Biosensors and Bioelectronics 80 (2016) 9–16

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Biosensors based on modularly designed synthetic peptides for recognition, detection and live/dead differentiation of pathogenic bacteria

Xiaobo Liu [a], Mouna Marrakchi [a,b,c,*], Dawei Xu [a], He Dong [a,*], Silvana Andreescu [a,*]

[a] Department of Chemistry & Biomolecular Science, Clarkson University, 8 Clarkson Avenue, Potsdam, NY 13699-5810, USA
[b] University of Carthage, National Institute of Applied Sciences and Technology (INSAT), Laboratoire d'Ecologie et Technologie Microbienne (LETMi), 1080 Tunis, Tunisia
[c] Tunis El Manar University, Higher Institute of Applied Biological Sciences (ISSBAT), 1006 Tunis, Tunisia

ARTICLE INFO

Article history:
Received 10 December 2015
Received in revised form
14 January 2016
Accepted 14 January 2016
Available online 15 January 2016

Keywords:
Impedimetric biosensor
Pathogenic bacteria
Antimicrobial peptides
Live/dead assay

ABSTRACT

Rapid and sensitive detection of bacterial pathogens is critical for assessing public health, food and environmental safety. We report the use of modularly designed and site-specifically oriented synthetic antimicrobial peptides (sAMPs) as novel recognition agents enabling detection and quantification of bacterial pathogens. The oriented assembly of the synthetic peptides on electrode surfaces through an engineered cysteine residue coupled with impedimetric detection facilitated rapid and sensitive detection of bacterial pathogens with a detection limit of 10^2 CFU/mL for four bacterial strains including *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Staphylococcus aureus* (*S. aureus*) and *Staphylococcus epidermidis* (*S. epidermidis*). The approach enabled differentiation between live and dead bacteria. The fabrication of the sAMPs functionalized surface and the importance of the sAMPs orientation for providing optimum recognition and detection ability against pathogens are discussed. The proposed methodology provides a universal platform for the detection of bacterial pathogens based on engineered peptides, as alternative to the most commonly used immunological and gene based assays. The method can also be used to fabricate antimicrobial coatings and surfaces for inactivation and screening of viable bacteria.

1. Introduction

Bacterial infection is one of the most threatening public health problems of significant social and economic impact. According to recent Centers for Disease Control and Prevention (CDC) reports, from 2009 to 2013, the number of illness cases caused by foodborne bacterial infection is 29,337. *Salmonella spp.*, *Campylobacter spp.* and *Escherichia coli* (*E. coli*) are the primary pathogens responsible for the major foodborne bacterial pathogen outbreaks in US (Centers for Disease Control and Prevention, 2013, 2014a, 2014b, 2015). The large number of reported outbreaks requires rapid and sensitive detection methods that can provide fast screening of samples for microbial contamination.

The most commonly used methods to detect bacteria are the colony counting assays with analysis of colony forming units (CFU), immunological assays and polymerase chain reaction (PCR). The CFU assay is labor intensive and time-consuming with some standardized procedures requiring up to 7 days (de Boer and Beumer, 1999). Immunological assays require expensive materials and involve labeling and multiple washing steps (Beumer and Brinkman, 1989; Palumbo et al., 2003). The nucleic acid probe-based technique is expensive and requires specialized facilities (Cai et al., 2014). Moreover, nonviable cells and single naked DNA, extraction and degradation of nucleic acids, and/or the direct inhibition of the PCR may lead to false positive/negative responses (Wilson, 1997). Except for the CFU assay, most methods cannot differentiate live/dead bacteria.

