# The state of the union is strong: A review of ASM's 6th conference on Cell-cell Communication in Bacteria

Sam P. Brown[1], Helen E. Blackwell[2] & Brian K Hammer[1]*

[1]School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA 30332

[2]Department of Chemistry, University of Wisconsin-Madison, Madison, WI 53706

*Corresponding author

Mailing address:

School of Biological Sciences

Georgia Institute of Technology

Atlanta, Georgia m30332-0230

Tel: 404-385-7701

Fax: 404-385-4759

brian.hammer@biosci.gatech.edu

**Abstract:**

The sixth American Society for Microbiology Conference on Cell-Cell Communication in Bacteria convened from 16-19 October 2017 in Athens, GA. In this minireview, we highlight some of the research presented at that meeting that addresses central questions emerging in the field: How are cell-cell communication circuits designed to generate responses? Where are bacteria communicating? And why are bacteria engaging in such behaviors?

**Introduction:**

From 16-19 October 2017 in Athens, GA, Beth Lazazzera and Eric Stabb co-chaired the sixth ASM Conference in Cell-Cell Communication in Bacteria (CCCB-6). The meeting showcased recent advances in our understanding of physical and chemical signaling mechanisms bacteria use for engagement with one another, and the communication networks coordinating these processes. The ~125 participants included senior scientists, postdoctoral researchers and students from diverse institutions in Asia, Australia, Europe, and North America. The program of 68 poster presentation and 42 talks was designed to promoted exchange of information and engagement among peers.

In 2001, the first Cell-Cell Communication in Bacteria conference, CCCB-1, in Snowbird UT highlighted quorum sensing (QS) as the archetype cell-cell communication process in bacteria (1). That meeting was an announcement of our field's emerging realization that bacteria were not asocial, but rather extroverted. The *Vibrio fischeri* LuxI/R system and *Pseudomonas aeruginosa* tandem LasI/LasR and RhlI/RhlR systems were a dominant topic at CCB-1. What followed that meeting has been an explosion of work on this topic. The field has continued to grow and to

mature. We have discovered a wide range of bacteria that, in varied settings, can communicate by diverse mechanisms, which can result from exchange of diffusible signals as well as contact-dependent interactions. As the contributions of cell-cell signaling in ecology and disease are deciphered, efforts continue to develop strategies aimed to thwart or augments these activities. Most current efforts seek to disable such communication in pathogenic bacteria that engage in collective virulence strategies.

Paul Williams from the University of Nottingham delivered the keynote address at CCCB-6 and launched the conference on a dynamic note. For nearly 25 years, the William's group has focused their efforts on the QS signal molecules used by several notable bacteria including *P. aeruginosa, Staphylococcus aureus*, and *Erwinia carotovora* (now *Pectobacterium carotovorum*), with an emphasis on the identification of inhibitors that may be used to thwart clinically relevant human pathogens. *P. aeruginosa* secretes an array of potent virulence factors that can contribute to chronic lung infections. Many of these factors and are under control of a complex quorum sensing circuit that includes the production of two acyl homoserine lactones (3-oxo-C12-HSL and C4-HSL) and their cognate receptors (LasR and RhlR, respectively), as well as quinolone signals (PQS) recognized by their transcription factor (PqsR, or MvfR) (2). Williams' talk reviewed the PQS signaling system and described a major study by his group to determine the crystal structure of PqsR with the natural agonist HHQ. Based on ligand binding features, a panel of antagonists was synthesized and characterized. He discussed identification of a PqsR inhibitor that alters virulence gene expression (3). This exciting advance has led to on-going *in silico* screening of nearly 100,000 drug-like compounds to identify additional candidate predicted to inhibit PqsR. Several such candidates are currently being studied. Ultimately, the

goal is to use these inhibitors in clinical settings - alone, or in conjunction with other therapeutics to alter disease progression. Williams showed attendees preliminary evidence that one inhibitor sensitized biofilms to the action of the antibiotic tobramycin. Finally, Williams discussed progress with colleagues to use QS autoinducers as biomarkers of disease progression in cystic fibrosis patients (4). It remains unclear whether QS autoinducer levels detected in body fluids correlate with disease outcome and are predictive of future *P. aeruginosa* colonization. Nonetheless, these advances highlight concrete efforts to translate knowledge of QS circuitry into therapy. At its core, Williams' keynote address also highlighted how many with deep roots in this field tackle cell-cell communication in bacteria from many angles; asking mechanistic questions regarding signal circuitry and inhibitor design, the environmental or clinical context in which chemical signaling is occurring, and the evolutionary and ecological consequences of microbial signaling. While many like Williams work at the intersection of these questions, we highlight the exciting presentation that also addresses these challenges and organize the oral presentations around these central questions: How? Where? And Why?

**How: Design principles for signals, networks and inhibitors**

As with prior CCCB meetings, LuxI/LuxR-type QS systems were a frequent topic at the 2017 meeting. Caroline Harwood outlined her lab's efforts to study the role of LuxI/LuxR-type QS in bacteria isolated from the roots of Populus cottonwood trees. Over the past decade, her team has shown that *luxI/luxR* genes are highly prevalent in root-associated Proteobacteria and in root rhizosphere metagenomes (5). Two subfamilies of LuxR-type receptors appear to be well-represented in these bacterial populations. One of these LuxR-subfamilies is linked to AHL

synthases (*i.e.*, LuxI homologs) that use coenzyme A (CoA), as opposed to the more common acyl carrier protein (ACP)-linked substrates, for AHL synthesis (6). In a series of beautiful studies, Harwood's lab has shown that these CoA-type AHL synthases produce QS signals with plant-derived acyl tails, or "side chains. These building blocks include a range of aromatic acids, including *p*-coumaroyl-HL (7) and cinnamoyl-HL (8) and likely others (**Figure 1**). The second LuxR-subfamily found in Populus proteobacterial isolates has similarity to OryR from the rice pathogen, *Xanthomonas oryzae*. Members of the OryR subfamily do not respond to acyl-HSL-type signals, but instead detect unknown plant-derived compounds. Harwood described recent work with Pete Greenberg's lab on the OryR-homolog PipR produced by Pseudomonas sp. GM79, a cottonwood endophyte, which suggested the plant signal for PipR may be small peptide and controls behaviors associated with growth while on the plant host (9). Collaborator Bruna Goncalves Coutinho from Greenberg's group, in a later session of the meeting, described more recent studies using gene fusions to GFP to determine the contribution of PipR to root colonization and discussed the possibility of determining the plant signal by exploiting its strong binding to the periplasmic binding component of the ABC-type transporter that imports the plant signal. Collectively, work from Harwood and Greenberg's labs demonstrates the substantial capacity for LuxR-type cell-cell communication in the microbiomes of other plants. In addition, the incorporation of plant-derived metabolites into QS signal synthesis suggests that there could be a sizable and diverse set of natural AHL signals waiting to be unearthed.

In synergy with Harwood's talk, Jannis Brehm described his research in the Heermann lab focused on Proteobacteria with non-standard LuxR-type QS systems. While the prototypical AHL quorum sensing system consists of a LuxI-like AHL synthase and a cognate LuxR-like

receptor, many Proteobacteria also possess further LuxR-family proteins that lack a cognate LuxI synthase in their genomes. These orphan receptors may not respond to AHLs (like Harwood's PipR) and are often called LuxR-type receptors "solos". *Photorhabdus* species contain a remarkably high number of LuxR solos, with certain bacteria containing 40 different solo receptors. The role of these receptors is largely unknown. Brehm shed some light on these mysterious systems in his presentation. First, he described the LuxR solo PluR from the insect pathogen *P. luminescens*. Instead of an AHL signal, PluR appears to sense photopyrones (PPYs, **Figure 1**) produced by the pyrone synthase PpyS (10). A PluR homolog, PauR, from the related insect and human pathogen *P. asymbiotica*, senses dialkylresorcinols (DARs) and the precursors cyclohexanediols (CHDs, **Figure 1**) instead (11). Brehm then added further complexity to the story as he described how the majority of the LuxR solos in *Photorhabdus* species have a "PAS4" signal domain that shares close structural homology with "PAS3" domains, which are hormone-binding domains in insects, such as *Drosophila melanogaster*. Brehm showed that some of these LuxR-type receptors sense compounds originating from the insect host. In addition, knockouts of these *luxR* genes show a decrease in pathogenicity in insects. He hypothesized that the PAS4-LuxR solos could play a central role in inter-kingdom-signaling between the bacteria and their eukaryotic hosts. Thus, Brehm's conclusions for LuxR receptors in *Photorhabdus* species beautifully built from those of Harwood's, but now extended them from plant to insect hosts for signal origination.

Peptide signaling in Gram-positive bacteria, many of which are human associated, was also well-represented at the 2017 meeting. Gary Dunny provided a update on his lab's long-term efforts to study peptide signing in the enterococci (12). Relatively little is known about their lifestyle in

healthy human hosts, and the role of cell-cell communication therein. Through a series of detailed mechanistic studies and single cell expression analyses (13), Dunny described the complex regulatory circuits associated with the induction of conjugative transfer of the enterococcal plasmid pCF10 by the peptide pheromones cCF10 and iCF10 (14). He provided compelling evidence from a recent study (15) with Helmut Hirt, who also presented at the conference. Their study demonstrated that pheromone signaling mediates efficient transfer of the plasmid in the gastrointestinal (GI) tract, and that the plasmid increases competitiveness of the host. In contrast, failure to limit the extent and duration of the pheromone response is lethal for the host bacterium. Dunny went on to hypothesize that it is these opposing pressures that drove the evolution of the complicated regulatory circuitry in enterococci featuring two competing peptide signals and stochastic variation in the response to cCF10 within donor populations exposed to the same inducing conditions. He closed by describing how the pCF10 pheromone response system likely has an evolutionary origin in other, more recently described Gram-positive peptide signaling systems (16, 17), but has several unique mechanistic features that are probably related to its linkage with a mobile element that had to co-evolve along with its bacterial host.

