**FEMS Microbiology Letters: Mini-Review**

# The expanding horizon of alkyl quinolone signalling and communication in polycellular interactomes

F. Jerry Reen[1]*, Gerard P. McGlacken[2], and Fergal O'Gara[3,4]

[1] School of Microbiology, University College Cork, Cork, Ireland.

[2] School of Chemistry and Analytical & Biological Chemistry Research Facility (ABCRF), University College Cork, Ireland.

[3] BIOMERIT Research Centre, School of Microbiology, University College Cork, Cork, Ireland.

[4] Human Microbiome Programme, School of Pharmacy and Biomedical Sciences, Curtin Health Innovation Research Institute, Curtin University, Perth, WA 6102

**Keywords:** *Pseudomonas aeruginosa*; Pseudomonas Quinolone Signal (PQS), Alkylquinolones; Anti-infectives; Interspecies signalling; Quorum sensing:

*****To whom correspondence should be addressed. Mailing address: School of Microbiology, University College Cork, Ireland. Phone number: + 353-21-4901330; Fax number: + 353-21-4903101; E. mail: j.reen@ucc.ie.

## ABSTRACT

Population dynamics within natural ecosystems is underpinned by microbial diversity and the heterogeneity of host-microbe and microbe-microbe interactions. Small molecule signals that intersperse between species have been shown to govern many virulence-related processes in established and emerging pathogens. Understanding the capacity of microbes to decode diverse languages and adapt to the presence of 'non-self' cells, will provide an important new direction to the understanding of the 'polycellular' interactome. Alkyl Quinolones (AQs) have been described in the ESKAPE pathogen *Pseudomonas aeruginosa*, the primary agent associated with mortality in patients with Cystic Fibrosis and the third most prevalent nosocomial pathogen worldwide. The role of these molecules in governing the physiology and virulence of *P. aeruginosa* and other pathogens has received considerable attention, while a role in interspecies and interkingdom communication has recently emerged. Herein we discuss recent advances in our understanding of AQ signalling and communication in the context of microbe-microbe and microbe-host interactions. The integrated knowledge from these systems-based investigations will facilitate the development of new therapeutics based on the AQ framework that serve to disarm the pathogenesis of *P. aeruginosa* and competing pathogens.

## BACKGROUND

Prokaryotes and eukaryotes have coexisted and coevolved for millions of years. Though higher order eukaryotes have evolved an array of communicative mechanisms, small molecule signalling remains an integral element in the microbe-microbe and microbe-host interactomes. Deciphering these molecular interactions, their spatio-temporal dynamics, and the hierarchical structure with which they modulate community behaviour remains a major

challenge. One of the best studied forms of bacterial small molecular communication is termed quorum sensing (Fuqua *et al.*, 1994). Initially described as a process characterised by auto-inducing small molecules that govern cellular behaviour in response to a particular cell density or quorum, our understanding of QS signalling has expanded rapidly in recent years (Whiteley *et al.*, 2017). In describing molecular interactions that govern microbial cell-cell communication, we are required to carefully assign behaviours and distinguish between signalling, coercion, and cues (Diggle *et al.*, 2007). This is particularly true where quorum sensing based interactions are not restricted to bacterial cell-cell communication, but are also involved in communication with higher order eukaryotes and mammalian cells.

Alkyl quinolones (AQs) are a species-specific class of quorum sensing molecule that have been described in *P. aeruginosa* (Pesci *et al.*, 1999, McGrath *et al.*, 2004), and related bacteria including *P. putida* and *Burkholderia* spp. (Diggle *et al.*, 2006). More than 55 distinct AQs are produced through the PqsABCDE biosynthetic pathway in *P. aeruginosa*, with the majority of the diversity arising from unsaturation, different alkyl chain lengths, and modification of the ring-substituted nitrogen (Deziel *et al.*, 2004, Dulcey *et al.*, 2013). An insight into the evolutionary basis of AQ diversity has emerged from *Burkholderia thailandensisis* where two AQ analogues (HQNO and a methylated HMNQ) were shown to act synergistically to inhibit bacterial growth (Wu & Seyedsayamdost, 2017). AQs exhibit a broad spectrum of functions including cell-cell signalling, redox activity, iron-chelation, and antimicrobial activity (Deziel *et al.*, 2004, Bredenbruch *et al.*, 2006, Diggle *et al.*, 2007, Diggle *et al.*, 2007). The two AQ quorum sensing signal molecules have been identified as 2-heptyl-3-hydroxy-4(1H)-quinolone (also known as the Pseudomonas Quinolone Signal, PQS) and its biological precursor 2-heptyl-4(1H)-quinolone (HHQ). PQS is generated from HHQ through the action of the distantly encoded PqsH monooxygenase (Deziel *et al.*, 2004). The role of other AQs such as HQNO and DHQ in *P. aeruginosa* physiology and pathogenesis is

unclear. In this mini-review, we summarise recent advances in understanding the regulation and mode of action of AQs. We discuss new knowledge that has changed perspectives on the interactivity of AQs and discuss innovative initiatives in the design of potent novel anti-infective therapies.

## MULTI-SYSTEM CONTROL OF AQ SIGNALLING

Control of AQ biosynthesis is controlled at the transcriptional level directly through the action of PqsR (referred to as MvfR in the PA14 strain). PqsR is a member of the LysR Type Transcriptional Regulator (LTTR), of which *P. aeruginosa* encodes approximately 125 individual proteins, with PqsR being amongst the most evolutionarily constrained (Reen *et al.*, 2013). The activity of PqsR is controlled via auto-induction by HHQ and PQS, while the *pqsR* promoter itself has been shown to fall under the control of two separate promoter sites, distal and proximal (Farrow & Pesci, 2017). LasR-mediated activation has been shown to occur at a distal promoter site, which can be antagonised by the activation of another LTTR, CysB (Farrow *et al.*, 2015). The proximal promoter site also contributes to activation of PqsR, with initiation at this site inhibited by a negative regulatory sequence element, and potentially by the H-NS family members MvaT and MvaU (Farrow & Pesci, 2017). The authors propose that this arrangement could allow for dual information processing from both environmental signals and cell-cell communication. Small RNAs (sRNAs) such as PhrS, PrrF and ReaL, are also known to fine tune AQ production in response to environmental signals. PqsR activation is influenced by PhrS, which responds to oxygen levels (Sonnleitner *et al.*, 2011). Iron homeostasis in *P. aeruginosa* is maintained in part by the PrrF sRNAs which were recently shown to promote the production of PQS through repression of another LTTR protein AntR, an activator of genes involved in degradation of the AQ precursor anthranilate (Reinhart *et al.*, 2015, Reinhart *et al.*, 2017). Yet another sRNA, ReaL, links Las and PQS signalling through post-transcriptional regulation of PqsC (Carloni *et al.*, 2017).

Complex regulation also extends to other elements of the AQ signalling system (**Figure 1**). RhlR was found to bind an alternative transcriptional start site to PqsR, resulting in the formation of secondary structure in the 5' untranslated region of the *pqsA* promoter (Brouwer *et al.*, 2014). A novel AraC regulator CdpR (*PA2588*) was found to regulate *pqsH* in addition to itself through interaction with the ClpAS-P system (Zhao *et al.*, 2016). A host of other regulatory elements have been shown to influence AQ production. These include RpoN [mediating carbapenem tolerance through PQS and PqsE (Viducic *et al.*, 2017)], DesB [controlling AQ synthesis through modulation of the MexEF-OprN efflux pump (Kim *et al.*, 2015)], the alarmone signal (p)ppGpp [significantly modulating the AHL and PQS quorum sensing hierarchy (Schafhauser *et al.*, 2014)], and QapR [modulating PQS production through the *qapR* operon and *PA5507* (Tipton *et al.*, 2015)]. Host factors such as serum, antimicrobial peptides, dynorphin, and bile have also been shown to promote PQS production (Zaborina *et al.*, 2007, Cummins *et al.*, 2009, Reen *et al.*, 2012, Strempel *et al.*, 2013, Kruczek *et al.*, 2014, Reen *et al.*, 2016) as has cigarette and e-cigarette smoke (Gallagher *et al.*, 2017). In contrast, the C-natriuretic peptide hormone suppressed PQS levels (Blier *et al.*, 2011).

Analysis of global transcriptomics has provided some further insights into the regulation of AQ signalling. PqsE emerged as a key factor involved in the regulation of a repertoire of diverse genes encoding factors involved in biofilm formation and virulence, while there was further evidence for the link between PQS and iron (Rampioni *et al.*, 2016). HQNO did not influence transcription, providing further evidence that perhaps it is not a true QS signal molecule (Rampioni *et al.*, 2016). Although the function of PqsR has been suggested to be restricted to the *pqsA* promoter, it has recently been suggested that multiple promoters may interact with the AQ master regulator (Maura *et al.*, 2016). It is therefore likely that the complexity of the regulation of AQ production in response to cell-cell communication and environmental factors is only beginning to be understood.

