Contents

1 Introduction

1.1 Antibiotic resistance

Antibiotics add, on average, twenty years to a person's life.[1] However, antibiotic resistance is increasing alarmingly and is now recognised as a major threat to global health.[1,2] Antibiotic discovery had its heyday in the 1940s to 60s, which saw the discovery of many new classes of antibiotic. Since then, the rate of discovery of new classes has slowed and resistance to existing treatments has increased.

The story of how Alexander Fleming discovered penicillin by accidentally allowing a Petri dish containing *Staphylococcus aureus* to become contaminated with *Penicillium* mould whilst he was on holiday in Suffolk[1] is well known to many scientists. The initial serendipitous discovery of penicillin occurred in 1928 and was reported in 1929,[3] but it was not until 1943 that the drug was mass produced thanks to the research of Ernst Chain and Howard Florey. However, bacterial resistance to penicillin was being found in hospitals by the late 1940s.[4,5] This alarmingly quick emergence of resistance is a common phenomenon for antibiotics (see Table 1) as bacteria have multiple resistance mechanisms against antibacterial agents. These mechanisms can be broken down into five main categories:

1. The bacterium may inactivate the drug before it can cause damage, for example the hydrolysis of β-lactam antibiotics such as penicillin by β-lactamase enzymes.

2. The bacterium may produce a membrane, cell wall or biofilm which does not allow the drug to pass through, for example biofilm formation may allow bacterial resistance to antibiotics to increase 1000-fold compared with bacteria in suspension culture.[6]

3. The bacterium may pump antibacterial molecules out of its cell membrane using efflux pumps, for example the mexAB and mexXY pumps used by *Pseudomonas aeruginosa*.[7]

4. Mutations may cause the target of the antibacterial molecule to alter such that the molecule no longer effectively binds the target, for example the alteration of penicillin binding proteins which are involved in the final stages of peptidoglycan biosynthesis in the cell walls of MRSA and other penicillin-resistant bacteria.[8]

5. The bacterium may switch to using a metabolic pathway which does not involve the target of the antibacterial molecule, for example sulfonamide resistance may be achieved by taking in folic acid from the environment rather than synthesising it using *p*-aminobenzoic acid - a process which is blocked by sulfonamides.[9]

Antibiotic	Introduction	Resistance
Sulfonamides	1930s	1940s
Penicillin	1943	1946
Streptomycin	1943	1959
Chloramphenicol	1947	1959
Tetracycline	1948	1953
Erythromycin	1952	1988
Vancomycin	1956	1988
Methicillin	1960	1961
Ampicillin	1961	1973
Trimethoprim	1962	1972
Cephalosporins	1960s	late 1960s
Ciprofloxacin	1987	1988
Linezolid	2000	1997
Daptomycin	2003	2005

Table 1: A timeline of when various antibiotics were first introduced and when resistance to them first appeared.[10–15]

1.2 Quorum sensing

A quorum is defined as 'A fixed minimum number of members of an assembly or society that must be present at any of its meetings to make the proceedings of that meeting valid.'[16] A similar concept is used in bacterial signalling, whereby group behaviour is only triggered when a certain minimum population of bacteria has been reached. Examples of group behaviour include bioluminescence, the production of virulence factors and biofilm formation. It is advantageous for bacteria to coordinate such behaviours as they would be ineffective, and therefore a waste of resources, when carried out by a single bacterium but effective when carried out as a group.

1.2.1 *Vibrio fischeri*

The first example of quorum sensing was discovered in *Vibrio fischeri*, a symbiotic bacterium that produces bioluminescence in the photophore of the Hawaiian bobtail squid, *Euprymna scolopes*[7,17,18] (see Figure 1a). This bacterium receives amino acids[19,20] from its host in exchange for producing light which the squid uses for counterillumination, to camouflage itself[21] (*V. fischeri* also has symbiotic relationships with other species, including the Japanese pinecone fish, *Monocentris japonica*[22])(see Figure 1b).

Figure 1: a) "Euprymna scolopes, South shore of Oahu, Hawaii" by Jamie Foster. Licensed under CC BY-SA 3.0 via Commons. b) "Monocentris japonica.1 - Aquarium Finisterrae" by Drow_male. Licensed under GFDL via Commons.

If a low population of *V. fischeri* is present in the photophore, the light that the bacteria could produce would be insufficient to attract prey. Therefore, the bacteria conserve resources by not producing light. However, if there is a high population of *V. fischeri* it is useful for them all to produce light, as this incentivises the squid to provide them with nutrients.

1.2.1.1 The LuxR-LuxI system

The bacteria sense the population of other *V. fischeri* in their vicinity by the detection of 3-oxo-C_6-HSL **1**[23] (see Figure 2), a freely diffusible[24] molecule which is secreted by all *V. fischeri* cells[25] at a low basal level.[17] When the bacterial population density, and hence the concentration of 3-oxo-C_6-HSL **1**, reaches a threshold, a response is triggered leading to expression of high levels of luciferase, and hence a 10,000-fold[26] increase in the production of (blue-green

The quorum sensing system of *V. fischeri* consists of two operons (see Figure 3). The left operon encodes just one gene, *luxR*, a transcription factor which binds 3-oxo-C_6-HSL **1**. The right operon encodes *luxICDABEG*. *luxI* encodes an enzyme (LuxI) which uses acyl-acyl carrier protein and *S*-adenosyl-L-methionine (SAM) to form 3-oxo-C_6-HSL **1** by lactonisation and acylation.[27,28] *luxCDABEG* encodes luciferase enzymes required for light production. Both operons are continuously expressed at low levels, leading to production of low concentrations of LuxI, 3-oxo-C_6-HSL **1** and LuxR, and low-level light production.[29]

V. fischeri can multiply to very high cell concentrations in the photophore of *E. scolopes* (around 10^9 cells,[30–32] or 10^{11} cells per mL[17] in the organ of a mature squid). As concentrations rise to these levels, the concentration of 3-oxo-C_6-HSL **1** also rises. At a threshold of around 1-10 μg/mL,[23] 3-oxo-C_6-HSL **1** binds to a N-terminal domain of LuxR,[33] leading to unmasking of the C-terminal transcriptional activator domain.[34,35] The LuxR-3-oxo-C_6-HSL complex can then bind to the *lux* operator, which is situated between the left and right operons and, unusually, affects the transcription of both operons in a bidirectional manner, involving both positive and negative regulation.[36] It is thought that the LuxR-3-oxo-C_6-HSL complex forms a homodimer,[37] but this has not been conclusively proven.[38,39]

Binding of LuxR-3-oxo-C_6-HSL complex to the *lux* operator activates transcription of the right operon, leading to production of both 3-oxo-C_6-HSL **1** and light. Production of more 3-oxo-C_6-HSL **1** enables a positive feedback loop, re-inforcing the effect of high population density on 3-oxo-C_6-HSL **1** concentration and hence light production.

Concurrently, transcription of the left operon is also affected by binding of the LuxR-3-oxo-C_6-HSL complex to the *lux* operator, but in a more complex manner. At low concentrations of 3-oxo-C_6-HSL **1** transcription

of the left operon is activated, leading to production of more LuxR. However, at high concentrations of 3-oxo-C_6-HSL **1** production of LuxR is inhibited in an autoinducer-dependent manner.[40] This effect is dependent on DNA sequences found upstream of the left operon, within the right operon, and without them LuxR has a stimulatory effect at all concentrations of LuxR and autoinducer.

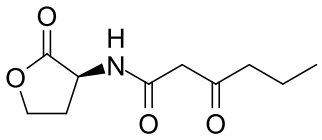


Figure 2: 3-oxo-C_6-HSL **1**.

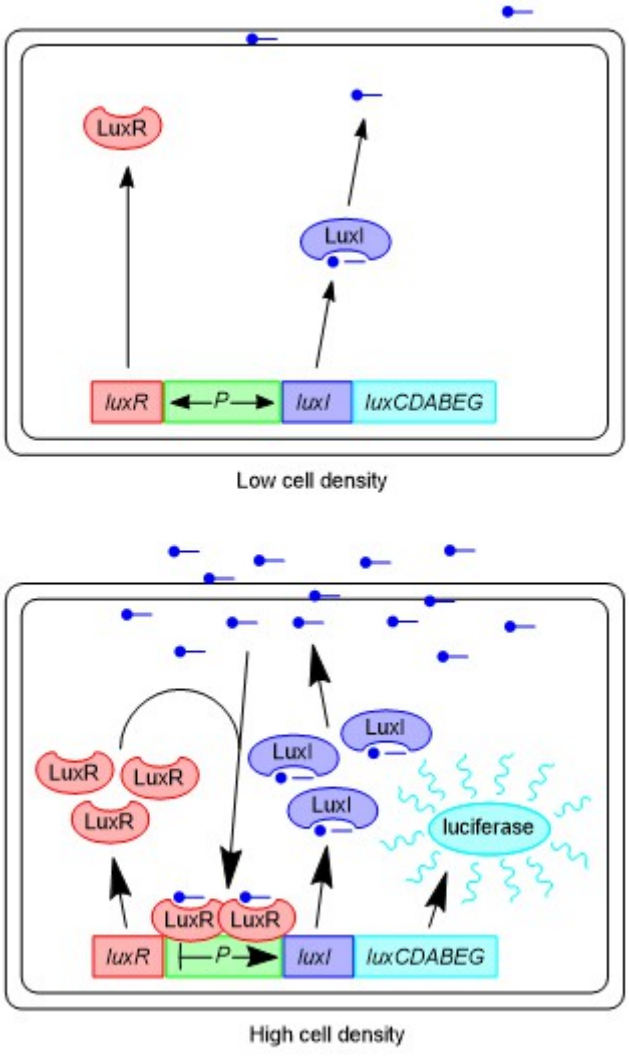


Figure 3: The LuxR-LuxI quorum sensing system in *V. fischeri*.