Todd Gray described the social process of conjugation between donor and recipient strains of *Mycobacterium smegmatis*, the only talk on mycobacteria at the meeting. Conjugation in mycobacteria is unusual, as it does not involve plasmids, is driven by the recipient strain, and creates mosaic genomes in a single event. Gray's team hypothesized that cell-cell contact in stable co-culture initiates transcriptional programs that coordinate conjugation between the participating cells (18). Through the analysis of thousands of transcribed polymorphisms

between conjugal strains, they monitored the response of each strain to its mating partner. One of the most highly induced loci was found to encode the ESX-4 secretion apparatus (19). ESX-4 was found to be required in the recipient strain for DNA transfer, thereby connecting transcriptional response and genetic requirement. This finding was significant, as it was the first demonstration of a functional role for ESX-4, which is the ancestral progenitor for all the other ESX systems encoded by mycobacteria. Gray went on to describe their discovery that the ESX-1 secretion systems from both donor and recipient control the transcriptional activation of ESX- 4 in the recipient strain. While many functions have been ascribed to ESX-1 secretion systems, this was the first sign of its involvement in intercellular communication in mycobacteria. Lastly, Gray reported on an extracytoplasmic sigma factor and anti-sigma factor that may be part of this cell-cell contact response network. This sigma factor, SigM, is required both for contact-dependent activation of recipient ESX-4 and to be in the recipient for conjugation. A model emerging from Gray's data is shown in **Figure 2** and suggests that ESX-1 systems in conjugal donor and recipient strains of *M. smegmatis* secrete cell surface identifiers that modulate coculture response networks, and in the recipient, this response network includes the SigM induction of ESX-4. This conjugal network could share principles and components with non-conjugal and QS networks in other mycobacteria.

In *Vibrio harveyi*, response to HAI-1 and AI-2 autoinduers triggers production of the transcription factor LuxR, which controls expression of hundreds of genes for behaviors including bioluminescence, virulence, motility, and biofilm formation (20). Interestingly, LuxR and its homolog in *V. cholerae*, can directly activate transcription, and also behave as direct transcriptional repressors. Alyssa Ball from Julia van Kessel's lab described progress made in

183 defining the molecular mechanism by which LuxR directly activates promoters under its control.
184 Using a combination of site-directed mutagenesis, co-IP assays, and an optical analytical
185 technique called BioLayer Interferometry, a domain on LuxR was identified for interactions with
186 the alpha subunit of RNAP. LuxR derivatives lacking this domain prevented transcription at
187 LuxR-activated but not LuxR-repressed promoters. Prior work showing that many active
188 promoters have multiple LuxR binding sites (21) suggests further work may uncover unexpected
189 interactions between RNAP and LuxR that tune the QS response of different behaviors.
190
191 A bacterial cell engaged in producing QS autoinducer signals within a population may
192 directly respond to self-produced autoinducers as well as those made by other members of the
193 population. To determine the contribution of self-sensing to cell physiology, Avigdor Eldar,
194 combined mathematical modeling with experiments using fluorescent reporters in *Bacillus*
195 *subtilis* strains impaired or proficient in autoinducer production to study the ComQXP and Rap-
196 Phr QS systems. The co-culture system design permitted simultaneous measurement of distinct
197 fluorescence reporters of otherwise isogenic self-sensing and non-sensing (synthase mutant)
198 strains in liquid co-culture at various cell densities. Self-sensing was apparent in both the
199 ComQXP and Rap-Phr QS systems, with secreting cells producing a stronger response than non-
200 secreting cells. This study (22) also demonstrates the physiological relevance of self-sensing.
201 Following transient exposure of the ComQXP co-cultures to ampicillin, the self-sensing strain
202 persisted better than the non-self-sensing strains at low density, with differences in the strains
203 diminishing at high density. Mathematical analyses predict that self-sensing is a product of the
204 design feature of many QS systems, and perhaps other similar mechanisms. It remains to be
205 determined whether selective pressure has led to evolution of self-sensing in QS circuitry.

Ilka Bischofs also presented work on the *B. subtilis* Rap-Phr QS system and its control of sporulation dynamics. Her group developed a reporter system based on FRET (Fluorescence Resonance Energy Transfer), which holds great promise for investigating bacterial cell-cell communication, since it allows quantitative analysis of key bacterial signaling processes. PhrA is produced by an active export-import circuit and is detected intracellularly by RapA receptors that inhibit the response regulator Spo0F of the sporulation phosphorelay. By measuring FRET between RapA-CFP and Spo0F-YFP, they were able to monitor the intra- and extracellular signal dynamics in response to PhrA-stimulation. Their results, in conjunction with mathematical modeling, suggests that active signal import by the oligopeptide permease Opp plays a central role in determining the high sensitivity and dynamics of Phr-signaling, while potentially also limiting its robustness in the presence of competing peptides(23).

In *P. aeruginosa*, the decision to stick to a surface in a biofilm or to explore new territory is controlled by changes in the levels of intracellular second messenger c-di-GMP (cyclic diguanylate). *P. aeruginosa* has numerous sensory systems for altering c-di-GMP in response to diverse environmental conditions and ligands. To clarify how bacteria like *P. aeruginosa* evolved a network to make the "right" choice to stick or swim in response to uncertain and changing conditions, Joao Xavier described work, similar to Eldar's, that includes complementary experimental and modeling approaches. Xavier presented a mathematical model termed "bowtie", based on the architecture of the c-di-GMP network that includes multiple inputs that converge on c-di-GMP to control a large set of phenotype outputs (24). Modeling was driven by phenotypic and genomic analyses of patient-derived isolates, as well as prior

experimental evolution study of *P. aeruginosa* in lab settings (25). In isolates from both studies, genetic variants in c-di-GMP related genes suggest the network is evolving by incremental changes in response to evolutionary pressures encountered. Parallels between machine learning and evolution suggest the capacity of microbes to "learn" through evolution.

Knowledge of QS signals and network architecture can lead to the development of useful tools to further dissect communication networks, engineer synthetic systems, and perhaps also to disable them in human pathogens that control virulence factor production via QS. The Meijler group studies QS in bacterial pathogens using a range of elegant chemical approaches. Michael Meijler first outlined the origins of his novel electrophilic probes that are designed to bind LuxR-type QS receptors covalently, leading to inhibition of QS regulated gene expression (26) (27). His lab has applied these probes, along with newer photoactivatable probes, as molecular tools to obtain new insights into the mechanisms of QS. Their current major focus is the deployment of probes to study the role of QS in specific interkingdom signaling events, such as the effects of the *P. aeruginosa* 3-oxo-C12-HSL (C12) signal on the mammalian immune system. Meijler next reported his chemical profiling platform (that combines tag-free photoactivatable probes with high throughput MS proteomics experiments) and its application to study eukaryotic interception of QS signaling. He presented exciting results in human bronchial epithelial cells and his discovery of a human receptor for 3-oxo-C12-HSL. He also described a similar profiling strategy to find previously unidentified sensors of PQS-like signals in *P. aeruginosa* (28). Meijler's tools and techniques should be broadly applicable to the growing interface of QS research at the host-microbe interface (recently reviewed here (29)).

LuxR/LuxI-type QS systems, are popular building blocks in systems biology to engineer new function into living cells, as their activity can be readily tuned by the addition of exogenous signals or the controlled production endogenous synthesis of signals. Cynthia Collins provided an introduction to her lab's engineering approach to the design of synthetic signaling systems and the harnessing of these systems to control the dynamics and make-up of microbial consortia (30). She described several systems based on LuxR/LuxI QS circuits, along with one based on the peptide-mediated Agr QS systems typical to Gram-positive bacteria. Notably, Collins was able to fully reconstitute the complex latter system in *Bacillus mageterium*, a bacterium used often on large scale for production, opening up novel approaches for the control of bioprocessing (31). She also described two synthetic "AND-gate" promoters that require both a QS signal and an exogenously added inducer to activate gene expression. The two promoters, LEE and TTE, contain binding sites for the LuxR-type protein, EsaR, and either LacI or TetR: they are then induced by addition of an AHL-type signal and IPTG or aTc, respectively. These new AND-gate promoters represent a model for new regulatory systems that integrate both QS and the presence of cellular metabolites or other cues, and thereby permit dynamic changes in gene expression for a wide range of metabolic engineering problems (32).

While many talks at the meeting discussed quorum sensing communication between kin, James Boedicker described how individual quorum sensing networks function in the presence of multispecies interference from non-kin. The work described includes a combination of theoretical models of signal exchange and experimental measurements with synthetic microbial networks. In a clever crosstalk plate assay, LuxI-*E. coli* "senders" secrete 3-oxo-C6 HSL apart from LuxR-expressing E. coli "receiver" that carry a fluorescent reporter gene under control of

the *luxI* promoter. *E. coli* "interactors" plated between the senders and receives led to excitatory crosstalk when secreting 3-oxo-C12 HSL via LasI and alternatively to inhibitory crosstalk when secreting C4-HSL via RhlI. Experimental measurements of signal interference revealed that the LuxI/R QS network is largely robust to high levels of interference from neighboring strains, and in models the amount of interference can be captured with a signal interaction weight for each signal/receptor combination. Boedicker's results (33) suggest that interference limits the spatial range of coordinated quorum sensing activation within mixed species populations.

