$_2$:Zn$_2$) to a species with one bound regulatory Zn(II) (Zur$_2$:Zn$_3$) and eventually to a fully loaded dimer (Zur$_2$:Zn$_4$) (Figure 1). We thus set out to test whether both the Zn$_3$ and Zn$_4$ forms of the repressor are functional in vivo and, if so, whether this provides a mechanism for a graded response in which the cell activates different genes at different thresholds of Zn(II) deprivation.

**Methods:** Zur protein and a C84S mutant derivative were purified for analysis of DNA-binding affinity in vitro. In the presence of Zn(II) ion, the wild-type protein forms the Zn$_4$ form of the dimer, whereas the C84S mutant protein forms only the Zn$_3$ form due to greatly enhanced negative cooperativity (the first Zn(II) binds with wild-type affinity, but the second binds with negligible affinity). The affinity of the purified Zur proteins was measured for all of the Zur- regulated promoters in *B. subtilis* using EMSA assays. In parallel, the derepression of each operon was measured as a function of available Zn(II) by addition of the Zn(II) chelator TPEN.

**Results:** The Zur-regulated operons in *B. subtilis* can be divided into two distinct groups. Two of the six Zur- regulated operons bind both wild-type and C84S Zur with high affinity in vitro, suggesting that formation of the Zn$_3$ form of the repressor is sufficient for repression. These same two operons are the last to be induced upon transition of cells from conditions of Zn(II) sufficiency to Zn(II) limitation.

**Conclusion:** As *B. subtilis* transitions from Zn(II) sufficiency to deficiency, the Zur-regulated operons are derepressed in an ordered manner (a graded response). These results provide insights into a complex ordered response that includes mobilization of stored zinc ions, expression of high affinity uptake systems, and derepression of Zn(II)-independent proteins.

![Figure 1. Zn(II) sensing (black circles) by the Zur dimer](image)
POSTER ABSTRACTS
INTERACTION OF TWO CHEMOTAXIS PATHWAYS PRODUCES AN INTEGRATED RESPONSE

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**Background:** Signal transduction during bacterial chemotaxis is initiated by the detection of extracellular cues by receptors which are assembled in a large array of interacting dimer-of-trimers at the cell poles. The draft genome sequence of *Azospirillum brasilense* indicates the presence of several homologs of chemotaxis (Che) pathways, which we have named Che1, Che2, Che3, and Che4. Recent experimental evidence indicates that Che1 and Che4 both function in chemotaxis and aerotaxis while Che2 and Che3 control cellular behaviors unrelated to chemotaxis. In addition, the genome encodes for 41 chemotaxis receptors and experimental evidence suggests that Che1 and Che4 integrate signaling at the level of chemotaxis receptors. Here, we present data supporting the hypothesis that proteins from the Che1 and Che4 pathways interact to coordinate chemotaxis responses. A model for chemotaxis signal transduction and processing by Che1 and Che4, including how these functionally interact to produce a coordinated chemotaxis response, will be presented.

**Methods:** Fluorescence microscopy and bacterial two-hybrid assays were used to assess localization of proteins as well as protein-protein interactions.

**Results:** Several key proteins from Che1 and Che4 interact. In addition, the subcellular localization of some chemotaxis receptors is affected by the lack of Che4 and/or Che1 cytoplasmic proteins.

**Conclusion:** Here, we identify several key interactions between Che1 and Che4 proteins and demonstrate their influence on receptor localization. Experimental evidence indicates that interaction of different CheA and CheW proteins within a single chemotaxis receptor cluster represents the mechanism by which Che1 and Che4 integrate signaling to coordinate chemotaxis responses.

![Figure 1. Che1 and Che4 pathways contribute to chemotaxis in *A. brasilense*.](image-url)
AZOSPIRILLUM BRASILENSE CONTROLS CHEMOTAXIS BY INTEGRATING SIGNALING FROM TWO CHEMOTAXIS PATHWAYS

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Background: Chemotaxis is a key determinant of the ability of diverse soil motile bacteria to colonize plant root surfaces. Recent comparative genomics analysis has revealed that genomes of free-living soil bacteria encode, on an average, more than two chemotaxis (Che) pathways and a large number of chemotaxis receptors. However, little is known regarding how multiple pathways contribute to chemotaxis behaviors. *Azospirillum brasilense*, a gram negative soil diazotroph, is motile by means of a single polar flagellum and able of chemotaxis toward various chemical compounds. During chemotaxis, *A. brasilense* cells transiently modulate both swimming velocity as well as changes in the swimming direction (reversals). While the chemotaxis system that controls changes in swimming speed during chemotaxis (so called Che1) has been characterized, the Che pathway controlling changes in the swimming direction remains unknown. The sequenced genome of *A. brasilense* encodes for 4 Che operons. Two of these Che pathways are either not expressed under laboratory conditions (Che2) or involved in controlling flocculation (Che3).

Methods: Spatial and temporal gradient chemo- and aerotaxis assays, computerized motion analyses, promoter expression, mutagenesis and fluorescence microscopy.

Results: Combining genetics and behavioral assays, we demonstrate that the Che4 pathway controls the probability of changes in the swimming direction during chemo-and aerotaxis. We provide evidence that CheA4 is the major kinase controlling chemotaxis in this species and uses multiple CheY as targets. We also correlate the functions of different CheY homologs in controlling swimming speed or reversal frequency with distinct subcellular localization.

Conclusion: Che1 and Che4 signaling outputs are expected to be distinct. Together with other evidence, the emerging model suggests that signaling via Che1 and Che4 is coordinated by cross-talk occurring at the levels of chemoreceptors.

**Fig:** A model showing roles of Che1 and Che4 pathways in *A. brasilense* chemotaxis.

**Lab:** Gladys Alexandre
C-DI-GMP AND CHEMOTAXIS RECEPTOR SIGNALING IN AZOSPIRILLUM

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**Background:** Azospirillum brasilense are motile alphaproteobacteria able of movement through an oxygen gradient by aerotaxis. Aerotaxis is the strongest behavioral response in this species and is facilitated by several chemotaxis receptors including Tlp1. Tlp1 is a protoypical transmembrane chemotaxis receptor that also possesses a c-di-GMP effector domain at the extreme C-terminus of the protein. The role of the c-di-GMP binding in Tlp1-mediated and Tlp1-independent aerotaxis responses has not been fully characterized and is the focus of the work presented here.

**Methods:** We used mutagenesis, recombinant protein expression and equilibrium dialysis to characterize c-di-GMP binding the Tlp1 PilZ domain. A novel optogenetic system comprised of a blue light-activated c-di-GMP phosphodiesterase and a red-light activated diguanylate cyclase was used to control intracellular c-di-GMP levels, which were monitored by mass spectrometry analysis. Aerotaxis was characterized in a temporal gradient assays and cells’ taxis responses were analyzed by computerized motion analysis.

**Results:** The Tlp1 PilZ domain binds c-di-GMP with high affinity and this binding affects the ability of cells to navigate in oxygen gradients. The novel optogenetic system for manipulating intracellular c-di-GMP levels permits modulation of intracellular c-di-GMP levels, using red and blue light. Using this system, we characterize the effect of changes in intracellular c-di-GMP concentrations in real time, during aerotaxis.

**Conclusions:** C-di-GMP modulates Tlp1 chemotaxis receptor sensitivity to oxygen gradient and thus affects behavioral responses.

**Lab:** Gladys Alexandre
SEGREGATION OF THE CYTOPLASMIC CHEMOSENSORY CLUSTER OF R. SPHAEROIDES

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Background: Rhodobacter sphaeroides has two spatially separate chemoreceptor arrays, one transmembrane and polar and the other cytoplasmic and mid-cell. Prior to cell division the cytoplasmic cluster is duplicated and positioned at one and three quarter positions in the cell, ensuring that on division each daughter cell inherits a cluster at mid-cell (See figure B). This process is dependent on a ParA homologue, PpfA (see figure A). ParA proteins are Walker A ATPases used in type I plasmid segregation systems as well as the segregation of the chromosomes of various bacterial species. Canonical type I plasmid segregation is dependent of just 4 factors: an ATPase, ParA; a centromeric DNA sequence, parS; an ATPase activating protein which binds the centromeric site, ParB; and a surface, the nucleoid. Together these elements combine to produce dynamic positioning of plasmids which results in segregation on division. Like other ParA proteins PpfA has been shown to bind the nucleoid non-specifically. It also forms a focus which coincides with the cytoplasmic cluster. The presence of PpfA foci is dependent on the N-terminal region of the soluble chemoreceptor TlpT, thus TlpT bridges the segregation machinery and its cargo, the chemosensory cluster.

Methods: Epifluorescence microscopy. Photoactivation localisation microscopy single molecule tracking. qPCR. ChIP-Seq.

Results: The use of super-resolution microscopy was used to track individual PpfA molecules revealed that PpfA molecules in the focus have slower diffusion coefficients than molecules far from the cluster. Data will also be presented tracking individual molecules of PpfA mutants, which are unable to bind DNA, bind ATP, or hydrolyse ATP. Thus revealing the diffusive behaviour of PpfA in different nucleotide bound states. Imaging of the cytoplasmic cluster along with the origin and terminus of both chromosomes shows that the cluster does not duplicate and segregate until the chromosomes have begun duplicating and segregating themselves. Experiments blocking the initiation of replication of the chromosomes will reveal whether the duplication and segregation of the cluster is dependent on the replication and segregation of the chromosome. In addition the replication fork will be followed using fluorescently labelled proteins to identify any temporal or spatial correlation between DNA replication and cluster splitting. ChIP-seq data will identify whether the DNA binding pattern of PpfA includes any sequence specific binding.

Conclusions: Data will be presented suggesting that the cytoplasmic cluster of R. sphaeroides does not occur by any mechanism currently described for either DNA or protein segregation by ParA systems.

Lab: Judith Armitage
STRUCTURE-FUNCTION STUDIES OF THE RESPONSE REGULATOR CHEY6 FROM RHODOBACTER SPHAEROIDES

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Background: *Rhodobacter sphaeroides* has a complex chemosensory network which comprises multiple homologous genes and involves metabolic sensing. Unlike most bacteria, the expressed motor displays a stop-and-go phenotype. *R. sphaeroides* genome encodes six different CheY proteins but only three (CheY3, CheY4 and CheY6) are expressed under laboratory conditions. Deletions of these CheYs result in the flagellar motor being unable to stop (smooth swimming). CheY6 has been shown to be essential for chemotaxis, whereas CheY3 and CheY4 have some functional redundancies. Although CheY6 can stop the motor alone, the presence of either CheY3 or CheY4 is required for a chemotactic phenotype. To date, not much is known about how these three CheY proteins interact to stop the flagellar motor.

Methods: NMR, Epi-fluorescence microscopy, Phosphotransfer assays, Tethering & swim plate assays

Results: Structural studies of CheY6 using NMR experiments, highlighted a flexible loop region (residues G109-K118) that is not present in the other CheYs. It was hypothesised that this flexible loop might confer to CheY6 a different mechanism compared to other CheY proteins. Loop deletion mutants were made to investigate its function in CheY6.

![A) Crystal structure of CheY6, taken from a complex with CheA3. The loop region (red) from S113-K119 is not present (no electron density - adapted from Bell et al, 2010). B) Proposed CheY6-ΔL structure.](image)

Phenotypical studies were performed using swim plates and tethering assays. The mutants showed smooth swimming, indicating that CheY6-ΔL has lost its function. Results also indicated that CheY6-ΔL is able to compete with wild type CheY6 for phosphorylation by the kinase protein CheA3. Phosphotransfer assays revealed that CheY6-ΔL can still be phosphorylated. Epi-fluorescence microscopy of the mutant showed co-localisation with the cytoplasmic cluster, as seen in wild type. NMR studies have been used to elucidate the solution structures of active and inactive CheY6, and a direct comparison to the crystal structure was made.

Conclusions
CheY6-ΔL is folded, retains the ability to be phosphorylated by CheA3, localises at the cytoplasmic chemoreceptor cluster, but appears unable to bind to the flagellar motor. Wild type CheY6 shows structural changes between the active and inactive conformations.

Lab: Judith Armitage
CHARACTERISATION OF METHYLATION SITES IN TlpT, A CYTOPLASMIC CHEMOSENSORY PROTEIN

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Background: Rhodobacter sphaeroides is a model organism used for the study of complex chemotaxis systems. It has a classical chemosensory pathway using a cluster of transmembrane chemoreceptors, as seen in Escherichia coli, plus an additional cytoplasmic chemosensory cluster providing complexity to the chemosensory pathway. Little is known of adaptations mechanisms when two pathways control one motor. Previous work on R. sphaeroides cytoplasmic chemosensory protein TlpT identified three adaptation sites by MS/MS (E296, E478, and Q485) and predicted an additional one from sequence alignments (E289). These four residues have been independently mutated to alanine and the chemotactic phenotype characterised by tethered cell assay. These mutations should not allow methylation or demethylation, therefore the receptor should have an altered ability to adapt after a response. In E. coli, loss of each methylation site causes a measurable loss of the ability to adapt.

Methods: Swimming behaviour in response to changes in environmental conditions can be measured by tethering a cell by its flagellum and tracking its rotation. Tethering was used to observe the response of individual cells to a step up, followed by a step down in the attractant, propionate. The assay allows the monitoring of cell-level behaviour in population-size samples.

Results: The membrane cluster remained unchanged, but the change of a single adaptation site on one cytoplasmic receptor, E289A, E296A, and E478A, caused a loss of chemotaxis while the Q485A mutant took much longer to respond and adapt.

Conclusion: Single mutations of the putative adaptation sites on a single cytoplasmic receptor altered the chemotactic response, suggesting the cytoplasmic cluster is dominant over the membrane cluster and the loss of any adaptation site has a major influence on R. sphaeroides chemotaxis. This suggests that, as in E.coli adaptation is critical for chemotaxis, but that, unlike E.coli, the failure of a single site on a single receptor to adapt can cause the complete loss of response.

Lab: Judith Armitage
MOLECULAR STRUCTURE OF FLIN-FLIY COMPLEX IN HELICOBACTER PYLORI

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\textbf{Background:} The motor switch complex is a nano-rotary machine essential for flagellar export, torque generation and rotational switching. In model microorganisms such as \textit{E. coli} and \textit{Salmonella}, the switch complex is composed of multiple copies of FliG, FliM and FliN. In \textit{B. subtilis} and \textit{T. maritima}, FliN is replaced by FliY which contains a FliM binding motif, CheC/CheX domain and a FliN-like domain. Interestingly, epsilon-proteobacteria \textit{H. pylori} possess both FliN and FliY. However the molecular basis of their co-existence is unclear.

\textbf{Methods:} Interaction of FliN and FliY were characterized by co-expression, pull down and size exclusion chromatography. The atomic details of their association was further studied by x-ray crystallography. We also applied molecular genetics to investigate the biological importance of FliY in \textit{H. pylori}.