1.2.1.2 Other quorum sensing systems

Since the discovery in 1970 of the LuxR-LuxI quorum sensing system in *V. fischeri*, several other mechanisms have also been discovered (it should be noted that several of the proteins mentioned in this section were first characterised in *Vibrio harveyi*, and functions may be assigned by analogy to a closely-related *V. harveyi* protein[39]). Systems using two other quorum sensing molecules, *N*-octanoyl-homoserine lactone (C_8-HSL **20**) and a furanosyl borate diester (AI-2 **21**) have been discovered, both of which act via the *luxCDABEG* system and

increase luminescence by increasing luciferase production[39,41] (see Figure 4 and Figure 5). Additional controls in the *lux* promoter region have also been discovered, which respond to O_2 and cAMP[39] (see 1.2.1.3 and 1.2.1.4).

The AinS–AinR system uses C_8-HSL **20**, synthesised by AinS, as its signalling molecule.[39,42,43] C_8-HSL **20** has two main effects on the quorum sensing network. Firstly, it can bind to LuxR,[25] albeit with lower affinity than 3-oxo-C_6-HSL **1**, leading to partial upregulation of lux operon transcription. Secondly, it binds to the histidine kinase AinR, inhibiting its ability to phosphorylate the histidine phosphotransferase LuxU, which links into the LuxR pathway by a less direct route (see later in this section).[44]

The LuxS–LuxP/Q system uses AI-2 **21**, synthesised by LuxS, as its signalling molecule[39,45,46] (this pathway is common to all *Vibrio* species[39]). The receptor of AI-2 **21** is a complex of a two proteins, LuxP and LuxQ. LuxP is a periplasmic protein which binds AI-2 **21**, LuxQ is an inner membrane histidine kinase of the two-component sensor kinase family.[47] It is likely that LuxQ is constitutively dimeric, although this has not yet been demonstrated.[48]

When AI-2 **21** is not bound to LuxP, LuxQ autophosphorylates a histidine residue.[46] This phosphoryl group is then transferred to an aspartic acid residue in LuxQ, and then to a histidine residue in LuxU.

When AI-2 **21** binds to LuxP, this causes a major conformational change in the LuxP/Q complex, replacing one set of contacts between the proteins with another[45,46] and causing the formation of an asymmetric complex of two LuxP/Q dimers. Formation of the asymmetric dimers switches the activity of LuxQ from kinase to phosphatase, which can then dephosphorylate LuxU.

At high cell density, and hence autoinducer concentration, both the AinS–AinR system and the LuxS–LuxP/Q system bring about a decrease in the amount of phosphorylated LuxU. LuxU is a phosphotransferase protein which transfers its phosphate group to an aspartic acid residue in LuxO.[49] Phosphorylated LuxO inhibits quorum sensing responses by via LuxR. Hence, at high cell densities there is a decreased amount of phosphorylated LuxU present, leading to a lack of phosphorylated LuxO, and hence increased quorum sensing responses, e.g. light production. This 'many-to-one' signalling pathway is common in bacterial two-component signalling systems[50] and is found in several other *Vibrionaceae*.[51]

LuxO phosphate inhibits quorum sensing responses via σ_{54}-dependent transcriptional activation of *qrr1*[52,53] (despite the proximity of the *luxOU* and *qrr* promoters, LuxO only affects the activates the production of Qrr1 and not itself[54]). Qrr1 is a small RNA molecule (a quorum regulatory RNA or Qrr) which, with the help of Hfq, can bind to LitR RNA, leading to its degradation.[53] Qrr1 is the only Qrr to regulate LitR expression in *V. fischeri*, and is conserved across all *Vibrionaceae*.[53] In contrast, in other *Vibrionaceae* a family of Qrrs is often used.[55]

Qrr1/Hfq-mediated degradation of LitR mRNA inhibits the production of LitR, an activator of the *lux* operon. LitR binds to a region of the *luxR* promoter, causing increased LuxR production and hence increased bioluminescence.[56]

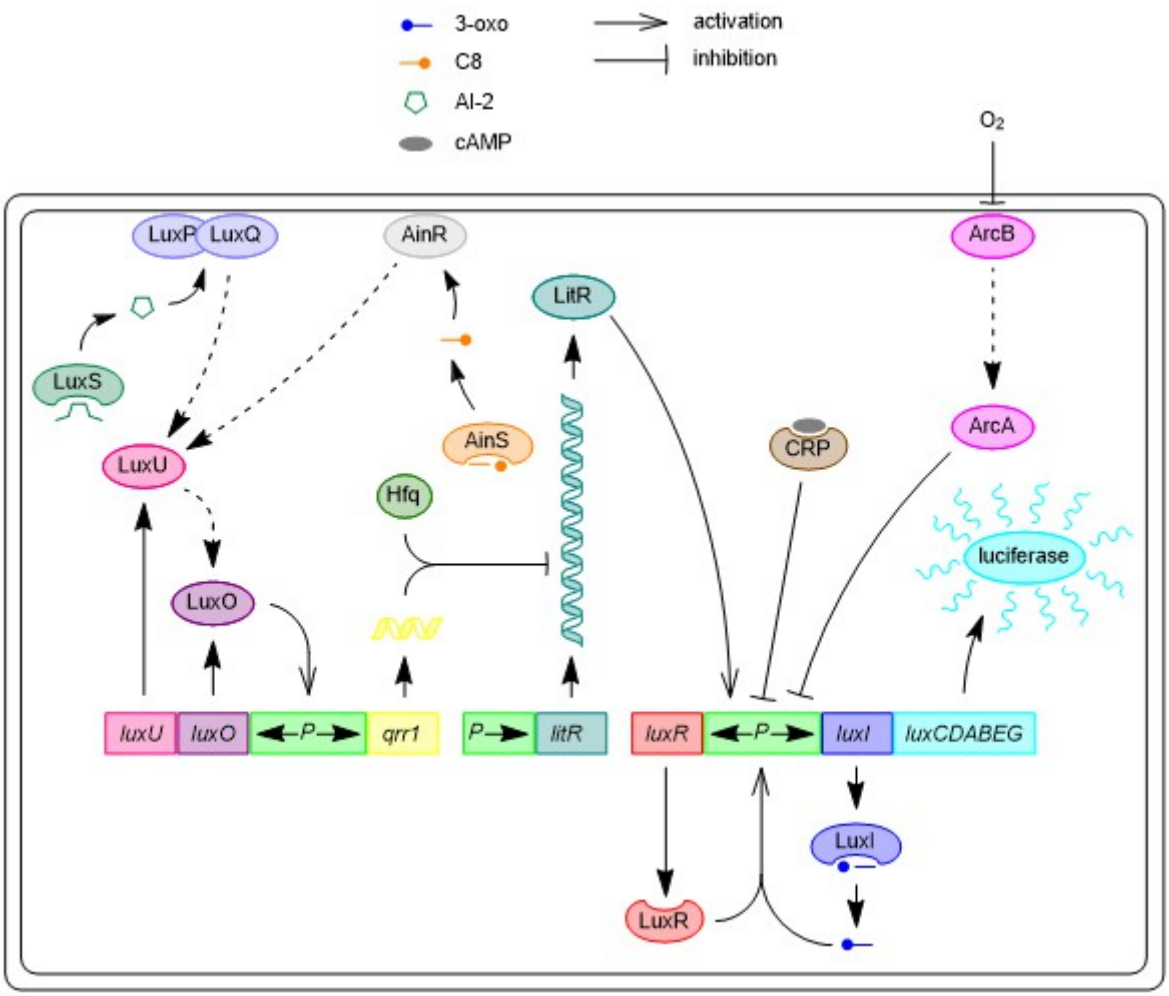


Figure 4: Quorum sensing in *V. fischeri*.

1.2.1.3 The effect of O_2

V. fischeri uses the ArcA-ArcB system to sense how oxygen-rich its environment is, by monitoring the redox state of various quinones produced by the cell. In an oxygen-rich environment, luminescence is stimulated. It is though that luminescence is used by *V. fischeri* make its environment less oxidising, as luminescence consumes oxygen.

ROS are bad and it doesn't want them[57]

ArcB is a histidine kinase which senses the redox state of the quinones. When the environment is low in oxygen or reactive oxygen species, the quinones are reduced and can stimulate ArcB to phosphorylate ArcA. ArcA is a response regulator which represses the transcription of *luxICDABEG* (and to a lesser extent, *luxR*).