## AQ CHEMICAL MESSAGING IN THE POLYCELLULAR INTERACTOME

### AQ signalling in nosocomial pathogens

AQs are known to control virulence and pathogenesis in *P. aeruginosa* both dependent and independent of the central regulator PqsR. In all, an estimated 12% of the genes present in the *P. aeruginosa* genome are known to fall under the control of the hierarchical QS network (Schuster *et al.*, 2003, Deziel *et al.*, 2005). The mechanism through which AQs achieve such an exquisite level of control within multicellular aggregates requires intercellular communication. While HHQ is reportedly able to diffuse into the extracellular environment or can be exported through the MexEF-OprN efflux pump (Lamarche & Deziel, 2011), export of PQS has been shown to be intrinsically linked to strain-dependent outer membrane vesicle (OMV) formation (Florez *et al.*, 2017). Several groups have reported a role for PQS in OMV formation in *P. aeruginosa* and other pathogens (Mashburn-Warren *et al.*, 2008, Tashiro *et al.*, 2010, Lin *et al.*, 2017). Schertzer and co-workers proposed the bilayer-couple model for OMV biogenesis, where PQS intercalates into the outer membrane causing expansion of the outer leaflet and consequently the induction of curvature in a strain dependent manner (Schertzer & Whiteley, 2012, Florez *et al.*, 2017). However, a number of other studies have reported that PQS is not an absolute requirement for MV production in planktonic cultures (Macdonald & Kuehn, 2013, Turnbull *et al.*, 2016), particularly under anaerobic conditions (Toyofuku *et al.*, 2014).

Much of the attention on AQ signalling in *P. aeruginosa* has focused on the interaction of HHQ and PQS with PqsR (reviewed recently by (Sams *et al.*, 2016)). Interplay between PQS and the quorum sensing regulator VqsR has also been reported and shown to mediate carbapenem tolerance through the action of the sigma factor RpoS (Viducic *et al.*, 2017). More recently, immobilised PQS probes were used to identify new interacting proteins, with

MexG and MgtA being implicated as binding partners of PQS (Hodgkinson *et al.*, 2016). In a subsequent study using photoaffinity probes, additional PQS binding partners were identified, including RhlR, PqsD, WbpB and FtsZ (Baker *et al.*, 2017). The latter two proteins were also found to interact with HHQ. These studies add a further layer of complexity to the AQ signalling system, and how these new signal-protein interactions impact on the classical models of QS regulation in *P. aeruginosa* remain to be seen.

The role of other AQs in modulating *P. aeruginosa* behaviour has been less extensively studied. The function of HQNO in *P. aeruginosa* cellular physiology remains to be elucidated, as does the mechanism by which self-poisoning is avoided (Rampioni *et al.*, 2016). Rather than simply protecting itself from the anti-respiratory activity of HQNO, it is possible that subpopulations of *P. aeruginosa* may be targeted, in a similar manner to the stochastic effects of PQS that have been described (Haussler & Becker, 2008). Hazan *et al* have described how auto poisoning of the respiratory chain by HQNO can promote biofilm formation and antibiotic tolerance (Hazan *et al.*, 2016). It is possible that HQNO may provide *P. aeruginosa* with an evolutionary mechanism to sacrifice the few for the greater benefit of the population.

Unlike PQS, DHQ production does not require oxygen, and therefore its synthesis in low-oxygen environments such as the lungs of patients with CF would be of particular interest. Lepine and colleagues reported that DHQ did not influence *pqsA-lacZ* activity in *P. aeruginosa*, while exogenous DHQ had no effect on the production of AQs or the blue phenazine pyocyanin (Lepine *et al.*, 2007). More recently, Gruber et al have shown that DHQ binds to PqsR, activating transcription of the *pqs* operon, and influencing pyocyanin production in *P. aeruginosa* (Gruber *et al.*, 2016). It is worth noting that 100 µM DHQ was required to elicit a 60% increase in *pqsA* expression relative to carrier control in an *E. coli*

reporter strain, compared with 110% activation by 1 μM PQS (Gruber *et al.*, 2016). The apparent disparity between these studies may be attributed to dose dependent effects.

**AQs as modulators of interspecies behaviour**

Apart from controlling cell-cell communication within the growing population of *P. aeruginosa* cells, AQs are also proposed to play a prominent role in facilitating the emergence of *P. aeruginosa* within complex microbial communities. PQS and HHQ were found to modulate the virulence behaviour of a range of bacterial pathogens. Swarming motility was affected in response to PQS, while HHQ suppressed biofilm formation in the gram positive pathogen *Bacillus atropheaus* (Reen *et al.*, 2011). HHQ was also shown to be selectively bacteriostatic to several gram negative species including *Vibrio* spp., although *V. parahaemolyticus* was unaffected. Structure activity relationship (SAR) analysis revealed the C-3 position of HHQ to be important in the anti-biofilm activity of this compound (McGlacken *et al.*, 2010, Reen *et al.*, 2012, Reen *et al.*, 2015). Although neither PQS nor HHQ had any effect on the growth of *Burkholderia cenocepacia*, the antagonistic activity of *P. aeruginosa* supernatants against this important pathogen was shown to be dependent on an intact AQ signalling system (Costello *et al.*, 2014). Fernández-Piñar and colleagues also reported an interspecies dimension to AQ signal molecules with biofilm formation, swarming, and iron uptake affected in *P. putida* (Fernandez-Pinar *et al.*, 2011), while Inaba et al., reported that PQS could inhibit biofilm formation in *Streptococcus mutans* (Inaba *et al.*, 2015). Furthermore, Toyufoku et al., showed that PQS could affect growth of gram negative and gram positive bacteria, owing at least in part to its iron-trap activity (Toyofuku *et al.*, 2010). Indeed, the ability of PQS to chelate or trap iron is a central factor in its biological activity, being also implicated in the ‘red-death’ killing of *Caenorhabditis elegans* (Zaborin *et al.*, 2009).

The dominance of *P. aeruginosa* within polymicrobial communities more than likely depends on the action of several AQs. The antimicrobial activity of *P. aeruginosa* against *Staphylococcus aureus* was shown to be enhanced by iron depletion and was dependent on multiple AQ metabolites (Filkins *et al.*, 2015, Nguyen *et al.*, 2015, Nguyen *et al.*, 2016). *P. aeruginosa* can scavenge iron through AQ-dependent lysis of *S. aureus* (Mashburn *et al.*, 2005), with AQ production itself stimulated by resulting peptidoglycan released from *S. aureus* (Korgaonkar *et al.*, 2013). *S. aureus* can mount its own challenge to *P. aeruginosa* competition through suppression of virulence, antibiotic tolerance, and growth, depending on the functionality of the strains QS signalling system (Korgaonkar *et al.*, 2013, Frydenlund Michelsen *et al.*, 2016). Furthermore, proto-cooperative interactions between *P. aeruginosa* and *S. aureus* have also been reported (Frydenlund Michelsen *et al.*, 2016), perhaps a reflection of the adaptive heterogeneity reported among clinical isolates (Markussen *et al.*, 2014, Winstanley *et al.*, 2016). Rather than the antagonistic interactions described above, the DK2-P2M24-2003 lung adapted isolate altered its AQ production and protected *S. aureus* from the killing effect of tobramycin. The ability of DK2-P2M24-2003 and *S. aureus* to co-exist could be due to the fact that no pyocyanin, rhamnolipids, or HQNO were detected in the former, in contrast to the model strains used in most studies (Frydenlund Michelsen *et al.*, 2016). However, Orazi & O'Toole have subsequently shown that *P. aeruginosa* supernatants protect *S. aureus* from vancomycin challenge, this time implicating HQNO as the active component (Orazi & O'Toole, 2017). Another study using clinical isolates from patients with CF implicated both PQS and HQNO in the stimulation of *S. aureus* biofilm formation, although this effect was lost in co-existing isolates (Fugere *et al.*, 2014). Together, these studies suggest specific adaptations occur within microbial communities, further emphasising the necessity to interrogate interactions in multiple clinical isolates before the dynamics of a particular interaction can be established. They also serve to highlight the impact that

interspecies interactions can have on antibiotic efficacy, of critical importance in light of the pending 'perfect storm' of antibiotic resistance and the insufficient antibiotic development pipeline (Cooper & Shlaes, 2011).

PQS has also recently been shown to promote the evolution of resistance to parasitic bacteriophages (Moreau *et al.*, 2017). Addition of exogenous PQS improved the growth of non-signalling bacteria in the presence of phage. It follows that the loss of QS within a population could have a previously unforeseen fitness cost whereby cells would be more prone to phage attack. Production of PQS could itself be driven by phage attack, as seen when *P. aeruginosa* was challenged with YuA, 14-1 and LUZ24 phage (De Smet *et al.*, 2016). Therefore, *P. aeruginosa* appears to utilise the AQ system as a primed attack response, much in the same way it does when faced with antibiotic challenge or microbial competition.

Evidence for the importance of AQ chemical messages in microbial communities has also come from the observation that co-colonising microbes have developed mechanisms to disarm the threat of AQ signalling and its associated secondary metabolite production (**Figure 2**). Pustelny et al described the action of an *Arthrobacter* dioxygenase HodC against PQS (Pustelny *et al.*, 2009). *Mycobacterium abcessus* and *Rhodococcus erythropolis* were subsequently shown to degrade HHQ and PQS, potentially through the action of a novel *aqd* gene cluster (Muller *et al.*, 2015, Birmes *et al.*, 2017), as could *Achromobacter xylosidans* (Soh *et al.*, 2015). *Arthrobacter*, *Rhodococcus* and *S. aureus* were all shown to modify HQNO by incorporation of a hydroxyl group at the C-3 position (Thierbach *et al.*, 2017). While quenching may be an attractive anti-virulence strategy, there are limitations. PQS quenching by HodC has been shown to increase *P. aeruginosa* biofilm formation as a result of increased iron availability (Tettmann *et al.*, 2016), a factor that may also influence the pathogenesis of competing organisms within the polymicrobial community.

