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RPPR Final Report
as of 27-Jan-2023

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Proposal Number: 73190BB **Agreement Number: W911NF-18-1-0254**
INVESTIGATOR(S):

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Organization: **University of California - Los Angeles**
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Final Report for Period Beginning 05-Jul-2018 and Ending 30-Jun-2021
Title: Emerging roles of the exopolysaccharide matrix and surface sensing in bacterial communication and ecology
Begin Performance Period: 05-Jul-2018 **End Performance Period:** 30-Jun-2021
Report Term: 0-Other
Submitted By: PhD Gerard Wong Email: gclwong@seas.ucla.edu
Phone: (310) 794-7684
Distribution Statement: 1-Approved for public release; distribution is unlimited.

STEM Degrees: 1 **STEM Participants:** 3

Major Goals: (1) To understand how the existence of a surface can drive the formation of heterogeneous populations of P. aeruginosa through surface sensing.
(2) To understand the role of surface sensing controlled EPS secretion during reversible attachment, the first stage of bacterial engagement with a surface, and quantitatively compare P. aeruginosa strains PA14 and PAO1
(3) To assess whether we can guide biofilm forming behavior of Shewanella oneidensis, a strain capable of extracellular electron conduction that has been proposed for devices
(4) Determine the breadth of EPS cross-linking activities for the P. aeruginosa biofilm matrix protein, CdrA.
(5) To understand how specific molecular details of EPS can influence sensing, cdiGMP signaling, and social behavior in P. aeruginosa

Accomplishments: Findings:
(1)
Major activity: Heterogeneity in surface sensing and EPS production suggest a division of labor in Pseudomonas aeruginosa populations

Specific objectives: To understand how the existence of a surface can drive the formation of heterogeneous populations of P. aeruginosa through surface sensing.

Significant results: The second messenger signaling molecule cyclic diguanylate monophosphate (c-di-GMP) drives the transition from planktonic to biofilm growth in many bacterial species. Pseudomonas aeruginosa has two surface sensing systems that produce c-di-GMP in response to surface adherence. The current thinking in the field is that once cells attach to a surface, they uniformly respond with elevated c-di-GMP. Here, we describe how the Wsp system generates heterogeneity in surface sensing, resulting in two physiologically distinct subpopulations of cells. One subpopulation has elevated c-di-GMP and produces biofilm EPS matrix, serving as the founders of initial microcolonies. The other subpopulation has low c-di-GMP and engages in surface motility, allowing for exploration of the surface. We also show that this heterogeneity strongly correlates to surface behavior for descendent cells. Together, our results suggest that after surface attachment, P. aeruginosa engages in a division of labor that persists across generations, accelerating early biofilm formation and surface exploration.
Key outcomes: This work was published in eLife in 2019.

(2)

Major activity: Parsing social cooperativity during surface attachment in young Pseudomonas aeruginosa biofilms

Specific objectives: To understand the role of surface sensing controlled EPS secretion during reversible attachment, the first stage of bacterial engagement with a surface, we performed a quantitative comparison between P. aeruginosa PA14 and PAO1.

Significant results: What are bacteria doing during “reversible attachment,” the period of transient surface attachment when they initially engage a surface, besides attaching themselves to the surface? Can an attaching cell help any other cell attach? If so, does it help all cells or employ a more selective strategy to help either nearby cells (spatial neighbors) or help its progeny (temporal neighbors)? Using community tracking methods at single-cell resolution, we suggest answers to these questions based on how reversible attachment progresses during surface sensing for Pseudomonas aeruginosa strains PAO1 and PA14, which have drastically different surface sensing circuits and EPS production profiles. Although PAO1 and PA14 exhibit similar trends of surface cell population increase, they show unanticipated differences when cells are considered at the lineage level and interpreted using the quantitative framework of an exactly solvable stochastic model. Reversible attachment comprises two regimes of behavior, processive and nonprocessive, corresponding to whether cells of the lineage stay on the surface long enough to divide or not before detaching. Stark differences between PAO1 and PA14 in the processive regime of reversible attachment suggest the existence of two surface colonization strategies, which are roughly analogous to “immediate-” vs “deferred-gratification” in a prototypical cognitive-affective processing system made possible by different uses of EPS: PAO1 lineages commit quickly to a surface compared to PA14 lineages, with early c-di-GMP mediated EPS production that can facilitate attachment of neighbors. PA14 lineages modulate their motility via cAMP, and retain memory of the surface so that their progeny are primed for improved subsequent surface attachment. Based on previous studies, we propose that the differences between PAO1 and PA14 are potentially rooted in downstream differences between Wsp-based and Pil-Chp-based surface sensing systems, respectively.
Key outcomes: This work was published in mBio in 2020. (We note that predictions we had made in this paper regarding the complex ecological competition landscape between strains of P. aeruginosa that use EPS differently has recently been confirmed by Kassety et al, J. Bact. (2021))

(3)

Major activity: Initiate prototypical studies using artificially patterned glycopolymers to mimic EPS

Specific objectives: To assess whether we can guide biofilm forming behavior of Shewanella oneidensis.