1.2.1.4 The effect of cAMP

cAMP

1.2.1.5 The purpose of multiple signalling pathways

It might reasonably be asked: why does *V. fischeri* use three quorum sensing pathways rather than just one? The answer to this question lies in the bacterium's relationship with its squid host. It has been shown that the LuxS-LuxP/Q and AinS-AinR systems are important in the medium cell densities found during early colonisation of the host, whereas the LuxR-LuxI system is important at the higher cell densities found in late colonisation.[42,58]

It has been shown that the LuxS-LuxP/Q system does not have an especially large effect on colonisation of the host squid, although it does have some effect on luminescence.[59] It has therefore been speculated that the LuxS-LuxP/Q system is more important in the colonisation of other marine invertibrates, either in their light organs[22,60] or as part of multi-species colonies in their guts.[59,61]

The AinS-AinR system has a larger effect on colonisation and luminescence, in that *ainS* mutants show only 10-20 % of wild-type luciferase activity at medium cell densities in culture (10_8 to 10_8 cells ml_{-1}).[42] At the higher cell densities in the squid host ($>10_{10}$ cells ml_{-1}), *ainS* mutants show 10-40 % of wild-type luciferase activity, an effect which can be partially attributed to failure of the mutants to colonise the host (bacterial cell numbers are down to 20-80 % compared to the wild type). This failure of *ainS* mutants to colonise the host is due to *ain* regulation of pathways involved in early colonisation. The AinS-AinR system controls around 30 genes via LuxO and LitR.[58] *ain* quorum sensing is thought to repress several motility genes, causing loss of flagella, which are initially required for normal colonisation of the host,[62,63] but are lost as the bacteria colonise the host.[30] *ain* quorum sensing also induces a putative exopolysaccharide, which could be important in biofilm formation inside the host, or evasion of its immune system,[64] as well as two unique. In addition, *ain* quorum sensing affects the transcription several genes involved in metabolism, and new genes of unknown function which could affect colonisation by an as yet unknown pathway.

In contrast to the AinS-AinR, the LuxI-LuxR system is only fully induced at the high cell densities found in the *E. scolopes* light organ. At medium cell densities, C_8-HSL **20** is thought to be the dominant autoinducer, partially activating transcription from the *lux* operon by binding to both AinR and LuxR.[42] At high cell densities, C_8-HSL **20** is displaced from LuxR by 3-oxo-C_6-HSL **1**, leading to full light production.

lux quorum sensing also affects the transcription of five non-*lux* proteins which could potentially act as late colonisation factors.[65,66] Three of these genes, *qsrP*, *acfA*, and *ribB*, are directly activated by LuxR/3-oxo-C_6-HSL[66] and a strain lacking *qsrP* is less effective at colonising *E. scolopes* than the wild type, providing good evidence that it is a late-stage colonisation factor.[65]

ain positive feedback, LuxPQ not[59]

Which do CRP and Arc act on? Add phos. LitR upregulates AinS[59]

Quorum sensing has since been observed in many species of bacteria, including *P. aeruginosa*, *Agrobacterium tumefaciens*, *Erwinia carotovora*, *Streptococcus pneumoniae*, *Bacillus subtilis*, *S. aureus*, *Vibrio harveyi*,

Escherichia coli, *Myxococcus xanthus*, *Salmonella enterica*, *Yersinia enterocolitica Aeromonas* sp. and *Acinetobacter* sp.[17,67–74] Many of these bacteria are significant causes of disease and death in humans, for example, it is estimated that in 2005 in the US *S. aureus* caused 477,927 hospitalisations and 11,406 deaths.[75] *S. aureus* uses a peptide autoinducer know as autoinducing peptide (AIP) (see **??** in **??**) which interacts with the *agr* system leading to increased protease and toxin production.[76] *P. aeruginosa* also uses quorum sensing to coordinate biofilm formation, swarming motility and virulence.

1.2.2 *Pseudomonas aeruginosa*

One of the most well-studied examples of QS is in *P. aeruginosa*. *P. aeruginosa* is a Gram-negative opportunistic pathogen which typically infects immunocompromised individuals such as those with cystic fibrosis, neutropenia and AIDS. It can infect the pulmonary and urinary tracts as well being the most frequent cause of burn wound infections and the most frequent conloniser of medical devices such as catheters.[77]

P. aeruginosa uses quorum sensing (QS) to coordinate biofilm formation, swarming motility and virulence. The autoinducers used by *P. aeruginosa* are shown in Figure 7 (HHQ **5** is a precursor to PQS **4** but can bind to its receptor, PQSr, and hence can act as a autoinducer[78]). QS in *P. aeruginosa* involves a complex interplay of the four signalling molecules and various proteins (see Figure 6).[79] QS regulates the production of virulence factors including elastase, alkaline protease, exotoxin A, rhamnolipids, pyocyanin, lectins and superoxide dismutases, as well as regulating biofilm formation.

P. aeruginosa has a low susceptibility to many antibiotics due to its chromosomally encoded multidrug efflux pumps: mexAB and mexXY.[7] It is also difficult for drugs to cross into cells due to low cell wall permeability and biofilm formation. *P. aeruginosa* may also acquire antibiotic resistance by mutation or horizontal gene transfer.[80] This high level of antibiotic resistance makes *P. aeruginosa* an important target for drug discovery.

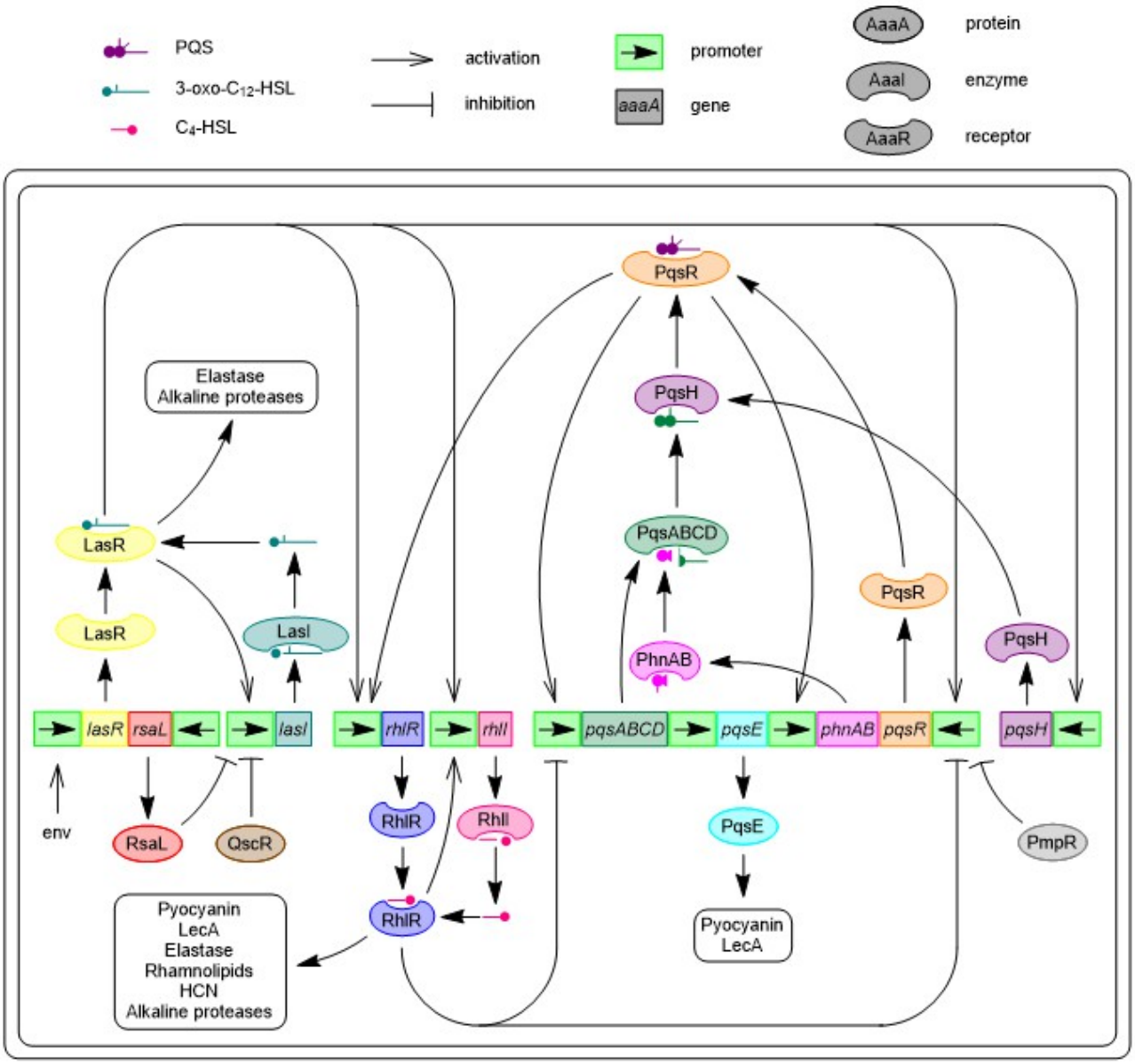


Figure 6: Quorum sensing in *P. aeruginosa*.[79]