The LuxR-type receptor, LasR, sits at the top of the QS hierarchy in *P. aeruginosa*, at least under certain environmental conditions (34), and as such has been a primary target for the development of non-native anti-QS compounds for over 20 years (35, 36) (37). However, most of these compounds have relatively low potencies (low micromolar $IC_{50}$ values at best) (38). Daniel Manson provided an overview of his work in Helen Blackwell's lab to develop new LasR inhibitors based on the scaffold of V-06-018, a small molecule first discovered in 2006 by Greenberg and co-workers (39), which represents one of the most potent reported LasR inhibitors. Surprisingly however, despite its activity profile, this compound has seen limited study from a structure-function perspective. Manson reported on his systematic study of this compound's structure-activity relationships (SARs) for LasR inhibition via the synthesis and biological evaluation of a focused library of novel V-06-018 derivatives. This work revealed structural features of the V-06-018 scaffold that appear to govern its ability to inhibit LasR. Mason applied these SAR data to design probes that inhibit LasR with greater potency and efficacy than V-06-018, with sub-micromolar $IC_{50}$ values. Biochemical experiments with V-06-018 and LasR revealed that it strongly destabilized the receptor in vitro. These experiments

support the hypothesis that V-06-018 (and related derivatives) function primarily by displacing the AHL signal from LasR, which leads to LasR unfolding. Manson's compounds serve to underscore the utility of the V-06-018 scaffold for probe design and represent valuable new chemical tools to study the role of LasR in *P. aeruginosa* QS and virulence.

Cyclic dinucleotides, introduced to the attendees by Xavier, regulate many physiological processes in both prokaryotes and eukaryotes (40). Herman Sintim provided a detailed overview of his research over the past decade on cyclic dinucleotide signaling (41, 42), along with the development of novel assays methods to sensitively detect these compounds (43, 44). C-di-AMP (**Figure 1**), found in Gram-positive bacteria and mycobacteria, regulates cell wall homeostasis as well as biofilm formation. As many current antibiotics also target bacterial cell wall formation, receptors of c-di-AMP could represent novel antibacterial targets (45). In turn, c-di-GMP (**Figure 1**), a second messenger in Gram-negative bacteria such as *P. aeruginosa*, appears to be a master regulator of biofilm formation (46). Immune cells of higher organisms also sense bacteria-derived cyclic dinucleotides. For instance, the binding of cyclic dinucleotides to the host's receptor protein STING leads to the production of cytokines, which could lead to pathogen clearance and/or deleterious inflammation and tissue damage. In a series of vignettes at the meeting, Sintim described both elegant chemistry and biology leading to a suite of synthetic compounds developed by his lab that strongly inhibit cyclic dinucleotide synthesis, signaling in bacterial pathogens and their associated biofilm formation. These compounds and chemical strategies represent valuable tools to dissect cyclic dinucleotide signaling, and with further development, could provide novel scaffolds for next-generation therapeutics.

Laurence Rahme presented research in her lab on the central role of the MvfR (PqsR) receptor in *P. aeruginosa* virulence. MvfR regulates functions important in both acute and persistent infections. Rahme outlined the development of synthetic inhibitors of MvfR that suppress both acute and persistent *P. aeruginosa* infections in mice without perturbing bacterial growth (for example, M64 (47); **Figure 1**). These compounds also perturb biofilm formation and can potentiate antibiotic-mediated biofilm disruption (48). Rahme went on to describe compounds that can inhibit PqsBC enzyme activity (i.e., the synthetic machinery responsible for the two MvfR activating ligands, HHQ and PQS; **Figure 1**) and the first to target both MvfR and PqsBC activity. Additional experiments revealed that MvfR remains the best target of this QS pathway, as antagonists of MvfR were found to concomitantly block acute infection and multiple persistence-related virulence functions in *P. aeruginosa* in several infection models. Understanding the interplay and possible synergies of these compounds with known antibiotics, along with inhibitors of the other QS pathways in *P. aeruginosa*, will be exciting avenues for the future (**Figure 3**) (49).

Viviana Gatta presented her work in the Tammela lab on the development of a new assay to uncover compounds that inhibit autoinducer-2 (AI-2) signaling, that was briefly discussed by Alyssa Ball for its role in inducing LuxR production in *Vibrio harveyi*. Numerous bacteria, besides *Vibrios*, produce AI-2, leading to its description as an inter-species QS signal (50, 51). The precursor to AI-2, (4*S*)-4,5-dihydroxy-2,3-pentanedione (DPD) has been found in over 70 species of bacteria (**Figure 1**). Gut enteric bacteria that generate AI-2 produce LsrK, a kinase responsible for DPD phosphorylation. As only the phosphorylated form of DPD is believed to be important for signaling (52), inhibition of LsrK represents a possible route to the eventual

blockade of AI-2 mediated QS. To this end, Gatta sought to identify small molecules capable of inhibiting LsrK and developed an automation compatible, high throughput screen to identify such compounds from chemical libraries. The assay was applied in the screening of two small libraries: 1) ~100 compounds selected by virtual screening of a commercial library, and 2) ~90 non-native DPD-analogues. The screening campaign yielded four, target-specific LsrK inhibitors with $IC_{50}$ values ranging from 100–500 μM. These new agents represent candidates to be further optimized for the development of LsrK inhibitors as a new class of research tools, and potential antivirulence agents.

**Where: Signaling During Infection and in Microbial Development**

The field of microbiology arose from successful isolation of microbes from complex natural environments and the development of methods to study them in isolation. Many of the talks at the conference highlighted discoveries regarding microbial communication that emerge from current culturing methods that more accurately mimic conditions where bacteria interact with other microbes and eukaryotes. On day two of the CCCB conference, Rosie Alegado gave a riveting talk of a serendipitous discovery with the closest living relative of animals, choanoflagellates, a flagellated eukaryote that can exist free living or in multi-celled rosettes (**Figure 4**). The talk began with a review of work by Alegado while in Nicole King's lab with collaborator Jon Clardy (53). She retold how the addition of antibiotic to the original ATCC culture containing the choanoflagellate *Salpingoeca rosetta* with environmental bacteria led to an unexpected disruption of rosette formation that was restored by addition of only one bacterial species from the ATCC culture, *Algoriphagus machipongonensis.* Recent study revealed that

sphingolipids produced by *A. machipongonensis* is sufficient to induce rosette formation (54), suggesting that the developmental switch of this animal from single cell to multicellular form is dictated by lipid-mediated signaling by a microbe. Studies by King (55) show that currents created by flagellar beating draw bacteria toward the choanoflagellates where they are phagocytosed. Alegado presented evidence that cells in multicellular rosettes have higher feeding rates than unicellular counterparts, suggesting that rosette formation may be advantageous by permitting more efficient feeding of bacterial prey. Any benefits to the bacteria for promoting rosette formation are unclear since these bacteria are also preyed upon by their eukaryotic partner.

Heidi Kaplan described her most recent results of an ongoing successful collaboration with physicist Oleg Ogoshin exploring the physics of social motility (S motility), which enables populations of *Myxococcus xanthus,* but not individual cells, to swarm across surfaces. S motility requires extension and contraction of type IV, as well as exopolysaccharide (EPS) secretions from neighboring cells that serve as scaffolds for the swarming population. Kaplan described that expansion rates across the agar surface are affected by the initial cell density. A reaction-diffusion model developed by the team predicted that a transient period of slow expansion during low density was due to the time required to accumulate sufficient EPS (56). Kaplan discussed recent work by Zhou and Nan showing an additional role for EPS beyond scaffolding (57). Whereas individual cells reverse direction frequently, high EPS levels minimize reversals within swarms, perhaps reducing the departure of individuals from swarms. Currently, the reversal rates at the expanding edge of a colony are being studied by Kaplan. Such studies underscore how physical mechanics play an important role in microbial communities.

Talks regarding QS in *V. fischeri* are a staple of the CCCB meetings for this microbe was one of the first organisms where cell-cell communication was described for its role in regulating bioluminescence within the crypts of the light organ of its symbiotic partner the bobtail squid (58). Lauren Speare, from Alecia Septer's lab, described an additional level of chemical communication among *V. fischeri* during squid colonization. Speare introduced meeting participants to the Type VI Secretion System (T6SS), a membrane spanning apparatus encoded by approximately 25% of gram negative bacteria, including *V. fischeri* (59-61). The spike of the T6SS is decorated with toxic effector proteins that can induce damage or lysis in adjacent cells lacking cognate immunity factors. Speare described recent work from the Septor and Miyashiro labs to discover whether the T6SS plays a role in host colonization by *V. fischeri*. Prior work revealed that squid co-colonized with multiple *V. fischeri* strains nonetheless harbor singly colonized crypts (62), suggesting competition between strains for access to crypts followed by expansion. Speare presented compelling evidence for a model that T6SS interactions are critical for segregating individual strains into distinct crypts. Co-colonization of T6-deficient strains led to mixed crypts, where T6-killing results in single colonized crypts. A search for factors controlling the T6SS of *V. fischeri* has also identified a putative VasR regulator that appears to be active in host conditions, as reported by Smith during the poster session.