Significant results: Using glycopolymer decorated surfaces, we have stimulated Shewanella oneidensis bacterial colonization and controlled bacterial attachment via artificial molecular patterns. When adherent bacteria were rinsed with methyl α-d-mannopyranoside, the glycopolymer-functionalized surfaces retained more cells than self-assembled monolayers terminated by a single mannose unit. These results suggest that the three-dimensional multivalency of the glycopolymers both promotes and retains bacterial attachment. When the methyl α-d-mannopyranoside competitor was codeposited with the cell culture, however, the mannose-based polymer was not significantly different from bare gold surfaces. The necessity for equilibration between methyl α-d-mannopyranoside and the cell culture to remove the enhancement suggests that the retention of cells on glycopolymer surfaces is kinetically controlled and is not a thermodynamic result of the cluster glycoside effect. The MshA lectin appears to facilitate the improved adhesion observed. Our findings that the surfaces studied here can induce stable initial attachment and influence the ratio of bacterial strains on the surface may be applied to harness useful microbial communities.
Key outcomes: This work was published in ACS Appl Mater Interfaces in 2020.

(4)
Major activity: Identifying CdrA as a versatile biofilm EPS matrix cross-linking protein that mediates interactions with multiple EPS types in Pseudomonas aeruginosa.
Specific Objectives: Determine the breadth of EPS cross-linking activities for the P. aeruginosa biofilm matrix protein, CdrA.
Significant Results: Depending upon the strain, Pseudomonas aeruginosa can use different exopolysaccharides (e. g., Psl, Pel, and alginate) to build its biofilm matrix. Previously, we demonstrated that the biofilm matrix protein CdrA binds to Psl, promoting biofilm formation and aggregate stability. As such, it was thought that CdrA might be important for biofilm assembly only in strains that rely upon Psl. However, past studies indicated that CdrA can interact with monosaccharides not present in Psl, including N-acetylglucosamine, a constituent of another EPS called Pel. We discovered that CdrA also binds to Pel and promotes biofilm formation by strains in which Psl is not dominant. Thus, our findings suggest that CdrA plays a common role as a biofilm matrix cross-linker across P.

aeruginosa isolates with different EPS types. This finding has significant implications for CdrA contributing to biofilm assembly by multiple species and additionally raises the key question as to whether it is present in Psl trails. Key Outcomes: This work was published in the Journal of Bacteriology in 2020 (Spotlight article).

(5)
Major activity: Demonstration that specific molecular motifs in artificial EPS can differentially drive surface sensing and c-di-GMP signaling in Pseudomonas aeruginosa

Specific objectives: To understand how specific molecular details of EPS can influence sensing, cdiGMP signaling, and social behavior in P. aeruginosa.

Significant Results: We designed artificial EPS consisting of glycomimetic polymers that mimic the known composition of Psl and can be “printed” on surfaces as defined spatial patterns of artificial EPS. In this work, we are able to compare artificial trails with naturally secreted trails, and assessed how P. aeruginosa can differentiate between EPS trails of different composition. Details are in the attachment.

Training Opportunities: Training opportunities were provided for the following graduate students.

Calvin Lee
Jaime de Anda
William Schmidt.

Calvin Lee has since graduated with his PhD and is now a postdoc.

Results Dissemination: 1. Catherine R. Armbruster*, Calvin K. Lee*, Jessica Parker-Gilham, Jaime De Anda, Aiguo Xia, Boo Shan Tseng, Lucas R. Hoffman, Fan Jin, Caroline Harwood, Gerard C. L. Wong, Matthew R. Parsek, “Heterogeneity in surface sensing suggests a division of labor in Pseudomonas aeruginosa populations”, eLife 8, e45084 (2019). (*co-1st authors). doi: 10.7554/eLife.45084. PMID: 31180327.

2. Calvin K. Lee*, Jérémy Vachier*, Jaime de Anda, Kun Zhao, Amy E. Baker, Rachel R. Bennett, Catherine R. Armbruster, Kimberley A. Lewis, Rebecca L. Tarnopol, Charles J. Lomba, Deborah A. Hogan, Matthew R. Parsek, George A. O’Toole, Ramin Golestanian, and Gerard C. L. Wong, “Social Cooperativity of Bacteria during Reversible Surface Attachment in Young Biofilms: a Quantitative Comparison of Pseudomonas aeruginosa PA14 and PAO1” (*co-1st authors). mBio 11, e02644-19 (2019). doi: 10.1128/mBio.02644-19. PMID: 32098815.

3. Calvin K. Lee, Alexander J. Kim, Giancarlo S. Santos, Peter Y. Lai, Stella Y. Lee, David F. Qiao, Jaime De Anda, Thomas D. Young, Yujie Chen, Annette R. Rowe, Kenneth H. Nealson, Paul S. Weiss, and G. C. L. Wong, “Evolution of Cell Size Homeostasis and Growth Rate Diversity during Initial Surface Colonization of Shewanella oneidensis”, ACS Nano 10 9183-9192 (2016). (See Perspectives article in same issue) PMID: 27571459.