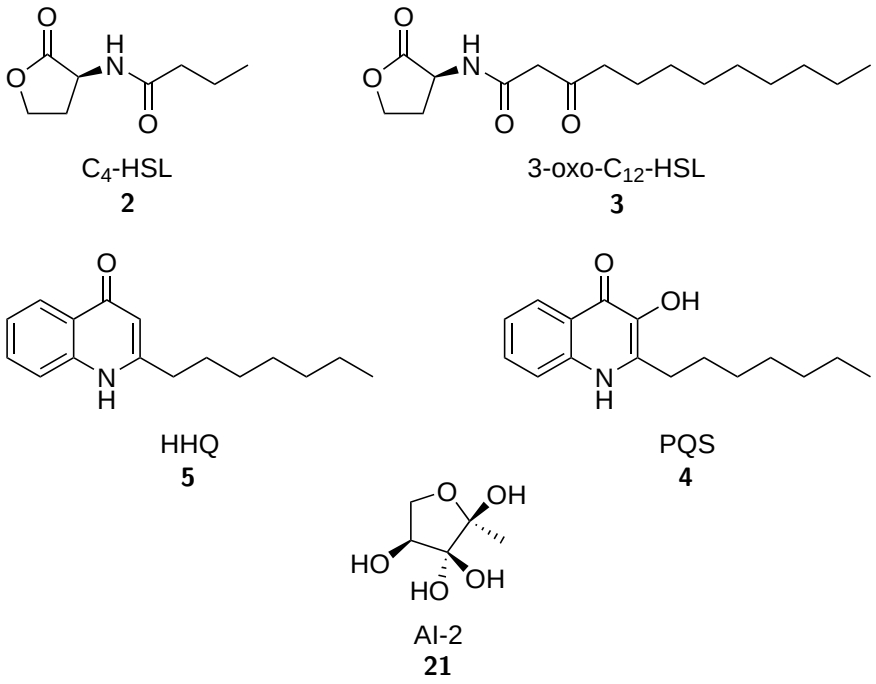
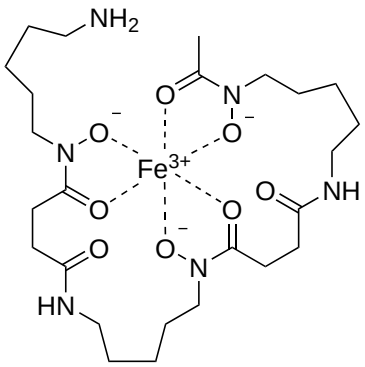


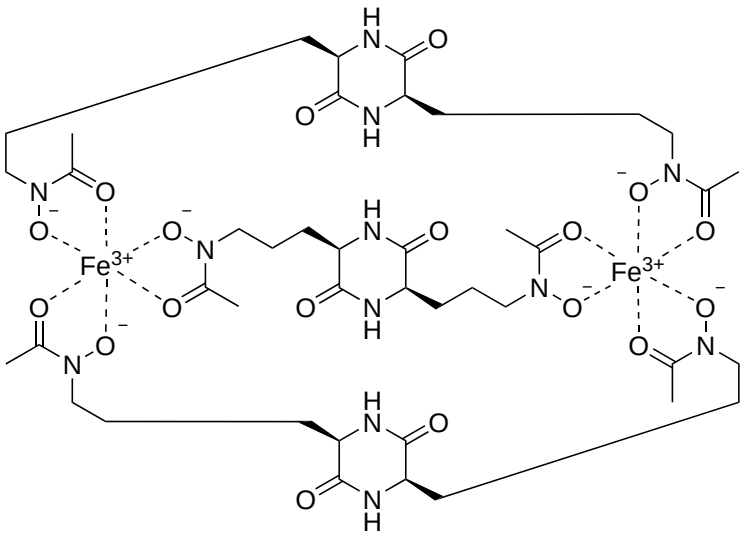
Figure 7: *P. aeruginosa* quorum sensing molecules.

1.3 Siderophores

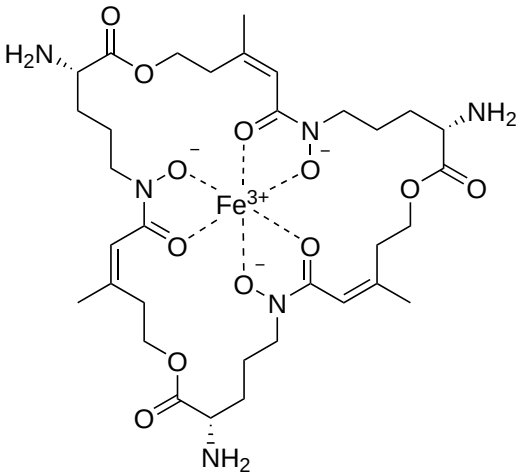
Siderophores are peptides or small molecules used by microorganisms to chelate iron for the purposes of 'iron mining'.[81] Soluble iron is often scarce but it is crucial for many cellular processes including respiration and DNA synthesis. Siderophores are synthesised by the microorganisms and secreted into the extracellular environment where they bind to Fe^{3+}, often with exceptionally high affinities. The iron-bound siderophores are then brought back into the cell by active transport and the iron is released, either by reduction of the Fe^{3+} to Fe^{2+} or by enzymatic degradation of the siderophore. Siderophores have a wide range of structures (see Figure 8 and Figure 9), possibly so one species can avoid its siderophores being taken up by another species.[82]



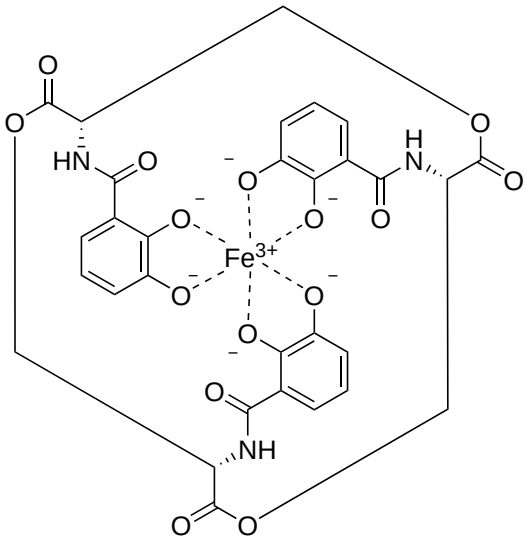
Deferoxamine B
6



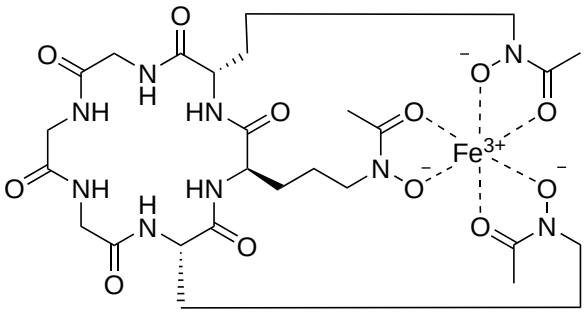
Rhodotorulic acid
7



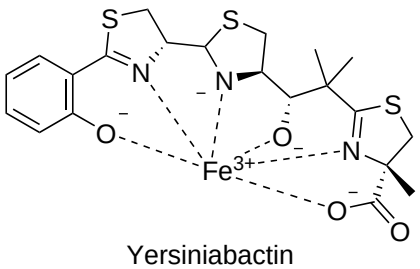
Fusarinine C
8



Enterobactin
9



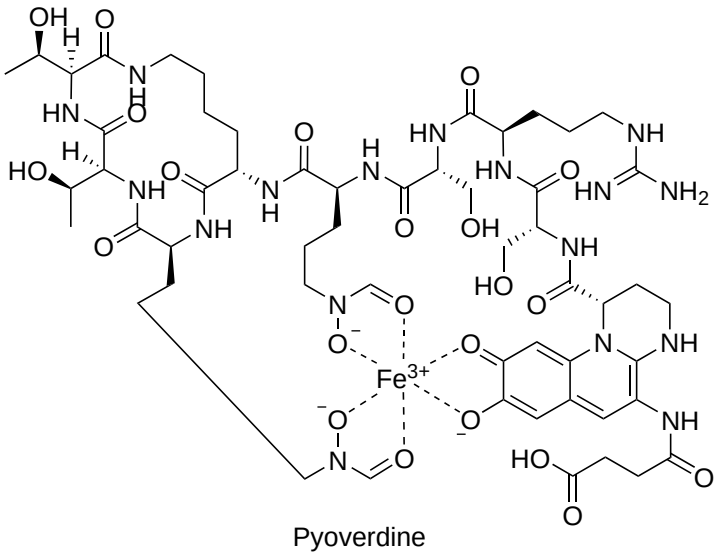
Ferrichrome
10



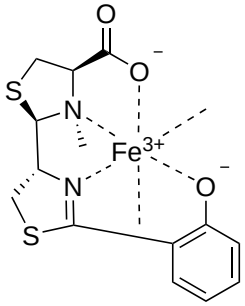
Yersiniabactin
11

Figure 8: Iron-siderophore complexes: Deferoxamine **6**[83] (*Streptomyces pilosus* and *Streptomyces coelicolor*), rhodotorulic acid **7**[84] (*Rhodotorula pilimanae*), fusarinine C **8**[85] (*Fusarium roseum*), enterobactin **9**[83] (*Escherichia coli* and enteric bacteria), ferrichrome **10**[86] (*Ustilago sphaerogena*), yersiniabactin **11**[83] (*Yersinia pestis*).