Work on the T6SS of the related waterborne gastrointestinal pathogen *Vibrio cholerae* was presented by Cristian Crisan, from the Hammer lab, which has traditionally studied quorum sensing, natural transformation, and the T6SS in clinical reference strain C6706 (63), with a more recent emphasis on host associations (64). The group recently characterized and sequenced

413 a set of *V. cholerae* isolates (65, 66) that encode diverse T6SSs. Prior work showed that co-
414 culturing of an environmental isolate and C6706, which have distinct effector/immunity pairs,
415 generates a spatially segregated microbial community by the process of phase separation (67).
416 Crisan briefly described a new bioinformatic tool used to identify several new putative T6SS loci
417 in another sequenced environmental isolate of *V. cholerae*. His talk focused on one novel T6
418 locus, termed Aux5 here. The Aux5 effector Crisan described appears similar to a *P. aeruginosa*
419 T6 lipase. When expressed alone in *E. coli*, the Aux5 effector induced toxicity, but not when co-
420 expressed with its cognate immunity factor. The novel Aux5 locus could also be transferred to
421 C6706 by natural transformation, which then could use the additional T6 locus to kill its kin.
422 These intriguing results suggests a diversity of cargo can be loaded onto a T6 harpoon, and that it
423 may be possible to repurpose a T6SS for delivery of customized effectors for therapeutic
424 purposes.
425
426 The talks by Speare and Crisan highlighted that delivery of lethal effector proteins, but not
427 cognate immunity factor by the T6SS, allows microbes to kill non-kin, but not kin. Martha
428 Zepeda-Rivera from the lab of Karine Gibbs framed the T6 as a system for communication that
429 can be viewed for its role in kin recognition. Zepeda-Rivera gave an update on the recognition
430 system used by *Proteus mirabilis*, which is well studied for its swarming motility but also
431 encoded a T6SS at its *tss* locus (68). Similar to observations reported for swarming *B. subtilis* by
432 Polonca Stefanic, the Gibbs group has been using genetic tools and elegant fluorescence
433 microscopy to decipher how the *P. mirabilis* recognition system generates visible kill zones
434 when non-self swarms encounter one another and permits mixing of self swarms. A separate
435 *idsA-F* operon discovered by Gibbs prior (69), encodes several T6-secreted proteins IdsA, IdsB

and IdsD. Zepeda-Rivera presented evidence from the Gibbs' group that the T6-dependent secreted effector IdsD interacts directly in recipient cells with IdsE, with IdsD and IdsE having shared and distinct characteristics of traditional T6 effector-immunity pairs. In contrast to traditional T6 lethal effectors, like Aux5 described by Crisan, delivery of IdsD to non-kin recipients does not lead to reduced viability, but rather a restriction of swarming. Surprisingly, overexpression of IdsE in recipient cells is also sufficient to restrict swarming, by mechanisms to be determined (70). Zepeda-Rivera described her own work on IdsC, (71), which appears to fulfill roles for TAP/TAAR proteins in other systems, acting to inhibit IdsD activity prior to secretion, and serving a chaperone function for loading of the effector to the apparatus.

In contrast to T6SS, which can mediate killing of non-kin by direct contact, secreted antimicrobial compounds like antibiotics produced can act on non-kin from a distance. Gabrielle Grandchamp from Elizabeth Shank's lab described work to identify novel compounds produced by member of soil microbial communities, based on methods established prior (72). Many commonly used antibiotics are derived from the fungus-like soil bacterium Actinomycetes, and genomic analysis predicts that this microbe produces a wide array of secondary metabolites. However, isolation of additional, novel, biologically-active secreted compounds remains challenging because lab conditions are typically insufficient to generate the signal(s) required for expression of novel biosynthetic gene clusters. To capture environmental interactions, Shank's group has developed co-culture methods to screen isolated Actinomycetes adjacent to bacteria such as *S. aureus*, which are obtained from the same soil samples (**Figure 5**). Several actinomycetes only show antibiotic activity against *S. aureus* when grown in coculture. Imaging mass spectrometry (73) to identify the mass of putative antibiotics, followed by fractionation,

may lead to the discovery of novel cell-cell communication molecules of therapeutic significance.

Following the talk by Grandchamp, Gabriel Lozano from Jo Handelsman's group described similar coculturing methods for the discovery of new antibiotics in another member of the actinomycetes. Prior, Handelsman's group isolated from Alaskan boreal forest soil *Streptomyces* sp. 2AW, and then used bioinformatics and biochemical analyses to uncover broad antimicrobial activity, including production of the antibiotic hygromycin A (74). Lorano described his follow-up experiments co-culturing *Streptomyces* sp. 2AW with another soil microbe, *Chromobacterium violaceum*, which produces the purple pigment violacein that has its own antimicrobial properties and is under control of the LuxI/R-like CviI/R QS system. Surprisingly, co-culturing did not lead to lethality of *C. violaceum*, but rather a hygromycin A-dependent stimulation of violaceum production. Screening of transposon mutants of *C. violaceum* with sublethal levels of the secondary metabolite hygromycin A identified a two-component signal transduction system termed *air* (antibiotic-induced response), encoding the AirS sensor and AirR response regulator. RNA-seq confirmed that AirR plays a role in modest upregulation in CviR sufficient for violaceum production. Lorenzo's talk illustrated one of the complexities in deciphering chemical signaling: namely, that secondary metabolites we define as antibiotics for inducing lethality in microbes at high concentrations may also serve as signaling molecules for inter-species interactions at sub-lethal concentrations.

Pseudomonads are also common members of the soil community, and Lucy McCully, from Mark Silby's lab described co-culture experiments with *Pseudomonas fluorescens* Pf0-1 and a

distantly related microbe, *Pedobacter* sp. V48. While both bacteria are sessile and each fail to swarm alone on a solid agar surface, social motility can be observed in co-culture with strains expressing distinct fluorescence tags. The social motility requires contact, as this behavior ceases when the two species are separated by a semi-permeable membrane barrier. High salt also prevents social motility, while enabling growth, providing insights into how microbial interactions are influenced by environmental conditions (75).

While *P. aeruginosa* is a member of polymicrobial soil communities, it receives much attention for its pathogenicity in the lungs of people afflicted with cystic fibrosis. Deborah Hogan showcased the evolving interdomain chemical signaling occurring in within the CF lung where *P. aeruginosa* is often found with the fungus *Candida albicans*, which is typically in a filamentous form rather than yeast cells during infection. Hogan remind the attendees of her earlier work with reference strains PA01 and PA14 showing that *P. aeruginosa* 3-oxo-C12 HSL inhibits *C. albicans* filamentation (76), while *C. albicans* farenesol production inhibits PQS signal production by *Pseudomonas* (77). Hogan then described two recent studies revealing how dynamic interaction are within hosts. In one recent survey by Kim et al. (78) of the fungal microbiome from CF patients, evidence suggests selective pressure of this interdomain signaling within the host. Specifically, many CF yeast isolates obtained from patients have mutations in a regulator, NGR1, which makes them resistant to the repressive effects of *P. aeruginosa* on filamentation. Hogan's group has also followed up on a curious observation in the field that despite the positive effects of LasI/R RhlI/R QS systems on *P. aeruginosa* virulence factor production, clinical isolates with LasR loss-of-function mutations often accumulate as lung function declines (79). Hogan's work (80) suggests that the inability of *lasR* mutants to repress

505 the Anr transcription factor may lead to worse infections, as Anr overexpression promotes
506 factors important for growth in low $O_2$. Hogan's talk, like others, was a poignant reminder of the
507 value of studying cell-cell signaling strains beyond the few lab reference strains that many study.
508
509 Bacteria in chronic infections, like *P. aeruginosa* within the CF lung, often reside in
510 communities comprised of micron-sized, highly dense aggregates (~$10^1$-$10^4$ cells). A critical
511 question is whether QS signaling occurs as a localized event (intra-aggregate) or at the
512 community level (inter-aggregate). To answer this question, Sophie Darch, from the Whiteley
513 lab, used a mixture of micro-3D-printed bacterial traps and naturally formed synthetic CF
514 sputum aggregates to explore the 'calling distance' of QS signals in *P. aeruginosa*. They found
515 that 2 pL traps (~2000 cells) containing signal-producing *P. aeruginosa* were unable to signal
516 neighboring aggregates, while *P. aeruginosa* containing traps with volumes of 5-20 pL signaled
517 aggregates as far away as 140 um. However, not all aggregates responded, suggesting that
518 individual aggregates have differential sensitivity to QS signals. These results show that
519 aggregates must be within 140 um to communicate in synthetic CF sputum, but even if they are
520 positioned within the calling distance of QS signals, not all respond and this is part due to the
521 regulation of *lasR* (81) (**Figure 6**). Using synthetic sputum medium as a model for studying
522 aggregates has allowed us to define the calling distance of QS signals in an environment that
523 closely recapitulates chronic infection inside the CF lung.
524
525 Ian Peak explored the mechanisms of immune-modulatory impacts of QS signal molecules
526 within host organisms. Acyl-homoserine lactones (AHLs) have profound effects on mammalian
527 cells, the best known being 3-O-C12 AHL from *P. aeruginosa*. The medium and longer chain

AHLs (C8-C14) are immunomodulatory, but short chain AHLs (less than C8) have limited immunomodulatory effects. A mammalian cell surface receptor mediating these effects has proved elusive, but a bitter taste receptor (Tas2R38) can respond to both 3-O-C12 and C4 AHL (82). Peak also studies a fatty acid receptor, GPR84, that is upregulated by inflammatory stimuli but appears to play no role in obesity (83). Recent work reveals that GPR84 is also up regulated in response to different AHLs; GPR84 binds immunomodulatory AHLs at high affinity and finally that mammalian cell responses are GPR84-dependent. Together these results suggest that GPR84 represents a pattern recognition receptor, where GPR84 binds and mediates responses to immunomodulatory AHLs.