\textbf{Results:} The C-terminal of FliY is found to form a heterodimer with FliN. A 2.1 Å crystal structure of the binary complex reveals the heterodimer mimicking the FliN homodimer in \textit{T. maritima}. \textit{In vivo} complementation further suggests that the FliN-FliY complex is critical for flagellation while the CheC/CheX-like domain of FliY is required for retaining wild type motility.

\textbf{Conclusion:} Co-existence of FliN and FliY in \textit{H. pylori} has a structural and functional significance for motor function. Our findings also provide insights to the diversity of flagellar motor.
A PARTITIONING-LIKE SYSTEM INVOLVED IN FLAGELLAR-BASED MOTILITY IN PSEUDOMONAS AERUGINOSA

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Background: *Pseudomonas aeruginosa* is polarly-flagellated and has two clusters of genes (*che* and *V*) that are involved in chemotaxis via flagellar-based motility. Within *che* are genes encoding two partitioning-like proteins: ParC (PA1462) and ParP (PA1463). In *Vibrio parahaemolyticus*, homologous proteins have been found that are responsible for the localization and inheritance of chemotaxis proteins to the poles of cells upon cell division. Deletion of these partitioning proteins results in a 30% decrease in swimming motility, 30% of cells having mislocalized chemotaxis protein clusters, and 30% of cells having no chemotaxis protein clusters (Ringgaard et al., 2014). Our goal is to determine the function of ParC and ParP in regulating flagellar-based motility in *P. aeruginosa*.

Methods: To determine the roles of these partitioning-like proteins, chemotaxis protein interactions and localizations were studied using a bacterial two-hybrid system and fluorescence microscopy, respectively. In-frame deletions were made and static biofilm and swimming motility assays were used to determine the resultant phenotypes. Flagellar staining and flagellin protein preparation protocols were used to observe the flagellation state of individual cells and the overall surface flagellation.

Results: Our preliminary results show that ParC and ParP interact with each other and with several methyl-accepting chemotaxis proteins (MCPs). In ΔparP, there is a reduction in the number of cells having chemotaxis protein clusters. ΔparC has a 30% reduction in swimming motility and ΔparP is null for swimming motility, which is similar to a *fliC* transposon mutant (non-flagellated) and a non-chemotactic strain (Δche). Interestingly, in static biofilm assays ΔparP behaves similarly to the non-chemotactic strain. Populations of ΔparC and ΔparP cells have a reduction in the percentage of flagellated cells compared to wild type.

Conclusion: ParC and ParP together may comprise a partitioning system that is important for flagellar-based motility. These results, particularly of ParP, are distinct from that seen in *V. parahaemolyticus*. Our work now focuses on the elucidating the role of ParP in flagellar function.

Lab: Sonia Bardy
FROM ELEMENTARY TO ELABORATE: INVESTIGATING THE EVOLUTION AND DIVERSITY OF BACTERIAL FLAGELLAR MOTORS

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Background: The bacterial flagellar motor is an intricate protein complex that is responsible for rotating the flagella filament and creating cell propulsion. Investigation of motor structures from organisms like *Escherichia coli* has provided us with a basic understanding of the core motor structure, and imaging of different bacteria has revealed a wide spectrum of motor complexity.

Methods: We are combining molecular phylogenetics with electron cryo-tomography to explore and understand the evolution of this complexity.

Results: Some increased complexity appears to correlate with an increased torque generation potential for these motors, suggesting that some bacteria have recruited additional proteins to "gear" their motors to be able to swim through more viscous environments. We have identified two such "geared up" motors that may represent incremental evolutionary steps towards increased torque generation. A complex motor from *Campylobacter* species and an intermediately complex motor from *Vibrio* species appear to build from the relatively simple motor found in *E. coli*. Preliminary phylogenetic comparisons based on conserved core motor proteins from a wide taxonomic range of motile bacteria is being undertaken in an attempt to understand the evolutionary and taxonomic relationships between flagellar motor components. This analysis is informing targeted acquisition and culturing of species suspected of possessing flagellar motors representing evolutionary intermediates between *E. coli*, *Vibrio* and *Campylobacter* structures. Imaging of these different flagellar motors is being undertaken using electron cryo-tomography, a technique that produces 3-D images of macromolecular machinery in situ.

Conclusions: The results of these investigations will generate an increased understanding of hypothesised evolutionary pathways leading to higher torque-generating motors and highlight processes involved in the evolution of complexity of macromolecular machines (Figure: a phylogenetic tree of flagellar motors).

Lab: Morgan Beeby
STRUCTURAL AND FUNCTIONAL ADAPTATION OF THE BACTERIAL FLAGELLAR MOTOR IN THE FAST-SWIMMING PATHOGEN CAMPYLOBACTER JEJUNI

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Background: Campylobacter jejuni is a gastrointestinal pathogen that exhibits remarkably fast swimming speeds in highly viscous environments. Its motility is powered by bipolar flagella, located one at each cell pole. Analysis of the C. jejuni flagellar motor using electron cryo-tomography reveals certain key variations in its structure compared with the well-studied model organisms E. coli and Salmonella. An important difference is an increase in the width of its cytoplasmic rotor ring. We hypothesize that this difference in rotor width corresponds to differences in stoichiometry of the torque-generating stator and rotor subunits of the C. jejuni motor, and that these differences in turn “gear” the C. jejuni motor to produce higher levels of torque that adapt it for efficient swimming in viscous environments.

Methods: To test our hypothesis, we are pursuing a hybrid approach of electron cryo-tomography with subtomogram averaging to directly visualize stator and rotor components in situ, in parallel with single-molecule biophysical measurements to test the mechanics of the motor in vivo. Briefly, our approach is to use tags or deletions of critical proteins in the C. jejuni stator and rotor rings to map their precise locations and stoichiometries within the overall motor structure. Then, by combining this information with torque measurements from observing the rotation of beads attached to the C. jejuni flagellum spinning at different viscosities, we aim to construct a structural model for rotary function of the C. jejuni motor.

Results and conclusions: By understanding the structural and functional adaptation of the C. jejuni flagellar motor, our results aim to provide insight into how bacteria adapt this versatile molecular motor to thrive in distinct environments.

The C. jejuni flagellar motor displays a wider rotor ring than E. coli or Salmonella.

\[
\text{Torque} = r \times F
\]

Does the wider rotor of the C. jejuni motor generate higher torque, allowing cells to swim more effectively at greater viscosities?

Lab: Morgan Beeby
BACKGROUND: To change swimming direction in response to the chemical environment, many bacterial cells contain Methyl-Accepting Chemotaxis Proteins (MCPs). MCPs bind external ligands and transduce signals across the cellular membrane to regulate the histidine kinase CheA. MCPs are transmembrane receptors that form trimers-of-dimers and cluster into large hexagonal arrays. The receptor arrays are stabilized by intracellular binding to a layer of CheA and the adaptor protein CheW. The molecular mechanism for receptor-based regulation of CheA remains to be fully understood. Structural and biochemical analysis of arrays containing receptors, CheA and CheW may elucidate the conformational changes that differentiate the CheA-on and CheA-off states.

METHODS: We have generated chimeric receptor variants that form a trimer-of-dimer arrangement and complex with CheA and CheW. Complexes of the receptor variant with CheA and CheW were isolated by co-incubation of the proteins followed by size-exclusion chromatography. The composition of the complexes were analyzed by multi-angle light scattering and their activities were established by radioisotope labeling assays. We are attempting to characterize the structure of the complexes by crystallography, pulsed-dipolar ESR spectroscopy and cryo-electron microscopy.

RESULTS: The chimeric receptor variants form a trimer-of-dimers and are capable of locking CheA into specific activity states in vitro. Isolated ternary complexes of the variant receptor with CheA and CheW are homogenous and stable—making them ideal for structural analysis. Comparison of the complexes containing CheA in different activity states may allow us to elucidate important aspects of CheA regulation by MCPs.

CONCLUSIONS: Reconstituting an in vitro chemotaxis system is difficult due to the insolubility and complex oligomeric state of chemotaxis receptors. Therefore, it is challenging to probe functional and structural aspects of this system. However, we have been able to produce in vitro homogenous complexes of a receptor variant with CheA and CheW. Using these complexes, we hope to elucidate structure-function relationships of the chemotaxis system that are currently unclear.
NON-GENETIC DIVERSITY RESULTS IN DIFFERENTIAL PERFORMANCE IN BACTERIAL CHEMOTAXIS

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**Background:** Genetically-identical *E. coli* in a uniform environment display different swimming behavior. Theory and simulations based on molecular models of the chemotaxis signaling pathway predict that these different phenotypes will perform best in different types of environments, and that a single phenotype cannot perform best in all environments. This suggests that non-genetic diversity in a clonal population could help a population survive unpredictable environments.

**Methods:** We have developed a microfluidics platform and single-cell analysis tools that allow us to extract phenotypic information from thousands of individual cells in stable gradients of attractant without cross-flow. All cells started in a small band at the gradient sink, allowing us to quantify how well different phenotypes climb the gradient. Using a technique known as φDM and a strain with inducible control of the chemotaxis protein CheR, we quantified the relationship between protein expression, swimming phenotype, and gradient-climbing performance.

**Results:** The wild-type population had a broad distribution of phenotypes and these phenotypes swam up the gradient at different rates. Altering the amount of the chemotaxis protein CheR altered both population phenotypic distributions and gradient-climbing performance. The relationship between gradient-climbing performance and gradient steepness revealed a performance trade-off between the phenotypes tested.

**Conclusions:** The swimming behavior of a cell determines its gradient-climbing performance, and altering the expression level of a single chemotaxis protein changes the proportions of these phenotypes in a population. It is therefore possible that natural selection could match population diversity to environmental diversity through mutations in regulatory regions that control chemotaxis protein expression without altering the chemotaxis signal transduction pathway itself. This lends credence to the idea that non-genetic diversity serves an adaptive function in genetically-identical populations.

**Lab:** Thierry Emonet
FUNCTIONAL ANALYSIS OF THE SALMONELLA TYPHIMURIUM FLAGELLAR TYPE THREE SECRETION APPARATUS PROTEIN FliO

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Background: Flagellar Motility plays a key role in \textit{Salmonella} pathogenesis. Flagella synthesis is dependent on the flagellar type-III secretion system (fT3SS), a nanomachine closely related to the type-III injectisome.

This apparatus is composed of six cytoplasmic and seven membrane proteins essential for export of the flagellar components. FliGMN and FliHIJ form the C-ring and the ATPase complex, respectively. Membrane proteins FlhBA, FliOPQR and FliF form the export gate. Six of these are highly conserved. FliO, however, shows less conservation and is absent in some species. Little is known about FliO function, but a previous study showed a genetic interaction with the FliP protein [1]. Here we investigate the function of FliO.

Methods: The role and the mechanism of FliO in fT3SS were analyzed by performing complementation, degradation and cell fractionation assays. Cellular localization of FliO was studied using super resolution microscopy.

Results: We showed that FliO and FliP are functionally linked with each other. Indeed, we observed that \textit{fliO} deletion can be bypassed by \textit{fliP} over-expression and that the presence of FliO stabilized FliP, although it did not influence the ability of FliP to integrate into the membrane. We also demonstrated that, unlike the other proteins of the export gate, FliO is not localized inside the flagellar basal body and appeared to be mobile in the cell membrane.

Conclusion: Our results suggest that FliO does not participate actively in the export process, but might act as an accessory protein for efficient FliP assembly into the export gate.

Reference:

Lab: Marc Erhardt
ISOLATION, PHOSPHONOMETHYLATION, AND CRYSTALLIZATION OF PHOSPHONO-CheY
FROM THERMOTOGA MARITIMA

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Background: Isolation, chemical modification, purification, crystallization, and x-ray diffraction of an
analog of the active form of the signal transduction protein, Thermotoga maritima CheY, are described.
Rendering the signal transduction protein permanently stable by chemical modification is accomplished
by replacing the active site aspartyl residue with a cysteinyl residue and reacting the protein with
phosphonomethyltriflate. Diffraction quality crystals of purified phosphono-CheY (P-CheY) were grown.
Binding between P-CheY and the FliM peptide was studied by ITC and fluorescence polarization
anisotropy.

Methods: Thermotoga maritima C54/S81 or C54/A81 CheY had been previously cloned into a
pet28a(+) vector encoded with a poly-his tag. Pure CheY is isolated by a Ni(II)-NTA column, followed
by thrombin digestion and a Sephadex G50 column. Isolated CheY is phosphonomethylated and
purified via CEX HPLC. The degree of purity is judged by RP HPLC, colorimetric phosphate assay, 31P-
NMR and mass spectrometry. Crystallization of a characterized P-CheY sample was performed. The
dissociation constant between P-CheY and FliM peptide was determined by isothermal titration
calorimetry and fluorescence polarization anisotropy.

Results and Conclusion: Phosphate assays, NMR, and mass spectral data suggested the desired
modification, had taken place. The peptide-binding data suggest that P-CheY is a good functional
mimic of phosphorylated CheY with respect to binding the FliM peptide. Crystallographic results
suggest that the structure of P-CheY resembles unmodified CheY, possibly due to the apparent
disorder of the phosphonomethyl group. Our working hypothesis is that the binding of the peptide
brings about a change in conformation of P-CheY.

Figure. In vivo activation of CheY by CheA and ATP (top). Phosphonomethylation of D54C/C81S CheY
(bottom). PMT = phosphonomethyl trifluoromethanesulfonate.

Lab: Christopher Halkides
FUNCTIONAL STUDIES AND STRUCTURAL SHOWCASING OF FLAGELLAR PROTEIN FlhE FROM
S. ENTERICA

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Background: The Type III Secretion System (T3SS) is a multicomponent apparatus that aids in the
transportation of macromolecules across the membranes of gram-negative bacteria. Pathogenic
bacteria employ the T3SS to infect human, plants and insects alike. T3SS builds cellular flagella and
offers a mechanism for the delivery of effector proteins into the host cell. In flagellar biogenesis,
cytoplasmic proteins FlhA and FlhB, members of the flhBAE operon, are well-studied components
of the T3SS; however, the function of periplasmic protein FlhE remains to be elucidated.

Methods: Gene knockout, motility assays, green plate assays, GFP fluorimetry, His-tag protein
expression and purification, protein crystallography

Results: The absence of FlhE results in a proton leak, inappropriate secretion patterns, and cell
death, indicating that FlhE regulates an important aspect of proper flagellar biogenesis. In
addition to functional studies, the structure of FlhE was solved to 1.5 Å-resolution using single-
wavelength anomalous dispersion (SAD). Structural searches revealed that FlhE is distantly related
to the P-domain of the kexin family of serine proteases and viral coat proteins. Structural homologs do not
reveal the exact biological role of FlhE but do provide some clues.

Conclusions: FlhE is essential for proper flagellar biogenesis and likely plays a structural role in the
periplasmic space.