Ferrichrome was originally isolated3a in 1952, from the fungus Ustilago sphaerogena, and has subsequently been de- tected in cultures of U. maydi ,) Aspergillus t iger, A. quadricintus, 3c A. duricaulis, 3c and Penicillium resticolo- sum. 3b The



Pyoverdine
12



Pyochelin
13

Figure 9: Iron-siderophore complexes: Pyochelin **12**[87] (*P. aeruginosa*), Pyoverdine **13**[83] (*P. aeruginosa*). Note that pyoverdine **13** is a tetradentate ligand, hence the iron ion has two sites which can bind other ligands.

1.4 Sideromycins

Siderophore-antibiotic conjugates are produced naturally by some bacteria and are known as sideromycins[88] (see Figure 10). Bacteria produce these molecules to attack other bacteria by hijacking their siderophore uptake mechanisms to introduce toxic compounds. Albomycin **14** (see Figure 10) is an example of a sideromycin produced by *Actinomyces subtropicus*.[89] It contains a siderophore coupled to a nuceloside antibiotic using a peptide linker. The siderophore is structurally similar to ferrichrome **10** (see Figure 8), and so is taken up by the same transport protein in *E. coli*. The linker is hydrolysed by a protease in the cytoplasm of the *E. coli*, releasing the active nuceloside antibiotic. This can lead to 500-fold concentration of the antibiotic within the *E. coli* cells, enough to have significant antibiotic activity.

and Streptomyces griseus? Others? albo Do they share ferri? Ferrichrome made by fungi but used by bacteria?

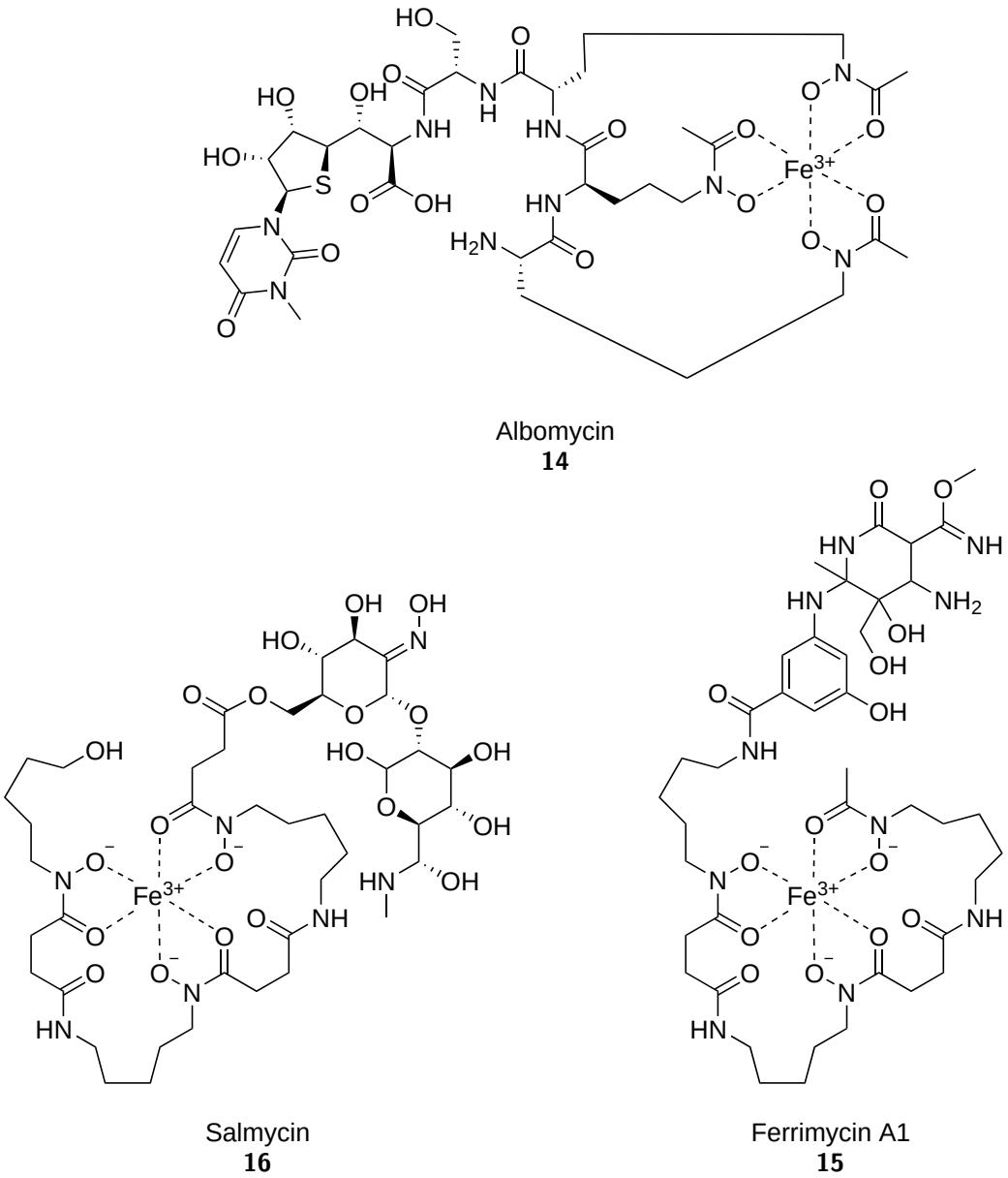


Figure 10: Sideromycins.

1.5 Synthetic siderophore-antibiotic conjugates

Sideromycins served as inspiration to Ghosh *et al.*,[90] prompting the design, synthesis and biological evaluation of the first synthetic siderophore-antibiotic conjugates using β-lactam,[91–93] nucleoside,[94] glycopeptides[95] and macrolide[90] antibiotics. Fluoroquinolone **158** conjugates with sideromycins have been studied by several groups,[96–98] including conjugates with linkers which can be cleaved[97,98] in a similar manner to albomycin.[89] Some of these showed comparable activity to the parent antibiotic, but it is not clear whether attachment of the siderophore improved uptake or whether the conjugates acted as classical prodrugs.

β-lactam-sideromycin conjugates have been more widely investigated[88] and show good activity *in vitro*, however, resistance can evolve by loss of the TonB transporter or of the relevant siderophore receptor, e.g. Cir and Fiu for catecholate siderophores or FhuA for hydroxamate siderophores. Initial studies used aminopenicillins and cephems, and recently a conjugate of enterobactin (see Figure 8) and ampicillin (see **??** in **??**) has been shown to have increased activity against pathogenic *E. coli* when compared to native ampicillin.[99] Other work has focused on monocyclic β-lactams, which show high potency against Gram-negative bacteria but low potency against Gram-positive bacteria.[100] Monocyclic β-lactams are generally fairly stable to β-lactamase activity,

which is an advantage compared with many β-lactams.

Siderophore-antibiotic conjugates are a promising strategy to deliver antibiotics across bacterial membranes, but it is worth noting that conjugation to a siderophore may lead to loss of activity, or resistance may be acquired by loss of transport proteins. Encouragingly, mutants lacking transport proteins tend to grow more slowly in low-iron conditions, but with an immunocompromised host siderophores may not always be required for infection as bacteria can obtain iron from other sources.[101]

BAL30072 **17** (see Figure 11) is a conjugate of a pyochelin **13** (see Figure 8) analogue and a monocyclic β-lactam. It is in early clinical trails and appears to have overcome many of the above hurdles as it is a poor substrate for β-lactamases and resistance due to loss of transport proteins is infrequent.[88] Building on this positive example of an antibiotic conjugate, it is hoped that the approach can be extended to conjugates of antibiotics and autoinducers

Kills liver[102]

MC-1[103,104] cephalosporin S-649266[105] LN-1-255[106,107]

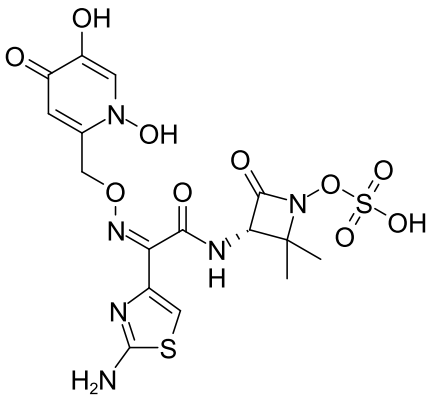


Figure 11: BAL30072 **17**.

1.6 Autoinducer-antibiotic conjugates

The success of the siderophore-antibiotic conjugate field lead us to speculate that a similar strategy might be employed with autoinducers, leading to autoinducer-antibiotic conjugates. It is hypothesised that attaching a autoinducer to a known antibiotic could lead to increased uptake or better localisation of the antibiotic and could restore function in resistant strains. We decided to focus initially on the autoinducers synthesised by *P. aeruginosa* due to the low activity of various antibiotics towards it. Ciprofloxacin **158** (see Figure 12) was chosen as the initial antibiotic half as it is often used to treat *P. aeruginosa*[108] but resistance is developing.[109] It is hoped that the quorum sensing molecules will deliver the attached ciprofloxacin into the cell in a 'Trojan horse'/citeMollmann2009 approach. This could kill or halt the growth of the bacteria. Alternatively, the bacteria might develop mutations in their quorum sensing machinery to avoid taking up the antibiotics, but this could stop them being able to communicate effectively, therefore decreasing virulence. We have access to some ciprofloxacin-resistant strains and aim to test the conjugates on them, as well as on susceptible strains, to test whether there is an increase in potency or even a restoration of activity against resistant strains.