A series of talks showcased the role of Gram-positive cell-cell communication in health and disease. The Gram-positive, facultative intracellular pathogen *Listeria monocytogenes* invades mammalian cells and escapes from the intracellular vacuoles into the cytosol through the activity of listeriolysin O (LLO). (84). Nancy Freitag discussed her group's discovery (85) that *L. monocytogenes* secretes a peptide pheromone, pPplA, derived from the secretion signal sequence of a lipoprotein of unknown. The pPplA pheromone may participate in diffusion sensing within the confines of the intracellular vacuole because *L. monocytogenes* mutants unable to produce pheromone are impaired in vacuolar escape. Specifically, pheromone deletion mutants are capable of perforating the vacuole through LLO but are defective in fully disrupting the vacuole to gain access to the cytosol. Deletion of *prgX*, encoding a protein that shares homology with an *Enterococcus faecalis* peptide responsive repressor protein PrgX, restores vacuole escape to a Listeria pheromone mutant, suggesting that PrgX may contribute to pheromone sensing within the vacuole.

Streptococcal species utilize a peptide signal termed the competence stimulating peptide (CSP). Using organic synthesis techniques along with cell-based reporter and phenotypic assays, Yftah Tal-Gan described his group's analysis of the CSP signals in *S. pneumoniae* and *S. mutans.* Recent work has revealed critical residues and conformational requirements for effective receptor binding and activation (86). In addition, co-culture competition assays led to the identification of antagonistic relationships between different oral streptococci and provided lead species to investigate for interspecies QS interference. These results highlight that peptide-based tools can be applied to modulate streptococci behavior. From a mechanistic stand point, an alpha helix conformation is required for CSP to effectively bind its transmembrane receptor, ComD in both *S. pneumoniae* and *S. mutans*. Moreover, the *N*-terminus of the CSP signals utilized by *S. pneumoniae* are only needed for receptor activation, thus modifications in these positions lead to competitive inhibitors. Lastly, many interactions between streptococci species that inhabit the same natural niches are antagonistic.

The key dental pathogen *Streptococcus mutans* displays complex regulation of natural genetic competence. Competence development in *S. mutans* is controlled by a peptide derived from ComS (*comX* inducing peptide, XIP); which along with the cytosolic regulator ComR controls the expression of the alternative sigma factor *comX*, the master regulator of competence development. ComR-XIP also activates *comS* to create a positive feedback loop. Justin Kaspar from Robert Burne's group described the discovery of a gene embedded within the coding region of *comX* designated *xrpA* (*comX* regulatory peptide A). XrpA was found to be an antagonist of ComX (87), but the mechanism was not established at the time. Kaspar reported recent progress

showing that XrpA impacts ComRS function, resulting in decreased expression of *comX* and late *com* gene expression, negatively impacting transformability. These results highlight XrpA as a new negative regulator of competence signaling and broaden our understanding of the complex regulatory mechanisms that modulate competence and virulence in *S. mutans*.

Katherine Lemon provided a community ecological perspective to pathogen dynamics, with a focus on 'mining the nasal microbiome'. She reminded attendees that carriage rates within the nasopharynx of bacterial pathogens, e.g., *S. aureus* and *Streptococcus* species are particularly high in children (88). Lemon asked the simple but important question – why do kids only rarely become sick from these organisms? She hypothesized that among the constituents of nostril and throat microbiota, there are beneficial microbes that interfere with pathogen carriage and/or pathogen invasion. Such beneficial bacteria could be the basis for novel small molecule and probiotic therapies to both prevent and treat infections. Working to understand the role and dynamics of human microbiota, Lemon's long-term goal, like many at the conference, is to develop new approaches to manage the composition of the human microbiota in order to prevent infections.

**Why: Ecology and Evolution of Bacterial Social Behaviors**

Recent CCB meetings have embraced the contributions to the field that come from an evolutionary perspective. Josie Chandler presented results studying QS evolution that were obtained with a clever laboratory co-culture model with two soil microbes, *Burkholderia thailandensis* and *C. violaceum. B thailandensis* produces bactobolin to compete with *C.*

*violaceum*; and although *C. violaceum* secretes the known antimicrobial, violacein, (89), another unknown antimicrobial is thought to be necessary for competing with *B thailandensis.* These two bacteria require quorum sensing to activate the production of their antimicrobials. Chandler showed prior that QS-controlled antimicrobial production can provide a competitive advantage to either organism in this co-culture model (90). Her recent work presented at the meeting (91), uncovered that *C. violaceum* also uses quorum sensing to increases transcription of a putative cell-localized multidrug efflux pump, which can be considered a private good. QS-cheaters that neither make bactobolin nor upregulate the presumptive efflux pump are restrained in this co-culture, supporting other studies (92) documenting that production of private goods, along with shared goods, may allow microbes to constrain the evolution of cheaters within populations.

The evolution of *P. aeruginosa* during long term chronic infections of the cystic fibrosis lung, was the topic discussed by Sheyda Azimi from the lab of Steve Diggle, who views QS through a distinctly evolutionary lens (93). Azimi described exciting results from a long term (50-day) evolution experiment with *P. aeruginosa* PA01 that was cultured in synthetic sputum medium that included serial passage of biofilms attached to beads, an approach pioneered by Vaugn Cooper (94). Phenotypically diverse morphotypes emerged quickly and subsequent pairwise co-culture experiments revealed varying levels of cooperation and conflict between the evolved morphotypes (**Figure 7**). Notably, certain populations also showed increased tolerance to certain antibiotics, despite no prior exposure. Whole genome sequencing analysis of select morphotypes identified candidate mutations that will be investigated for their role in mediating interaction that may contribute to increased antibiotic tolerance. This study highlighted the sobering reality that

619 microbial evolution in a host may require customized therapeutic intervention to combat long
620 term chronic infections.
621
622 Since microbes commonly engage in behaviors like secretion that are costly and beneficial to
623 neighbors, microbes have evolved mechanisms to recognize relatives (kin) (95). Like *P.*
624 *mirabilis* discussed by Zepeda-Rivera, Polonca Stefanic detailed how *B. subtilis* cells also
625 engage in kin discrimination, using mechanisms still poorly understood. Like *P. aeruginosa*, *B.*
626 *subtilis* is a common soil bacterium that evolves in the presence of other microbes, both kin and
627 non-kin. Earlier observations revealed that genetically related kin strains of *B. subtilis* swarming
628 on a solid agar plate merge, while non-kin with less genetic similarity collide to create visible
629 boundaries (kill-zones). Dead cells common at the border suggested antagonism between the
630 non-kin (96). In the roots of a host plant, *Arabidopsis thaliana*, electron microscopy indicated
631 that kin and non-kin pairs of strains formed mixed biofilms, where competition between non-kin
632 led to root colonization by a single strain, similar to results presented by Spear on squid
633 colonization by competing T6SS$^{+}$ *V. fischeri*.
634
635 Sam Brown reviewed the functional roles of quorum-sensing in a mixed genotype context and
636 illustrated that the canonical 'positive feedback' QS regulatory architecture allows bacteria to
637 sense the genotypic composition of high-density populations, and limit co-operative
638 investments to social environments enriched for co-operators. Brown and colleagues
639 demonstrated mathematically and experimentally that the observed response rule of 'co-
640 operate when surrounded by co-operators' allows bacteria to match their investment in co-
641 operation to the composition of the group, therefore allowing the maintenance of co-operation

642 at lower levels of population structuring (that is, lower relatedness) (97). Similar behavioural
643 responses have been described in vertebrates under the banner of ‘generalised reciprocity'.
644 These results suggest that mechanisms of reciprocity are not confined to taxa with advanced
645 cognition and can be implemented at the cellular level via positive feedback circuits.
646
647 Wai-Leung Ng opened a broad metabolic perspective on microbial social interactions,
648 highlighting that the consequences or producing toxic metabolic biproducts vary drastically with
649 cell density. Using unbiased metabolomics, Ng's group discovered that *V. cholerae* mutants
650 genetically locked in a low cell density (LCD) QS state are unable to alter the pyruvate flux to
651 convert fermentable carbon sources into neutral acetoin and 2,3-butanediol molecules to offset
652 organic acid production. As a consequence, LCD-locked QS mutants rapidly lose viability when
653 grown with fermentable carbon sources. This key metabolic switch relies on the QS-regulated
654 small RNAs Qrr1-4 but is independent of downstream QS regulators AphA and HapR. Qrr1-4
655 dictate pyruvate flux by translational repression of the enzyme AlsS, which carries out the first
656 step in the biosynthesis of 2,3-butanediol and acetoin. Thus, QS enables *V. cholerae* to switch
657 from a low cell density energy-generating metabolism that is beneficial to individuals at the
658 expense of the environment to a high cell density mode that preserves environmental habitability
659 (98).
660
661 Wrapping up the social evolution session, Joe Sexton from the Schuster group highlighted the
662 role of nutrient limitation in determining the cost of cooperative investment, using siderophores
663 as an experimental model. Using metabolic modeling, Sexton showed that pyoverdine, although
664 energetically costly, incurs a fitness cost only when its building blocks carbon or nitrogen are