Lab: Rasika Harshey
A NEW PLAYER AT THE FLAGELLAR MOTOR: FliL CONTROLS BOTH MOTOR OUTPUT AND BIAS

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Background: The bacterial flagellum is driven by a bidirectional rotary motor, which propels bacteria to swim through liquids or swarm over surfaces. While the function of the major structural and regulatory components of the flagellum is known, the function of the well conserved FliL protein is not. In Salmonella and E. coli, absence of FliL leads to a small defect in swimming, but complete elimination of swarming. The specific location, interacting partners and contribution to motor output remain unclear.

Methods: In addition to classical swim and swarm assays, we assess the behavior of single-motors, characterizing speed and switching patterns. Protein interactions were monitored with two-hybrid assays, GST-Pulldowns and fluorescence microscopy. Additional experiments to measure motor component levels in the presence/absence of FliL used western blots and FRAP.

Results: We show that absence of FliL decreases motor speed as well as switching frequency. We demonstrate that FliL interacts strongly with itself, the MS-ring protein FliF, the stator proteins MotA and MotB, and weakly with the rotor switch protein FliG.

Conclusion: These and other experiments suggest that FliL increases motor output either by recruiting or stabilizing the stators or by increasing their efficiency, and contributes additionally to torque generation at higher motor loads. The increased torque enabled by FliL explains why this protein is essential for swarming on an agar surface expected to offer increased resistance to bacterial movement.

Lab: Rasika Harshey
STRUCTURE AND CONFORMATIONAL SIGNALING IN THE CYTOPLASMIC DOMAIN OF BACTERIAL CHEMORECEPTORS CHARACTERIZED BY EPR SPECTROSCOPY

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Background: Transmembrane bacterial chemoreceptors couple ligand recognition in the periplasmic domain and adaptational modification in the cytoplasmic domain to receptor conformation and allosteric control of a receptor-associated histidine kinase. At present there is an incomplete understanding of chemoreceptor cytoplasmic domain structure, conformational dynamics, and how those features relate to signal transduction.

Methods: We employed site-directed spin labeling and electron paramagnetic resonance (EPR) spectroscopy to probe features of structure and conformational signaling of the cytoplasmic domain of the *Escherichia coli* aspartate chemoreceptor Tar. By attaching spin labels to cysteines at surface positions along the length of the extended, helical coiled-coil cytoplasmic domain, we probed features of helical backbone dynamics on the nanosecond timescale. Via this technique we assessed the relative extent of region-specific helical stability along the length of the Tar cytoplasmic domain as a consequence of detergent solubilization, lipid association, ligand recognition, and adaptational modification.

Results: We found that detergent solubilization perturbed much of the Tar cytoplasmic domain, that certain regions of bilayer-inserted, functional receptors remain highly dynamic, and that adaptational modification altered helical backbone stability in the modification region. Furthermore, we found that the effects of ligand recognition were distinct from the effects of adaptational modification.

Conclusion: Bacterial chemoreceptors demonstrate varying helical stability along the length of their cytoplasmic domain structure and helical stability is modulated by adaptational modification. We propose these are common features of bacterial chemoreceptors.

Lab: Gerald Hazelbauer
SELECTIVE ALLOSTERIC COUPLING IN CORE CHEMOTAXIS SIGNALING COMPLEXES

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Background: Bacterial chemotaxis is mediated by signaling complexes that sense chemical gradients and direct bacteria to favorable environments by controlling a histidine kinase as a function of chemoreceptor ligand occupancy. Core signaling complexes contain two trimers of transmembrane chemoreceptor dimers, each trimer binding a coupling protein CheW and a protomer of the kinase dimer. Core complexes assemble into hexagons and these form hexagonal arrays. The notable cooperativity and amplification in bacterial chemotaxis is thought to reflect allosteric interactions in cores, hexagons and arrays, but little is known about this presumed allostery.

Methods: We investigated allostery in core complexes assembled with two chemoreceptor species, each recognizing a different ligand. Chemoreceptors were inserted in Nanodiscs, which rendered them water-soluble and allowed isolation of individual complexes.

Results: Neighboring dimers in receptor trimers influenced one another’s operational ligand affinity, indicating allosteric coupling. However, this coupling did not include the key function of kinase inhibition. Our data indicated only one receptor dimer could inhibit kinase as a function of ligand occupancy. This selective allosteric coupling corresponded with previously identified structural asymmetry: only one dimer in a trimer contacts kinase and only one CheW. We suggest one of these dimers couples ligand occupancy to kinase inhibition. Additionally, we found that kinase protomers are allosterically coupled, conveying inhibition across the dimer interface.

Conclusion: We found that in core chemotaxis signaling complexes the three dimers in a trimer of dimers are not functionally equivalent. Instead only one of the three inhibits kinase as a function of ligand concentration. This functional asymmetry is consistent with previous observations of structural asymmetry among the dimers of a timer in signaling complexes. In addition, we observed allosteric coupling in core complexes between the protomers of the kinase dimer. Since kinase dimers connect core complex hexagons, allosteric communication across dimer interfaces could provide a pathway for receptor-generated kinase inhibition in one hexagon to spread to another, providing a crucial step for the extensive amplification characteristic of chemotactic signaling.

Lab: Gerald Hazelbauer
STRUCTURAL AND FUNCTIONAL ANALYSIS OF THE DNAJ FAMILY PROTEIN SFLA, THAT IS INVOLVED IN REGULATION OF FLAGELLATION IN VIBRIO ALGINOLYTICUS

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Background: Marine bacterium \textit{Vibrio alginolyticus} usually has a single polar flagellum, and its number and location are regulated by FlhF and FlhG. We isolated a suppressor mutant from the \textit{ΔflhFG} strain that occasionally tends to have peritrichous flagella in a few cells. The mutation was mapped onto non-flagella-related gene, named \textit{sflA}, which is speculated to encode a single transmembrane protein with a DnaJ domain. The \textit{sflA} deletion strain didn’t affect the flagellation, so the role of SflA is enigmatic. In this study, we attempt to clarify the structure and function of SflA.

Methods: To clarify the function of SflA, we constructed a C-terminal GFP fusion of SflA and observed its localization in the \textit{ΔsflA} and \textit{ΔflhFGΔsflA} under the fluorescence microscopy. To clarify the structure of SflA, we constructed and purified SflA\textsubscript{N} (N-terminal periplasmic region), SflA\textsubscript{C} (C-terminal cytoplasmic region with DnaJ domain) and SflA\textsubscript{F} (Full length).

Results: C-terminally GFP-fused SflA is localized at cell pole in the \textit{ΔsflA} cells, whereas in the \textit{ΔflhFGΔsflA} cells it is found not only at cell pole but also all around the cell surface. We can obtain the crystal structure of the SflA\textsubscript{N} at about 2Å resolution. One of the homologous structure is SycD, that is known as a type III secretion chaperon in the \textit{Yersinia enterocolitica}.

Conclusion: SflA seems to work as a chaperon at the cell surface, and its cytoplasmic region may interact with FlhF or FlhG. Therefore SflA may help flagellation at a certain condition, like a cofactor. In order to test our hypothesis, we investigate the binding protein(s) of SflA and would like to clarify the structure of SflA\textsubscript{C} and SflA\textsubscript{F}.
MOTILITY INHIBITION BY PlzD, A c-di-GMP BINDING YCGR HOMOLOG IN VIBRIO alginolyticus

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Background: A small molecule, cyclic diguanylate (c-di-GMP) has been emerging as a second messenger that is involved in diverse bacterial signal transduction pathways. YcgR, a c-di-GMP binding protein in Escherichia coli, is known to inhibit flagellar motility by binding to the motor. In marine bacterium, Vibrio alginolyticus, swims in aqueous medium by using a Na⁺-driven polar flagellum, and swarms on the surface by multiple H⁺-driven lateral flagella. It has a YcgR homolog, PlzD. In this study, we tested whether PlzD affects motility of Vibrio alginolyticus.

Methods: We cloned PlzD and tested whether the overproduction of PlzD from the plasmid or the deletion of plzD affects the polar flagellar motility. We raised antibody against purified recombinant PlzD, and examined cellular fractionation of PlzD. GFP-fused variant of PlzD is expressed to investigate its subcellular localization.

Results: Overproduction of PlzD could affect polar flagellar motility at nutrient-poor condition, and it localized at flagellated cell pole. On the other hand, deletion of plzD did not affect polar flagellar motility. Subcellular fractionation showed that overproduced PlzD seems to form inclusion body, but N-terminally truncated variants were found in soluble fraction. Purified PlzD and immunoblots revealed that endogenous PlzD was actually shorter, synthesized from Met33.

Conclusion: PlzD of Vibrio alginolyticus seems not to be strongly related to the polar flagellar motility. We need to consider the possibility that N-terminal extension of “full-length PlzD” seems to cause motility inhibition. Testing the effect of PlzD on the lateral flagella is the ongoing project.

Lab: Michio Homma
ATP BINDING MOTIF OF FLHG, A MIND HOMOLOG, IS IMPORTANT FOR NEGATIVE REGULATION OF FLAGELLAR NUMBER AND FLHF POLAR LOCALIZATION.

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Background: Marine bacterium Vibrio alginolyticus has a single polar flagellum whose number is regulated positively by FlhF and negatively by FlhG. Our previous study showed that FlhG interacts with FlhF to prevent the localization of FlhF at cell pole. FlhG is a homolog of bacterial cell division regulator (MinD) and putative ATPase. However, we do not know how ATP is used for the FlhF function to inhibit the flagellation.

Methods: We introduced mutations in the ATPase motif of FlhG and examined their effects on motility, flagellation, subcellular localization and ATPase activity.

Results: The K31A and K36Q mutants, which are expected to abolish FlhG ATP binding, could not complement a flhG mutant, reduced their polar localization and lost the ATPase activity. The D60A mutants which is expected to abolish ATP hydrolysis had the ability of flagellar number regulation as wild-type FlhG. The D171A mutant, which is expected to impair the stimulation of ATPase activity, severely reduced the motility and the flagellar number, and increased its localization at the flagellated cell pole. Purified wild-type FlhG exhibited low ATPase activity, whereas D171A mutant showed ten-times higher activity than the wild type.

Conclusion: We propose that ATPase motif of FlhG is involved in regulating flagellar number. ATP-bound/ATP hydrolysis states of FlhG can be recruited to the flagellated cell pole, and reduced polar localization of FlhF.
LOCALIZED TRANSCRIPTION OF fliC IN Salmonella enterica

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**Background:** The flagellar regulon is complex, involving more than 60 genes transcribed in a hierarchical fashion from three distinct classes of promoters (I, II, III). First, the class I-regulated fliDC is regulated by a host of mechanisms. FlhDC then directs $\sigma^70$-mediated transcription of class II promoters. The products of class II transcription build the flagellar hook-basal-body (HBB) in a cascaded assembly process (2, 4, 5). The cytoplasmic C-ring rotor of the flagellum is composed of FliG, FliM, and FliN and assembled onto the membrane-embedded MS-ring (6). After HBB construction, up to 30,000 flagellin molecules are secreted through the flagellar type 3 secretion system at a rate of 10,000 amino acids per second through a 2 nm pore (4, 7). There are two flagellin genes in Salmonella that undergo phase variation, where fljBA is under an invertible promoter and FljA inhibits fliC translation (1). The flagellin monomers polymerize at the distal end of the filament, capped by FliD. The question under investigation is how Salmonella enterica achieves the high rate of secretion for flagellin molecules.

**Methods:** A strain was constructed to co-express fliC and fljB. Also, the ParB-parS system was utilized to tag the chromosomal locus of fliB, adjacent to the fliC locus. The ParB-parS system works by exploiting parS sites common in plasmids and a ParB protein, which binds the parS site (3). The ParB protein then polymerizes on the parS site, providing a high signal-to-noise ratio. Further, we utilized phase variation in Salmonella to create a fljA::mCherry construct to label the fliC mRNA. The co-expression of fliC and fliB showed distinct lengths of FliC and FljB on the filament, but not a mixture of both, by immunohistochemistry. The ParB-parS system showed co-localization of fliB chromosomal locus with the HBB, suggesting localized transcription for fliC. A fusion protein FljA::mCherry allowed us to label the mRNA of fliC 5'UTR (Fig 1). This technique allowed us to visualize localized translation of fliC to the HBB.

**Results:** Co-expression of fliC and fljB showed distinct lengths of FliC and FljB on the filament, but not a mixture of both, by immunohistochemistry. The ParB-parS system showed co-localization of fliB chromosomal locus with the HBB, suggesting localized transcription for fliC. A fusion protein FljA::mCherry allowed us to label the mRNA of fliC 5'UTR (Fig 1). This technique allowed us to visualize localized translation of fliC to the HBB.

**Conclusion:** The co-expression of fliC and fljB showed distinct lengths of FliC and FljB on the filament, but not both. These findings suggest that there is a spatial-temporal organization of flagellar genes to the HBB. To this end, a chromosomal parS site was constructed near the fliC flagellin gene in a FliM::GFP background. Our findings suggested localized transcription of the fliC gene occurs near the HBB. To investigate localized translation, a fljA::mCherry construct was created in a FliM::GFP background to show localized translation of fliC in Salmonella enterica.

**WORKS CITED**


**Lab:** Kelly Hughes
3,4-DIHYDROXYMANDELIC ACID: A CHEMOATTRACTANT AND VIRULENCE FACTOR IN THE GUT

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**Background:** Norepinephrine (NE), the predominant neurotransmitter of the enteric sympathetic nervous system, is an inter-kingdom signaling molecule that induces growth, motility and virulence in enteric bacteria. Intestinal locations rich in NE are favored sites for colonization and invasion by pathogens. The mechanism of this directed response is therefore of interest in understanding the role of signaling and chemotaxis in pathogenesis.

**Methods:** Chemotactic response of *E. coli* RP437 was measured using the microfluidic assay. Relative expression of the locus of enterocyte effacement (LEE) genes in Enterohaemorrhagic *E. coli* 86-24 was determined by RT-qPCR.

**Results:** *E. coli* shows a chemotactic response to NE only when previously exposed it. This response requires the QseC sensor kinase, and involves the induction of the *tynA* and *feaB* genes which encode a primary amine oxidase and an aldehyde dehydrogenase, respectively. These enzymes potentially metabolize NE to produce 3,4-dihydroxymandelic acid (DHMA). DHMA is a potent chemoattractant with sensitivity in the nM range. It is sensed by the Tsr chemoreceptor at the serine-binding site, and it gives a peak attractant response at 50 µM. DHMA also induces LEE gene expression in Enterohemorrhagic *E. coli* (EHEC) and increases attachment of the pathogen to HeLa cells.

**Conclusion:** Enteric bacteria can metabolize NE to produce DHMA, which acts as a potent chemoattractant to recruit bacteria to sites of NE leakage. This signaling mechanism may be involved in the infections caused by enteric pathogens.