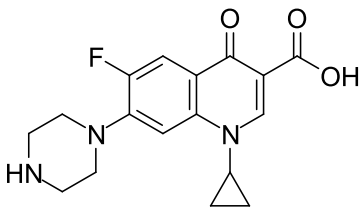


Figure 12: Ciprofloxacin **158**.

Why Cip, Tri, PA AIs?

2 References

[1] S. C. Davies, *The Drugs Don't Work: A Global Threat*, Penguin Books Limited. 2013.

[2] *Antibiotic Resistance Threats in the United States*. 2013.

[3] A. Fleming. ON THE ANTIBACTERIAL ACTION OF CULTURES OF A PENICILLIUM , WITH SPECIAL REFERENCE TO THEIR USE IN THE ISOLATION OF B . INFLUENZ ? 1E . *The British Journal of Experimental Pathology*, 10(3):226–236. 1929.

[4] M. Barber. Staphylococcal infection due to penicillin-resistant strains. *British Medical Journal*, 2(4534):863–865. 1947.

[5] P. M. Rountree and E. F. Thomson. Incidence of penicillin-resistant and streptomycin-resistant staphylococci in a hospital. *The Lancet*, 254(6577):501–504. 1949.

[6] P. S. Stewart and J. W. Costerton. Antibiotic resistance of bacteria in biofilms. *The Lancet*, 358(9276):135–138. 2001.

[7] K. Poole. Efflux-mediated multiresistance in Gram-negative bacteria. *Clinical Microbiology and Infection*, 10:12–26. 2004.

[8] C. Fuda, M. Suvorov, S. B. Vakulenko and S. Mobashery. The basis for resistance to beta-lactam antibiotics by penicillin-binding protein 2a of methicillin-resistant Staphylococcus aureus. *The Journal of Biological Chemistry*, 279(39):40802–40806. 2004.

[9] O. Sköld. Sulfonamide resistance: mechanisms and trends. *Drug Resistance Updates*, 3:155–160. 2000.

[10] A. E. Clatworthy, E. Pierson and D. T. Hung. Targeting virulence: a new paradigm for antimicrobial therapy. *Nature Chemical Biology*, 3(9):541–548. 2007.

[11] S. R. Palumbi. Humans as the World's Greatest Evolutionary Force. *Science*, 293:1786–1790. 2001.

[12] J. W. Ogle, L. B. Reller and M. L. Vasil. Development of resistance in Pseudomonas aeruginosa to imipenem, norfloxacin, and ciprofloxacin during therapy: proof provided by typing with a DNA probe. *The Journal of infectious diseases*, 157(4):743–748. 1988.

[13] P. Huovinen. Resistance to Trimethoprim-Sulfamethoxazole. *Antimicrobial Resistance*, 32(11):1608–1614. 2001.

[14] M. C. Birmingham, C. R. Rayner, A. K. Meagher, S. M. Flavin, D. H. Batts and J. J. Schentag. Linezolid for the Treatment of Multidrug-Resistant , Gram-Positive Infections: Experience from a Compassionate-Use Program. *Clinical Infectious Diseases*, 36(2):159–168. 2003.

[15] D. K. Lee, Y. Kim, K. S. Park, J. W. Yang, K. Kim and N. J. Ha. Antimicrobial activity of mupirocin, daptomycin, linezolid, quinupristin/dalfopristin and tigecycline against vancomycin-resistant enterococci (VRE) from clinical isolates in Korea (1998 and 2005). *Journal of biochemistry and molecular biology*, 40(6):881–887. 2007.

[16] *Oxford English Dictionary*, Oxford University Press. 2014.

[17] M. B. Miller and B. L. Bassler. QUORUM SENSING IN BACTERIA. *Annual Review of Microbiology*, 55:165–199. 2001.

[18] K. L. Visick and E. G. Ruby. Vibrio fischeri and its host: it takes two to tango. *Current Opinion in Microbiology*, 9(6):632–638. 2006.

[19] J. Graf and E. G. Ruby. Host-derived amino acids support the proliferation of symbiotic bacteria. *Proceedings of the National Academy of Sciences of the United States of America*, 95(4):1818–1822. 1998.

[20] J. D. Lemus and M. J. McFall-Ngai. Alterations in the proteom of the Euprymna scolopes light organ in presonse to symbiotic Vibrio fischeri. *Applied and Environmental Microbiology*, 66(9):4091–4097. 2000.

[21] B. W. Jones and M. K. Nishiguchi. Counterillumination in the Hawaiian bobtail squid, Euprymna scolopes Berry (Mollusca: Cephalopoda). *Marine Biology*, 144(6):1151–1155. 2004.

[22] E. G. Ruby and K. H. Nealson. Symbiotic association of Photobacterium fischeri with the marine luminous fish Monocentris japonica; a model of symbiosis based on bacterial studies. *Biological Bulletin*, 151:574–586. 1976.

[23] a. Eberhard, a. L. Burlingame, C. Eberhard, G. L. Kenyon, K. H. Nealson and N. J. Oppenheimer. Structural identification of autoinducer of Photobacterium fischeri luciferase. *Biochemistry*, 20(9):2444–2449. 1981.

[24] H. B. Kaplan and E. P. Greenberg. Diffusion of autoinducer is involved in regulation of the Vibrio fischeri luminescence system. *Journal of bacteriology*, 163(3):1210–1214. 1985.

[25] A. L. Schaefer, B. L. Hanzelka, A. Eberhard and E. P. Greenberg. Quorum sensing in Vibrio fischeri: probing autoinducer-LuxR interactions with autoinducer analogs. *Journal of Bacteriology*, 178(10):2897–2901. 1996.

[26] J. H. Devine, G. S. Shadel and T. O. Baldwin. Identification of the operator of the lux regulon from the Vibrio fischeri strain ATCC7744. *Proceedings of the National Academy of Sciences*, 86(15):5688–5692. 1989.

[27] M. R. Parsek, D. L. Val, B. L. Hanzelka, J. E. Cronan and E. P. Greenberg. Acyl homoserine-lactone quorum-sensing signal generation. *Proceedings of the National Academy of Sciences of the United States of America*, 96(8):4360–4365. 1999.

[28] W. T. Watson, T. D. Minogue, D. L. Val, S. B. Von Bodman and M. E. a. Churchill. Structural basis and specificity of acyl-homoserine mactone signal production in bacterial quorum sensing. *Molecular Cell*, 9(3):685–694. 2002.

[29] J. Engebrecht, K. Nealson and M. Silverman. Bacterial bioluminescence: isolation and genetic analysis of functions from Vibrio fischeri. *Cell*, 32(3):773–781. 1983.

[30] E. G. Ruby and L. M. Asato. Growth and flagellation of Vibrio fischeri during initiation of the sepiolid squid light organ symbiosis. *Archives of Microbiology*, 159(2):160–167. 1993.

[31] E. G. Ruby and K. H. H. Lee. The Vibrio fischeri-Euprymna scolopes light organ association: Current ecological paradigms. *Applied and Environmental Microbiology*, 64(3):805–812. 1998.

[32] S. V. Nyholm and M. J. Mcfall-Ngai. Sampling the light-organ microenvironment of Euprymna scolopes: Description of a population of host cells in association with the bacterial symbiont Vibrio fischeri. *Biological Bulletin*, 195(2):89–97. 1998.

[33] B. L. Hanzelka and E. P. Greenberg. Evidence That the N Terminal Region of the Vibrio Fischeri Luxr Protein Constitutes an Autoinducer Binding Domain. *Journal of Bacteriology*, 177(3):815–817. 1995.

[34] S. H. Choi and E. P. Greenberg. The C-terminal region of the Vibrio fischeri LuxR protein contains an inducer-independent lux gene activating domain. *Proceedings of the National Academy of Sciences of the United States of America*, 88(24):11115–11119. 1991.

[35] S. H. Choi and E. P. Greenberg. Genetic dissection of DNA binding and luminescence gene activation by the Vibrio Genetic Dissection of DNA Binding and Luminescence Gene Activation by the Vibrio fischeri LuxR Protein. 174(12):4064–4069. 1992.

[36] G. S. Shadel and T. O. Baldwin. The Vibrio fischeri LuxR protein is capable of bidirectional stimulation of transcription and both positive and negative regulation of the luxR gene. *Journal of bacteriology*, 173(2):568–74. 1991.

[37] S. Choi and E. Greenberg. Genetic evidence for multimerization of LuxR, the transcriptional activator or Vibrio fischeri luminescence. *Molecular Marine Biology and Biotechnology*, 1:408–413. 1992.

[38] L. C. M. Antunes, R. B. R. Ferreira, C. P. Lostroh and E. P. Greenberg. A mutational analysis defines Vibrio fischeri LuxR binding sites. *Journal of Bacteriology*, 190(13):4392–4397. 2008.

[39] T. Miyashiro and E. G. Ruby. Shedding light on bioluminescence regulation in Vibrio fischeri. *Molecular Microbiology*, 84(5):795–806. 2012.