4. Courtney Reichhardt, Holly M Jacobs, Michael Matwichuk, Cynthis Wong, Daniel J Wozniak, Matthew R Parsek, “The Versatile Pseudomonas aeruginosa Biofilm Matrix Protein CdrA Promotes Aggregation through Different Extracellular Exopolysaccharide Interactions”, J. Bacteriology 202(19):e00216-20 (2020). doi: 10.1128/JB.00216-20. PMID: 32661078. PMCID: PMC7484184. DOI: 10.1128/JB.00216-20

See attachment for description of final project.

Honors and Awards: Gerard C. L. Wong gave the Goll Memorial Lecture at Northwestern University on the intersection between microbiology and materials science.

Protocol Activity Status:

Technology Transfer: Nothing to Report

PARTICIPANTS:

Participant Type: PD/PI
Participant: Gerard C. L. Wong

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Person Months Worked: 1.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Co PD/PI
Participant: Matthew Parsek
Person Months Worked: 1.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Graduate Student (research assistant)
Participant: Calvin K Lee
Person Months Worked: 12.00 **Funding Support:**
Project Contribution:
National Academy Member: N

International Travel:

CHN	8 days
SGP	8 days
BRA	4 days
ITA	5 days
ISR	5 days
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International Collaboration:

DEU
DEU
JPN
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Article Title: Social Cooperativity of Bacteria during Reversible SurfaceAttachment in Young Biofilms: a Quantitative Comparison ofPseudomonas aeruginosaPA14 and PAO1
Authors: Calvin K. Lee, Jér&eacute&#
Keywords: Pseudomonas aeruginosa, bacterial biofilms, reversible attachment,stochastic model, surface sensing
Abstract: What are bacteria doing during “reversible attachment,” the period oftransient surface attachment when they initially engage a surface, besides attachingthemselves to the surface? Can an attaching cell help any other cell attach? If so,does it help all cells or employ a more selective strategy to help either nearby cells(spatial neighbors) or its progeny (temporal neighbors)? Using community trackingmethods at the single-cell resolution, we suggest answers to these questions basedon how reversible attachment progresses during surface sensing forPseudomonasaeruginosastrains PAO1 and PA14. Although PAO1 and PA14 exhibit similar trendsof surface cell population increase, they show unanticipated differences when cellsare considered at the lineage level and interpreted using the quantitative frameworkof an exactly solvable stochastic model. Reversible attachment comprises two re-gimes of behavior, processive and nonprocessive, corresponding to whether cells ofthe lineage stay on the surface
Distribution Statement: 2-Distribution Limited to U.S. Government agencies only; report contains proprietary info
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Article Title: Selective Promotion of Adhesion of Shewanella oneidensis on Mannose-Decorated Glycopolymer Surfaces
Authors: Thomas D. Young, Walter T. Liau, Calvin K. Lee, Michael Mellody, Gerard C. L. Wong, Andrea M. Kask
Keywords: Shewanella glycopolymer adhesion biofilm mannose electrode cell density patterning
Abstract: Using glycopolymer surfaces, we have stimulated Shewanella oneidensis bacterial colonization and induced where the bacteria attach on a molecular pattern. When adherent bacteria were rinsed with methyl ?-d-mannopyranoside, the glycopolymer-functionalized surfaces retained more cells than self-assembled monolayers terminated by a single mannose unit. These results suggest that the three-dimensional multivalency of the glycopolymers both promotes and retains bacterial attachment. When the methyl ?-d-mannopyranoside competitor was codeposited with the cell culture, however, the mannose-based polymer was not significantly different from bare gold surfaces. The necessity for equilibration between methyl ?-d-mannopyranoside and the cell culture to remove the enhancement suggests that the retention of cells on glycopolymer surfaces is kinetically controlled and is not a thermodynamic result of the cluster glycoside effect. The MshA lectin appears to facilitate the improved adhesion observed.
Distribution Statement: 2-Distribution Limited to U.S. Government agencies only; report contains proprietary info
Acknowledged Federal Support: **Y**

RPPR Final Report
as of 27-Jan-2023

Partners

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I certify that the information in the report is complete and accurate:
Signature: Gerard Chee Lai Wong
Signature Date: 1/24/23 8:34PM

Emerging roles of the exopolysaccharide matrix and surface sensing in bacterial communication and ecology