Lab: Arul Jayaraman
ARCHITECTURES OF DIVERSE CHEMORECEPTOR ARRAYS AS SEEN BY ELECTRON CRYOTOMOGRAPHY

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Background: The structural basis of chemotaxis is well understood in the paradigm of the *E. coli* membrane-bound array. There, methyl-accepting chemotaxis proteins (MCPs) are networked into an extended hexagonal lattice by kinase (CheA) and coupling (CheW) proteins. This architecture is universal among chemotactic bacteria and underlies many remarkable properties of the system. Recently, we have become interested in the structure of other, less well-characterized chemotaxis systems.

Methods: To study the structure of chemoreceptor arrays we use electron cryotomography (ECT).

Results and Conclusions: Soluble, or cytoplasmic, arrays are found in distantly related bacterial species. We show that soluble MCPs form hexagonal lattices, as in membrane-bound arrays, two of which interact to form a sandwiched array, bounded on either side by CheA/CheW baseplates. Similarly, it was known that many archaeal species have chemotaxis systems, but their structure was unknown. We describe the structure of both membrane-bound and soluble arrays in distantly related archaeal species, revealing the universal conservation of this architecture across bacteria and archaia.

Structure of membrane-bound and cytoplasmic receptor complexes. (A) Schematic showing the topology of receptor-trimers-of-dimers, CheA and CheW in membrane-bound arrays. The methylation region of each receptor dimer is indicated by a star. IM = inner membrane. (B) Top-view of the arrangement of the array components showing the interaction sites between the receptors, CheA and CheW, colored as in (A). (C) Schematic showing the topology of receptor, CheA, and CheW complexes in cytoplasmic arrays, colored as in (A). Cytoplasmic chemoreceptors assemble into two hexagonally packed arrays interacting at their presumably ligand-binding tips.

(Briegel et al., eLife 2014)

Lab: Grant Jensen
NUTRIENT-SENSING NETWORK IN *ESCHERICHIA COLI*

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**Background:** Two-component signal transduction systems represent a prevalent bacterial mechanism to respond to changing environmental conditions. Recently we identified the YehU/YehT system and the YpdA/YpdB system as nutrient-sensing network in *E.coli* (Behr *et al.*, 2014).

**Results:** Both systems are activated at the transition to stationary phase. YehU/YehT putatively responds to peptides/amino acids and induces transcription of *yjiY* encoding a CstA-like transport protein (Kraxenberger *et al.*, 2012). YpdA/YpdB responds to pyruvate and induces *yjhX* (Fried *et al.*, 2013). Furthermore, induction of YehU/YehT has a negative effect on YpdA/YpdB mediated *yjhX* expression, while activation of YpdA/YpdB stimulates expression of *yjiY*. Biochemical studies verify ligands and substrates of the signaling network. The effect of different environmental cues, eg. pyruvate/lactate/serine, on signal transduction is currently under investigation.

**Methods:** These effects were confirmed in mutants lacking any of the genes for the three primary components of either system. In vivo interaction studies identified heteromeric interactions between the membrane-bound components, suggesting the formation of a larger signaling unit.

**Conclusion:** We have identified a nutrient-sensing signaling network in *E. coli*, which may facilitate optimal carbon usage, when nutrients become limiting for growth.

**Lab:** Kirsten Jung
THE PHOSPHORYLATION FLOW OF THE VIBRIO HARVEYI QUORUM SENSING CASCADE DETERMINES LEVELS OF PHENOTYPIC HETEROGENEITY IN THE POPULATION

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Background: Quorum sensing is a communication process enabling a bacterial population to coordinate and synchronize specific behaviors. It involves the production, excretion and perception of low-molecular, diffusible molecules called autoinducers (AIs). In Vibrio harveyi, quorum sensing relies on three parallel systems involving three autoinducers: AI-2, HAI-1, CAI-1. Perception of the AIs is channeled into the same signaling cascade; the information is transduced via phosphorelay and sRNAs to the master regulator LuxR which controls numerous AIs-dependent genes and phenotypes.

Methods: Using a new set of V. harveyi mutants lacking genes of the AIs synthases and/or sensors, we assayed the activity of the quorum sensing cascade at population level using bioluminescence as a direct readout and at single cell level using a fluorescent reporter fusion from a quorum sensing activated gene.

Results: The expression of the reporter fusions in different genetic backgrounds corresponding to different designs of the signaling cascade showed that the strength of the phosphorylation flow in the cascade drives the homogeneity of the quorum sensing response. In conditions where the quorum sensing cascade is not fully activated, for example upon depletion of one AI, single cells show heterogeneity in quorum sensing activation ranging from non-activated cells to fully activated cells.

Conclusion: The pools of phosphorylated LuxU/LuxO per cells directly determine the amounts of sRNAs produced and consequently the copy number of LuxR, generating a heterogeneous quorum sensing activation at the single cell level. We believe that in natural conditions where nutrients are poorly abundant, this heterogeneous behavior is beneficial for the bacterial population to express both quorum sensing negative traits (e.g. siderophore production) and quorum sensing positive traits (e.g. proteolytic activity).

Lab: Kirsten Jung
A FRET-BASED DNA BIOSENSOR TRACKS OmpR-DEPENDENT ACIDIFICATION OF SALMONELLA DURING MACROPHAGE INFECTION

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Background: The EnvZ/OmpR two-component system is required for Salmonella to survive in the macrophage vacuole. During the intracellular phase of infection, Salmonella switches from secreting type three secretion structural components to secreting effectors into the macrophage cytoplasm, enabling Salmonella to replicate in the phagocytic vacuole. The questions we are interested in are how do bacteria survive the acidified vacuole and how does acidification affect bacterial secretion.

Methods: To address these questions, we employed a DNA-based FRET biosensor (“I-switch”) to measure bacterial cytoplasmic pH and immunofluorescence to monitor effector secretion during infection.

Results: We observed an EnvZ/OmpR-dependent rapid drop in bacterial cytoplasmic pH upon phagocytosis. Microarray analysis highlighted the cadC/BA operon, and additional experiments confirmed that it was repressed by OmpR. The absence of CadB/A proteins prevented recovery from acid stress. Acid-dependent activation of OmpR stimulated type three secretion; blocking acidification resulted in a neutralized cytoplasm that was defective for SPI-2 secretion.

Conclusion: We propose a novel model for Salmonella infection involving an acid-dependent secretion process in which the translocon SseB is released (detached) from the SPI-2 needle, driving the subsequent secretion of effectors such as SseJ. Supported by the Mechanobiology Institute, NUS and VA 5101BX000372 to LJK.

Lab: Linda Kenney
A ROLE FOR FORMIN FHOD1 IN EPEC PEDESTAL FORMATION

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BACKGROUND: Enteropathogenic E. coli are extracellular pathogens that cause dense puncta of polymerized actin at the site of bacterial attachment known as ‘actin pedestals’. The polymerization of actin pedestals is mediated by the WASp-Arp2/3-dependent pathway. Formins are a class of actin nucleators that lead to both nucleation and elongation of actin filaments. Recent evidence suggests that in structures like lamellipodia and cell junctions actin nucleators such as Arp2/3 and formins act in a temporally coordinated fashion. It was therefore of interest to examine whether there was a role for formins in EPEC actin pedestals.

METHODS: We infected NIH3T3 fibroblasts with EPEC. To study the effect of formins on pedestal formation, we used shRNA knockdowns and small molecule inhibitors. Fixed and live cell confocal microscopy was performed following EPEC infection. We measured actin and formin dynamics in pedestals using FRAP and compared it to the cytoplasm.

RESULTS: Interestingly, formins FHOD1 and mDia1 were discovered to be localized to the base of the pedestal. Inhibition of formin FHOD1 via the small molecule inhibitor SMIFH2, resulted in a drastic reduction of pedestal surface area. FHOD1 knockdown led to a similar size reduction of pedestals, which was restored by supplying FHOD1 in trans.

CONCLUSION: This is the first report of a role for formins in EPEC pedestal formation. Our findings suggest that EPEC employs multiple pathways for coordination in the Arp2/3 complex. It raises further questions as to how these actin nucleation pathways of Arp2/3 and formins are spatio-temporally regulated upon EPEC infection. We are now examining downstream effects of formin-dependent actin pedestal formation in stimulating transcription factors MRTF-A and NFkB, as these are major targets of EPEC infection.

Support: The Mechanobiology Institute, NUS and 5IO1BX1000372 to LJK.
CHARACTERIZATION OF DUPLICATED PILSR TWO COMPONENT SYSTEMS FOR REGULATION OF MOTILITY IN MYXOCOCCUS XANTHUS

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Background: Two-component signaling systems display remarkable specificity at the level of protein-protein interaction between the sensor kinase and cognate response regulator. However, mechanisms which allow for cross-regulation between highly conserved systems remain elusive. A further complication exists where specificity of signal inputs and downstream outputs also remain largely unknown for most TCS. M. xanthus encodes 147 TCS kinases, thus serving as a model organism for the study of signaling fidelity. A major subset of the TCS in M. xanthus are NtrB/C pairs, many of which have arisen through gene duplication events. We are currently characterizing the duplicated TCS comprising PilSR and PilS2R2 for their putative coordinated regulation of Type 4 pilus production and motility.

Methods: We have combined bioinformatic, biochemical and genetic approaches. Specifically, we are assaying PilS, PilR, PilS2, and PilR2 and combinations for phosphotransfer specificity in vitro. We are also quantifying gene expression for both pilA and pilA2 (encoding both major and minor pilins, respectively) in strains carrying deletions for pilS, pilR, pilS2 and pilR2. Subsequently, we are measuring effects on surface motility.

Results: Gene duplication of PilSR has occurred relatively recently within the delta-proteobacteria. PilS2 possesses a truncated sensing domain that should affect signaling for this kinase. A deletion of pilR leads to reduced motility, consistent with previous data. Deletion of pilR2 also displays reduced motility. These preliminary findings are consistent with reduced pil expression, or altered function of the T4P machinery. LacZ reporter fusions have also allowed us to assess a minimal pilA promoter and a putative PilR binding site has been identified. Additional results will be presented.

Conclusions: It is well established that PilSR is necessary for expression of pilA and therefore T4P production. From our latest data, it appears that PilS2R2 also regulates aspects of T4P production and function. Our goal is to determine how these systems coordinately regulate motility, whether cross-regulation occurs via sensing, protein interactions, or phosphotransfer specificity and promoter targets.

Lab: John Kirby
THE HBM DOMAIN: INTRODUCING BIMODULARITY TO BACTERIAL SENSING

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Background: We have recently reported the three dimensional structure of the McpS chemoreceptor sensor domain in complex with its cognate ligands. The domain was characterized by a bimodular architecture, where ligand binding to each module caused a chemotactic response. This is a novel small molecule binding domain, which, however, is un-annotated in relevant databases.

Methods: An alignment of a set sequences with homology to the McpS sensor domain was performed and their secondary structure predicted. The profile HMM generated was then used to perform a search in protein databases, which resulted in the retrieval of the full collection of sequences of protein containing the HBM domain.

Results: We report the domain signature of the family of McpS-like sensor domains, which was termed helical bimodular (HBM) domain. The HBM domain was identified in Bacteria and Archaea and forms part of chemoreceptors and histidine kinases.

Conclusion: The novelty of McpS sensor domain resides in its bimodular architecture and the fact that ligand binding at each module causes a response. The conservation of residues in both ligand binding sites suggests that the capacity to recognize ligands at the different modules is a characteristic common to the HBM family.
MOLECULAR ARCHITECTURE AND ASSEMBLY OF THE EXPORT APPARATUS IN BORRELIA BURGDORFERI

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Background: Type III secretion systems are widely utilized by Gram-negative bacteria to assemble flagella or to transport virulence effectors into eukaryotic cells. The central component is known as a type III secretion machine, which consists of a group of integral membrane proteins and three soluble proteins. Powered by the proton motive force and ATP hydrolysis, the secretion machine is responsible for substrate recognition and export. However, efforts to uncover the secretion mechanism have been hindered by a poor understanding of the structure and subunit organization of the intact machines.

Methods: We used the Lyme disease spirochete Borrelia burgdorferi as a model system and constructed a series of single and quintuple deletion variants of the export apparatus (flhA, flhB, fliP, fliQ, fliR, fliZ [a fliO homolog], and flhA-flhB-fliP-fliQ-fliR). High-throughput cryo electron tomography and sub-tomogram averaging were utilized to visualize the export apparatus in situ.

Results: We determined the motor structures from the deletion mutants that lack one or many integral membrane proteins (FlhA, FlhB, FliP, FliQ, FliR and FliZ) and revealed the profound changes in both flagellar motor assembly and substrate secretion. Together with comparative structural analysis and molecular modeling, we characterized the multi-protein organization of the intact export apparatus and provided structural evidences that a hexameric ATPase complex is optimally aligned with the export gate through multiple interactions.

Conclusion: The interconnected cytoplasmic network of the export apparatus may function as a docking platform critical for recruitment and export of substrates across the cell membrane.

Figure. A cartoon model of the intact flagellar motor and the export apparatus in B. burgdorferi.

Lab: Jun Liu
THE PERIPLASMIC LSRB PROTEIN INTERACTS WITH THE PERIPLASMIC DOMINAN OF THE E. COLI TSR CHEMORECEPTOR TO GENERATE A CHEMOTAXIS RESPONSE TO THE QUORM-SENSING MOLECULE AI-2

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Background: Bacterial population density is assessed by monitoring the accumulation of autoinducers (AIs) through a process called quorum sensing. AI-2 is a broad-spectrum autoinducer produced by both gram-negative and gram-positive bacteria. AI-2 is generated from S-ribosylhomocysteine and is exported to the periplasm, from which it then equilibrates with the external environment. The E. coli Tsr chemoreceptor interacts with the AI-2-binding protein LsrB to initiate an attractant response. AI-2 bound to LsrB is also taken up into the cell by the LsrACD transporter. The lsrACDBFG operon is transcriptionally repressed by the LsrR protein. Cytoplasmic AI-2 is phosphorylated by the LsrK kinase, and phospho-AI-2 binds to LsrR to derepress transcription. LsrF and LsrG degrade phospho-AI-2.

Methods: Chemotaxis was measured by capillary, MicroPlug, and MicroFlow assays. Western blot analysis was used to quantify levels of LsrB. AI-2 levels were monitored by intramolecular FRET using CFP/YFP tagged LuxP protein from Vibrio harveyi.

Results: An E. coli strain carrying a Kanamycin-cassette knockout of the lsrB gene was totally defective for AI-2 chemotaxis. Strains producing intact LsrB but missing Tsr were also defective in AI-2 chemotaxis. In contrast, an lsrC knockout strain was fully competent for AI-2 chemotaxis. Here, we report the dependence of AI-2 chemotaxis on the periplasmic concentration of AI-2 and describe a mutational analysis of the LsrB/Tsr interaction predicted by a computer docking model. We also show that tsr mutations that eliminate chemotaxis to L-serine have no effect on AI-2 chemotaxis.