**An inter-kingdom and host-microbe dimension to AQ-based communication**

The interspecies dimension to AQ communication suggested a central role in the competitiveness of *P. aeruginosa* within the complex polycellular ecosystem that is the human host (**Figure 2**). To be truly effective, AQs would require the ability to govern cellular behaviour at the interkingdom level, and studies in *Candida albicans* provided evidence that this may indeed be the case (Cugini *et al.*, 2007, McAlester *et al.*, 2008, Cugini *et al.*, 2010). Cugini and co-workers reported that farnesol, a sesquiterpene compound secreted by *C. albicans* could suppress AQ signalling in *P. aeruginosa* by binding to PqsR resulting in a non-optimal complex (Cugini *et al.*, 2007). A follow on study by the same group showed that farnesol could enhance PQS production and restore pyocyanin in a *lasR* mutant, this time through RhlR-dependent stimulation of *pqsH* transcription (Cugini *et al.*, 2010). HHQ was subsequently shown to antagonise *C. albicans* biofilm formation, while PQS elicited a marginal increase (Reen *et al.*, 2011). More recently, both HHQ and PQS were shown to suppress biofilm formation in clinical isolates of the respiratory fungal pathogen *Aspergillus fumigatus* (Reen et al., 2016).

AQ molecules have also been shown to play an important role in the host-microbe interaction, particularly in disrupting immune homeostasis in host cells. PQS was first shown to modulate cell proliferation, the production of interleukin-2 (IL-2) and TNFα in mitogen-stimulated human peripheral blood mononuclear cells (Hooi *et al.*, 2004). PQS was later shown to inhibit the production of IL-12 by LPS-stimulated bone marrow derived dendritic cells (Skindersoe *et al.*, 2009). Kim et al provided some insight into the transcriptional changes elicited by AQs revealing that both HHQ and PQS down-regulated the host immune response through inhibition of NF-κB (Kim *et al.*, 2010). At the same time, PQS was shown to destabilise the HIF-1α subunit of the hypoxia inducible factor-1 (HIF-1) which is known to govern the hypoxic response in host cells, in addition to being a key regulatory element of the

immune response (Legendre *et al.*, 2012). Secretion of PQS may therefore facilitate evasion of host defences and prevent the HIF-1 dependent resolution of acute inflammation (Campbell *et al.*, 2014). Mutation of *pqsA* led to a reduction in, but not loss of, HIF-1α destabilisation activity, thus implicating other but as yet uncharacterised AQs in this interaction. (Legendre *et al.*, 2012). Loss of *pqsA-E* resulted in attenuation of inflammation, tissue destruction, and reduced levels of pro-inflammatory cytokines at the site of infection in mice (Bala *et al.*, 2014). Neutrophil infiltration was influenced by PQS via the MAPK and p38 signalling pathways in a concentration dependent manner (Hansch *et al.*, 2014). More recently, PQS was shown to induce oxidative stress and inhibit haeme oxygenase-1 expression (Abdalla *et al.*, 2017). The induction of ROS is not a new phenomenon for PQS, with both anti-oxidant and pro-oxidant activities reported in *P. aeruginosa* (Haussler & Becker, 2008). Taken together, these studies all support a role for AQ signals in the host environment following acquisition of *P. aeruginosa*.

Understanding the role of AQs in the host-microbe interaction will require us to monitor the production of AQ profiles in clinically relevant samples. Though it is clear from *in vitro* studies that AQs can significantly modulate critical pathways in host cells, the presence of PQS, HHQ, and other AQs in clinical samples is yet to be established. While some studies have reported PQS positivity in biological samples (Collier *et al.*, 2002, Guina *et al.*, 2003, Barr *et al.*, 2015), others (including ourselves) have not been successful, even in samples from patients that were culture positive for *P. aeruginosa* (Buzid *et al.*, 2016, Quinn *et al.*, 2016, Buzid *et al.*, 2017). Advances in LC-MS/MS (Turnpenny *et al.*, 2017), *lux*-based biosensors (Fletcher *et al.*, 2018) and electrovoltammetric based detection (Zhou *et al.*, 2011, Seviour *et al.*, 2015, Buzid *et al.*, 2016, Buzid *et al.*, 2017) will provide more effective technologies for monitoring small molecule signalling both *in vitro* and *in situ*. Rapid and

early detection of *P. aeruginosa* would also enhance the clinical management of these infections.

## INTEGRATING NEW KNOWLEDGE FOR AQ BASED THERAPEUTICS

Quorum sensing has traditionally been viewed as being dispensable for survival in most natural ecosystems. Therefore, it follows that targeting QS would be an attractive anti-infective strategy, being neutral with regard to the selective pressure for the emergence of resistance. This view has been questioned somewhat by recent reports that describe resistance mechanisms against QS inhibitors (Defoirdt *et al.*, 2013, García-Contreras *et al.*, 2016), while intra-species heterogeneity (Markussen *et al.*, 2014, Winstanley *et al.*, 2016) and adaptive co-evolution (Frydenlund Michelsen *et al.*, 2016) also have implications for the effectiveness of anti-infective therapeutics (Whiteley *et al.*, 2017). Nonetheless, inactivation of AQ signalling in microbial pathogens remains an attractive strategy that continues to receive considerable attention (**Figure 3**).

Several enzymes in the PQS biosynthetic pathway have been the target of inhibitor development studies. The fact that PqsA is required for the synthesis of AQs in both *P. aeruginosa* and *Burkholderia thailandensis* makes it an attractive drug target, particularly as its interference would not only affect AQ synthesis but also block the accumulation of anthranilic acid and DHQ (Lesic *et al.*, 2007, Drees *et al.*, 2016, Gruber *et al.*, 2016, Witzgall *et al.*, 2017). Analogues of anthranilic acid were shown to increase survival and lower bacterial dissemination in mice (Lesic *et al.*, 2007). Sulfonyladenosine-based mimics of the anthranilyl-AMP reaction intermediate were shown to decrease HHQ and PQS levels in *P. aeruginosa*, although pyocyanin production was unaffected (Ji *et al.*, 2016). A SAR approach to the design of (2-nitrophenyl)methanol derivatives, known to be effective inhibitors of

PqsD, resulted in the generation of fluorescent compounds with improved cellulo activity (Storz *et al.*, 2014).

Given the central role of PqsR in mediating the signalling effects of the AQ system, it is unsurprising that this protein has been the target of inhibition of several studies. The development of PqsR inhibitors [reviewed recently in (Ó Muimhneachain *et al.*, 2018)] has benefited from the availability of crystal structure (Ilangovan *et al.*, 2013), and detailed SAR analyses by several groups (Mashburn-Warren *et al.*, 2009, Hodgkinson *et al.*, 2010, Lu *et al.*, 2014, Shanahan *et al.*, 2017). The outcome of these studies suggests that a highly lipophilc alkyl 'tail' and a polar 'head' are essential for effective antagonism, which is further enhanced by the presence of an electron-withdrawing group at C6 and a halide at C7. The therapeutic application of these HHQ/PQS-like antagonists will likely be hampered by their poor aqueous solubility. Efforts are ongoing to overcome this drawback (Lu *et al.*, 2014, Nafee *et al.*, 2014). The concept of dual target inhibition of both the PqsA-E and PqsR systems has gained traction in recent years. The Hartmann group have adopted this approach for the synthesis of compounds that inhibit PqsR and PqsD (Thomann *et al.*, 2016), while Welsh *et al*. have proposed the design of chemical agents that disrupt crosstalk between the PQS and Rhl pathways (Welsh *et al.*, 2015). A recent study by Allegretta *et al*. revealed an interesting divergence between the downstream effects of PqsR and PqsBC inhibitors on AQ compound profiles (Allegretta *et al.*, 2017). PqsR antagonists suppressed the production of 2-AA, HQNO, HHQ, PQS, and DHQ (at higher concentrations). In contrast, while PqsBC inhibitors did suppress HHQ and PQS, they also resulted in increased production of 2-AA, DHQ and HQNO. This divergence may underpin the need for a polypharmacology approach described by the Rahme group, whereby both PqsBC and PqsR are targeted in tandem (Maura & Rahme, 2017). In addition to targeting both acute and persistence-related

functions, this dual approach may also sustain the therapeutic intervention in the face of evolved resistance.

The importance of host-associated microbiota in maintaining homeostasis and health has been highlighted in recent years, as has the structural complexity and heterogeneity (both spatial and species) therein. The integrative 'holobiont' theory has received considerable attention, viewing microbe and host as a single organism or 'hologenome' (Bordenstein & Theis, 2015). Thus, the AQ-based anti-*Pseudomonas* strategies described above must be viewed in the context of an interactome that involves bacterial, fungal, viral and host cells. This requires us to look at how interventions influence the orchestra, rather than simply silencing what may be the loudest instrument. As described earlier, several microbes have developed strategies to degrade the AQ signal. While evidence of AQ analogue degradation by competing organisms has not yet been reported, the possible biotransformation of AQ anti-infectives within a community must be considered, and further work is needed in this regard. The impact of PQS inactivation on iron availability within the community could have implications for competing organisms, as well as on the host cell, one of the key players in the polycellular interactome. The collective influence of AQ signals on HIF-1 and NFκB signalling, oxidative stress, and neutrophil stimulation amongst other effects, will need to be assessed as part of the implementation strategy of AQ based therapies.