Gerard C L Wong, Matthew R Parsek

i. Abstract

Biofilms are surface-adhered, organized multicellular communities that are fundamental to the microbiology and ecology of bacteria. A canonical feature of bacterial biofilms is exopolysaccharides (EPS), which are secreted by bacteria during biofilm formation in response to increased c-di-GMP signaling. EPS molecules are known to function as cell-to-surface and cell-to-cell adhesins ('molecular glues'), and as structural components of the extracellular matrix. What is missing in microbiology is an understanding of precisely how EPS impacts bacterial surface sensing, signaling, intercellular communication, and motility behavior that ultimately lays the foundations of the bacterial biofilm, especially at a sufficient level of detail that allows deterministic engineering of bacterial social behavior. In our early work on *Pseudomonas aeruginosa*, the Psl EPS has been shown to be secreted on surfaces and act as trails for bacteria to follow during the initial stages of biofilm formation. At present, control of the exact structure, composition, and spatial location of bacterial EPS is an intractable problem, which makes it difficult to study how bacteria interact with their EPS at molecular resolution.

ii. Objectives:

To meet these challenges, we designed the program of research which was executed in the last 3 years. We combined state-of-the-art microbiology (M R Parsek) with state-of-the-art bioengineering in the form of glycopolymer synthesis, engineered surfaces for modifying bacterial social behavior, and artificial intelligence assisted tracking of entire bacterial communities at single cell resolution, so that we can answer the necessary fundamental science questions and demonstrate some of the engineering possibilities. The major objectives are as follows:

(1) To understand how the existence of a surface can drive the formation of heterogeneous populations of *P. aeruginosa* through surface sensing.

(2) To understand the role of surface sensing controlled EPS secretion during reversible attachment, the first stage of bacterial engagement with a surface, and quantitatively compare *P. aeruginosa* strains PA14 and PAO1

(3) To assess whether we can guide biofilm forming behavior of *Shewanella oneidensis,* a strain capable of extracellular electron conduction that has been proposed for devices

(4) Determine the breadth of EPS cross-linking activities for the *P. aeruginosa* biofilm matrix protein, CdrA.

(5) To understand how specific molecular details of EPS can influence sensing, cdiGMP signaling, and social behavior in *P. aeruginosa*

Our collaboration finished five major projects, four of which have been published in flagship journals in microbiology and engineering (eLife, mBio, ACS Appl Mater Interfaces, J. Bacteriology), and one that we are preparing to submit to a high profile journal. These individual projects are summarized below.

iii. Findings:

(1)

Major activity: Heterogeneity in surface sensing and EPS production suggest a division of labor in *Pseudomonas aeruginosa* populations

Specific objectives: To understand how the existence of a surface can drive the formation of heterogeneous populations of *P. aeruginosa* through surface sensing.

Significant results: The second messenger signaling molecule cyclic diguanylate monophosphate (c-di-GMP) drives the transition from planktonic to biofilm growth in many bacterial species. *Pseudomonas aeruginosa* has two surface sensing systems that produce c-di-GMP in response to surface adherence. The current thinking in the field is that once cells attach to a surface, they uniformly respond with elevated c-di-GMP. Here, we describe how the Wsp system generates heterogeneity in surface sensing, resulting in two physiologically distinct subpopulations of cells. One subpopulation has elevated c-di-GMP and produces biofilm EPS matrix, serving as the founders of initial microcolonies. The other subpopulation has low c-di-GMP and engages in surface motility, allowing for exploration of the surface. We also show that this heterogeneity strongly correlates to surface behavior for descendent cells. Together, our results suggest that after surface attachment, P. aeruginosa engages in a division of labor that persists across generations, accelerating early biofilm formation and surface exploration.

Key outcomes: This work was published in *eLife* in 2019.

(2)

Major activity: Parsing social cooperativity during surface attachment in young *Pseudomonas aeruginosa* biofilms

Specific objectives: To understand the role of surface sensing controlled EPS secretion during reversible attachment, the first stage of bacterial engagement with a surface, we performed a quantitative comparison between *P. aeruginosa* PA14 and PAO1.

Significant results: What are bacteria doing during "reversible attachment," the period of transient surface attachment when they initially engage a surface, besides attaching themselves to

the surface? Can an attaching cell help any other cell attach? If so, does it help all cells or employ a more selective strategy to help either nearby cells (spatial neighbors) or help its progeny (temporal neighbors)? Using community tracking methods at single-cell resolution, we suggest answers to these questions based on how reversible attachment progresses during surface sensing for *Pseudomonas aeruginosa* strains PAO1 and PA14, which have drastically different surface sensing circuits and EPS production profiles. Although PAO1 and PA14 exhibit similar trends of surface cell population increase, they show unanticipated differences when cells are considered at the lineage level and interpreted using the quantitative framework of an exactly solvable stochastic model. Reversible attachment comprises two regimes of behavior, processive and nonprocessive, corresponding to whether cells of the lineage stay on the surface long enough to divide or not before detaching. Stark differences between PAO1 and PA14 in the processive regime of reversible attachment suggest the existence of two surface colonization strategies, which are roughly analogous to "immediate-" vs "deferred-gratification" in a prototypical cognitive-affective processing system made possible by different uses of EPS: PAO1 lineages commit quickly to a surface compared to PA14 lineages, with early c-di-GMP mediated EPS production that can facilitate attachment of neighbors. PA14 lineages modulate their motility via cAMP, and retain memory of the surface so that their progeny are primed for improved subsequent surface attachment. Based on previous studies, we propose that the differences between PAO1 and PA14 are potentially rooted in downstream differences between Wsp-based and Pil-Chp-based surface sensing systems, respectively.