[40] P. V. Dunlap and J. M. Ray. Requirement for autoinducer in transcriptional negative autoregulation of the Vibrio fischeri luxR gene in Escherichia coli. *Journal of bacteriology*, 171(6):3549–52. 1989.

[41] S. C. Verma and T. Miyashiro. Quorum sensing in the squid-Vibrio symbiosis. *International journal of molecular sciences*, 14(8):16386–16401. 2013.

[42] C. Lupp, M. Urbanowski, E. P. Greenberg and E. G. Ruby. The Vibrio fischeri quorum-sensing systems ain and lux sequentially induce luminescence gene expression and are important for persistence in the squid host. *Molecular microbiology*, 50(1):319–331. 2003.

[43] L. Gilson, A. Kuo and P. V. Dunlap. AinS and a new family of autoinducer synthesis proteins. *Journal of bacteriology*, 177(23):6946–51. 1995.

[44] M. Timmen, B. L. Bassler and K. Jung. AI-1 influences the kinase activity but not the phosphatase activity of LuxN of Vibrio harveyi. *Journal of Biological Chemistry*, 281(34):24398–24404. 2006.

[45] M. B. Neiditch, M. J. Federle, S. T. Miller, B. L. Bassler and F. M. Hughson. Regulation of LuxPQ receptor activity by the quorum-sensing signal autoinducer-2. *Molecular Cell*, 18(5):507–518. 2005.

[46] M. B. Neiditch, M. J. Federle, A. J. Pompeani, R. C. Kelly, D. L. Swem, P. D. Jeffrey, B. L. Bassler and F. M. Hughson. Ligand-Induced Asymmetry in Histidine Sensor Kinase Complex Regulates Quorum Sensing. *Cell*, 126(6):1095–1108. 2006.

[47] B. L. Bassler, M. Wright and M. R. Silverman. Multiple signalling systems controlling expression of luminescence in Vibrio harveyi: Sequence and function of genes encoding a second sensory pathway. *Molecular Microbiology*, 13(2):273–286. 1994.

[48] A. M. Stock, V. L. Robinson and P. N. Goudreau. Two-Component Signal Transduction. *Annual Review of Biochemistry*, 69(1):183–215. 2000.

[49] J. A. Freeman and B. L. Bassler. Sequence and Function of LuxU : a Two-Component Phosphorelay Protein That Regulates Quorum Sensing in Vibrio harveyi Sequence and Function of LuxU : a Two-Component Phosphorelay Protein That Regulates Quorum Sensing in Vibrio harveyi. 181(3):899–906. 1999.

[50] M. T. Laub and M. Goulian. Specificity in two-component signal transduction pathways. *Annu Rev Genet*, 41:121–145. 2007.

[51] D. L. Milton. Quorum sensing in vibrios: Complexity for diversification. *International Journal of Medical Microbiology*, 296(2-3):61–71. 2006.

[52] C. M. Miyamoto, Y. H. Lin and E. A. Meighen. Control of bioluminescence in Vibrio fischeri by the LuxO signal response regulator. *Molecular Microbiology*, 36(3):594–607. 2000.

[53] T. Miyashiro, M. S. Wollenberg, X. Cao, D. Oehlert and E. G. Ruby. A single qrr gene is necessary and sufficient for LuxO-mediated regulation in Vibrio fischeri. *Molecular microbiology*, 77(6):1556–67. 2010.

[54] B. N. Lilley and B. L. Bassler. Regulation of quorum sensing in Vibrio harveyi by LuxO and Sigma-54. *Molecular Microbiology*, 36(4):940–954. 2000.

[55] D. H. Lenz, K. C. Mok, B. N. Lilley, R. V. Kulkarni, N. S. Wingreen and B. L. Bassler. The small RNA chaperone Hfq and multiple small RNAs control quorum sensing in Vibrio harveyi and Vibrio cholerae. *Cell*, 118(1):69–82. 2004.

[56] P. M. Fidopiastis, C. M. Miyamoto, M. G. Jobling, E. a. Meighen and E. G. Ruby. LitR, a new transcriptional activator in Vibrio fischeri, regulates luminescence and symbiotic light organ colonization. *Molecular Microbiology*, 45(1):131–143. 2002.

[57] K. L. Visick, J. Foster, J. Doino, M. McFall-Ngai and E. G. Ruby. Vibrio fischeri lux genes play an important role in colonization and development of the host light organ. *Journal of Bacteriology*, 182(16):4578–4586. 2000.

[58] C. Lupp and E. G. Ruby. Vibrio fischeri Uses Two Quorum-Sensing Systems for the Regulation of Early and Late Colonization Factors Vibrio fischeri Uses Two Quorum-Sensing Systems for the Regulation of Early and Late Colonization Factors. 187(11):3620–3629. 2005.

[59] C. Lupp and E. G. Ruby. Vibrio fischeri LuxS and AinS: Comparative Study of Two Signal Synthases. *Journal of Bacteriology*, 186(12):3873–3881. 2004.

[60] J. L. REICHELT, K. NEALSON and J. W. HASTINGS. The Specificity of Symbiosis: Pony Fish and Luminescent Bacteria. *Arch. Microbiol.*, 112:157–161. 1977.

[61] A. Ramesh, R. Nandakumar and V. K. Venugopalan. Enteric Luminous Microflora of the Pond-Cultured Milk Fish Chanos chanos (Forskal). pages 231–235. 1986.

[62] D. S. Millikan and E. G. Ruby. Alterations in Vibrio fischeri motility correlate with a delay in symbiosis initiation and are associated with additional symbiotic colonization defects. *Applied and Environmental Microbiology*, 68(5):2519–2528. 2002.

[63] J. Graf, P. V. Dunlap and E. G. Ruby. Effect of transposon-induced motility mutations on colonization of the host light organ by Vibrio fischeri. *Journal of Bacteriology*, 176(22):6986–6991. 1994.

[64] I. S. Roberts. The biochemistry and genetics of capsular polysaccharide production in bacteria. *Annual Review of Microbiology*, 50(1):285–315. 1996.

[65] S. M. Callahan and P. V. Dunlap. lux Genes Define a Quorum-Sensing Regulon in Vibrio fischeri LuxR- and Acyl-Homoserine-Lactone-Controlled Non- lux Genes Define a Quorum-Sensing Regulon in Vibrio fischeri. *Journal of bacteriology*, 182(10):2811–2822. 2000.

[66] N. Qin, S. M. Callahan, P. V. Dunlap and A. M. Stevens. Analysis of LuxR regulon gene expression during quorum sensing in Vibrio fischeri. *Journal of Bacteriology*, 189(11):4127–4134. 2007.

[67] W. C. Fuqua, S. C. Winans and E. P. Greenberg. MINIREVIEW Quorum Sensing in Bacteria : the LuxR-LuxI Family of Cell Density-Responsive Transcriptional Regulatorst. *Journal of Bacteriology*, 176(2):269–275. 1994.

[68] C. M. Waters and B. L. Bassler. Quorum sensing: cell-to-cell communication in bacteria. *Annual Review of Cell and Developmental Biology*, 21:319–346. 2005.

[69] S. Atkinson, C.-Y. Chang, R. E. Sockett, M. Cámara and P. Williams. Quorum Sensing in Yersinia enterocolitica Controls Swimming and Swarming Motility. *Journal of Bacteriology*, 188(4):1451–1461. 2006.

[70] K.-G. Chan, S. D. Puthucheary, X.-Y. Chan, W.-F. Yin, C.-S. Wong, W.-S. S. Too and K.-H. Chua. Quorum sensing in Aeromonas species isolated from patients in Malaysia. *Current Microbiology*, 62(1):167–72. 2011.

[71] K. Sauer, A. K. Camper, G. D. Ehrlich, J. W. Costerton and D. G. Davies. Pseudomonas aeruginosa Displays Multiple Phenotypes during Development as a Biofilm. *Journal of Bacteriology*, 184(4):1140–1154. 2002.

[72] B. Michael, J. N. Smith, S. Swift and F. Heffron. SdiA of Salmonella enterica Is a LuxR Homolog That Detects Mixed Microbial Communities. *Journal of Bacteriology*, 183(19):5733–5742. 2001.

[73] B. M. M. Ahmer. Cell-to-cell signalling in Escherichia coli and Salmonella enterica. *Molecular Microbiology*, 52(4):933–945. 2004.

[74] K. H. Nealson, T. Platt and J. W. Hastings. Cellular Control of the Synthesis and Activity of the Bacterial Luminescent System. *Journal of bacteriology*, 104(1):313–322. 1970.

[75] E. Klein, D. L. Smith and R. Laxminarayan, *Hospitalizations and Deaths Caused by Methicillin-Resistant Staphylococcus aureus, United States, 1999-2005.* 2007.

[76] L. C. M. Antunes, R. B. R. Ferreira, M. M. C. Buckner and B. B. Finlay. Quorum sensing in bacterial virulence. *Microbiology*, 156:2271–2282. 2010.

[77] G. P. Bodey, R. Bolivar, V. Fainstein and L. Jadeja. Infections caused by *Pseudomonas aeruginosa*. *Reviews of Infectious Diseases*, 5(2):279–313. 1983.