The ability of HHQ, PQS, and their derivatives to modulate the behaviour of bacterial and fungal pathogens, as well as host signalling, may see the AQ signal itself provide a potential therapeutic strategy to control virulence. A suite of AQ analogues were shown to suppress biofilm formation in a range of bacterial and fungal pathogens (Reen *et al.*, 2015, Reen *et al.*, 2016, Reen *et al.*, 2016). Importantly, these compounds were non-agonists in *P. aeruginosa* and less cytotoxic than the parent molecule (Reen *et al.*, 2016). Further modification of the AQ framework to encompass suppressive activity towards PqsR, while retaining anti-

infective activity against competing pathogens, would prove a significant advance towards targeted therapeutic intervention. Combinatorial therapies may ultimately be required, for example where immunomodulators would be administered in tandem with AQ anti-infectives. Long-term low dose macrolide antibiotics have been shown to inhibit the expression of *P. aeruginosa lasR* and *pqsA* within the airways of patients with non-cystic fibrosis bronchiectasis, without reducing the bacterial load (Burr *et al.*, 2016). The activity of immunomodulatory macrolide therapeutics is the subject of significant interest at the moment, with the basis of anti-inflammatory and anti-infective outcomes poorly understood. Dual targeting of AQ signalling with tandem enhancement of the host immune response may prove to be a particularly attractive therapy. However, significant pharmacological and pharmacokinetic challenges remain before such interventions could become a reality.

## SUMMARY

While the challenge of translating these microbial chemical messages into a readable code remains, unlocking the elements that govern cell-cell signalling and communication in natural ecosystems will provide a framework upon which this goal can be pursued. Many important concept rich papers have been published in the sphere of microbial signalling and socio-biology, with clear distinctions drawn between signalling, coercion, and cues. While some of the interactions described in this review do not fulfil the strict criteria of evolved signals, they are nonetheless important in understanding the complexity of inter-cellular interactions. Microbe-microbe and microbial-host communication may be just as prone to dysfunction as their more developed eukaryotic counterparts, whereby community homeostasis is overtaken by subversion of chemical messages resulting in the emergence of a dominant force to the detriment of the common good.

**Funding**

FJR, GMG, and FOG acknowledge support from Enterprise Ireland (CF-2017-0757-P).This research was also supported in part by grants awarded to FOG by the European Commission (FP7-PEOPLE-2013-ITN, 607786; FP7-KBBE-2012-6, CP-TP-312184; FP7-KBBE-2012-6, 311975; OCEAN 2011-2, 287589; EU2020-634486-2015), Science Foundation Ireland (SSPC-2, 12/RC/2275; 13/TIDA/B2625; 12/TIDA/B2411; 12/TIDA/B2405; 14/TIDA/2438; 15/TIDA/2977), the Department of Agriculture and Food (FIRM 1/F009/MabS; FIRM 13/F/516), the Irish Research Council for Science, Engineering and Technology (GOIPG/2014/647), the Health Research Board/Irish Thoracic Society (MRCG-2014-6) and grants awarded to GMG by Science Foundation Ireland (SFI/12/TIDA/B2405, SFI/12/IP/1315 and SSPC2 12/RC/2275).

**Acknowledgements**

The authors thank Stephanie Flynn and David Woods for suggestions and critical reading of the manuscript.

**Conflict of interest**

The authors have no conflict of interest to declare.

**References**

Abdalla MY, Hoke T, Seravalli J, Switzer BL, Bavitz M, Fliege JD, Murphy PJ & Britigan BE (2017) Pseudomonas Quinolone Signal induces oxidative stress and inhibits heme oxygenase-1 expression in lung epithelial cells. *Infect Immun* **85**. doi: 10.1128/IAI.00176-17

Allegretta G, Maurer CK, Eberhard J, Maura D, Hartmann RW, Rahme L & Empting M (2017) In-depth profiling of MvfR-regulated small molecules in *Pseudomonas aeruginosa* after quorum sensing inhibitor treatment. *Front Microbiol* **8**: 924.

Baker YR, Hodgkinson JT, Florea BI, Alza E, Galloway W, Grimm L, Geddis SM, Overkleeft HS, Welch M & Spring DR (2017) Identification of new quorum sensing autoinducer binding partners in *Pseudomonas aeruginosa* using photoaffinity probes. *Chem Sci* **8**: 7403-7411.

Bala A, Chhibber S & Harjai K (2014) Pseudomonas quinolone signalling system: a component of quorum sensing cascade is a crucial player in the acute urinary tract infection caused by Pseudomonas aeruginosa. *Int J Med Microbiol* **304**: 1199-1208.

Barr HL, Halliday N, Camara M, *et al.* (2015) *Pseudomonas aeruginosa* quorum sensing molecules correlate with clinical status in cystic fibrosis. *Eur Respir J* **46**: 1046-1054.

Birmes FS, Wolf T, Kohl TA, Ruger K, Bange F, Kalinowski J & Fetzner S (2017) *Mycobacterium abscessus* subsp *abscessus* is capable of degrading *Pseudomonas aeruginosa* Quinolone Signals. *Front Microbiol* **8**. 339.

Blier AS, Veron W, Bazire A, *et al.* (2011) C-type natriuretic peptide modulates quorum sensing molecule and toxin production in *Pseudomonas aeruginosa*. *Microbiol* **157**: 1929-1944.

Bordenstein SR & Theis KR (2015) Host biology in light of the microbiome: ten principles of holobionts and hologenomes. *PLoS Biol* **13**: e1002226.

Bredenbruch F, Geffers R, Nimtz M, Buer J & Haussler S (2006) The *Pseudomonas aeruginosa* quinolone signal (PQS) has an iron-chelating activity. *Environ Microbiol* **8**: 1318-1329.

Brouwer S, Pustelny C, Ritter C, Klinkert B, Narberhaus F & Haussler S (2014) The PqsR and RhlR transcriptional regulators determine the level of Pseudomonas quinolone signal

synthesis in *Pseudomonas aeruginosa* by producing two different *pqsABCDE* mRNA isoforms. *J Bacteriol* **196**: 4163-4171.

Burr LD, Rogers GB, Chen AC, Hamilton BR, Pool GF, Taylor SL, Venter D, Bowler SD, Biga S & McGuckin MA (2016) Macrolide treatment inhibits *Pseudomonas aeruginosa* quorum sensing in non-Cystic Fibrosis bronchiectasis. An analysis from the bronchiectasis and low-dose erythromycin study trial. *Ann Am Thorac Soc* **13**: 1697-1703.

Buzid A, Reen FJ, Langsi VK, Muimhneachain EO, O'Gara F, McGlacken GP, Luong JHT & Glennon JD (2017) Direct and rapid electrochemical detection of *Pseudomonas aeruginosa* quorum signaling molecules in bacterial cultures and Cystic Fibrosis sputum samples through cationic surfactant-assisted membrane disruption. *Chemelectrochem* **4**: 533-541.

Buzid A, Shang F, Reen FJ, Muimhneachain EO, Clarke SL, Zhou L, Luong JH, O'Gara F, McGlacken GP & Glennon JD (2016) Molecular signature of *Pseudomonas aeruginosa* with simultaneous nanomolar detection of quorum sensing signaling molecules at a boron-doped diamond electrode. *Sci Rep* **6**: 30001.

Campbell EL, Bruyninckx WJ, Kelly CJ, *et al.* (2014) Transmigrating neutrophils shape the mucosal microenvironment through localized oxygen depletion to influence resolution of inflammation. *Immunity* **40**: 66-77.

Carloni S, Macchi R, Sattin S, Ferrara S & Bertoni G (2017) The small RNA ReaL: a novel regulatory element embedded in the *Pseudomonas aeruginosa* quorum sensing networks. *Environ Microbiol* **19**: 4220-4237.

Collier DN, Anderson L, McKnight SL, Noah TL, Knowles M, Boucher R, Schwab U, Gilligan P & Pesci EC (2002) A bacterial cell to cell signal in the lungs of cystic fibrosis patients. *FEMS Microbiol Lett* **215**: 41-46.

Cooper MA & Shlaes D (2011) Fix the antibiotics pipeline. *Nature* **472**: 32-32.

Costello A, Reen FJ, O'Gara F, Callaghan M & McClean S (2014) Inhibition of co-colonizing cystic fibrosis-associated pathogens by *Pseudomonas aeruginosa* and *Burkholderia multivorans*. *Microbiol* **160**: 1474-1487.

Cugini C, Morales DK & Hogan DA (2010) *Candida albicans*-produced farnesol stimulates Pseudomonas quinolone signal production in LasR-defective *Pseudomonas aeruginosa* strains. *Microbiol* **156**: 3096-3107.

Cugini C, Calfee MW, Farrow JM, Morales DK, Pesci EC & Hogan DA (2007) Farnesol, a common sesquiterpene, inhibits PQS production in *Pseudomonas aeruginosa*. *Mol Microbiol* **65**: 896-906.

Cummins J, Reen FJ, Baysse C, Mooij MJ & O'Gara F (2009) Subinhibitory concentrations of the cationic antimicrobial peptide colistin induce the pseudomonas quinolone signal in *Pseudomonas aeruginosa*. *Microbiol* **155**: 2826-2837.

De Smet J, Zimmermann M, Kogadeeva M, Ceyssens PJ, Vermaelen W, Blasdel B, Jang HB, Sauer U & Lavigne R (2016) High coverage metabolomics analysis reveals phage-specific alterations to *Pseudomonas aeruginosa* physiology during infection. *ISME J* **10**: 1823-1835.

Defoirdt T, Brackman G & Coenye T (2013) Quorum sensing inhibitors: how strong is the evidence? *Trends Microbiol* **21**: 619-624.