Key outcomes: This work was published in *mBio* in 2020. (We note that predictions we had made in this paper regarding the complex ecological competition landscape between strains of *P. aeruginosa* that use EPS differently has recently been confirmed by Kassety et al, *J. Bact.* (2021))

(3)

Major activity: Initiate prototypical studies using artificially patterned glycopolymers to mimic EPS

Specific objectives: To assess whether we can guide biofilm forming behavior of *Shewanella oneidensis*.

Significant results: Using glycopolymer decorated surfaces, we have stimulated *Shewanella oneidensis* bacterial colonization and controlled bacterial attachment via artificial molecular patterns. When adherent bacteria were rinsed with methyl α-d-mannopyranoside, the glycopolymer-functionalized surfaces retained more cells than self-assembled monolayers terminated by a single mannose unit. These results suggest that the three-dimensional multivalency of the glycopolymers both promotes and retains bacterial attachment. When the methyl α-d-mannopyranoside competitor was codeposited with the cell culture, however, the mannose-based polymer was not significantly different from bare gold surfaces. The necessity for equilibration between methyl α-d-mannopyranoside and the cell culture to remove the

enhancement suggests that the retention of cells on glycopolymer surfaces is kinetically controlled and is not a thermodynamic result of the cluster glycoside effect. The MshA lectin appears to facilitate the improved adhesion observed. Our findings that the surfaces studied here can induce stable initial attachment and influence the ratio of bacterial strains on the surface may be applied to harness useful microbial communities.

Key outcomes: This work was published in *ACS Appl Mater Interfaces* in 2020.

(4)

Major activity: Identifying CdrA as a versatile biofilm EPS matrix cross-linking protein that mediates interactions with multiple EPS types in *Pseudomonas aeruginosa*.

Specific Objectives: Determine the breadth of EPS cross-linking activities for the *P. aeruginosa* biofilm matrix protein, CdrA.

Significant Results: Depending upon the strain, *Pseudomonas aeruginosa* can use different exopolysaccharides (e.g., Psl, Pel, and alginate) to build its biofilm matrix. Previously, we demonstrated that the biofilm matrix protein CdrA binds to Psl, promoting biofilm formation and aggregate stability. As such, it was thought that CdrA might be important for biofilm assembly only in strains that rely upon Psl. However, past studies indicated that CdrA can interact with monosaccharides not present in Psl, including *N*-acetylglucosamine, a constituent of another EPS called Pel. We discovered that CdrA also binds to Pel and promotes biofilm formation by strains in which Psl is not dominant. Thus, our findings suggest that CdrA plays a common role as a biofilm matrix cross-linker across *P. aeruginosa* isolates with different EPS types. This finding has significant implications for CdrA contributing to biofilm assembly by multiple species and additionally raises the key question as to whether it is present in Psl trails.

Key Outcomes: This work was published in the Journal of Bacteriology in 2020 (Spotlight article).

(5)

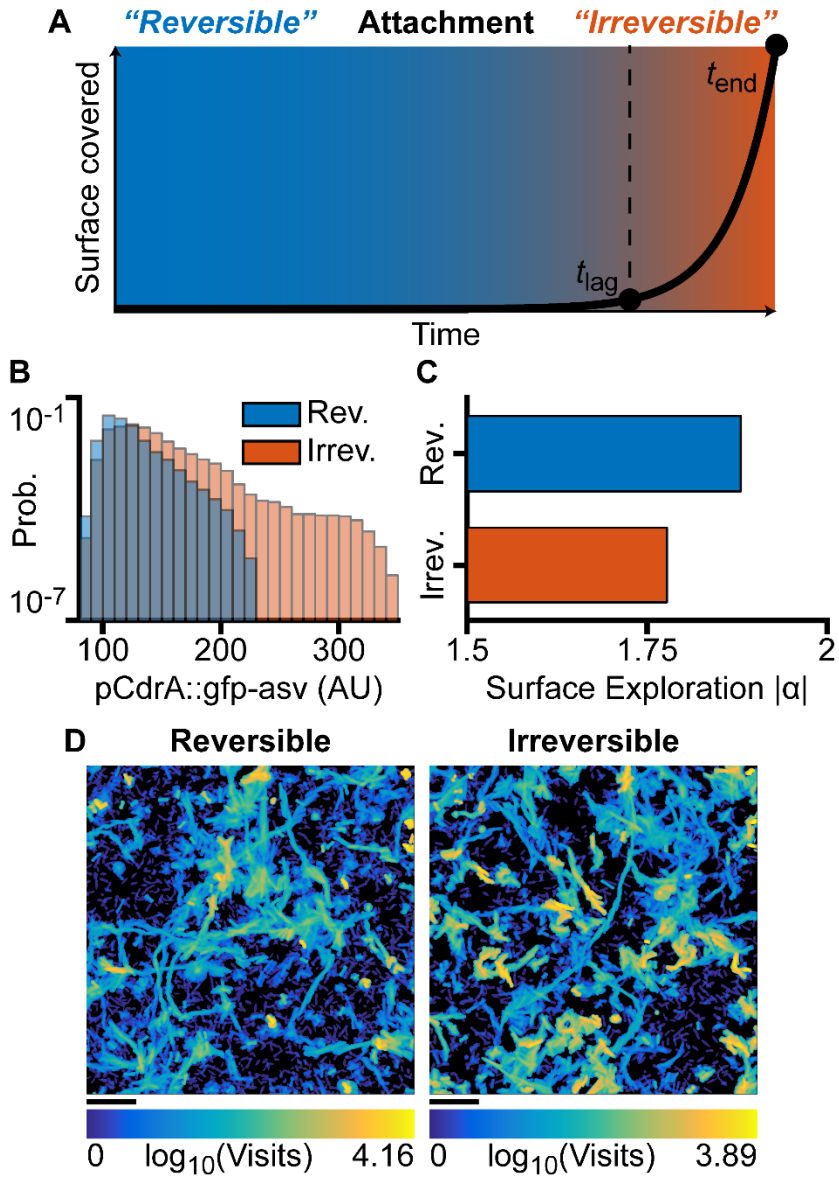
Major activity: Demonstration that specific molecular motifs in artificial EPS can differentially drive surface sensing and c-di-GMP signaling in *Pseudomonas aeruginosa*