[78] G. Xiao, E. Déziel, J. He, F. Lépine, B. Lesic, M.-H. Castonguay, S. Milot, A. P. Tampakaki, S. E. Stachel and L. G. Rahme. MvfR, a key Pseudomonas aeruginosa pathogenicity LTTR-class regulatory protein, has dual ligands. *Molecular Microbiology*, 62(6):1689–99. 2006.

[79] J.-F. Dubern and S. P. Diggle. Quorum sensing by 2-alkyl-4-quinolones in *Pseudomonas aeruginosa* and other bacterial species. *Molecular BioSystems*, 4(9):882–888. 2008.

[80] P. Cornelis, *Pseudomonas: Genomics and Molecular Biology*, Caister Academic Press. 2008.

[81] R. C. Hider and X. Kong. Chemistry and biology of siderophores. *Natural Product Reports*, 27(5):637–657. 2010.

[82] M. R. Seyedsayamdost, S. Cleto, G. Carr, H. Vlamakis, M. João Vieira, R. Kolter and J. Clardy. Mixing and matching siderophore clusters: structure and biosynthesis of serratiochelins from Serratia sp. V4. *Journal of the American Chemical Society*, 134(33):13550–135503. 2012.

[83] T. Zheng and E. M. Nolan. Siderophore-based detection of Fe(III) and microbial pathogens. *Metallomics*, 4:866–880. 2012.

[84] C. J. Carrano and K. N. Raymond. Synthesis and characterization of iron complexes of rhodotorulic acid: a novel dihydroxamate siderophore and potential chelating drug. *Journal of the Chemical Society, Chemical Communications*, (12):501. 1978.

[85] M. B. Hossain, D. L. Eng-Wilmot, R. A. Loghry and D. van der Helm. Circular Dichroism , Crystal Structure , and Absolute Configuration of the Siderophore Ferric N,N',N"-Triacetylfusarinine, FeC39H57N60. *Journal of the American Chemical Society*, 102:5766–5773. 1980.

[86] D. van der Helm, J. R. Baker, D. L. Eng-Wilmot, M. B. Hossain and R. A. Loghry. Crystal Structure of Ferrichrome and a Comparison with the Structure of Ferrichrome A. *Journal of the American Chemical Society*, 102(12):4224–4231. 1980.

[87] K. Schlegel, J. Lex, K. Taraz and H. Budzikiewicz. The X-ray structure of the pyochelin Fe3+ complex. *Zeitschrift für Naturforschung*, 61c:263–266. 2006.

[88] M. G. P. Page. Siderophore conjugates. *Annals of the New York Academy of Sciences*, 1277:115–126. 2013.

[89] A. Hartmann, H.-P. Fiedler and V. Braun. Uptake and conversion of the antibiotic albomycin by Escherichia coli K-12. *European Journal of Biochemistry*, 99(3):517–24. 1979.

[90] M. Ghosh and M. J. Miller. Design, synthesis, and biological evaluation of isocyanurate-based antifungal and macrolide antibiotic conjugates: iron transport-mediated drug delivery. *Bioorganic & Medicinal Chemistry*, 3(11):1519–1525. 1995.

[91] U. Möllmann, L. Heinisch, A. Bauernfeind, T. Köhler and D. Ankel-Fuchs. Siderophores as drug delivery agents: application of the "Trojan Horse" strategy. *Biometals*, 22(4):615–624. 2009.

[92] C. Dini and J. Aszodi. Synthesis of a dihydroxythiophene analogue of catechosporines. *Bioorganic & Medicinal Chemistry Letters*, 10(4):349–352. 2000.

[93] T. Kline, M. Fromhold, T. E. Mckennon, S. Cai, J. Treiberg, N. Ihle, D. Sherman, W. Schwan, M. J. Hickey, P. Warrener, P. R. Witte, L. L. Brody, L. Goltry, L. M. Barker, S. U. Anderson, S. K. Tanaka, R. M. Shawar, L. Y. Nguyen, M. Langhorne, A. Bigelow, L. Embuscado and E. Naeemi. Antimicrobial Effects of Novel Siderophores Linked to -Lactam Antibiotics. *Bioorganic & Medicinal Chemistry*, 8(2000):73–93. 1999.

[94] Y. Lu and M. J. Miller. Syntheses and Studies of Multiwarhead Siderophore-5-fluorouridine Conjugates. *Bioorganic & Medicinal Chemistry*, 7(1999):3025–3038. 1999.

[95] M. Ghosh and M. J. Miller. Synthesis and in vitro antibacterial activity of spermidine-based mixed catechol- and hydroxamate-containing siderophore–vancomycin conjugates. *Bioorganic & Medicinal Chemistry*, 4(1):43–48. 1996.

[96] S. R. Md-Saleh, E. C. Chilvers, K. G. Kerr, S. J. Milner, A. M. Snelling, J. P. Weber, G. H. Thomas, A.-K. Duhme-Klair and A. Routledge. Synthesis of citrate-ciprofloxacin conjugates. *Bioorganic & Medicinal Chemistry Letters*, 19(5):1496–1498. 2009.

[97] F. Rivault, C. Liébert, A. Burger, F. Hoegy, M. A. Abdallah, I. J. Schalk and G. L. A. Mislin. Synthesis of pyochelin-norfloxacin conjugates. *Bioorganic & Medicinal Chemistry Letters*, 17(3):640–644. 2007.

[98] C. Ji and M. J. Miller. Chemical syntheses and in vitro antibacterial activity of two desferrioxamine B-ciprofloxacin conjugates with potential esterase and phosphatase triggered drug release linkers. *Bioorganic & Medicinal Chemistry*, 20(12):3828–3836. 2012.

[99] T. Zheng and E. M. Nolan. Enterobactin-Mediated Delivery of β-Lactam Antibiotics Enhances Antibacterial Activity Against Pathogenic Escherichia coli. *Journal of the American Chemical Society*. 2014.

[100] G. E. Zurenko, S. E. Truesdell, B. H. Yagi, R. J. Mourey and A. L. Laborde. In vitro antibacterial activity and interactions with beta-lactamases and penicillin-binding proteins of the new monocarbam antibiotic U-78608. *Antimicrobial Agents and Chemotherapy*, 34(5):884–8. 1990.

[101] H. Takase, H. Nitanai, K. Hoshino and T. Otani. Impact of siderophore production on Pseudomonas aeruginosa infections in immunosuppressed mice. *Infection and Immunity*, 68(4):1834–9. 2000.

[102] F. Paech, S. Messner, J. Spickermann, M. Wind, A. Hortense, S. Hoffmann, A. Therese, W. Brett, A. H. Rachel, J. C. Jeff, W. Marc, S. Krähenbühl and M. Maurer. Mechanisms of hepatotoxicity associated with the monocyclic β - lactam antibiotic BAL30072. *Archives of Toxicology*, 91(11):3647–3662. 2017.

[103] I. J. Schalk and G. L. A. Mislin. Bacterial Iron Uptake Pathways: Gates for the Import of Bactericide Compounds. 2017.

[104] G. S. Tillotson. Trojan Horse Antibiotics - A Novel Way to Circumvent Gram-Negative Bacterial Resistance ? pages 45–52. 2016.

[105] A. Ito, T. Sato, M. Ota, M. Takemura, T. Nishikawa, S. Toba, N. Kohira, S. Miyagawa, N. Ishibashi, S. Matsumoto, R. Nakamura, M. Tsuji and Y. Yamanoa. In Vitro Antibacterial Properties of Cefiderocol, a Novel Siderophore Cephalosporin, against Gram-Negative Bacteria. *Antimicrobial Agents and Chemotherapy*, 62(1):1–11. 2018.

[106] P. Pattanaik, C. R. Bethel, A. M. Hujer, K. M. Hujer, A. M. Distler, M. Taracila, V. E. Anderson, T. R. Fritsche, R. N. Jones, S. Ram, R. Pagadala, F. V. D. Akker, J. D. Buynak and R. A. Bonomo. Strategic Design of an Effective β-Lactamase Inhibitor. *The Journal of B*, 284(2):945–953. 2009.

[107] G. Bou, E. Santillana, A. Sheri, A. Beceiro, J. M. Sampson, M. Kalp, C. R. Bethel, O. A. M. Distler, S. M. Drawz, S. Ram, R. Pagadala, F. V. D. Akker, R. A. Bonomo, O. A. Romero and J. D. Buynak. Design, Synthesis, and Crystal Structures of 6-Alkylidene-2' -Substituted Penicillanic Acid Sulfones as Potent Inhibitors of Acinetobacter baumannii OXA-24 Carbapenemase. *Journal of the American Chemical Society*, 132:13320–13331. 2010.

[108] A. P. Macgowan, M. Wootton and H. A. Holt. The antibacterial efficacy of levofloxacin and ciprofloxacin against *Pseudomonas aeruginosa* assessed by combining antibiotic exposure and bacterial susceptibility. *Journal of Antimicrobial Chemotherapy*, 43:345–349. 1999.

[109] H.-C. Su, K. Ramkissoon, J. Doolittle, M. Clark, J. Khatun, A. Secrest, M. C. Wolfgang and M. C. Giddings. The development of ciprofloxacin resistance in *Pseudomonas aeruginosa* involves multiple response stages and multiple proteins. *Antimicrobial Agents and Chemotherapy*, 54(11):4626–4635. 2010.

Todo list