665 growth-limiting and are diverted from cellular biomass production, but not when other nutrients
666 are limiting such that building blocks are in relative excess. The results were confirmed
667 experimentally, demonstrating that pyoverdine non-producers (cheaters) enjoy a large fitness
668 advantage in co-culture with producers (co-operators) when limited by carbon, but not when
669 limited by phosphorus (99). The principle of nutrient-dependent fitness costs has implications for
670 the stability of cooperation in contexts beyond pyoverdine.
671
672 Vittoria Venturi discussed the two QS systems that regulate virulence in the plant pathogen
673 *Pseudomonas fuscovaginae,* one of several microbes capable of causing rice sheath brown rot
674 disease. Prior work by his group had identified a locus for both a PfsI/R and PfvI/R QS systems
675 and the biologically active AHLs produced by each QS system (100). Unlike typical LuxI/R–
676 type QS systems, between each synthase and regulator gene is the gene for a repressor protein.
677 Genetic evidence demonstrated the RsaM repressor negatively regulates the PfsR/I locus in
678 which it is encoded, while the RsaL repressor negatively regulates both systems. These repressor
679 are thought to be responsible for observations that the two QS systems were not active in planta
680 but not under lab conditions. The inability to construct a double repressor mutant also suggested
681 that the two QS systems controlled many genes important for interactions within a plant host.
682 Indeed, RNA-seq experiments Venturi described reveal that the RsaM regulon controls hundreds
683 of genes, many of which are predicted to encode virulence factors, including genes for a T6SS
684 (101). Understanding how *P. fuscovaginae* uses its QS systems to interacts with commensal
685 partners and the host plant is valuable for understanding plant disease and may provide insights
686 regarding the cell-cell interactions occurring with the microbiome of the human gut.
687

Phr signaling peptides first introduced to the conference by Eldar and Bischofs are secreted by *B. subtilis* and then transported back into the cell where they interact with Rap phosphatase receptors, which in turn dephosphorylate numerous response regulators controlling behaviors including quorum sensing, natural competence and sporulation. Sequenced *B. subtilis* reference strain 168 (102) has eleven chromosomally encoded two-component Rap-Phr systems predicted to allow adaptation of populations to diverse ecological niches (103). Recently, a *B. subtilis* gastrointestinal strain was isolated that sporulates with high efficiency due to the loss of several *rap* genes that modulate phosphorylation of the Spo0A transcription factor of sporulation (104). Akos Kovacs described a Herculean experimental competition set-up to study conditions where particular Rap-Phr systems provided an advantage. This was accomplished by competition of bar-coded single and double *rap-phr* mutants with wildtype *B. subtilis* under different conditions for various amounts of time. Results of high throughput sequencing of the evolved populations suggest certain sets of Rap-Phr favored by distinct selective pressures.

The final speaker for the CCCB-6 conference was Marvin Whiteley, who described his lab's on-going efforts to develop robust model systems that accurately reflect in vivo conditions encountered by *P. aeruginosa* during infection within the CF lung. Whiteley frankly and unabashedly admitted his skepticism regarding how well in vitro monoculture results in test tubes can reflect the dynamic complex in vivo environments. His group itself has evolved from explorations of the contributions of CF sputum cues (105) to the utilization of synthetic sputum medium (106), and current co-culture experiments with other microbes *P. aeruginosa* encounters in the lung. Like the work of Hogan and other speakers described here, Whiteley's group is discovering different sets of genes required by *P. aeruginosa* is grown alone or when in co-

culture with *S. aureus*, which is often found in chronic lung infections. Whitely also introduced the attendees to the concept he has termed the "biogeography of polymicrobial infections", reflecting not only the importance of microbial composition, but also the spatial organization of community members (107). Indeed, Whiteley's talk was a fitting bookend to the 6$^{th}$ CCCB meeting that showed how much we have progressed in the field, and also how much more there is to discover regarding cell-cell communication among bacteria.

**Concluding Remarks:**

The sixth Cell-Cell Communication in Bacteria conference highlighted that the study of microbial interactions cuts across diverse areas of microbiology and is enriched by different approaches and insights drawn from chemistry, physics, mathematics, and computer science. The oral presentations described here, as well as the poster presentations on display, showcased the broad consequences of cell-cell communication for numerous human concerns, from the healthy functioning of human and environmental microbiomes to the causes and novel treatments of infectious disease. At the first conference in 2001, quorum sensing and cell-cell communication were largely viewed as synonymous. In the ensuing years we have begun to appreciate that perhaps “quorum sensing” and indeed “communication” are components of the ubiquitous, varied interactions in which microbes engage. Future conferences on this theme will invariably reveal more astounding revelations regarding the challenges and potential of this exciting field of study.

**Acknowledgements:**

We thank the program cochairs Eric Stabb and Beth Lazazzera, the American Society for Microbiology, and members of the program advisory committee for developing an exciting program and for securing the necessary funding. We appreciate the assistance provided by Rosie Alegado, James Boedicker, Ilka Bischofs, Nancy Freitag, and Paul Williams in the preparation of the manuscript. S.P.B is supported by the Centers for Disease Control (2017-OADS-01), the Simons Foundation (396001), and the Human Frontier Science Program (RGP0011). H.E.B is supported by H.E.B is supported by the National Institutes of Health (GM109403; AI135745), the National Science Foundation (CHE-1708714), and the Office of Naval Research (N00014-16-1-2185). B.K.H is supported by the National Science Foundation (MCB-1149925), the Binational Science Foundation (2015103), and the Gordon and Betty Moore Foundation (6790.13).

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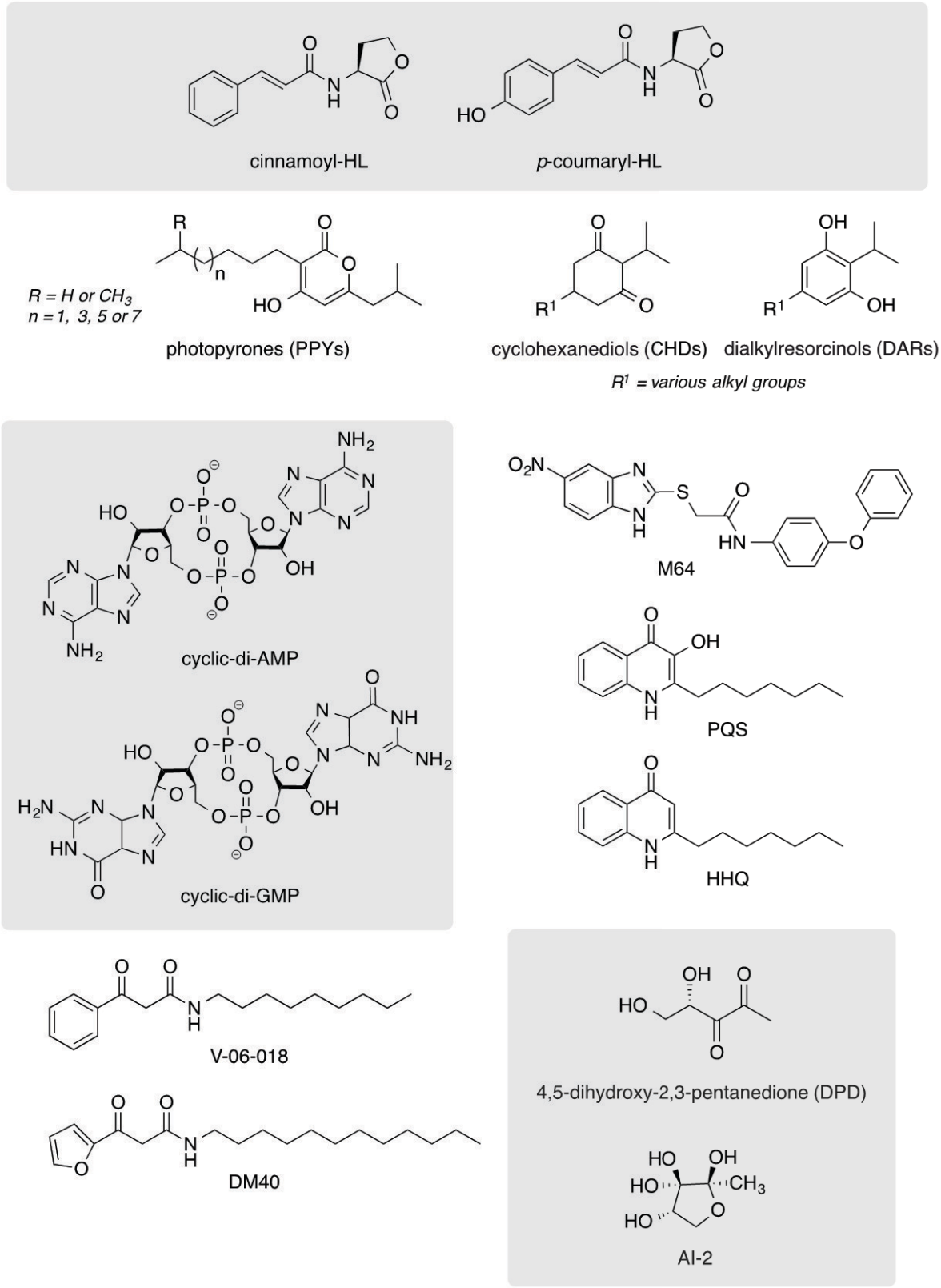