Specific objectives: To understand how specific molecular details of EPS can influence sensing, cdiGMP signaling, and social behavior in *P. aeruginosa*.

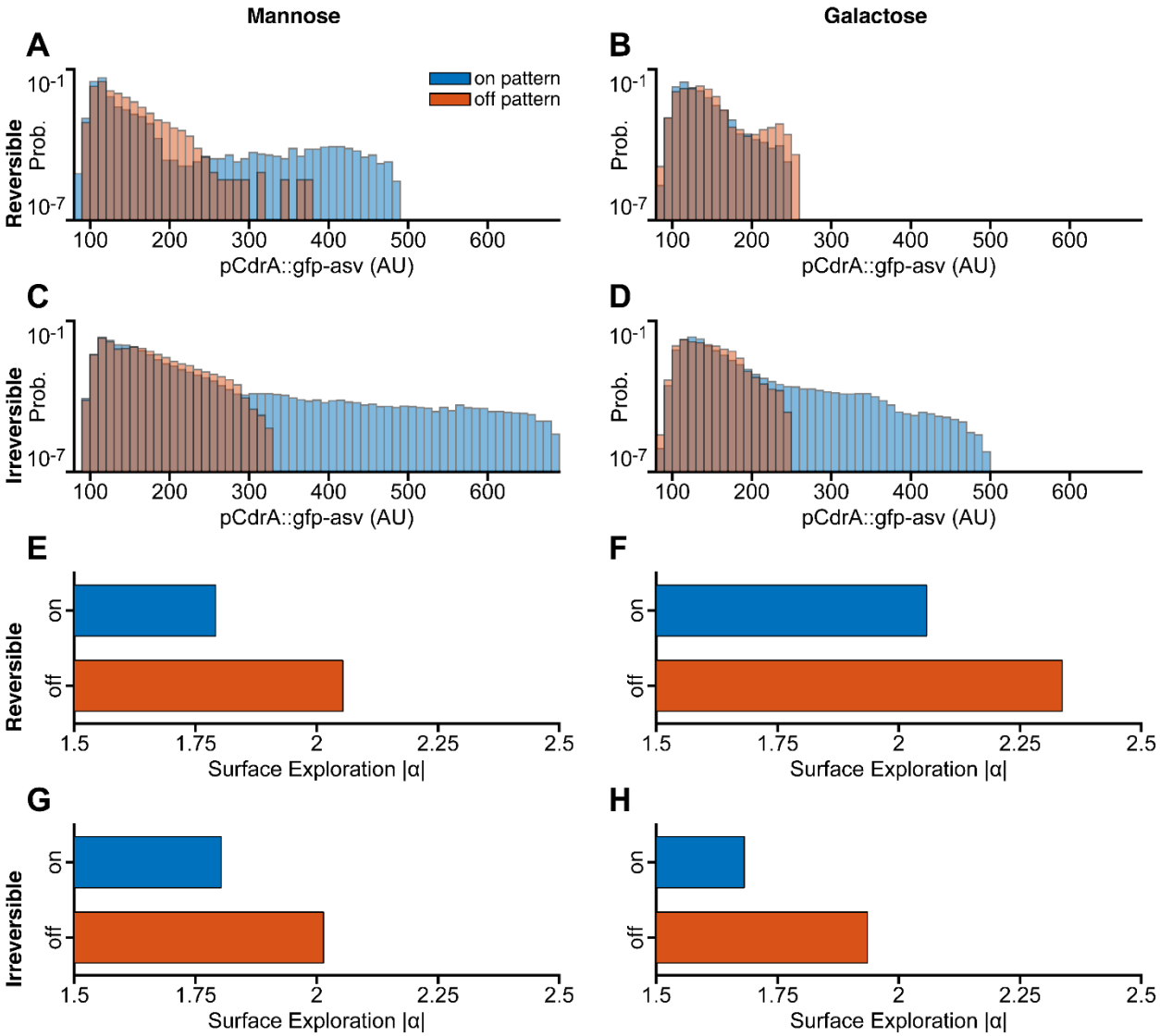
Significant Results: We designed artificial EPS consisting of glycomimetic polymers that mimic the known composition of Psl and can be "printed" on surfaces as defined spatial patterns of artificial EPS. In this work, we are able to compare artificial trails with naturally secreted trails, and assessed how *P. aeruginosa* can differentiate between EPS trails of different composition. It is well known that during the earliest stages of biofilm formation, known as "reversible" attachment, the surface cell density is roughly constant, and cells that land on the surface tend to