Deziel E, Lepine F, Milot S, He JX, Mindrinos MN, Tompkins RG & Rahme LG (2004) Analysis of *Pseudomonas aeruginosa* 4-hydroxy-2-alkylquinolines (HAQs) reveals a role for 4-hydroxy-2-heptylquinoline in cell-to-cell communication. *P Natl Acad Sci USA* **101**: 1339-1344.

Deziel E, Gopalan S, Tampakaki AP, Lepine F, Padfield KE, Saucier M, Xiao GP & Rahme LG (2005) The contribution of MvfR to *Pseudomonas aeruginosa* pathogenesis and quorum sensing circuitry regulation: multiple quorum sensing-regulated genes are modulated without

affecting *lasRI*, *rhlRI* or the production of N-acyl-L-homoserine lactones. *Mol Microbiol* **55**: 998-1014.

Diggle SP, Gardner A, West SA & Griffin AS (2007) Evolutionary theory of bacterial quorum sensing: when is a signal not a signal? *Philos T R Soc B* **362**: 1241-1249.

Diggle SP, Lumjiaktase P, Dipilato F, Winzer K, Kunakorn M, Barrett DA, Chhabra SR, Camara M & Williams P (2006) Functional genetic analysis reveals a 2-alkyl-4-quinolone signaling system in the human pathogen *Burkholderia pseudomallei* and related bacteria. *Chem Biol* **13**: 701-710.

Diggle SP, Matthijs S, Wright VJ, *et al.* (2007) The *Pseudomonas aeruginosa* 4-quinolone signal molecules HHQ and PQS play multifunctional roles in quorum sensing and iron entrapment. *Chem Biol* **14**: 87-96.

Drees SL, Li C, Prasetya F, Saleem M, Dreveny I, Williams P, Hennecke U, Emsley J & Fetzner S (2016) PqsBC, a condensing enzyme in the biosynthesis of the *Pseudomonas aeruginosa* Quinolone Signal: crystal structure, inhibition, and reaction mechanism. *J Biol Chem* **291**: 6610-6624.

Dulcey CE, Dekimpe V, Fauvelle D-A, Milot S, Groleau M-C, Doucet N, Rahme LG, Lépine F & Déziel E (2013) The end of a long-standing hypothesis: the Pseudomonas signalling molecules 4-hydroxy-2-alkylquinolines are derived from fatty acids, not 3-ketofatty acids. *Chem Biol* **20**: 10.1016/j.chembiol.2013.1009.1021.

Farrow JM, 3rd & Pesci EC (2017) Distal and proximal promoters co-regulate pqsR expression in *Pseudomonas aeruginosa*. *Mol Microbiol* **104**: 78-91.

Farrow JM, 3rd, Hudson LL, Wells G, Coleman JP & Pesci EC (2015) CysB negatively affects the transcription of *pqsR* and Pseudomonas Quinolone Signal production in *Pseudomonas aeruginosa*. *J Bacteriol* **197**: 1988-2002.

Fernandez-Pinar R, Camara M, Dubern JF, Ramos JL & Espinosa-Urgel M (2011) The *Pseudomonas aeruginosa* quinolone quorum sensing signal alters the multicellular behaviour of *Pseudomonas putida* KT2440. *Res Microbiol* **162**: 773-781.

Filkins LM, Graber JA, Olson DG, Dolben EL, Lynd LR, Bhuju S & O'Toole GA (2015) Coculture of *Staphylococcus* aureus with *Pseudomonas aeruginosa* drives *S. aureus* towards fermentative metabolism and reduced viability in a Cystic Fibrosis model. *J Bacteriol* **197**: 2252-2264.

Fletcher MP, Diggle SP, Cámara M & Williams P (2018) Detection of 2-Alkyl-4-Quinolones using biosensors. *Quorum Sensing: Methods and Protocols,*(Leoni L & Rampioni G, eds.), pp. 25-34. Springer New York, New York, NY.

Florez C, Raab JE, Cooke AC & Schertzer JW (2017) Membrane distribution of the Pseudomonas Quinolone Signal modulates outer membrane vesicle production in *Pseudomonas aeruginosa*. *MBio* **8**(4): e01034-17

Frydenlund Michelsen C, Hossein Khademi SM, Krogh Johansen H, Ingmer H, Dorrestein PC & Jelsbak L (2016) Evolution of metabolic divergence in *Pseudomonas aeruginosa* during long-term infection facilitates a proto-cooperative interspecies interaction. *ISME J* **10**: 1323-1336.

Fugere A, Seguin DL, Mitchell G, Deziel E, Dekimpe V, Cantin AM, Frost E & Malouin F (2014) Interspecific small molecule interactions between clinical isolates of *Pseudomonas aeruginosa* and *Staphylococcus aureus* from adult Cystic Fibrosis patients. *Plos One* **9**(1): e86705

Fuqua WC, Winans SC & Greenberg EP (1994) Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators. *J Bacteriol* **176**: 269-275.

Gallagher K, McGown KA, Bengoechea J, Dumigan A, Tunney M, Carson G & Gilpin DF (2017) Cigarette and e-cigarette effects on *Pseudomonas aeruginosa* and A549 cells. *J Cystic Fibrosis* **16**(S1): S88.

García-Contreras R, Maeda T & Wood TK (2016) Can resistance against quorum-sensing interference be selected? *ISME J* **10**: 4-10.

Gruber JD, Chen W, Parnham S, Beauchesne K, Moeller P, Flume PA & Zhang YM (2016) The role of 2,4-dihydroxyquinoline (DHQ) in *Pseudomonas aeruginosa* pathogenicity. *PeerJ* **4**: e1495.

Guina T, Purvine SO, Yi EC, Eng J, Goodlett DR, Aebersold R & Miller SI (2003) Quantitative proteomic analysis indicates increased synthesis of a quinolone by *Pseudomonas aeruginosa* isolates from cystic fibrosis airways. *Proc Natl Acad Sci U S A* **100**: 2771-2776.

Hansch GM, Prior B, Brenner-Weiss G, Obst U & Overhage J (2014) The Pseudomonas quinolone signal (PQS) stimulates chemotaxis of polymorphonuclear neutrophils. *J Appl Biomater Func* **12**: 21-26.

Haussler S & Becker T (2008) The pseudomonas quinolone signal (PQS) balances life and death in *Pseudomonas aeruginosa* populations. *PLoS Pathogens* **4**(9): e1000166.

Hazan R, Que YA, Maura D, Strobel B, Majcherczyk PA, Hopper LR, Wilbur DJ, Hreha TN, Barquera B & Rahme LG (2016) Auto poisoning of the respiratory chain by a quorum-sensing-regulated molecule favors biofilm formation and antibiotic tolerance. *Curr Biol* **26**: 195-206.

Hodgkinson J, Bowden SD, Galloway WR, Spring DR & Welch M (2010) Structure-activity analysis of the Pseudomonas quinolone signal molecule. *J Bacteriol* **192**: 3833-3837.

Hodgkinson JT, Gross J, Baker YR, Spring DR & Welch M (2016) A new Pseudomonas quinolone signal (PQS) binding partner: MexG. *Chem Sci* **7**: 2553-2562.

Hooi DS, Bycroft BW, Chhabra SR, Williams P & Pritchard DI (2004) Differential immune modulatory activity of *Pseudomonas aeruginosa* quorum-sensing signal molecules. *Infect Immun* **72**: 6463-6470.

Ilangovan A, Fletcher M, Rampioni G, *et al.* (2013) Structural basis for native agonist and synthetic inhibitor recognition by the *Pseudomonas aeruginosa* quorum sensing regulator PqsR (MvfR). *PLoS Pathog* **9**: e1003508.

Inaba T, Oura H, Morinaga K, Toyofuku M & Nomura N (2015) The Pseudomonas Quinolone Signal inhibits biofilm development of *Streptococcus mutans*. *Microbes Environ* **30**: 189-191.

Ji C, Sharma I, Pratihar D, Hudson LL, Maura D, Guney T, Rahme LG, Pesci EC, Coleman JP & Tan DS (2016) Designed small-molecule inhibitors of the anthranilyl-CoA synthetase PqsA block quinolone biosynthesis in *Pseudomonas aeruginosa*. *ACS Chem Biol* **11**: 3061-3067.

Kim K, Kim YU, Koh BH, Hwang SS, Kim SH, Lepine F, Cho YH & Lee GR (2010) HHQ and PQS, two *Pseudomonas aeruginosa* quorum-sensing molecules, down-regulate the innate immune responses through the nuclear factor-kappa B pathway. *Immunol* **129**: 578-588.

Kim S, Yoon Y & Choi KH (2015) *Pseudomonas aeruginosa* DesB Promotes *Staphylococcus aureus* growth inhibition in coculture by controlling the synthesis of HAQs. *PLoS One* **10**(7): e0134624.

Korgaonkar A, Trivedi U, Rumbaugh KP & Whiteley M (2013) Community surveillance enhances *Pseudomonas aeruginosa* virulence during polymicrobial infection. *Proc Natl Acad Sci U S A* **110**: 1059-1064.

Kruczek C, Qaisar U, Colmer-Hamood JA & Hamood AN (2014) Serum influences the expression of *Pseudomonas aeruginosa* quorum-sensing genes and QS-controlled virulence genes during early and late stages of growth. *Microbiologyopen* **3**: 64-79.

Lamarche MG & Deziel E (2011) MexEF-OprN efflux pump exports the Pseudomonas quinolone signal (PQS) precursor HHQ (4-hydroxy-2-heptylquinoline). *PLoS One* **6**: e24310.