Conclusion: We conclude that both LsrB and Tsr are necessary for AI-2 chemotaxis, but that uptake of AI-2 into the cytoplasm not required for chemotaxis to AI-2. Chemotaxis to AI-2 and L-serine are independent responses. Our mutational analysis suggests the AI-2-bound LsrB interacts with the periplasmic domain of E. coli Tsr similarly to the way that maltose-binding protein (MBP) interacts with the periplasmic domain of E. coli Tar. However, much lower periplasmic levels of LsrB are needed to evoke the chemotaxis response to AI-2 than the concentrations of MBP required to support good taxis to maltose.
UNTANGLING FLAVOBACTERIUM GLIDING MOTILITY AND PROTEIN SECRETION

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Background: Flavobacterium johnsoniae exhibits rapid gliding motility over surfaces. At least twenty proteins are involved in this process. Eight of these, GldK, GldL, GldM, GldN, SprA, SprE, SprF, and SprT, function in the type IX protein secretion system (T9SS). The T9SS is required for surface localization of the motility adhesin SprB and for secretion of many other proteins. Gliding involves the rapid movement of SprB along the cell surface. The functions of the other Gld proteins are less clear but the phenotypes of cells lacking these proteins suggest that they may be needed for secretion. The T9SS and the motility apparatus may be two faces of the same machinery or separate systems that can be disentangled from each other.

Methods: Genetic and molecular experiments coupled with analysis of motility and protein secretion functions identified proteins of the T9SS, proteins of the gliding motility apparatus, and proteins that function in both systems.

Results: Analysis of motility mutants revealed that GldA, GldB, GldD, GldF, GldG, GldH, GldI and GldJ are essential for T9SS function. Cells with mutations in the genes encoding any of these proteins had normal levels of gldK mRNA but dramatically reduced levels of GldK protein. GldK is required for T9SS function and the absence of GldK may explain the secretion defects of the motility mutants. Each mutant also lacked GldJ protein, and bacterial 2-hybrid analysis revealed an apparent interaction between GldJ and GldK. This suggested the possibility that GldJ may stabilize GldK. gldJ mutants were isolated that exhibited T9SS function but lacked motility. F. johnsoniae cells that produced truncated GldJ lacking thirteen amino acids from the C-terminus accumulated GldK protein and had functional T9SSs but were deficient in gliding motility. SprB was secreted by these cells but was not propelled along the cell surfaces. The C-terminal thirteen amino acids of GldJ are thus required for gliding motility but not for secretion.

GldA, GldF, and GldG constitute an apparent ABC transporter whose exact function is not known. Cells with a point mutation that disrupted the GldA ATP-binding site resulted in loss of gliding motility and secretion. The mutant cells failed to accumulate GldJ or GldK proteins suggesting that a functional Gld ABC transporter is needed to stabilize the motility protein GldJ and the T9SS protein GldK.

Conclusion: The F. johnsoniae T9SS and gliding machines appear to be intertwined, and many mutations in 'motility' genes disrupt both processes. The identification of gldJ mutants that are defective for motility but competent for secretion begins to untangle the F. johnsoniae gliding motility machinery from the T9SS.
FLAVOBACTERIUM COLUMNARE TYPE IX SECRETION SYSTEM MUTATIONS RESULT IN DEFECTS IN GLIDING MOTILITY AND VIRULENCE

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Background: The gliding bacterium Flavobacterium columnare causes columnaris disease in wild and aquaculture-reared freshwater fish. The mechanisms responsible for columnaris disease are not known. The related bacterium Flavobacterium johnsoniae uses a type IX secretion system (T9SS) to secrete enzymes, adhesins, and proteins involved in gliding motility. F. columnare has all of the T9SS components, and this system may have a role in virulence. Genetic techniques were recently developed for F. columnare, including procedures to make in-frame deletions. We used these techniques to test the hypothesis that the T9SS is required for virulence.

Methods: In-frame deletion mutations in the T9SS genes gldN and porV were constructed in F. columnare strain C#2. The mutants were examined for motility, secretion, and ability to kill zebrafish and yellow perch.

Results: An F. columnare gldN deletion mutant was constructed. GldN is a core component of the F. johnsoniae T9SS and is required for secretion. gldN mutants are unable to secrete the motility adhesin SprB to the cell surface, and thus are also defective in gliding motility. The F. columnare ΔgldN mutant was deficient in the secretion of several extracellular proteins and lacked gliding motility. The ΔgldN mutant exhibited greatly reduced virulence on zebrafish and yellow perch, and complementation restored virulence. Cell-free spent media from cultures of wild type and complemented cells caused rapid mortality in zebrafish, while cell-free spent media from ΔgldN mutant cells did not. F. johnsoniae PorV is required for secretion of a subset of proteins targeted to its T9SS and is not required for motility. A ΔporV mutant of F. columnare was constructed to determine whether motility or secretion was associated with virulence. The ΔporV mutant exhibited gliding motility but in preliminary tests it had decreased virulence similar to the ΔgldN mutant.

Conclusion: The F. columnare T9SS appears to be required for virulence. Future studies will identify the secreted proteins responsible for disease and test the efficacy of avirulent mutants as vaccine strains to prevent columnaris disease in aquaculture fish.

Lab: Mark McBride
HELICOBACTER PYLORI FlhA BINDS SENSOR KINASE AND FLAGELLAR GENE REGULATORY PROTEIN FlgS WITH HIGH AFFINITY

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Background: Flagellar biogenesis is a complex process that involves the coordinated expression of over 50 structural and regulatory genes with assembly of the nascent flagellum. In Helicobacter pylori, FlgS, a histidine kinase that with response regulator FlgR comprises a two-component signaling system, regulates the RpoN regulon, which includes the middle genes. In response to an unknown signal, FlgS autophosphorylates and transfers the phosphate to FlgR, initiating transcription from RpoN-dependent promoters. In the present study, export apparatus protein FlhA was examined as to its potential role as an activating signal protein.

Methods: Deletion of the N-terminal cytoplasmic sequence from FlhA dramatically decreased expression of RpoN-dependent genes flaB and flgE in two different H. pylori strains as measured by qRT-PCR. Biosensor interferometry (BLI), an optical biosensing technique that yields data similar to SPR, was used to examine interactions between FlgS and a peptide consisting of residues 1-25 of FlhA. Fragments of both FlgS and the FlhA peptide were also examined for binding.

Results: Deletion of the N-terminal segment of flhA eliminated motility and lowered flaB and flgE expression ~6-fold. K d for FlhA NT -FlgS binding was 21 nM and characterized by fast-on (k on = 2.9 x 10 4 M -1 s -1) and slow-off (k off = 6.2 x 10 -4 s -1) kinetics. Analysis of binding to purified fragments of FlgS demonstrated that the C-terminal portion of the protein containing the kinase domain binds FlhA NT with virtually identical kinetics. Substantially all of the FlhA sequence is required as fragmentary peptides exhibited no binding. FlhA NT did not stimulate autophosphorylation of FlgS under the conditions examined.

Conclusions: The present results indicate that FlhA, via its N-terminal segment is necessary for both motility and activation of RpoN-dependent genes. The lack of stimulation of FlgS autophosphorylation suggests that activation is more complex than pairwise interaction, perhaps involving other proteins, e.g. FliF, FliG, etc. We plan to investigate these possibilities.

Lab: Jonathan McMurry
THE FLAGELLAR BIOSYNTHETIC PROTEIN, FliR, AFFECTS COLONY MORPHOLOGY AND BIOFILM PRODUCTION IN THE SOIL BACTERIUM, PANTOEA SP. YR343

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Background: Interactions between plants and microbes have been an active area of research for many years; however, much of the underlying mechanism(s) behind these interactions remain unclear. We are particularly interested in microbes that promote plant growth for a variety of reasons, including: 1) they can improve the growth of both food crops and biofuel crops, and 2) they have potential to be commercialized and used as a natural bio-fertilizer that is less harmful to the environment. Our lab is studying Pantoea sp. YR343, a soil bacterium isolated from the roots of Populus deltoides, and working to decipher the mechanistic details behind root colonization by this organism. Among many interesting factors that contribute to root colonization, we are interested in studying the role of cyclic di-GMP, particularly in terms of exopolysaccharide production and biofilm formation along plant roots.

Methods: Briefly, we utilized techniques including genetic tools for gene expression and labeling, motility assays, transposon mutagenesis, and immunofluorescence. We have also performed RNAseq analysis with collaborators from Carrie Harwood's lab at the University of Washington.

Results: We constructed a strain of Pantoea sp. YR343 over-expressing a diguanylate cyclase (PMI39_02884) that we found to be expressed on plant roots. The over-expression strain exhibited phenotypes very different from the wild type, including formation of dry, wrinkly colonies, increased exopolysaccharide production, and reduced motility – phenotypes commonly associated with production of high levels of cyclic di-GMP. We next utilized this over-expression strain to perform transposon mutagenesis and screen for colonies that no longer exhibited these phenotypes associated with high levels of cyclic di-GMP. We isolated 137 mutants and have sequenced approximately 130 of them. In addition, we have used RNAseq analysis on this strain to identify genes affected by production of high levels of cyclic di-GMP. Among the many interesting genes identified, we will describe work done here on characterizing the role of a flagellar biosynthetic protein, FliR.

Conclusions: Interestingly, we have found that motility genes are down-regulated in the Pantoea sp. YR343 strain over-expressing the diguanylate cyclase (PMI39_02884), likely explaining the reduced motility phenotype; however, a FliR:Tn5 mutant does not show any motility defects, but is affected in biofilm production and colony morphology.

Figure 1. Colonization of wheat roots (in red) by green fluorescent protein (GFP)-labeled Pantoea sp. YR343.

Lab: Jennifer Morrell-Falvey
NOVEL PROTEIN INVOLVED WITH COLLAR STRUCTURE OF THE PERIPLASMIC FLAGELLA IN
BORRELIA BURGDORFERI

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Background. Borrelia burgdorferi is the causative agent of Lyme disease, the most prevalent vector-
borne disease in the United States infecting as many as 300,000 people each year. There are no
classically-defined virulence factors within the genome however, we recently reported that motility is
crucial for the infectious life cycle of B. burgdorferi, vital for transmission within and between arthropod
and vertebrate hosts. Since motility is so vital to infection, our goal is to further our understanding of the
mechanism involved in B. burgdorferi’s motility by examining the flagellar motor rotation or assembly.
As a spirochete the flagella is internal, contained within the periplasm. As such, this apparatus is
different to the well described externally located flagella and several of the main proteins in this motor
are still unidentified. For example, the collar structure is unique to spirochetes.

Methods. We used bioinformatics, gene inactivation followed by Cryo-electron tomography (Cryo-ET)
to identify novel proteins encoding spirochete-specific flagellar structures in B. burgdorferi.

Results. In the Cryo-ET images show the collar appears as a large organelle interconnected with the
MS-ring and stator. We identified the 1824bp B. burgdorferi gene bb0526 as a potential candidate
encoding the collar structure, as it has an unknown function with no significant homology to other
bacterial species outside of spirochetes. It is not located near any other motility or chemotaxis genes.
Once inactivated, complemented, and confirmed, the bb0526 mutant bacteria were observed under a
dark-field microscope. The mutant cells were motile and had normal flat-wave morphology, but, their
swimming velocity was significantly slower. Also, their flagellar apparatus was examined using Cryo-ET
and we discovered that they lacked a portion of the collar structure seen in wild-type cell, between the
 collar and stator.

Conclusions. These results indicate that novel protein BB0526 is involved in motility, potentially as a
linker between the collar and stator structures. We are investigating this mutant to identify if it exhibits
phenotype related to periplasmic flagellar assembly.

Lab: Md. Motaleb
ROLE OF BORRELIA BURGDORFERI CheD IN CHEMOTAXIS AND INFECTIOUS LIFE CYCLE

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Background: Motility and chemotaxis were reported to be crucial for the infectious life cycle of Borrelia burgdorferi, the Lyme disease spirochete. However, our knowledge on how the spirochete achieves its distinctive asynchronous motility is limited. Most importantly, the roles of most of the B. burgdorferi putative chemotaxis proteins are still elusive. B. burgdorferi has a putative gene encoding CheD which is relatively well-characterized in Bacillus subtilis, where it plays an important role in chemotaxis by deamidation of methyl-accepting chemotaxis protein receptors (MCPs), by increasing the receptor-kinase activity or by enhancing CheC phosphatase activity, thereby regulating the levels of the CheY response regulator.

Methods: To determine if B. burgdorferi CheD is able to stimulate CheX phosphatase activity, we initially performed protein-protein interaction analysis followed by phosphorylation-dephosphorylation assays. In vitro motility and chemotaxis phenotypes of B. burgdorferi ΔcheD mutant were verified by dark-field microscopy and swarm plate assays. Furthermore, we performed mouse and tick-mouse infection assays to determine the role of CheD in B. burgdorferi enzootic life cycle.

Results: CheD specifically interacts with CheX that significantly enhanced the CheX phosphatase activity. The cheD mutant exhibited defects in chemotaxis as well as in motility in vitro; whereas the wild-type cells had a run-flex-reverse swimming pattern, the cheD mutant exhibited an incomplete reverse phenotype. Our preliminary studies indicate that the cheD mutant was not significantly attenuated in C3H/HeN mice infection compared to wild-type or complemented strain via needle inoculation. Mouse-tick-mouse infection assays indicate that CheD is not required for acquisition (from infected mice to ticks) or transmission of spirochetes (from infected ticks to naïve mice), but burden of mutant spirochetes in ticks is significantly reduced than wild-type or complemented strain. Moreover, the mice fed by the mutant-infected ticks displayed a significantly lower immune responses.

Conclusion: CheD plays important role in motility, chemotaxis, and pathogenesis of B. burgdorferi. Delineating the role of CheD in B. burgdorferi will provide insights into not only the chemotaxis pathway of this spirochete but also its asymmetric swimming and infectious life cycle.

Lab: Md. Motaleb
BORRELIA burgdorferi CheY1, CheY2, AND CheY3 DISPLAY NON-OVERLAPPING VIRULENCE PROPERTIES IN THE NATURAL INFECTION CYCLE FOR LYME DISEASE

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Background. In the Lyme disease spirochete Borrelia burgdorferi, chemotaxis is believed to play a critical role in the life cycle of this organism, both by promoting migration of the spirochetes from the midgut of ticks into the dermis of the mammalian host, as well as facilitating spirochetal migration from infected vertebrates into uninfected ticks, perpetuating new vectors for disease transmission. We postulate that spirochetal motility governed by chemotaxis is essential for dissemination between these hosts, however the mechanisms allowing this distinctive motility is unknown. The chemotaxis system of B. burgdorferi is well-conserved to those of Escherichia coli. However, the B. burgdorferi chemotaxis system is much more complex as its genome encodes multiple homologs of several chemotaxis genes (e.g. two cheA, three cheY, three cheW, and two cheB genes). In prototypic bacteria, the CheY protein is the response regulator in the chemotaxis signal transduction system, 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) 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 CheY proteins during the mouse-tick-mouse infection cycle of B. burgdorferi.