**Figure 1**. Selected structures of compounds described at the meeting.

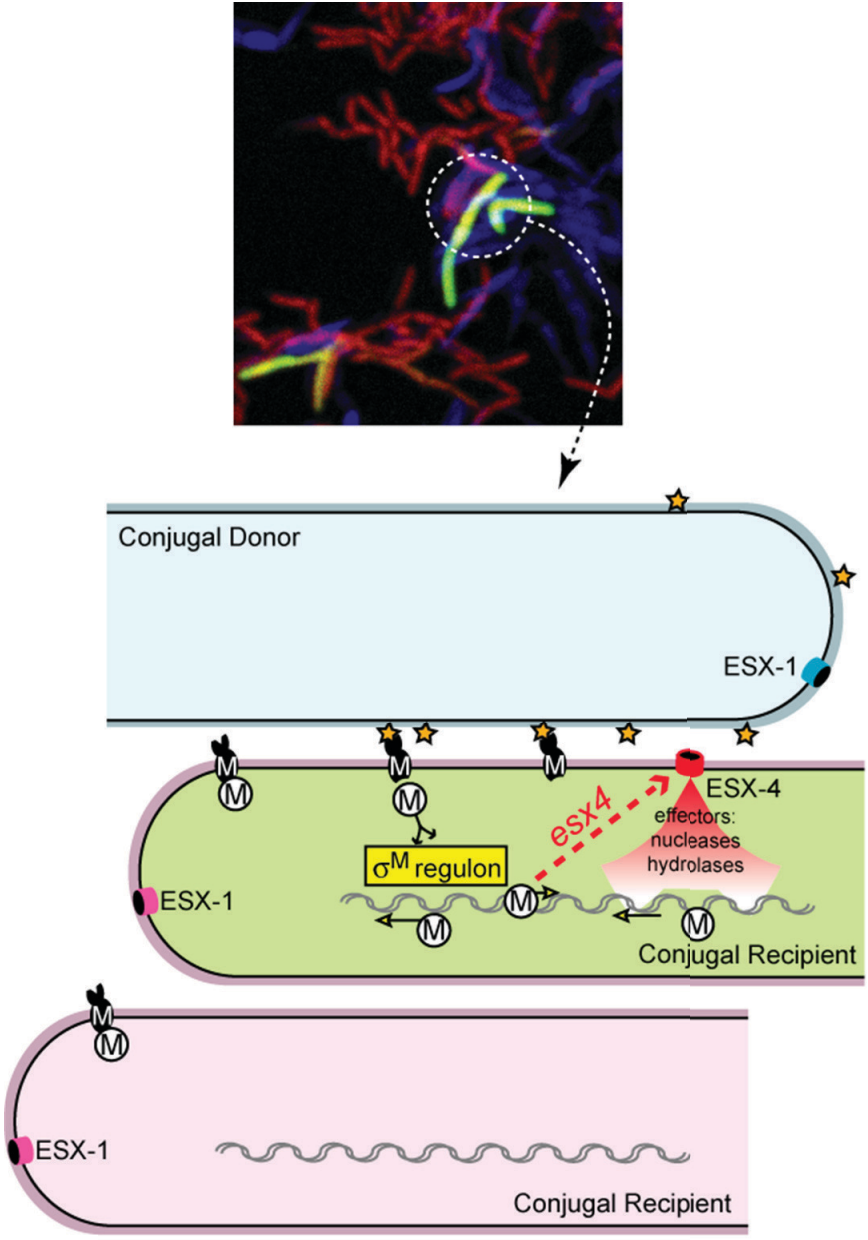


**Figure 2.** Schematic of conjugation process in M. smegmatis. The confocal image shows two strains of *Mycobacterium smegmatis* that engage in chromosomal conjugation. The blue (EBFP2) strain is the conjugal donor. The conjugal recipient constitutively expresses mCherry, and also has EGFP driven by a promoter from the ESX-4 secretion system gene cluster that responds to the extracytoplasmic sigma factor, SigM. The schematic interpretation shows a working model of 1) donor and recipient recognition is determined by their respective ESX-1 secretion systems, 2) the transmembrane anti-SigM (black with white M) in the recipient detects donor contact, releasing SigM, 3) SigM induces a small regulon focused on the ESX-4 secretion system, and 4) SigM's activation of ESX-4 is required for acquisition of donor DNA by the recipient cell. In summary, it shows that anti-SigM detects cell-cell contact and that SigM generates an appropriate response by inducing the ESX-4 secretion system required for conjugation between the two strains. Graphic courtesy of T. Gray.

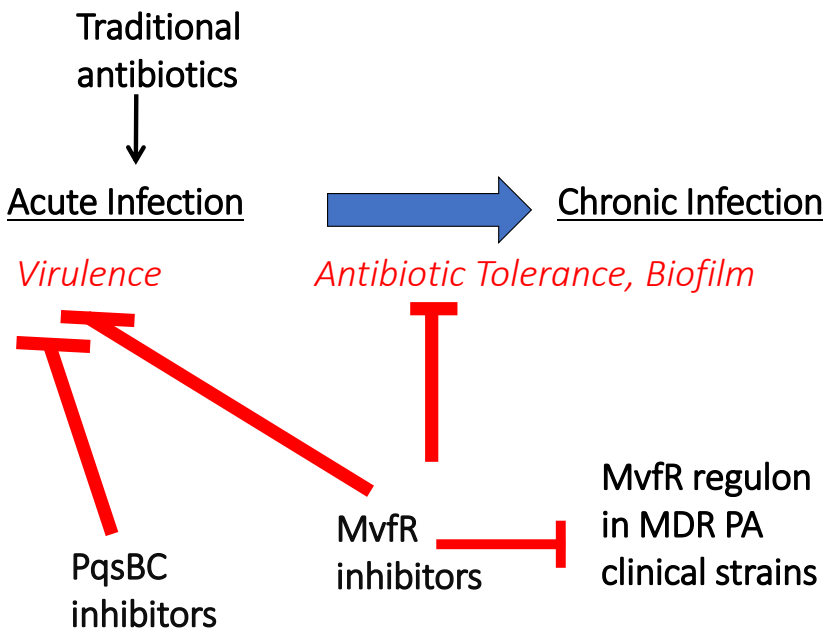


**Figure 3.** Pathways to block virulence via MvfR (PqsR) in *P. aeruginosa* contrasted with traditional antibiotic pathways. Graphic courtesy of L. Rahme.

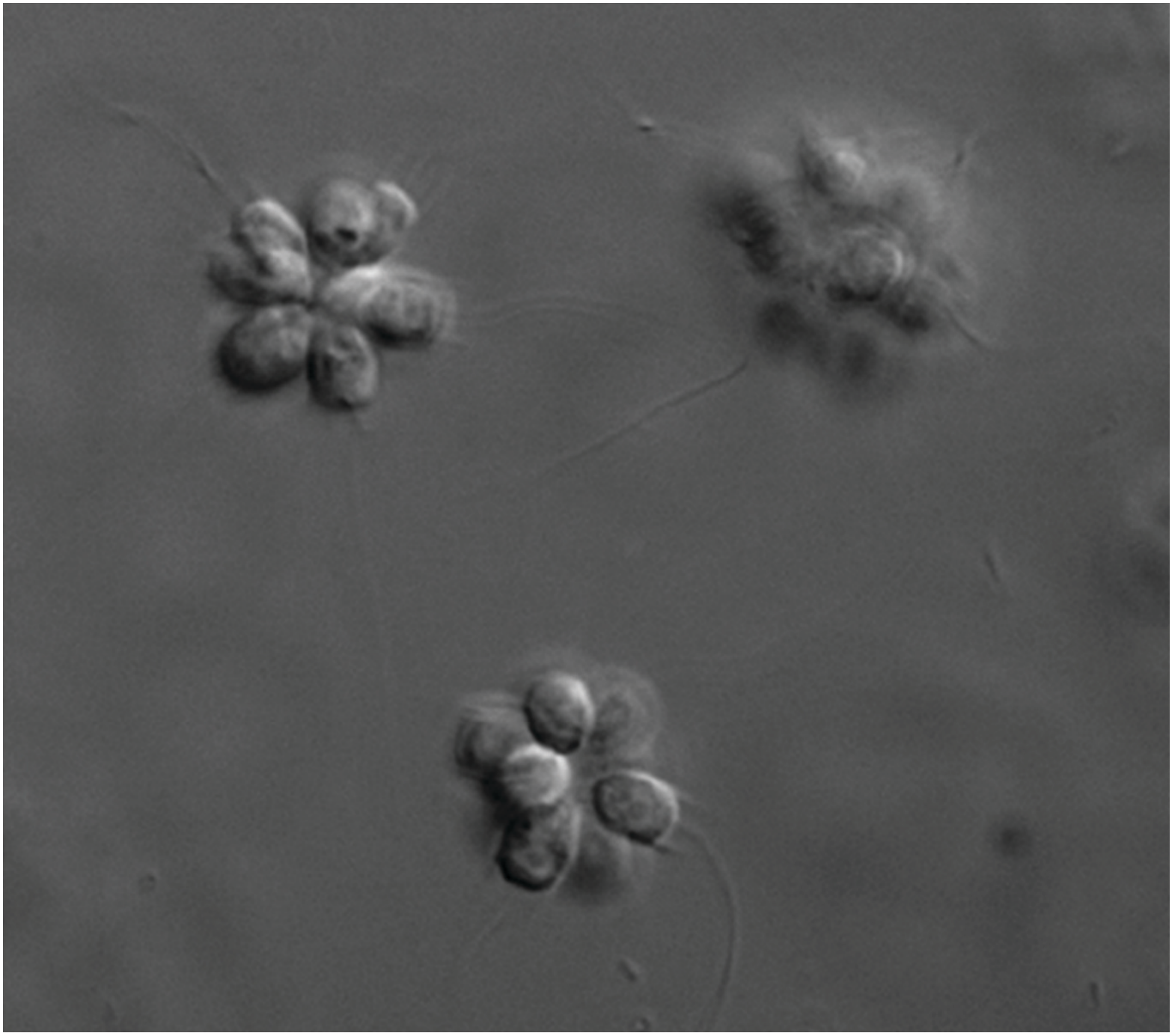

**Figure 4.** The bacterium *Algoriphagus machipongonensis* induces multicellular rosette development in the eukaryotic choanoflagellate *Salpingoeca rosetta*. Photo courtesy of R. Alegado.