In this work, we report a rapid, sensitive and label free detection of pathogenic bacteria, using synthetic antimicrobial peptides (sAMPs) as novel biorecognition elements for the detection and differentiation of live/dead bacteria. The method provides a simplified and cost effective alternative to the most commonly used immunological and DNA based procedures. As compared to other biorecognition entities, modularly designed sAMPs provide several advantages including: facile, low cost and large scale production, high stability even in harsh environments (Friedrich et al., 1999; Rydlo et al., 2006), ease of functionalization enabling design of

* Corresponding authors at: Department of Chemistry & Biomolecular Science, Clarkson University, 8 Clarkson Avenue, Potsdam, NY 13699-5810, USA.
E-mail addresses: mouna.marrakchi@issbat.rnu.tn (M. Marrakchi), hdong@clarkson.edu (H. Dong), eandrees@clarkson.edu (S. Andreescu).

http://dx.doi.org/10.1016/j.bios.2016.01.041
0956-5663/

specific binding sites and sequences with improved recognition function. We took advantage of these unique characteristics to develop a biosensor using sAMPs with site specifically engineered amino acids for the detection of bacterial pathogens. To our knowledge, this is the first platform for bacteria detection based on site specifically oriented attachment of synthetic sAMPs with selective microbial recognition ability.

To design the sensor, we use previously reported cationic Multidomain Peptides (MDPs) which can be modularly designed to form various protein secondary structures and demonstrate tunable, structure-dependent antimicrobial activities (Dong et al., 2007; Xu et al., 2015; Yang et al., 2014). MDPs have a general formula of $K_x(QL)_yK_z$ where the middle domain contains variable number (y) of alternating hydrophilic (glutamine, Q)-hydrophobic (leucine, L) repeating units terminated with two oligolysine (K) domains. The relative ratio of the repeating number (i.e. *x*, *y*, *z*) of each domain dictates the molecular secondary structures of MDPs and further their antimicrobial activity (Xu et al., 2015; Yang et al., 2014). In this work, $WK_3(QL)_6K_2$ was primarily used for bacterial detection to validate the designed sensor platform. In the sequences, tryptophan (W) was appended at the N-terminus to allow an accurate concentration measurement. The peptide forming mixed secondary structures of beta sheets and random coils (Fig. 1A) has been tested for its antimicrobial activity against *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus epidermidis* in broth medium with minimum inhibitory concentration (MIC) value in the range of 5–20 μM (Xu et al., 2015; Yang et al., 2014). In this work, we will demonstrate the use of these peptides as recognition sites for bacterial detection. To design the sensor, the peptides were modified with an external cysteine (C) residue that allows targeted attachment and site-specific orientation to a gold surface using the affinity of cysteine for gold (Fig. 1). We use electrochemical impedance spectroscopy (EIS) as transduction technique to quantify the interaction between the synthetic sAMPs and bacteria. The effect of the orientation of peptides on the electrode surface was investigated by comparing the impedimetric responses of the sensors modified with the engineered sAMPs with and without cysteine. Sensors modified with peptides without significant antimicrobial activity were also tested to link the peptide sequence with the antimicrobial activity and establish the recognition ability against *E. coli*, *P. aeruginosa*, *S. aureus* and *S. epidermidis*. The method was employed to quantify four bacterial strains (both gram-positive and gram negative) and to differentiate between live and dead bacteria.

This work demonstrates the potential of engineered sAMPs for rapid and sensitive detection of bacteria. This study also lays the foundation for a new technology of using designed peptides for fabricating functionalized surfaces with antimicrobial activity for biodetection and potential therapeutic applications (e.g. antimicrobial coatings).

2. Materials and methods

2.1. Reagents and equipment

Potassium ferricyanide, ACS grade was purchased from Fisher Scientific (Hampton, NH, USA), 20 mM Tris–HCl buffer, pH=7.5 was prepared from Trizma™ hydrochloride purchased from Sigma (St Louis, MO, USA). Glutaraldehyde, 50% in water, reagent grade was purchased from Spectrum Chemical (Brunswick, NJ, USA). Deionized (DI) water from Direct-Q system (Millipore, Billerica, MA) with a resistivity of 18.2 MΩ cm was used to prepare all solutions. All electrochemical experiments were conducted using a CHI 920C analyzer (CH Instruments, Austin, TX, USA). FMOC-amino acids and resin were purchased from NovaBiochem. Peptide synthesis grade solvents, deprotection reagent and piperidine were purchased from Sigma. Trifluoroacetic acids and diethyl ether were from Fisher.