Methods. To demonstrate the function of the three cheY genes, we have created individual cheY mutants in B. burgdorferi, and performed in vitro and in vivo (mouse and tick-mouse) phenotypic assays.

Results. Preliminary data suggests that cheY2 and cheY3 single mutants are significantly attenuated or non-infectious in mice via needle inoculation or tick bite, whereas the cheY1 mutant appears similar to wild-type in infecting mice. Regarding tick infection, the burden of ΔcheY3 spirochetes in fed ticks is significantly lower compared to wild-type whereas ΔcheY2 bacterial burden is normal. We are currently completing studies assessing the importance of the cheY1 and cheY2 in tick colonization and transmission of B. burgdorferi from tick to mouse.

Conclusion. Overall, our preliminary data suggests that the different CheY proteins are important for infection of or transmission between a different subset of hosts in the natural life cycle of B. burgdorferi.
UNIQUE CHEMOTAXIS-LIKE RECEPTOR TRIMER OF DIMER CONTACT RESIDUES

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Background. Motile bacteria perform chemotaxis, which initiates when ligand binds the periplasmic domain of chemotaxis receptors (methyl accepting chemotaxis proteins or MCPs) and is sensed by proteins bound to MCP cytoplasmic signaling domains. MCPs form a polar lattice of homodimers that form trimers of dimers. The MCP cytoplasmic tips have eleven nearly invariant residues for trimer formation when facing “inside” and may contact signaling proteins when facing “outside.” Numerous bacteria have MCP-like proteins that lack periplasmic and transmembrane domains and contain two linked cytoplasmic signaling domains (in essence linked MCP-like dimers). These proteins, named binary output only or BOOs, have conserved unique residues in the nearly invariant MCP cytoplasmic tip with each BOO domain containing a different set of substitutions. We hypothesize that these unique contact residues may force inside/outside orientation of the BOO domains.

Methods. Full-length *Escherichia coli* serine sensing MCP, Tsr, was constructed with either the BOO first domain substitution set or the second domain substitutions (*E. coli* do not naturally express BOO proteins). These constructs (individually or together) were tested for swarming when expressed in *E. coli* lacking MCPs.

Results. Sequence searching showed that BOO proteins have highly conserved substitutions in the cytoplasmic tips with each domain having a unique set. *E. coli* Tsr with either the first BOO substitutions or the second BOO substitutions were significantly reduced for chemotaxis function. When co-expressed BOO substitutions chemotaxis function increased.

Conclusions. BOO substitutions may act to complement function. We will explore whether these substitutions force BOO receptor orientation.

**BOO protein model.** A) Full-length MCPs (half circles) form homodimers (large circles) that form trimers through the binding of the nearly invariant cytoplasmic tip contact sites (black dots). B) BOO proteins have linked cytoplasmic domains (large circles) with some unique contact residues (blue and yellow dots) that may orient one domain “inside” for trimer formation (black and blue dots) and one domain “outside” for signaling molecules contact

Lab: Patricia Mowery
STRUCURAL CHARACTERIZATION OF FLIP, A COMPONENT OF THE TYPE III FLAGELLAR PROTEIN EXPORT APPARATUS

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**Background:** Bacteria can swim in liquid environment by rotating flagella. The bacterial flagellum is composed of about 30 different proteins with copy numbers ranging a few from to a few tens of thousands. For flagellar construction, most of flagellar proteins are transported by a specific export apparatus from the cytoplasm to the growing distal end of the flagellum through a 2 nm diameter central channel. The export apparatus consists of a water-soluble ATPase complex (FliH/I/J) and a proton-driven export gate made of six membrane proteins (FlhA/B, FliO/P/Q/R). Although the ATPase complex is well characterized, the structure and roles of each export gate component remain unknown. Recent genetic and biochemical analyses have shown that FliP is thought to assemble at an earliest stage of export gate formation. Therefore, FliP seems to be important not only for flagellar protein export but also export gate construction.

**Methods:** Protein expression/purification, Electron microscope, X-ray crystallography

**Results:** We overexpressed and purified FliP of *Salmonella enterica*. Electron microscopic observation revealed that purified FliP formed a ring-shaped oligomer with a central pore with a diameter of 2 nm. The crystal structure of the periplasmic domain of FliP of *Thermotoga maritima* (Tm-FliPᵣ) was determined at 2.4 Å resolution.

**Conclusion:** FliP self-assembles into a hexameric ring structure, suggesting that this ring structure is a functional relevant unit in the export gate. The crystal structure of Tm-FliPᵣ and the following mutational analyses of *Salmonella* FliP suggest that the FliPᵣ-FliPᵣ interaction contributes to efficient FliP ring formation.

Representative 2D classified image of FliP ring

*Lab: Keiichi Namba*
THE HELICOBACTER PYLORI CHEZ PROTEIN LOCALIZES TO THE POLE INDEPENDENT OF THE TYPICAL PROTEINS (CHEA OR FLIY) AND INSTEAD DEPENDS ON CHEPEP

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Background: Chemotaxis is important for Helicobacter pylori to colonize the stomach. Like other bacteria, H. pylori uses chemoreceptors and conserved chemotaxis machinery to drive phosphorylation of the flagellar regulator, CheY, and modulate flagellar rotational direction. To return phosphoryl CheY to the non-phosphorylated state, H. pylori employs a CheZ chemotaxis phosphatase. Chemotaxis phosphatases localize to the cellular poles by interactions with either the chemotaxis signaling proteins such as CheA, or with flagellar motor proteins such as FliY. H. pylori CheZ (CheZHP) however is quite divergent from other studied CheZ proteins, so we were curious whether it would use the same localization mechanisms.

Methods: Immunofluorescence and co-immunoprecipitation were used to analyze the localization and interactions of CheZHP.

Results: H. pylori chemotaxis signaling proteins CheA, CheV1 and chemoreceptors localize to the poles, as predicted but not yet shown. CheA and CheV1 depend on the chemoreceptors for polar localization. CheZHP localizes to the poles as well, but is independent of the chemoreceptors and all chemotaxis signaling proteins (CheA, CheW, CheV1, CheV2, CheV3, CheY). CheZHP polar localization is also independent of all tested flagellar proteins including FliM, FliN, FliY, FliG, FliF, MotB, FlhF, FlhG and FlhA. Instead, CheZHP localization depends on a chemotaxis regulatory protein called ChePep that is unique to the Epsilon Proteobacter. ChePep similarly localizes to the pole (Howitt et al. mBio, 2011 vol. 2 (4) pp. e00098-11-e00098-11), and requires CheZHP for its polar localization. Functional domain mapping of CheZHP determined the polar localization motif lies within its central domain.

Conclusion. CheZHP and ChePep appear to form a complex that is separate from the flagellar motor and chemoreceptor signaling complexes. These results suggest the intriguing idea that some chemotaxis regulatory proteins localize independently of the other motility proteins, possibly to confer unique regulation on these protein’s activities.

Fig. 1. Model of H. pylori protein-protein interactions of the chemotaxis system.
The chemotaxis proteins are actually at the same end as the flagella, but are shown at the opposite end for space considerations. Tlp’s are chemoreceptors; V, CheV; W, CheW; A, CheA; Y, CheY; Z, CheZ; Pep, ChePep.

Lab: Karen Ottememann
HOW MOTILITY AND CHEMOTAXIS PROMOTE HELICOBACTER PYLORI PATHOGENESIS

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Background: *Helicobacter pylori* chronically infects more than 50% of the human population worldwide, living in the gastrointestinal tract. Motility is essential for normal stomach colonization. While a non-motile mutant shows a severe defect in colonizing mouse stomachs, a chemotaxis mutant shows only slightly reduced numbers compared to wildtype. Interestingly, this reduction is predominantly due to a defect of colonization in a part of the stomach called the antrum. However, after one month of infection, both wildtype and non-chemotactic strains show similar colonization. In this situation, despite reaching wild-type numbers, the non-chemotactic mutant causes less inflammation.

Methods: infections of AGS cells, mice and gastric organoids with different *H. pylori* mutant strains deficient in chemotaxis or toxin (VacA) delivery as well as GFP expressing mutants to compare colonization, attachment, localization and host cell apoptosis; colonization and attachment levels: plating and flow cytometry; localization: confocal microscopy using GFP expressing strains; host cell apoptosis: propidium iodide staining,

Results: We first focused on two well established systems, AGS cell tissue culture and the *in vivo* system using C57BL6/N mice as a reference for our results obtained from gastric organoids. We found that in tissue culture chemotaxis does not play a significant role in apoptosis, while VacA is required for this outcome. In this system the double mutant shows no additive effects. In contrast, the *in vivo* experiments showed that both chemotaxis and VacA play a role for apoptosis in mice. In the gastric organoid model we were able to track the mutants during colonization and found that chemotaxis plays a role in attachment to the inner surface of the organoid structure.

Conclusions: We hypothesize that chemotaxis plays a role in positioning the bacterium for toxin delivery. To track if chemotaxis is involved in attaching to its target cells to deliver toxins we are studying chemotaxis mutants in gastric organoids. Our results suggest that gastric organoids maintain key facets of intact tissue and will allow dissection of chemotaxis-dependent processes.

**Figure.** Three model systems to study the effects of chemotaxis on host cell apoptosis and toxin delivery: (1) AGS cells, (2) mice, (3) gastric organoids
MUTAGENESIS OF THE *STAPHYLOCOCCUS AUREUS* SENSOR KINASE SaeS REVEALS EXTRACELLULAR RESIDUES IMPORTANT FOR TOXIN PRODUCTION AND PATHOGENESIS

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**Background:** *Staphylococcus aureus* is a ubiquitous pathogen that can cause both acute and chronic diseases in a variety of human tissues. *S. aureus*’s ability to cause such a wide range of diseases is made possible by the large arsenal of toxins, exoenzymes and adhesins encoded in its genome. Transcription of many of these virulence factors is regulated by the *sae* two-component system. Despite its importance, the mechanism by which the histidine kinase SaeS recognizes specific host stimuli, such as human neutrophil protein 1 (HNP-1) is unknown.

**Methods:** TOPCONS and the substituted cysteine accessibility method (SCAM) were used to determine the topology of SaeS. Fluorescent reporters, real-time quantitative PCR and QuantiGene 2.0 assays (Affymetrix) were used to analyze expression of *sae*-target genes in SaeS extracellular point mutants after exposure to HNP-1 and human neutrophils, signals known to activate the *sae* system. To assay for functional bacterial toxin production, neutrophil permeabilization assays analyzing propidium iodide (PI) uptake were used.

**Results:** After mutagenizing the predicted extracellular loop of SaeS, we discovered one methionine residue (M31) was important for the ability of *S. aureus* to transcribe *sae*-target genes, including α-toxin (*hla*), γ-toxin (*hlgA, hlgCB*) and leukotoxin (*lukAB*). The strain carrying the M31A mutation also caused significantly less cytotoxicity in neutrophils compared to wild-type, similar to Δ*saeS*. Another important finding was that mutation of two aromatic anchor residues (W32A and F33A) disrupted the normal basal signaling of SaeS in the absence of inducing signals, yet both mutant kinases had appropriate activation of effector genes following exposure to neutrophils. Although the transcriptional profile of aromatic mutation W32A was consistent with that of wild-type in response to purified HNP-1, mutant kinase F33A did not upregulate transcription of the γ-toxin genes in response to this stimulus.

**Conclusions:** Taken together, our results provide evidence for how SaeS recognizes specific host signals and triggers activation of select virulence factors to facilitate evasion of innate immunity.

**Lab:** John Parkinson
ß-PHENYLETHYLAMINE AND ACETOACETIC ACID AS INHIBITORS OF BIOFILM

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Background: Bacterial biofilm exerts many impacts in clinical, natural, environmental, and bio-industrial settings. Biofilms are notoriously difficult to remove and diminish the effectiveness of commonly used antibiotics. In an attempt to identify inhibitors of biofilm, we had previously screened 95 carbon and 95 nitrogen sources for the effectiveness in reducing Escherichia coli O157:H7 cell counts and biofilm amounts, when added as a supplement to beef broth medium [Lynnes et al., Meat Science, 2014]. In this study, we identify additional bacterial pathogens that ß-phenylethylamine (PEA) and acetoacetic acid (AAA) are effective against.

Methods: Overnight cultures of the E. coli K-12 strain AJW678, E. coli O157:H7, E. coli O26, Salmonella enterica Typhimurium, Salmonella enterica Newport, Pseudomonas aeruginosa, Cronobacter sakazakii and Yersinia enterocolitica were inoculated into tryptic soy broth, supplemented with a range of concentrations of PEA or AAA and incubated at 37°C on 24 well polystyrene plates. Maximal growth rates were determined, as well as biofilm amounts after 16 h of growth. The inhibitory concentrations that were needed to achieve 50% reduction (IC50) were determined.

Results: The sensitivity of the bacterial strains towards the respective supplement varied across the strains (Figure to the right). The E. coli K-12 strain, S. typhimuriuma, and P. aeruginosa had the lowest IC50 values for treatment with PEA, indicating a good inhibitory effect of PEA. Intriguingly, the two pathogenic E. coli strains, as well as S. Newport had higher IC50 values for biofilm than for growth. C. sakazakii and Y. enterocolitica were treated with AAA. The high IC50 values indicate a lower effectiveness of the supplement.

Conclusion: Supplements, such as PEA or AAA can be used to reduce bacterial growth and biofilm amounts. The effectiveness of the treatment is dependent on the bacterial species and the environmental conditions.

Lab: Birgit M. Prüß
STRUCTURAL AND DYNAMIC STUDY OF THE RESPONSE REGULATOR CHEY3 FROM THE R. SPHAEROIDES CHEMOTAXIS NETWORK

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Background: The chemotaxis signalling network of *E. coli* depends on autophosphorylation of a histidine protein kinase (HPK) in response to a signal from a sensor domain, with subsequent transfer of the phosphoryl group to an aspartate on response regulator (RR) proteins that bind to the flagellar motor and alter its rotation. CheY is a simple 14kDa single domain RR that is conserved across motile species. It is formed by 5 α-helices and 5 β-strands surrounding a conserved phosphoryl accepting aspartate residue, and once phosphorylated diffuses to the flagellar motor, binding to its FliM component to cause switching of rotational direction. The photosynthetic bacterium *Rhodobacter sphaeroides* has multiple chemosensory pathways formed by homologues of the *E. coli* chemosensory proteins. It has six homologues of the response regulator CheY with different effects on chemotaxis. In this work we have used solution-state NMR methods to answer questions about the structure, dynamics and function of CheY3.