detach before dividing. As the biofilm progresses, cells begin to stay long enough to divide on the surface, and this coincides with the surface cell density increasing exponentially and the transition to "irreversible" attachment. With naturally secreted EPS trails, as cells transition from reversible to irreversible attachment, their c-di-GMP levels tend to increase, while their surface exploration tends to decrease. In this work with synthetic EPS with well-defined glycol-motifs, we find that the composition of the EPS trails can strongly modulate biofilm sensing, signaling, and social behaviors, and do so in a time-dependent manner. Psl is a type of EPS found in *P. aeruginosa* which incorporates mannose and galactose motifs as the two majority components in its polysaccharide structure. Interaction with a mannose-rich glycopolymer pattern results in a larger increase in c-di-GMP and do so earlier in the biofilm development process. These changes influence behavior during both reversible and irreversible attachment. In contrast, interaction with a galactose glycopolymer pattern results in a larger increase in c-di-GMP *late*r, during irreversible, but not reversible, attachment. We show that these surface sensing and signaling differences have profound implications in the transition between motile and sessile bacterial communities. These results demonstrate umambiguously that molecular details of EPS play a central role in bacteria signaling and motility, with each component of EPS having a time-dependent impact on bacteria behavior during the biofilm development timeline. Moreover, the results suggest that bacteria may be able to coordinate motility and biofilm development via intercellular communication mediated by changes in the EPS composition.

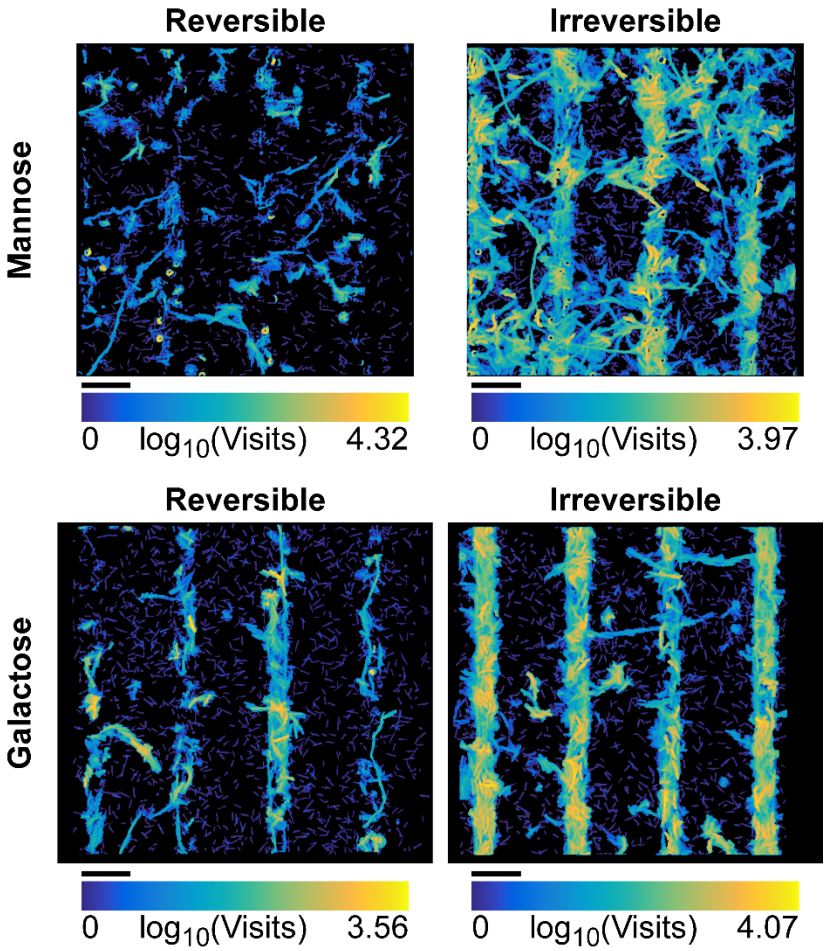
Key Outcomes: This work represents a major advance in our understanding of EPS and is being prepared for a joint high profile publication between our labs.