Legendre C, Reen FJ, Mooij MJ, McGlacken GP, Adams C & O'Gara F (2012) *Pseudomonas aeruginosa* alkyl quinolones repress hypoxia-inducible factor 1 (HIF-1) signaling through HIF-1alpha degradation. *Infect Immun* **80**: 3985-3992.

Lepine F, Dekimpe V, Lesic B, Milot S, Lesimple A, Mamer OA, Rahme LG & Deziel E (2007) PqsA is required for the biosynthesis of 2,4-dihydroxyquinoline (DHQ), a newly identified metabolite produced by *Pseudomonas aeruginosa* and *Burkholderia thailandensis*. *Biol Chem* **388**: 839-845.

Lesic B, Lépine F, Déziel E, *et al.* (2007) Inhibitors of pathogen intercellular signals as selective anti-infective compounds. *PLOS Pathogens* **3**: e126.

Lin J, Zhang W, Cheng J, Yang X, Zhu K, Wang Y, Wei G, Qian PY, Luo ZQ & Shen X (2017) A *Pseudomonas* T6SS effector recruits PQS-containing outer membrane vesicles for iron acquisition. *Nat Commun* **8**: 14888.

Lu C, Maurer CK, Kirsch B, Steinbach A & Hartmann RW (2014) Overcoming the unexpected functional inversion of a PqsR antagonist in *Pseudomonas aeruginosa*: an *in vivo* potent antivirulence agent targeting pqs quorum sensing. *Angew Chem Int Ed Engl* **53**: 1109-1112.

Macdonald IA & Kuehn MJ (2013) Stress-induced outer membrane vesicle production by *Pseudomonas aeruginosa*. *J Bacteriol* **195**: 2971-2981.

Markussen T, Marvig RL, Gomez-Lozano M, Aanaes K, Burleigh AE, Hoiby N, Johansen HK, Molin S & Jelsbak L (2014) Environmental heterogeneity drives within-host diversification and evolution of *Pseudomonas aeruginosa*. *MBio* **5**: e01592-01514.

Mashburn-Warren L, Howe J, Brandenburg K & Whiteley M (2009) Structural requirements of the Pseudomonas quinolone signal for membrane vesicle stimulation. *J Bacteriol* **191**: 3411-3414.

Mashburn-Warren L, Howe J, Garidel P, Richter W, Steiniger F, Roessle M, Brandenburg K & Whiteley M (2008) Interaction of quorum signals with outer membrane lipids: insights into prokaryotic membrane vesicle formation. *Mol Microbiol* **69**: 491-502.

Mashburn LM, Jett AM, Akins DR & Whiteley M (2005) *Staphylococcus aureus* serves as an iron source for *Pseudomonas aeruginosa* during *in vivo* coculture. *J Bacteriol* **187**: 554-566.

Maura D & Rahme LG (2017) Pharmacological inhibition of the *Pseudomonas aeruginosa* MvfR quorum-sensing system interferes with biofilm formation and potentiates antibiotic-mediated biofilm disruption. *Antimicrob Agents Chemother* **61**(12): e01362-17.

Maura D, Hazan R, Kitao T, Ballok AE & Rahme LG (2016) Evidence for direct control of virulence and defense gene circuits by the *Pseudomonas aeruginosa* quorum sensing regulator, MvfR. *Sci Rep* **6**: 34083.

McAlester G, O'Gara F & Morrissey JP (2008) Signal-mediated interactions between *Pseudomonas aeruginosa* and *Candida albicans*. *J Med Microbiol* **57**: 563-569.

McGlacken GP, McSweeney CM, O'Brien T, Lawrence SE, Elcoate CJ, Reen FJ & O'Gara F (2010) Synthesis of 3-halo-analogues of HHQ, subsequent cross-coupling and first crystal structure of Pseudomonas quinolone signal (PQS). *Tetrahedron Lett* **51**: 5919-5921.

McGrath S, Wade DS & Pesci EC (2004) Dueling quorum sensing systems in *Pseudomonas aeruginosa* control the production of the Pseudomonas quinolone signal (PQS). *FEMS Microbiol Lett* **230**: 27-34.

Moreau P, Diggle SP & Friman VP (2017) Bacterial cell-to-cell signaling promotes the evolution of resistance to parasitic bacteriophages. *Ecol Evol* **7**: 1936-1941.

Muller C, Birmes FS, Ruckert C, Kalinowski J & Fetzner S (2015) *Rhodococcus erythropolis* BG43 genes mediating *Pseudomonas aeruginosa* Quinolone Signal degradation and virulence factor attenuation. *Appl Environ Microb* **81**: 7720-7729.

Nafee N, Husari A, Maurer CK, Lu C, de Rossi C, Steinbach A, Hartmann RW, Lehr CM & Schneider M (2014) Antibiotic-free nanotherapeutics: ultra-small, mucus-penetrating solid lipid nanoparticles enhance the pulmonary delivery and anti-virulence efficacy of novel quorum sensing inhibitors. *J Control Release* **192**: 131-140.

Nguyen AT, Jones JW, Ruge MA, Kane MA & Oglesby-Sherrouse AG (2015) Iron depletion enhances production of antimicrobials by *Pseudomonas aeruginosa*. *J Bacteriol* **197**: 2265-2275.

Nguyen AT, Jones JW, Camara M, Williams P, Kane MA & Oglesby-Sherrouse AG (2016) Cystic Fibrosis isolates of *Pseudomonas aeruginosa* retain iron-regulated antimicrobial activity against *Staphylococcus aureus* through the action of multiple alkylquinolones. *Front Microbiol* **7**: 1171.

Ó Muimhneacháin E, Reen FJ, O'Gara F & McGlacken GP (2018) Analogues of *Pseudomonas aeruginosa* signalling molecules to tackle infections. *Org Biomol Chem* **16**: 169-179.

Orazi G & O'Toole GA (2017) *Pseudomonas aeruginosa* alters *Staphylococcus* aureus sensitivity to vancomycin in a biofilm model of Cystic Fibrosis infection. *MBio* **8**(4): e00873-17.

Pesci EC, Milbank JBJ, Pearson JP, McKnight S, Kende AS, Greenberg EP & Iglewski BH (1999) Quinolone signaling in the cell-to-cell communication system of *Pseudomonas aeruginosa*. *P Natl Acad Sci USA* **96**: 11229-11234.

Pustelny C, Albers A, Buldt-Karentzopoulos K, Parschat K, Chhabra SR, Camara M, Williams P & Fetzner S (2009) Dioxygenase-mediated quenching of quinolone-dependent quorum sensing in *Pseudomonas aeruginosa*. *Chem Biol* **16**: 1259-1267.

Quinn RA, Phelan VV, Whiteson KL, Garg N, Bailey BA, Lim YW, Conrad DJ, Dorrestein PC & Rohwer FL (2016) Microbial, host and xenobiotic diversity in the cystic fibrosis sputum metabolome. *ISME J* **10**: 1483-1498.

Rampioni G, Falcone M, Heeb S, Frangipani E, Fletcher MP, Dubern JF, Visca P, Leoni L, Camara M & Williams P (2016) Unravelling the genome-wide contributions of specific 2-alkyl-4-quinolones and PqsE to quorum sensing in *Pseudomonas aeruginos*a. *PLoS Pathog* **12**: e1006029.

Reen FJ, Woods DF, Mooij MJ, Adams C & O'Gara F (2012) Respiratory pathogens adopt a chronic lifestyle in response to bile. *PLoS One* **7**: e45978.

Reen FJ, Barret M, Fargier E, O'Muinneachain M & O'Gara F (2013) Molecular evolution of LysR-type transcriptional regulation in *Pseudomonas aeruginosa*. *Mol Phylogenet Evol* **66**: 1041-1049.

Reen FJ, Shanahan R, Cano R, O'Gara F & McGlacken GP (2015) A structure activity-relationship study of the bacterial signal molecule HHQ reveals swarming motility inhibition in *Bacillus atrophaeus*. *Org Biomol Chem* **13**: 5537-5541.

Reen FJ, Mooij MJ, Holcombe LJ, McSweeney CM, McGlacken GP, Morrissey JP & O'Gara F (2011) The Pseudomonas quinolone signal (PQS), and its precursor HHQ, modulate interspecies and interkingdom behaviour. *FEMS Microbiol Ecol* **77**: 413-428.

Reen FJ, Clarke SL, Legendre C, McSweeney CM, Eccles KS, Lawrence SE, O'Gara F & McGlacken GP (2012) Structure-function analysis of the C-3 position in analogues of microbial behavioural modulators HHQ and PQS. *Org Biomol Chem* **10**: 8903-8910.

Reen FJ, Phelan JP, Woods DF, Shanahan R, Cano R, Clarke S, McGlacken GP & O'Gara F (2016) Harnessing bacterial signals for suppression of biofilm formation in the nosocomial fungal pathogen *Aspergillus fumigatus*. *Front Microbiol* **7**: 2074.

Reen FJ, Flynn S, Woods DF, Dunphy N, Chroinin MN, Mullane D, Stick S, Adams C & O'Gara F (2016) Bile signalling promotes chronic respiratory infections and antibiotic tolerance. *Sci Rep* **6**: 29768.

Reen FJ, Phelan JP, Gallagher L, Woods DF, Shanahan RM, Cano R, E OM, McGlacken GP & O'Gara F (2016) Exploiting interkingdom interactions for development of small-molecule inhibitors of *Candida albicans* biofilm formation. *Antimicrob Agents Chemother* **60**: 5894-5905.