Methods: We have performed residual dipolar coupling (RDC) experiments to validate the available crystal structures of CheY3. We have performed a detailed study on how conditions such as pH and Mg$^{2+}$ concentration affect the structure and function of CheY3. We have assigned the backbone resonances of CheY3 using triple-resonance NMR methods and information about secondary structure has been obtained using TALOS-N. We have also investigated backbone dynamics of CheY3 using heteronuclear NOE experiments. Finally, we have used CPMG relaxation dispersion experiments to detect invisible, “active-like” conformations.

Results: Under conditions where CheY3 is inactive and at low pH we have detected one minor and one major protein conformation and upon pH increase these populations are inverted and finally only one species is present. We have observed that only at high pH is Mg$^{2+}$ required for binding of BeF$_3$, and the affinity for Mg$^{2+}$ is considerably higher than at low pH. Analysis of $^1$H, $^{13}$C and $^{15}$N chemical shifts using TALOS-N predicts a ‘typical’ CheY profile for high content in α and β secondary structure for all solution conditions. Two available X-ray structures for CheY3 give good agreement with experimental RDCs in most of the core α/β structure but do not reproduce the changes in RDCs observed between the inactive and active states in solution. The heteronuclear NOE experiment shows that there are no significant differences in fast timescale dynamics between the active and inactive forms of the protein. The CPMG relaxation dispersion experiments show that several residues, mainly located close to the phosphoryl binding site, undergo concerted motions under inactive conditions.

Conclusion: The available crystal structures do not represent the active and inactive conformations of CheY3. We have evidence for the presence of two binding sites for Mg$^{2+}$. The flexibility of the inactive and active conformations is very similar. The secondary structure present in the protein is in good agreement with the typical (α/β)$_5$ topology of all CheYs. We have evidence for the presence of an ‘invisible’ excited-state conformation for inactive CheY3.

Crystal Structure of CheY3 with residues involved in the intermediate state conformation displayed in red.

Lab: Christina Redfield
A NOVEL PAIR OF METHYL-ACCEPTING CHEMOTAXIS PROTEINS FOUND IN VIBRIO FISCHERI

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Background: Chemotaxis is an important and widely conserved mechanism by which microbes navigate their environment. The bioluminescent bacterium Vibrio fischeri is the monospecific symbiont of the Hawaiian bobtail squid, Euprymna scolopes. The bacteria are present as a planktonic population in the waters surrounding Hawaii, from which each generation of newly hatched squid isolate their symbiont. During colonization, the bacteria must navigate several complex environments to reach epithelium-lined crypts in the interior of the host’s light organ, where they multiply and provide light for the host. In chemotaxis, attraction/repulsion signaling is initiated by the binding of ligands to the periplasmic domain of methyl-accepting chemotaxis proteins (MCPs). These proteins are found in many different families of microbes, and the number of MCPs each species of bacteria carries is highly variable. V. fischeri encodes 42 MCPs in its genome, and many of the ligand-binding domains of these MCPs appear to be redundant. Previous work has shown that MCPs are able to sense a wide range of ligands, including metabolic intermediates, amino acids, and sugars.

Methods: The chemotactic cues sensed by V. fischeri have primarily been investigated using soft-agar swim plates and a capillary migration assay. Briefly, the capillary assay measures the number of bacterial cells that migrate up a chemical gradient containing a ligand of interest. The assay is performed by drawing a ligand solution into a glass capillary that is sealed at one end, then by exposing the open end to a suspension of actively motile cells. After exposure, the contents of the capillary are expelled and plated; the number of colonies arising provides a measure of the strength of attraction. By creating in-frame deletions of MCPs of interest in V. fischeri, the loss of attraction towards any known chemoattractant can be assessed by comparison to wild-type behavior. Genetic complementation of observed phenotypes was achieved by cloning the gene encoding the deleted MCPs of interest under constitutive expression within the MCP− mutant background, and showing that attraction to the ligand of interest was restored.

Results: Bioinformatic analysis of the ligand-binding domains of the V. fischeri MCP repertoire shows that there exists a high degree of apparent redundancy amongst these receptors. Two of these, termed VfcB (Vibrio fischeri chemotaxis protein B) and VfcB2, were found to be responsible for mediating attraction to several short- and mid-chain length fatty acids. While these MCPs were not found to be required in the early stages of symbiotic colonization, previous work suggests that they are likely involved in later stages.

Conclusions: V. fischeri contains a wide diversity of MCPs, and the ligands sensed by many of these are unknown. Here, I show initial characterization of a class of MCPs responsible for sensing fatty acids. This represents a novel set of these receptors that has not been previously characterized.
DETERMINING MOTILITY AND CHEMOTACTIC PROPERTIES OF THE TUMOR SEEKING STRAIN
SALMONELLA ENTERICA SEROVAR TYPHIMURIUM VNP20009

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Background: Strains of Salmonella Typhimurium have been used with high success as anticancer therapies in animal models because of their facultative anaerobic nature and ease of genetic modification. VNP20009 was engineered from the parental strain ATCC 14028S to have attenuation and selective colonization of tumors. The role of bacterial chemotaxis and motility in tumor approach and eradication remains unclear. Consequently, the goal of this project is to assess these bacterial traits using tumor targeting VNP20009 as a model. We first sequenced and de novo assembled the full genome. In addition to previously noted genomic changes, 50 non- synonymous SNPs were discovered differentiating it from the parental strain. Interestingly, one SNP occurred in the chemotaxis gene, cheY, which prevents phosphorylated CheY from interacting with the flagellar switch protein, FliM, rendering VNP20009 deficient in chemotaxis.

Methods: Restoration of the chemotaxis phenotype was employed by lambda-red genetic engineering. The VNP20009 cheY⁺ mutant was then assessed on swim plates, in comparison to the parental strain, 14028. In an effort to eliminate growth as a factor in testing, we implemented traditional Adler capillary assays to test the chemotactic sensitivity of the cheY⁺ mutant to the attractant aspartate compared to 14028.

Results: On swim plate the VNP20009 cheY⁺ mutant displayed a swim ring of 45% compared to 14028. After capillary assay testing, it was discerned that the number of VNP20009 cheY⁺ cells which entered the capillary was almost 70% of the parental strain.

Conclusions: Upon restoring this cheY mutation found in VNP20009, the chemotaxis assay performance remained partially impaired in relation to the parental strain. Possible explanations, and current focus of work, for this include differences in swimming speed and sensitivity to attractant concentrations. In future studies, the role of motility and chemotaxis will be deciphered in vivo using a mouse model, in which derivatives of VNP20009 with the genotypes of cheY⁺, cheY⁻, fla⁻ and mot⁻ will be assessed on their rate of tumor colonization.

Lab: Birgit Scharf
BOTH ROTATING FLAGELLA AND LIPOPOLYSACCHARIDES ARE REQUIRED FOR INFECTION OF AGROBACTERIUM SP. H13-3 BY THE FLAGELLOTROPIC BACTERIOPHAGE 7-7-1

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Background: The host range of bacteriophage 7-7-1 is exclusive to Agrobacterium sp. H13-3. Bacteriophage 7-7-1 attaches to the rotating flagellum of Agrobacterium sp. H13-3 and is hypothesized to make its way down the flagellum via a nut and bolt mechanism. Once at the flagellar base, phage 7-7-1 interacts with cell surface receptors and injects its DNA into the bacterial cell. However, cell surface receptors enabling injection of viral DNA have not been recognized. In this study, we identified motile, phage resistant Agrobacterium sp. H13-3 mutants and investigated genetic variations within their genomes underlying resistance. Bioinformatics analysis of these variations identified one cell surface receptor and one inner membrane protein that allow infection by phage 7-7-1.

Methods: Transposon mutagenesis was used to enable transposon insertion into Agrobacterium sp. H13-3 DNA. Phage-resistant mutants were then selected via patching of 1000 colonies on two sets of Bromfield swim plates, one set with phage and the other without. Screening of the 1000 colonies yielded 17 motile, phage-resistant mutants. Identification of transposon insertion sites within these mutants was achieved via arbitrary polymerase chain reaction to obtain flanking sequences from the transposon insertion sites.

Results: Bioinformatics analysis of these data revealed 8 mutants with transposon insertions in 6 distinct sites of AGROH133_07737, coding for a glycosyl transferase 25 family member, which catalyzes the transfer of various sugars onto the growing lipopolysaccharide chain during its biosynthesis. Nine mutants with transposon insertions in 5 distinct sites were found in AGROH133_08824, which encodes an integral, 4-transmembrane protein, predicted to be located in the inner membrane.

Conclusion: With these data we propose that after attachment to and propulsion down the Agrobacterium sp. H13-3 flagellum, phage 7-7-1 binds to and degrades the lipopolysaccharide, punctures through the outer membrane, and then associates with the protein in the inner membrane, which initiates release of DNA into the cytoplasm.

Lab: Birgit Scharf
ROLE OF PHOSPHORYLATION FEEDBACK IN *E. coli* CHEMOTAXIS PROBED BY SINGLE-CELL FRET

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**Background:** Bacterial chemotaxis has served as a paradigm for studies of intracellular signal transduction. The pathway is remarkably robust against cell-to-cell variations in protein abundance, arising from stochastic gene expression [1,2].

*E. coli* motility consists of a random walk, which the chemotaxis pathway can bias to navigate chemical gradients. The network activity is modulated by temporal comparisons between the rapid excitation response to ligand by the receptor-kinase complex, and the slower adaptation reactions mediated by two receptor-modification enzymes. The latter achieves precise adaptation by negative integral feedback, implemented via the dependence of their catalytic activities on the conformational state of the substrate receptors [1]. Interestingly, one of these two enzymes, CheB, is phosphorylated by the receptor-associated kinase, which in turn enhances its catalytic activity. Effectively, this adds an extra negative feedback loop to the adaptation module (see figure).

This additional feedback loop is a feature conserved across bacterial species, yet its functional significance is unclear. It has been shown that precise adaptation is achieved even in mutant strains defective in CheB phosphorylation [2], raising the question why the system has evolved this additional layer of phosphorylation-mediated feedback. It has been hypothesized that this additional feedback loop might play a role in reducing cell-to-cell variability in the pathway [2].

**Methods:** Förster Resonance Energy transfer (FRET) microscopy enables *in vivo* measurement of the network activity [3]. It has been used extensively to study the chemotaxis system mainly in ensemble-averaged measurements involving hundreds of cells. FRET measurement of chemotactic signaling has been previously achieved at the single-cell level [4], but not for determining parameters of dynamical models of pathway activity.

**Results:** We established a new protocol for FRET experiments and data analysis suited to measuring network activity over extended times in single cells, and investigated how pathway design affects cell-to-cell variability in signaling, using mutant strains with altered network topologies. We changed the network topology by measuring cells expressing CheB mutants defective in phosphorylation feedback. These data indicate that the variation in kinase activity (std/mean) is higher for cells expressing these CheB mutants, indicating that the additional layer of feedback involving CheB phosphorylation enables cells to reduce cell-to-cell variability.

**Conclusion:** Our single cell FRET data provides experimental evidence for the role of CheB phosphorylation feedback in bacterial chemotaxis, namely in attenuating cell-to-cell variability in the steady-state kinase activity.

**References**


**Lab:** Thomas Shimizu
LIGAND-DEPENDENT INVERSION OF THE THERMOTACTIC RESPONSE IN *ESCHERICHIA COLI*

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**Background:** An efficient way for bacteria to navigate towards a favorable gradient of nutrients and a physiological optimum of temperature is flagella-mediated motility. The classical unidirectional mode of bacterial chemotaxis relies on temporal comparison of physiological relevant chemical ligand concentrations. Although chemotaxis and thermotaxis in *Escherichia coli* are mediated by the same pathway, the interplay of these two responses is not well understood. It is also not known whether and why cells accumulate at a preferred temperature.

**Methods:** Here we apply an in-vivo FRET assay and microfluidics to monitor pathway activity and to analyze dependence of the thermotactic response on ambient temperature as well as on ligand stimulation. Biochemical analyses were performed to monitor receptor modification states.

**Results:** We show that in absence of chemoattractants when cells adapt to a higher temperature range the thermophilic (warm-seeking) response decreases, while the adaptive methylation of chemoreceptors increases. We further investigated the influence of chemoattractants on the thermotactic response and found that it reduces or inverts the response to be cryophilic (cold-seeking) in a dose-dependent manner. Interestingly, when cells were adapted to an intermediate concentration of ligands, we observed an inverted bidirectional thermotactic response, resulting in an accumulation temperature. Additionally, by changing the Tar to Tsr receptor ratio the accumulation temperature was shifted.

**Conclusions:** A model is proposed where the response to thermotactic and chemotactic stimuli is coordinated by receptor methylation of the two major chemoreceptors, Tar and Tsr, in an additive fashion. We further demonstrate that the interplay of Tar and Tsr enables bacteria to accumulate at a preferred temperature.

**Lab:** Victor Sourjik
HYDROGEN EXCHANGE DIFFERENCES BETWEEN CHEMORECEPTOR SIGNALING COMPLEXES LOCALIZE TO FUNCTIONALLY IMPORTANT SUBDOMAINS

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Background: The transmembrane signaling mechanism of bacterial chemotaxis receptors begins with a subtle ligand-induced piston of an alpha helix in the periplasmic and transmembrane domains, but it is unclear how the signal is then propagated through the cytoplasmic domain to control the activity of the associated kinase CheA. It is difficult to test recent proposals that signaling in the cytoplasmic domain involves opposing changes in dynamics in different subdomains, because the functional system consists of extended arrays of receptor complexes with two other proteins, CheA and CheW.

Methods: We have developed an approach to measure structure and dynamics of the receptor cytoplasmic domain within functional native-like arrays, by combining hydrogen exchange mass spectrometry with assembly of functional complexes of the receptor cytoplasmic fragment (CF) with CheA and CheW on vesicles, mediated by binding of His-tagged CF to nickel-chelating lipids.

Results: Comparison of CF$_{4E}$ complexes assembled at high and low density reveals signaling-associated changes in hydrogen exchange that localize to functionally important subdomains. In a kinase-on state, the methylation subdomain (top, orange) exhibits complex changes that include slower hydrogen exchange, and the signaling subdomain (bottom, green) exhibits significant protection from hydrogen exchange.

Conclusions: The increased protection in the signaling subdomain suggests a tighter and/or larger interaction interface with CheA and CheW in the kinase-on state. To resolve the complexity of the observed changes in the methylation subdomain, experiments are in progress to compare hydrogen exchange of kinase-on and kinase-off complexes assembled at the same density. These measurements of the stability of protein subdomains within functional signaling complexes demonstrate the promise of this approach for measuring functionally important protein dynamics within the various physiologically relevant states of multi-protein complexes.