2.2. Preparation of the engineered peptides

The preparation of the engineered peptides followed the same procedure as previously described (Xu et al., 2015). For this study, we have used the $WK_3(QL)_6K_2$ peptide unit at which we have added a cysteine residue. Briefly, $WK_3(QL)_6K_2G_3C$ was synthesized on a PS3 peptide synthesizer (Protein Technologies Inc., Tucson, AZ) using standard FMOC (fluorenylmethyloxycarbonyl)-solid phase peptide synthesis method. The sequence without significant antimicrobial activity, $WK_2(QL)_6K_2G_3C$, was used as control peptide. $WK_3(QL)_6K_2$ was used as a negative control to determine the role of the cysteine residue in the immobilization process. The sequences $WK_2(QL)_6K_2$ and $CE_2(QL)_6E_2$ (glutamic acid, E) were used to demonstrate the role of antimicrobial activity in bacteria-sAMP recognition process. The sequence and the design characteristics of all peptides used in this study are listed in Table S1.

2.3. Fabrication and characterization of the AMP based biosensor

The biosensor was developed on a gold disk electrode (1.6 mm diameter, MF-2014, Bioanalytical Systems, Inc., West Lafayette, IN). Prior to use, the gold disk electrode was polished using 0.3 μm

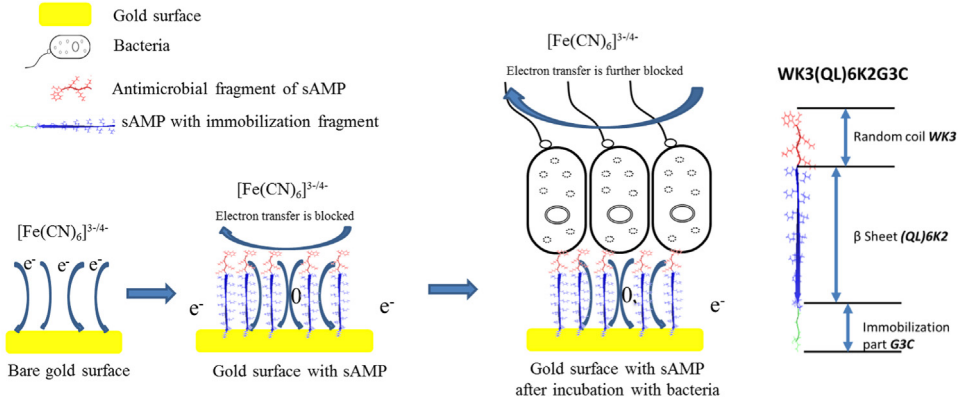

Fig. 1. A. Design and sensing principle of the cysteine modified sAMP impedimetric biosensor. B. Sequence and orientation of the active $WK_3(QL)_6K_2G_3C$ peptide on the gold surface.

alumina slurry, sonicated for at least 10 min in distilled water and then incubated in Piranha solution for 15 min and washed with DI water. Before modification, the gold disk electrode was sonicated in acetone and ethanol for 15 min consecutively, and dried under nitrogen gas flow. To immobilize the sAMP, the cleaned gold electrode was immersed in Tris–HCl buffer (20 mM), containing the peptide (0.5 mg/mL or 1 mg/mL) for 1 h. Afterward, the electrode was washed using Tris–HCl buffer and then immersed into the same buffer for 20 min to remove the weakly attached peptides. The surface of the electrode prior to and after deposition of peptides and after the binding of bacteria was characterized by cyclic voltammetry (CV) using the model reversible redox couple $Fe^{II}(CN)_6^{4-}/Fe^{III}(CN)_6^{3-}$ (10 mM) in phosphate buffer saline (PBS) buffer, pH = 7. The potential was scanned between −0.6 and 0.6 V at a scan rate of 100 mV/sec. Evaluation of non-specific adsorption was performed with a peptide lacking cysteine with the sequence $WK_3(QL)_6K_2$. In experiments comparing the sensing responses of antimicrobial active sAMP and control peptides ($WK_2(QL)_6K_2G_3C$ and $WK_3(QL)_6K_2$), a washing buffer containing 20 mM Tris–HCl at pH = 7.5 with 0.1% Tween 20 and 0.2 M NaCl was used to minimize non-specific adsorption.