Reinhart AA, Powell DA, Nguyen AT, O'Neill M, Djapgne L, Wilks A, Ernst RK & Oglesby-Sherrouse AG (2015) The prrF-encoded small regulatory RNAs are required for iron homeostasis and virulence of *Pseudomonas aeruginosa*. *Infect Immun* **83**: 863-875.

Reinhart AA, Nguyen AT, Brewer LK, Bevere J, Jones JW, Kane MA, Damron FH, Barbier M & Oglesby-Sherrouse AG (2017) The *Pseudomonas aeruginosa* PrrF small RNAs regulate iron homeostasis during acute murine lung infection. *Infect Immun* **85**.

Sams T, Baker Y, Hodgkinson J, Gross J, Spring D & Welch M (2016) The Pseudomonas Quinolone Signal (PQS). *Isr J Chem* **56**: 282-294.

Schafhauser J, Lepine F, Mckay G, Ahlgren HG, Khakimova M & Nguyen D (2014) The stringent response modulates 4-hydroxy-2-alkylquinoline biosynthesis and quorum-sensing hierarchy in *Pseudomonas aeruginosa*. *J Bacteriol* **196**: 1641-1650.

Schertzer JW & Whiteley M (2012) A bilayer-couple model of bacterial outer membrane vesicle biogenesis. *MBio* **3**(2): e00297-11.

Schuster M, Lostroh CP, Ogi T & Greenberg EP (2003) Identification, timing, and signal specificity of *Pseudomonas aeruginosa* quorum-controlled genes: a transcriptome analysis. *J Bacteriol* **185**: 2066-2079.

Seviour T, Doyle LE, Lauw SJ, Hinks J, Rice SA, Nesatyy VJ, Webster RD, Kjelleberg S & Marsili E (2015) Voltammetric profiling of redox-active metabolites expressed by *Pseudomonas aeruginosa* for diagnostic purposes. *Chem Commun (Camb)* **51**: 3789-3792.

Shanahan R, Reen FJ, Cano R, O'Gara F & McGlacken GP (2017) The requirements at the C-3 position of alkylquinolones for signalling in *Pseudomonas aeruginosa*. *Org Biomolec Chem* **15**: 306-310.

Skindersoe ME, Zeuthen LH, Brix S, *et al.* (2009) *Pseudomonas aeruginosa* quorum-sensing signal molecules interfere with dendritic cell-induced T-cell proliferation. *FEMS Immunol Med Microbiol* **55**: 335-345.

Soh EY, Chhabra SR, Halliday N, Heeb S, Muller C, Birmes FS, Fetzner S, Camara M, Chan KG & Williams P (2015) Biotic inactivation of the *Pseudomonas aeruginosa* quinolone signal molecule. *Environ Microbiol* **17**: 4352-4365.

Sonnleitner E, Gonzalez N, Sorger-Domenigg T, Heeb S, Richter AS, Backofen R, Williams P, Huttenhofer A, Haas D & Blasi U (2011) The small RNA PhrS stimulates synthesis of the *Pseudomonas aeruginosa* quinolone signal. *Mol Microbiol* **80**: 868-885.

Storz MP, Allegretta G, Kirsch B, Empting M & Hartmann RW (2014) From *in vitro* to *in cellulo*: structure-activity relationship of (2-nitrophenyl)methanol derivatives as inhibitors of PqsD in *Pseudomonas aeruginosa*. *Org Biomol Chem* **12**: 6094-6104.

Strempel N, Neidig A, Nusser M, Geffers R, Vieillard J, Lesouhaitier O, Brenner-Weiss G & Overhage J (2013) Human host defense peptide LL-37 stimulates virulence factor production and adaptive resistance in *Pseudomonas aeruginosa*. *PLoS One* **8**: e82240.

Tashiro Y, Ichikawa S, Nakajima-Kambe T, Uchiyama H & Nomura N (2010) Pseudomonas quinolone signal affects membrane vesicle production in not only gram-negative but also gram-positive bacteria. *Microbes Environ* **25**: 120-125.

Tettmann B, Niewerth C, Kirschhofer F, Neidig A, Dotsch A, Brenner-Weiss G, Fetzner S & Overhage J (2016) Enzyme-mediated quenching of the Pseudomonas Quinolone Signal (PQS) promotes biofilm formation of *Pseudomonas aeruginosa* by increasing iron availability. *Front Microbiol* **7**: 1978.

Thierbach S, Birmes FS, Letzel MC, Hennecke U & Fetzner S (2017) Chemical modification and detoxification of the *Pseudomonas aeruginosa* toxin 2-heptyl-4-hydroxyquinoline N-oxide by environmental and pathogenic bacteria. *ACS Chem Biol* **12**: 2305-2312.

Thomann A, Martins AGGD, Brengel C, Empting M & Hartmann RW (2016) Application of dual inhibition concept within looped autoregulatory systems toward antivirulence agents against *Pseudomonas aeruginosa* infections. *ACS Chem Biol* **11**: 1279-1286.

Tipton KA, Coleman JP & Pesci EC (2015) Post-transcriptional regulation of gene PA5507 controls Pseudomonas quinolone signal concentration in *P. aeruginosa*. *Mol Microbiol* **96**: 670-683.

Toyofuku M, Nakajima-Kambe T, Uchiyama H & Nomura N (2010) The effect of a cell-to-cell communication molecule, Pseudomonas Quinolone Signal (PQS), produced by *P. aeruginosa* on other bacterial species. *Microb Environ* **25**: 1-7.

Toyofuku M, Zhou SM, Sawada I, Takaya N, Uchiyama H & Nomura N (2014) Membrane vesicle formation is associated with pyocin production under denitrifying conditions in *Pseudomonas aeruginosa* PAO1. *Environ Microbiol* **16**: 2927-2938.

Turnbull L, Toyofuku M, Hynen AL, *et al.* (2016) Explosive cell lysis as a mechanism for the biogenesis of bacterial membrane vesicles and biofilms. *Nat Commun* **7**: 11220

Turnpenny P, Padfield A, Barton P, Teague J, Rahme LG, Pucci MJ, Zahler R & Rubio A (2017) Bioanalysis of *Pseudomonas aeruginosa* alkyl quinolone signalling molecules in infected mouse tissue using LC-MS/MS; and its application to a pharmacodynamic evaluation of MvfR inhibition. *J Pharm Biomed Anal* **139**: 44-53.

Viducic D, Murakami K, Amoh T, Ono T & Miyake Y (2017) Role of the interplay between quorum sensing regulator VqsR and the Pseudomonas quinolone signal in mediating carbapenem tolerance in *Pseudomonas aeruginosa*. *Res Microbiol* **168**: 450-460.

Welsh MA, Eibergen NR, Moore JD & Blackwell HE (2015) Small molecule disruption of quorum sensing cross-regulation in *Pseudomonas aeruginosa* causes major and unexpected alterations to virulence phenotypes. *J Am Chem Soc* **137**: 1510-1519.

Whiteley M, Diggle SP & Greenberg EP (2017) Progress in and promise of bacterial quorum sensing research. *Nature* **551**: 313-320.

Winstanley C, O'Brien S & Brockhurst MA (2016) *Pseudomonas aeruginosa* evolutionary adaptation and diversification in Cystic Fibrosis chronic lung infections. *Trends Microbiol* **24**: 327-337.

Witzgall F, Ewert W & Blankenfeldt W (2017) Structures of the N-terminal domain of PqsA in complex with anthraniloyl- and 6-fluoroanthraniloyl-AMP: substrate activation in Pseudomonas Quinolone Signal (PQS) biosynthesis. *Chembiochem* **18**: 2045-2055.

Wu Y & Seyedsayamdost MR (2017) Synergy and target promiscuity drive structural divergence in bacterial alkylquinolone biosynthesis. *Cell Chem Biol* **24**(12):1437-1444.

Zaborin A, Romanowski K, Gerdes S, *et al.* (2009) Red death in *Caenorhabditis elegans* caused by *Pseudomonas aeruginosa* PAO1. *Proc Natl Acad Sci U S A* **106**: 6327-6332.

Zaborina O, Lepine F, Xiao G, *et al.* (2007) Dynorphin activates quorum sensing quinolone signaling in *Pseudomonas aeruginosa*. *PLoS Pathog* **3**: e35.

Zhao J, Yu X, Zhu M, Kang H, Ma J, Wu M, Gan J, Deng X & Liang H (2016) Structural and molecular mechanism of CdpR involved in quorum-sensing and bacterial virulence in *Pseudomonas aeruginosa*. *PLoS Biol* **14**: e1002449.

Zhou L, Glennon JD, Luong JHT, Reen FJ, O'Gara F, McSweeney C & McGlacken GP (2011) Detection of the Pseudomonas Quinolone Signal (PQS) by cyclic voltammetry and amperometry using a boron doped diamond electrode. *Chem Commun* **47**: 10347-10349.