**Figure 5.** A *Streptomyces* sp. (left) co-cultured on solid growth medium with a *Nocardia* sp (right). Photo courtesy of G. Grandchamp & E. Shank.

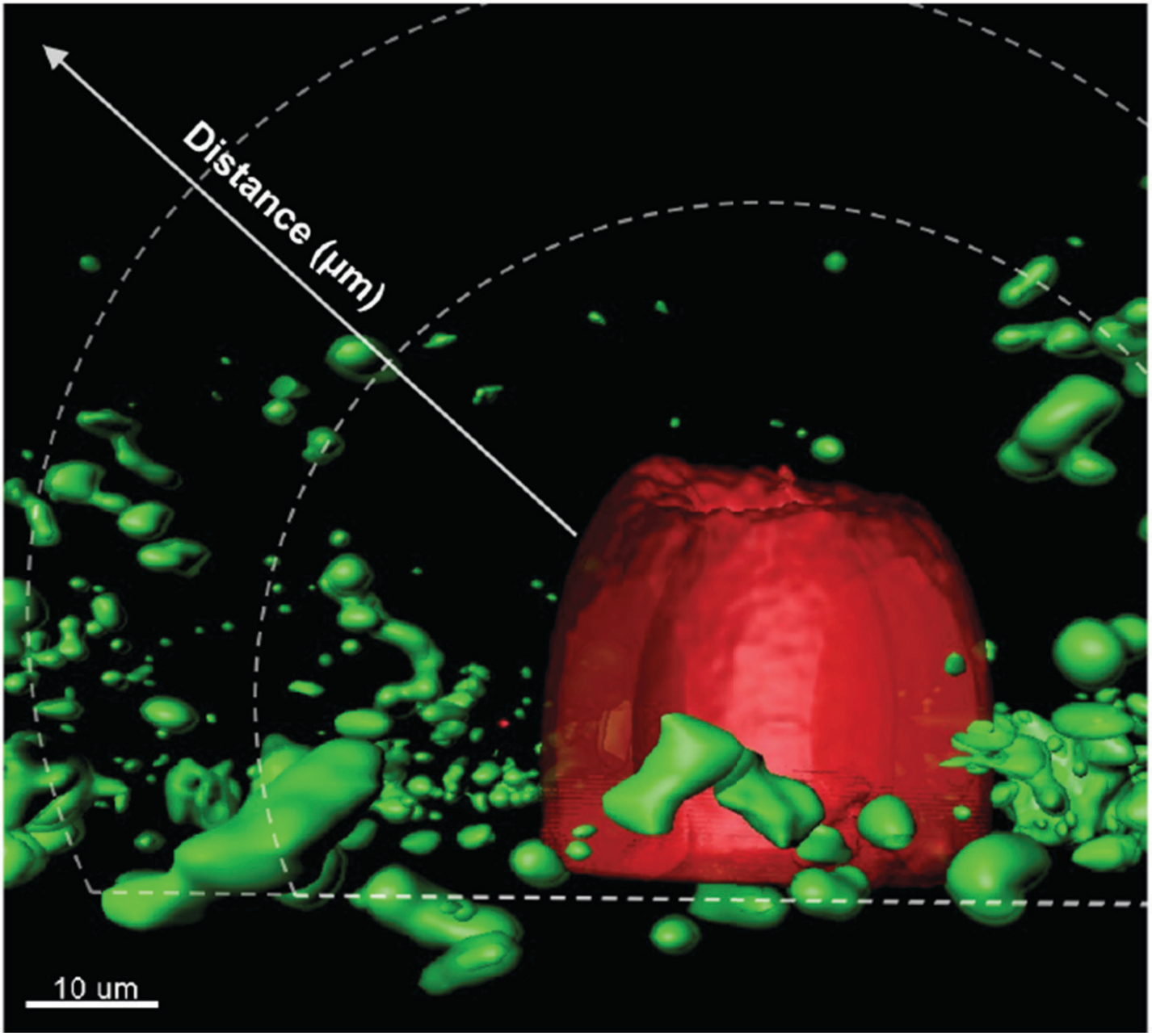


**Figure 6.** In synthetic CF sputum, *P. aeruginosa* responder aggregates express green fluorescent protein (green) in response to QS signals made by micro-3D-printed *P. aeruginosa* producer aggregates (red), allowing the determination of QS calling distances, as described in (81). Image courtesy of S. Darch and M. Whiteley.

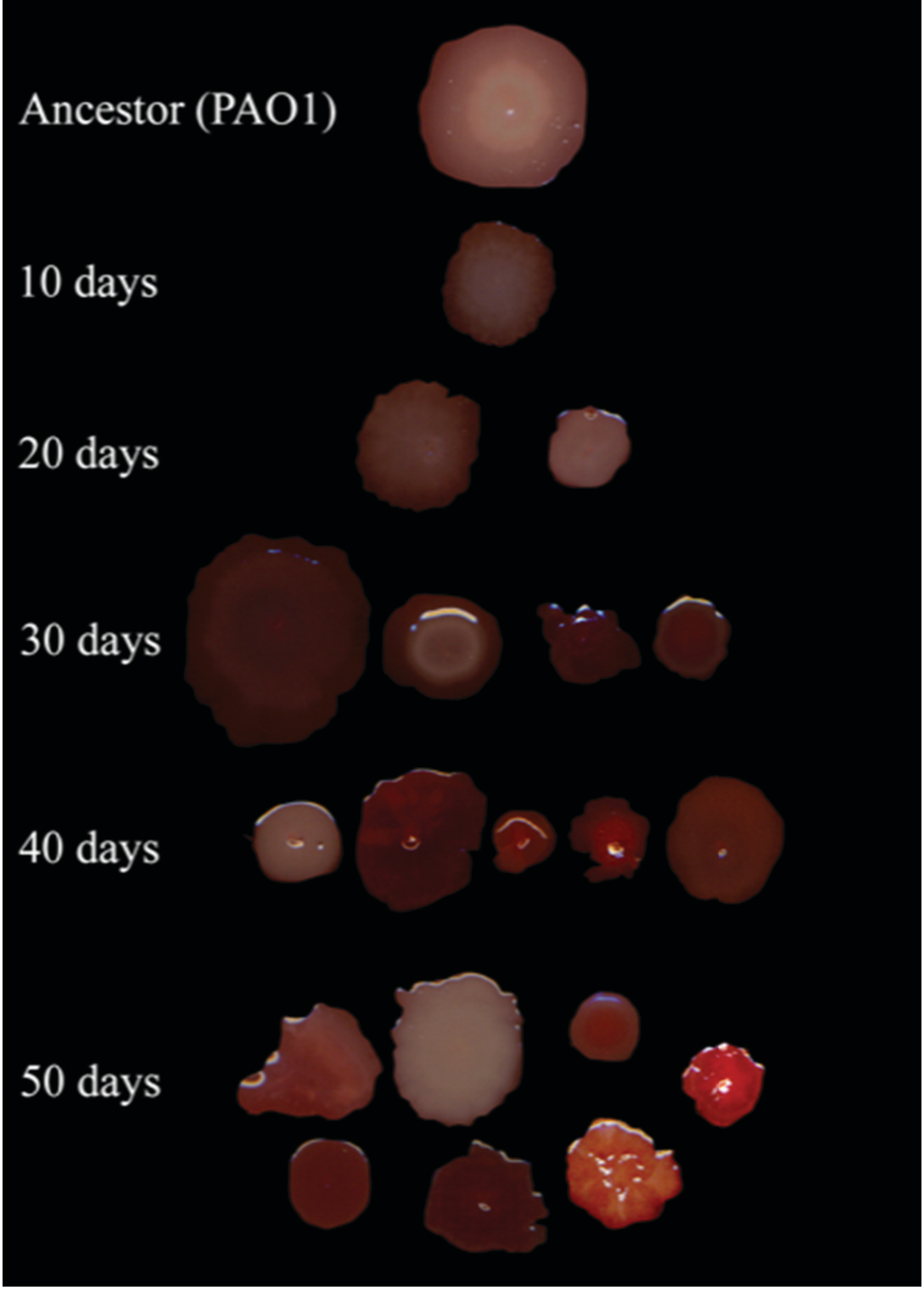


**Figure 7.** *Pseudomonas aeruginosa* strain PAO1 was evolved in a synthetic CF sputum media and plastic beads biofilms for 50 days. Using a Congo Red agar-based media, an increase in number of various colony morphologies was observed after 20 days of selection on biofilms. Image courtesy of S. Azimi and S. Diggle.