To detect bacteria, 100 μL bacteria dilutions with different concentrations prepared in Tris–HCl buffer was dropped on the sAMP modified gold electrode surface, incubated for 30 min. The electrode was then washed twice using Tris–HCl buffer. Detection was accomplished by probing the Faradaic electron impedance of the potassium ferricyanide redox pair at the sAMP modified electrode before and after incubation with bacteria, as described in supplementary information (SI). Unless otherwise noted, all characterization experiments were performed with the cysteine modified peptide, $WK_3(QL)_6K_2G_3C$. All electrochemical measurements were carried out in sterile buffer and inside a Faraday cage. The impedance variation percentages (Δlog Z (%)) registered at 12.12 Hz were used to show the surface change of the sensor surface at different conditions (Bayoudh et al., 2008; Mejri et al., 2010) (Eq. (1) in SI and Fig. S1). Control experiments using $WK_3(QL)_6K_2$ and $WK_2(QL)_6K_2G3C$ were performed as described above. The bare gold electrode (without peptides) incubated with 10^2–10^6 CFU/mL *P. aeruginosa* was also tested as control. JSM-7400F field emission scanning electron microscope (JEOL Ltd., Tokyo, Japan) was used to characterize the gold slide surface before and after modification with sAMP and visualize the presence of bacteria as described in the SI section.

3. Results

3.1. Study of the binding and recognition behavior of the site specifically immobilized sAMPs

One of the challenges on the use of immobilized biomolecular receptors is to conserve the conformation flexibility and activity for optimum recognition ability. The sAMP (sequence: $WK_3(QL)_6K_2G_3C$ where three glycine (G) residues act as a spacer to impart chain flexibility) used in this work is engineered to attach to gold via an external cysteine tag leaving the antimicrobial site exposed for providing enhanced activity against its target bacteria. This specific binding, combined with the use of impedance spectroscopy was employed in this work for the construction of an impedimetric biosensor for the detection of bacteria. The schematic of this concept is illustrated in Fig. 1A.

CV and EIS were used to demonstrate the attachment of the sAMP and bacteria onto the surface of the gold electrode. Fig. 2a shows CVs of the bare and sAMP modified gold electrodes recorded in 10 mM potassium ferricyanide. After immobilization of sAMP, a decrease in the current intensity is observed. This indicates partial insulation of the electrochemically active area due to peptide binding. It is expected that the cysteine modified