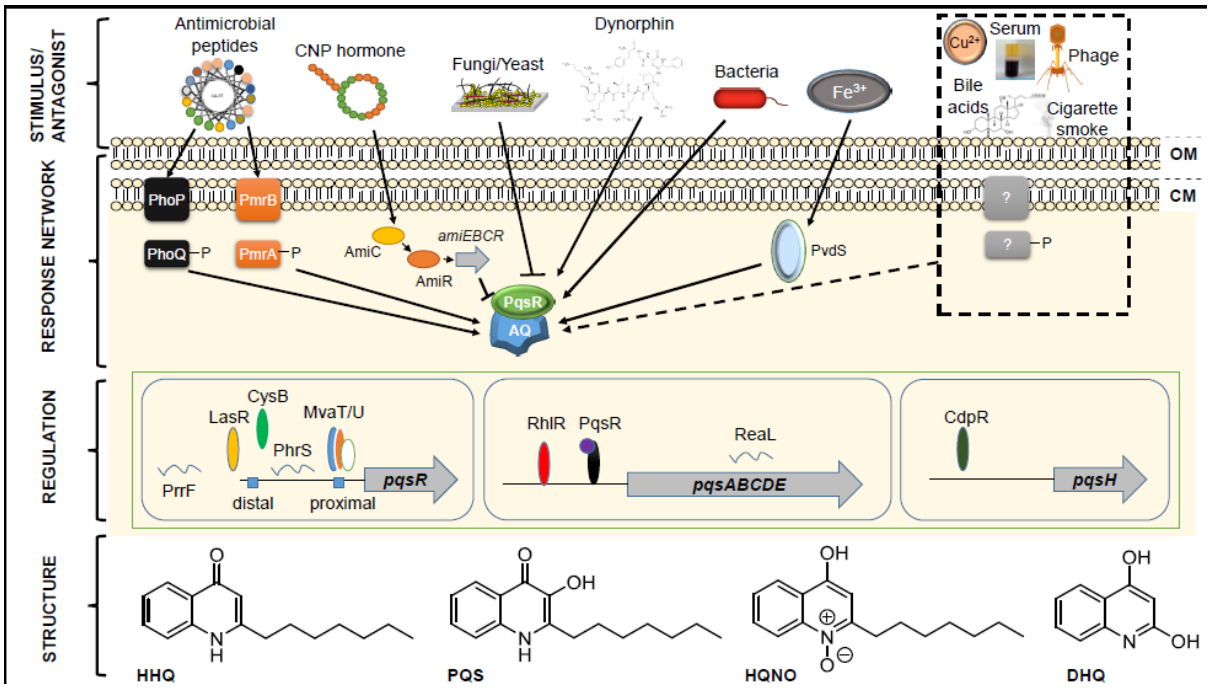


**Figure 1: Overview of environmental and host modulators of AQ production in *P. aeruginosa*.** The growing complexity of transcriptional and post-transcriptional regulation at the *pqsA-E*, *pqsR*, and *pqsH* promoters governs the levels of AQs produced, with HHQ, PQS, HQNO, and DHQ being the best studied to date. Dashed arrows indicate stimuli for which the molecular mechanism of AQ activation remains to be elucidated.

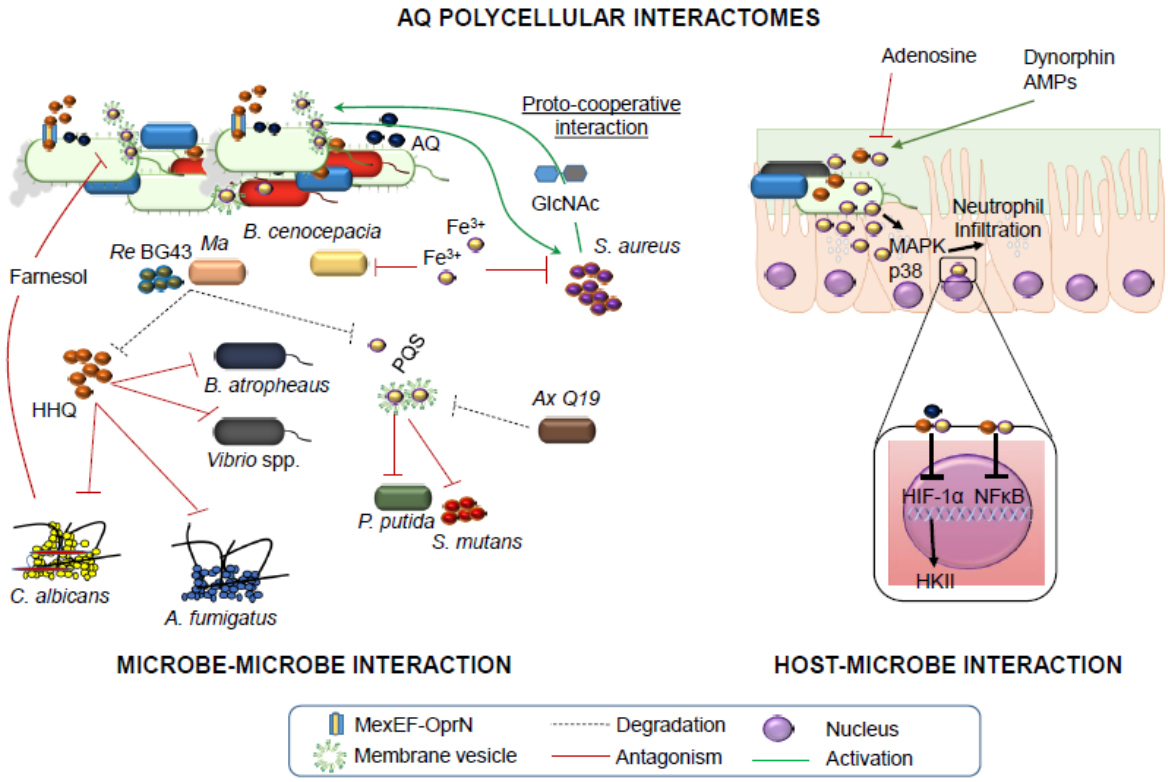


**Figure 2: AQ signalling in *P. aeruginosa* interfaces between microbe-microbe and microbial-host interactions.** Competition or co-operation between co-colonising pathogens can shape the dynamics of host microbiota, while communication with host cells can subvert the immune response.

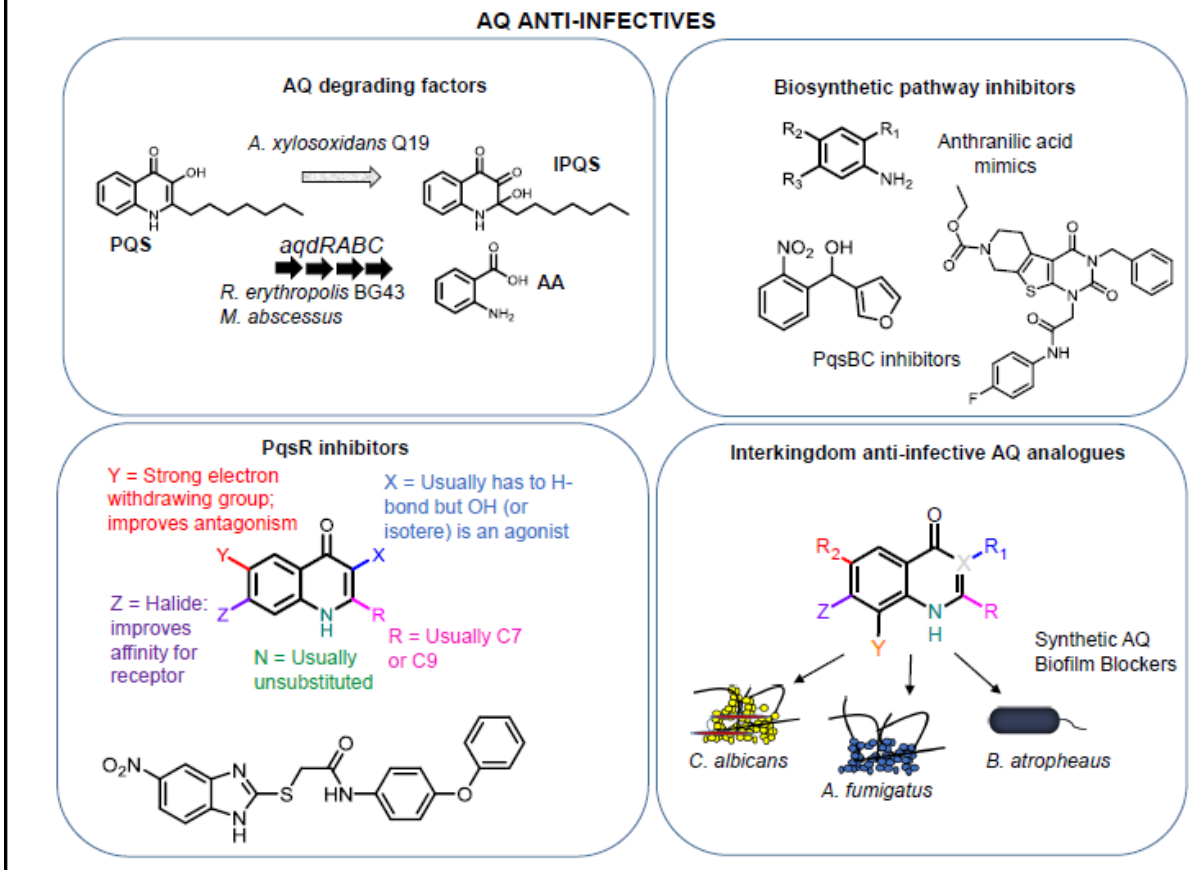


**Figure 3: AQ signals as the basis for innovative anti-infective strategies.** In light of the current antibiotic shortage, the AQ framework is proving an excellent target for anti-infective development, both against *P. aeruginosa* and other pathogens. Suppression of AQ production, either by targeting the biosynthetic process or its regulation, is a promising anti-infective strategy for the control of *P. aeruginosa* pathogenesis. Synthetic modification of the AQ framework also has significant potential for control of important bacterial and fungal pathogens based on the interkingdom dimension to AQ-based cell-cell communication.