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Lab: Lynmarie Thompson
REGULATION OF FLAGELLAR NUMBER IN DUAL FLAGELLAR SYSTEMS

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Background: Flagella are organelles of locomotion, which differ in number and arrangement in different bacterial species. Together with the signal recognition particle (SRP) -GTPase FlhF, the ATPase FlhG seems to regulate flagellation patterns in polarly as well as in peritrichously flagellated bacteria. Flagella number drastically increases when FlhG is depleted. However, the molecular mechanisms how FlhG establishes these patterns are widely unknown. Shewanella putrefaciens CN-32 possesses two distinct flagellar systems which are encoded in two different gene clusters. flhG is only present in the cluster for the polar flagellar system, but is absent in the lateral flagellar gene cluster.

Methods: Using biochemical approaches in concert with physiological and fluorescent microscopy approaches on various mutants we explored the role of FlhG in the dual flagellar system of S. putrefaciens.

Results: We demonstrated that, in S. putrefaciens, FlhG exclusively regulates the expression and number of the polar flagellar system and shows apparently no effect on expression and assembly of the 1 – 2 lateral flagella. Furthermore, we found evidence for interaction of FlhG with a complex of the polar flagellar basal body proteins FliM\textsubscript{1} and FliN\textsubscript{1}, while this interaction is absent with respective proteins of the secondary flagellar system. Deletion of FliM\textsubscript{1} or the N-terminal part of FliM\textsubscript{1} phenocopies a \(\Delta\text{flhG}\) deletion mutant.

Conclusion: Mechanistically, FlhG appears to interact with FliM/FliN by interaction with the N-terminal EIDAL motif of FliM. This domain is also known to interact with the chemotaxis response regulator CheY. Since FliM\textsubscript{2} of the secondary flagellar system lacks this motif, discrimination between the two flagellar systems is likely due to FlhG–FliM interaction.

These findings enable new insights into the control of flagellar number and assembly and show the specificity of regulatory elements in dual flagellar systems.

Lab: Kai Thormann
DECRYPTING KEY ELEMENTS IN THE Aer2 PAS SIGNALING PATHWAY

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Background: Pseudomonas aeruginosa is a common environmental organism and a significant cause of human disease. Its ability to survive in complex environments is aided by four chemosensory systems that sense environmental conditions and modify bacterial behavior. One of the chemosensory systems, Che2, contains a single receptor named Aer2, which contains a heme-binding PAS domain that detects diatomic gases like O₂ and CO. Crystal structures of the Aer2 PAS domain in ligand bound (Fe³⁺-CN, a proxy for O₂), and non-ligand bound (Fe³⁺) states have revealed a unique residue, W283, that may stabilize O₂ binding and residue L264 that may initiate conformational signaling (see Figure below). We hypothesize that amino acid substitutions in these and other conserved PAS residues will alter ligand binding and/or conformational signaling.

Methods: Aer2 PAS residues were mutated by site-specific random or alanine mutagenesis. Signal on/off phenotypes were determined by hijacking the E. coli chemotaxis system in an otherwise receptor-less E. coli strain and observing swimming behavior with different gases. PAS peptides were created for Aer2 mutants that had altered phenotypes, and then purified on Ni-NTA agarose columns. O₂ and CO affinities were determined by titrating air- or CO-saturated buffer into deoxy protein.

Results: Abnormal signaling behavior was observed for bacteria expressing Aer2 receptors with 15 different substitutions at W283, 13 different substitutions at L264, and for 12 of 14 alanine replacements. Most of the Aer2 mutants with altered behavior were signal-off in both the presence and absence of oxy-gas. As expected, replacements L264V and L264I, which are found in other Aer2-like PAS domains, had minimal effects on Aer2 function. Our preliminary data suggests that some W283 mutants, e.g., W283L, cannot stably bind O₂ but do bind CO. O₂ titrations with those mutants results in rapidly oxidized protein. Unexpectedly, some W283 mutants appear to have heme-binding defects. In contrast, L264 mutants can bind both O₂ and CO with varying affinities.

Conclusions: We have found that conserved Aer2 PAS residues are crucial for PAS sensing and signaling. L264 is necessary for normal O₂ signaling, but as yet, not for O₂ binding. In contrast, W283 is necessary for O₂-binding and for PAS signaling. Once complete, these analyses will help clarify the mechanisms used by Aer2-PAS domains to regulate ligand binding and initiate conformational signaling.

Lab: Kylie Watts
HOLOGRAPHIC MICROSCOPY FOR CELL TRACKING AND ANALYSIS

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\textbf{Background:} Tracking bacteria as they swim in three dimensions is a challenge in experimental biophysics. Bacterial tracks show how cells respond to each other, and to environmental stimuli such as chemical or thermal gradients. Since Berg’s pioneering experiments in the early 1970s\textsuperscript{1}, few techniques to study this behavior in three dimensions have emerged. Most operate at low temporal and spatial resolution, or (like Berg’s original experiments) require specialist apparatus.

\textbf{Methods:} We use high-speed holographic microscopy to track multiple cells in three dimensions. Our technique is designed to give information on the location and orientation of cells\textsuperscript{2}, and to investigate cell-cell interactions in planktonic samples. The method is computationally inexpensive: processing was performed on a desktop computer.

\textbf{Results:} Tracks have been observed extending over hundreds of microns (though the track in the figure below is on the order of 100 µm in length), with time resolution limited by the camera frame rate. Temporal resolution down to the millisecond level or below is easily achievable, but good results are attainable at standard frame rates (24-30 frames per second). The volume that can be imaged is set by the magnification and image sensor size in the lateral direction, and by the illumination source in the axial direction. LED illumination gives a depth range of ±150µm from the focal plane, while laser illumination can extend this to almost a millimeter\textsuperscript{3}. Position resolution is set by the microscope optics, but is usually comparable to diffraction-limited particle tracking. The equipment required is largely non-specialist, requiring an upright or inverted microscope, a standard CMOS or CCD camera and a monochromatic light source.

\textbf{Conclusion:} We have shown that holographic microscopy can be used to track bacterial cells in three dimensions, and with standard microscopy equipment. The next stages will be to look at cell-cell interactions, and to compare the swimming patterns of various strains.

\begin{center}
\textbf{Top left:} Raw holographic data from a chain of Streptococcus cells; the image measures 30 µm x 30 µm. \textbf{Bottom left:} Holographic reconstruction of the Streptococcus cells. \textbf{Right:} Three-dimensional track of an E. coli cell. Sections interpreted as run events are discretely coloured, and the squares on the ground measure 10 µm on a side.
\end{center}

3) C.B. Giuliano \textit{et al.}, \textit{J. Vis. Exp.} 84 e50488(2014)

\textbf{Lab: Laurence Wilson}
Ne-LYSINE ACETYLATION CAN REDUCE CRP AR2-DEPENDENT TRANSCRIPTION IN RESPONSE TO OVERFLOW CARBON METABOLISM

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Background: Bacteria use a variety of methods to sense and respond to changes in the environment. Post-translational modifications (PTMs) are one such method that can alter the function of a modified protein. In the presence of excess carbon, Escherichia coli cells metabolize much of that carbon into acetylphosphate (acP), which is the primary acetyl group donor for non-enzymatic Ne-lysine protein acetylation. Based on quantitative mass spectrometry data, one target of acP-dependent acetylation is K100 of cAMP Receptor Protein (CRP), the major transcription regulator of carbon flux. K100 lies adjacent to Activating Region 2 (AR2), a charged surface on CRP that interacts with RNA polymerase to promote transcription at certain CRP-dependent promoters. Previous work by others had shown that mutation of K100 to alanine reduced AR2-dependent transcription, suggesting that K100 may play a role in AR2-dependent transcription. We hypothesized that K100 acetylation, which would neutralize K100’s positive charge, could exert the same inhibitory effect as the alanine mutation.

Methods: To investigate the role of K100 and its acetylation on AR2-dependent transcription, we made use of point mutations commonly used to mimic lysine acetylation in histones. We overexpressed CRP variants [WT, K100A, K100Q (which mimics acetylated lysine), or K100R (which mimics unacetylated lysine)] and used a β-galactosidase assay to monitor transcription from the semi-synthetic AR2-dependent CC(-41.5) promoter fused to lacZ. We also measured the growth of these strains in minimal media supplemented with galactose, a carbon source whose import and metabolism almost exclusively require AR2-dependent transcription.

Results: We found that the K100R strain had CC(-41.5) activity equivalent to the WT CRP strain. In contrast, the K100A and K100Q strains had equivalent CC(-41.5) activity that was lower than the WT CRP strain. All four strains had identical activity at an AR2-independent promoter. When grown in galactose as the sole carbon source, the K100R strain displayed a moderate increase in time spent in lag phase compared to the WT CRP strain, while the K100A and K100Q strains displayed a drastic increase in lag phase. These defects were enhanced in the presence of a mutation that increases accumulation of acP.

Conclusions: We confirm that K100 plays a role at AR2-dependent promoters, likely due to its positive charge. The masking of this positive charge by acetylation could reduce transcription from these promoters, and an appropriate balance between the acetylated and unacetylated state may be required to optimally initiate transcription from galactose-related promoters. This could be one mechanism by which cells sense and respond to excess carbon.
SILENCING OF THE GAD CLUSTER GENES BY H-NS IN ESCHERICHIA COLI

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Background: Genes for the glutamic acid-dependent (GAD) acid resistance system in E. coli consist of at least 15 genes that form a large cluster (~13 kbp) on the E. coli genome. Under normal growth conditions, expression of all genes on the GAD cluster is silenced by H-NS, a nucleoid associated protein. Our aim is to understand the molecular mechanism of GAD-silencing and relief of GAD-silencing by H-NS.

Methods: We constructed a set of H-NS mutants with single Cys substitution. The in vivo expression levels of GAD genes were measured using a reporter assay system. In parallel, a combination of single-DNA manipulation with magnetic tweezers and atomic force microscopy (AFM) was employed to detect interactions in vitro between H-NS and single-DNA molecules.

Results: Using a whole set of single Cys-substitution mutants of H-NS (AA residue 2 to 137), we identified H-NS mutants that failed to silence the GAD genes in vivo. AFM and magnetic tweezers analyses in vitro indicated the formation of abnormal DNA complexes with these H-NS mutants.

Conclusions: The linker domain of H-NS is required for silencing of the GAD cluster genes. H-NS mutants containing a single amino acid substitution in the linker domain did not form DNA-H-NS stiffening structures. H-NS forms both oligomer and stiffening structures on the GAD cluster, leading to gene silencing of ~15 genes within the GAD cluster.
GENOME-WIDE IDENTIFICATION OF MUTANTS WITH ENHANCED SWARMING MOTILITY IN RHIZOBIUM LEGUMINOSARUM USING TRANSPOSON INSERTION SEQUENCING

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Background. *Rhizobium leguminosarum* biovar *viciae* is an agriculturally important bacteria that nodulates plant species belonging to the genera *Pisum*, *Vicia*, *Lathyrus*, and *Lens*. Vegetative cells of *R. leguminosarum* bv. *viciae* strain 3841 (Rlv3841) are propelled by one or two subpolar flagella. Under optimized conditions, Rlv3841 differentiates into a swarmer cell that exhibits increased expression of flagellar genes and a subsequent increase in flagellation. Swarming cells move outwards from the point of inoculation, forming short dendrite patterns on an agar surface. The center of the swarming colony is composed of vegetative cells while the dendrite arms contain swarmer cells. Little is known about the genetic control of swarming motility in rhizobia, thus genome-wide transposon mutagenesis followed by transposon junction sequencing was used to identify genes that interfere with swarming in Rlv3841.

Methods. A transposon mutant library was constructed using an MmeI-adapted mariner transposon, which inserts specifically at ‘TA’ dinucleotide motifs. Pools of mutants were inoculated on a swarming medium and cultures were incubated for 3 weeks. Vegetative cells and swarmer cells were collected from the center and the edge of the swarming colonies, respectively. Transposon genome junctions were sequenced using an Ion Torrent PGM. The Hidden Markov Model was used to identify mutations that result in 4 different states: growth defective, growth advantage, neutral, and essential. Designation of the genes to the different states is based on the relative decrease or increase in the number of reads that were mapped to the Rlv3841 genome. We focused on mutations that conferred a growth advantage under swarming conditions.

Results. Approximately 78,000 mutants were generated from the transposon mutagenesis experiment. *In silico* analysis of the Rlv3841 genome identified 140,056 TA insertion sites. A 36% transposon insertion density was obtained for the vegetative cells. Housekeeping genes and flagellar genes that are expected to be involved in motility were identified by the HMM as essential genes for swarming. Mutations that resulted in the enhancement of swarming were identified based on the abundance of sequencing reads. Six chromosomal genes and 6 plasmid-encoded genes exhibited reads that are higher than the average read counts. We identified a locus belonging to the MarR family of transcriptional regulator that binds DNA through a helix-turn-helix motif. MarR has been demonstrated to acts as a repressor of the multiple antibiotic resistance operon in *Escherichia coli* but the *R. leguminosarum* locus has not been characterized to date. Additional genes that were identified code for a putative helix-turn-helix transcriptional activator, a methyl-accepting chemotaxis protein, a transmembrane efflux protein, peptidases, dehydrogenases, and a cellulose synthesis enzyme. Future experiments will focus on characterization of these genes.

Conclusion. Genome-wide mutagenesis using a mariner transposon coupled with massive parallel sequencing of vegetative and swarming cells can identify genes that influence bacterial swarming behavior.

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COORDINATION OF MULTIPLE OUTPUTS IN BACTERIAL CHEMOTAXIS

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Background: In the bacterial chemotaxis signaling network, receptor clusters process input, and flagellar motors generate output. Coordination of these multiple outputs on a cell can be induced by two possible mechanisms: one being the stochastic fluctuation of the intracellular level of the chemotaxis signaling protein CheY-P, the other being extracellular mechanical coupling among bundled flagella.

The motor coordination due to intracellular [CheY-P] (CheY-P concentration) fluctuation has been explored experimentally, by measuring correlation between two motors on a cell with truncated flagellar filaments (thus no coordination due to mechanical coupling among bundled flagella), but the possible mechanisms were still unclear. The magnitude of motor coordination due to mechanical coupling among bundled flagella has not been studied, neither experimentally nor theoretically.

Methods: In the current study, we utilize E. coli strains exhibiting different magnitudes of [CheY-P] fluctuation. We measured their motor behavior by single-motor assays and swimming behavior by 3-d tracking.

Results: We measured both single-motor and swimming behaviors for E. coli strains with various levels of [CheY-P] fluctuation, and compared the experimental results with model simulations.

Conclusion: We experimentally study the behavioral consequence of flagella coordination, and also dissect the relative contributions of these two mechanisms of flagella coordination by correlating motor and swimming behaviors.

![Fig 1. Two possible mechanisms of flagella coordination: [CheY-P] fluctuation and mechanical coupling of bundled flagella](image-url)
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