(A) Schematic data for biofilm surface growth following 2 attachment phases. In the first phase, surface cell density is roughly constant. This corresponds to the "reversible" attachment phase, where most cells land and detach before dividing. As the biofilm progresses, cells begin to stay long enough to divide, and this coincides with the surface cell density increasing exponentially. This corresponds to the "irreversible" attachment phase. The transition from reversible to irreversible is broad and difficult to measure exactly, but we have approximated the transition time point t_{lag} through a fit of the surface coverage to an exponential function. (B) Histogram of c-di-GMP reporter intensities for cells in either the reversible or irreversible attachment phases. Cells in the irreversible attachment phase tend to have higher c-di-GMP levels. (C) Measurement of surface exploration behavior by fitting the spatial visit distribution to a power law. The value $|\alpha|$ characterizes the amount of surface exploration: higher values correspond to more surface exploration and vice versa. Cells in the irreversible attachment phase tend to have less surface exploration. (D) Visit maps indicate how many times bacteria have visited a given pixel location.



(A-D) Histograms of c-di-GMP reporter intensities for cells in either reversible or irreversible attachment phases and either on or off a mannose or galactose pattern. Cells off any pattern have a similar distribution of intensities compared to cells on natural trails. (A, C) In both reversible and irreversible attachment, more cells on a mannose pattern have higher c-di-GMP levels compared to cells off the pattern, as indicated by the longer tail of the distribution. (B) Cells on a galactose pattern during reversible attachment have a similar distribution of intensities compared to cells off the pattern. (D) More cells on a galactose pattern have higher c-di-GMP levels during irreversible attachment compared to cells off the pattern. (E-H) Measurements of surface exploratory behavior value $|\alpha|$, which characterize the amount of surface exploration: higher values correspond to more surface exploration and vice versa. Cells off any pattern tend to explore the surface more compared to cells off the pattern. (E, G) Cells on a mannose pattern tend to explore the surface similarly to cells on natural trails, and there is no change in exploration when cells transition from reversible to irreversible attachment. (F, H) Compared to natural trails, cells on a galactose pattern tend to explore the surface more during reversible attachment and less during irreversible attachment.



Visit maps for mannose and galactose patterns during reversible and irreversible attachment. The galactose pattern has a much stronger impact on bacteria visits compared to mannose during both reversible and irreversible attachment, since bacteria tend to visit the galactose pattern stripes more.

Publications:

1. Catherine R. Armbruster*, Calvin K. Lee*, Jessica Parker-Gilham, Jaime De Anda, Aiguo Xia, Boo Shan Tseng, Lucas R. Hoffman, Fan Jin, Caroline Harwood, Gerard C. L. Wong, Matthew R. Parsek, "Heterogeneity in surface sensing suggests a division of labor in *Pseudomonas aeruginosa* populations", *eLife* **8**, e45084 (2019). (*co-1st authors). doi: 10.7554/eLife.45084. PMID: 31180327.

2. Calvin K. Lee*, Jérémy Vachier*, Jaime de Anda, Kun Zhao, Amy E. Baker, Rachel R. Bennett, Catherine R. Armbruster, Kimberley A. Lewis, Rebecca L. Tarnopol, Charles J. Lomba, Deborah A. Hogan, Matthew R. Parsek, George A. O'Toole, Ramin Golestanian, and Gerard C. L. Wong, "Social Cooperativity of Bacteria during Reversible Surface Attachment in Young Biofilms: a Quantitative Comparison of Pseudomonas aeruginosa PA14 and PAO1" (*co-1st authors). *mBio* **11**, e02644-19 (2019). doi: 10.1128/mBio.02644-19. PMID: 32098815.

3. Calvin K. Lee, Alexander J. Kim, Giancarlo S. Santos, Peter Y. Lai, Stella Y. Lee, David F. Qiao, Jaime De Anda, Thomas D. Young, Yujie Chen, Annette R. Rowe, Kenneth H. Nealson, Paul S. Weiss, and G. C. L. Wong, "Evolution of Cell Size Homeostasis and Growth Rate Diversity during Initial Surface Colonization of *Shewanella oneidensis*", *ACS Nano* **10** 9183-9192 (2016). (See Perspectives article in same issue) PMID: 27571459.

4. Courtney Reichhardt, Holly M Jacobs, Michael Matwichuk, Cynthis Wong, Daniel J Wozniak, Matthew R Parsek, "The Versatile Pseudomonas aeruginosa Biofilm Matrix Protein CdrA Promotes Aggregation through Different Extracellular Exopolysaccharide Interactions", *J. Bacteriology* 202(19):e00216-20 (2020). doi: 10.1128/JB.00216-20. PMID: 32661078. PMCID: PMC7484184. DOI: 10.1128/JB.00216-20