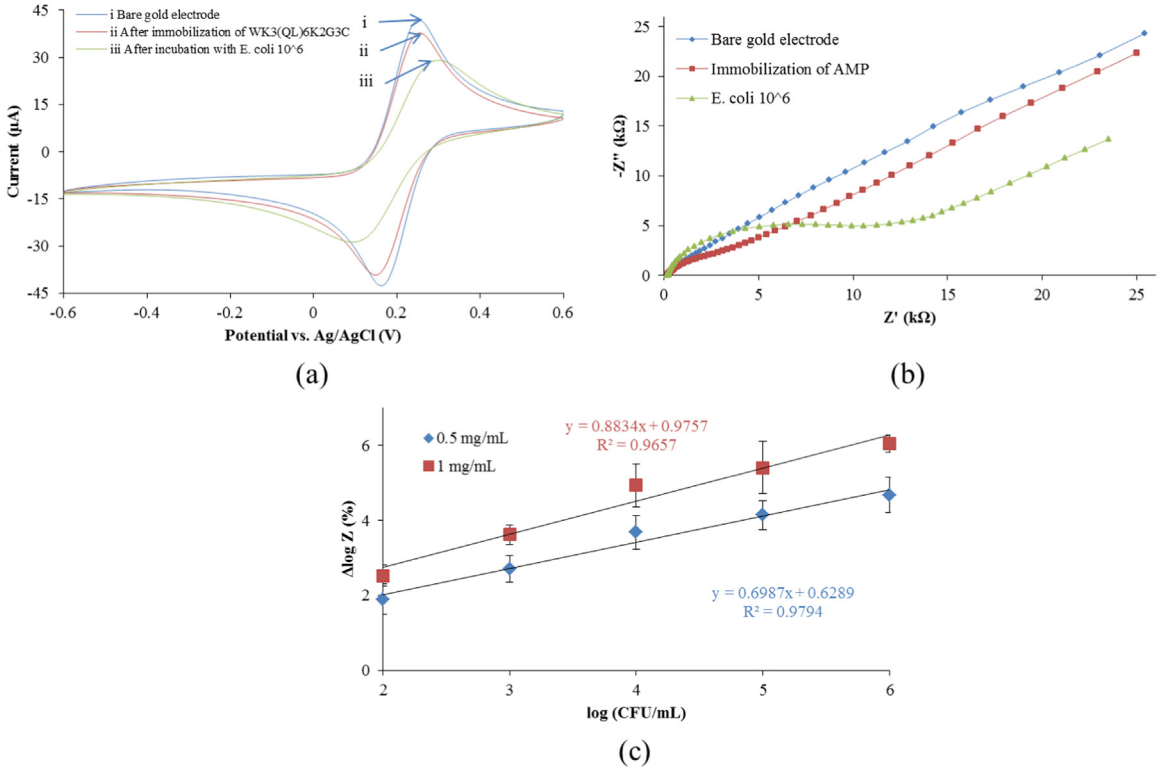

Fig. 2. (a) Cyclic voltammogram (CV) and (b) Nyquist plot of the sAMP impedimetric sensor at different stages, (c) impedimetric responses of gold electrode modified with different concentrations (0.5 and 1 mg/mL) of sAMP after incubation with *E. coli* at various concentrations in terms of impedance variation percentages (Δlog Z (%)) registered at 12.12 Hz.

peptide will attach to the gold surface through the formation of S–Au bond, which will hamper the electron transfer of the redox probe. In the presence of bacteria, a greater decrease in intensity is observed further blocking the electron transfer rate. This can be due to the binding of bacteria through the immobilized sAMP. The same trend is observed by EIS (Fig. 2b).

EIS was further used to investigate the concentration dependency of the sAMP-cysteine biosensor against bacteria using *E. coli* as a model. Fig. 2c shows the impedance variation percentages ($\Delta\log Z$ (%)) of gold electrodes modified with two different concentrations of sAMP (0.5 and 1 mg/mL) against *E. coli*. For both sAMP concentrations, the change in $\Delta\log Z$ (%) increases with the increase of bacterial concentration. This demonstrates the capability of this method to be used as a quantitative tool for the detection of bacterial pathogens.

3.2. Comparing biosensor responses against varying pathogens

To further establish the sensitivity and detection capability of the sAMP bacterial sensor, the sensor was exposed to varying concentrations of bacteria. Fig. 3a illustrates the Nyquist plot showing a mixed electron transfer and diffusion resistance process that varies gradually with increasing the concentration of *E. coli*. As it showed in Fig. S1, in the Bode plots, the impedance of the sensor increased from incubation with bacteria in the low frequency range. The Nyquist plots (Fig. 3a) shows that the difference in the real and imaginary impedance values at a given frequency between the spectra after immobilization of peptides and after incubation of bacteria can be clearly observed at low frequencies. Additionally, at higher frequencies in Bode plot, no significant difference is found between the impedance spectra, indicating that the detection of bacteria is more sensitive in the low frequency range (Fig. S1). Therefore, 12.12 Hz was used for the calculation of the impedance variation percentages (Bayoudh et al., 2008). This trend corresponds to the increase of $\Delta\log Z$ (%) obtained from Bode plots. Beyond a concentration of 10^6 CFU/mL *E. coli* there is no significant increase of $\Delta\log Z$ (%) (data not shown) indicating that the sensor surface reached a saturation point at concentrations higher than 10^6 CFU/mL bacteria. Fig. 3b showed cyclic voltammograms of the biosensor after each addition of bacteria. With increasing bacteria concentrations, a decrease in the direct current is observed due to the insulating properties of the bacteria attached on the biosensor surface. A similar trend and saturation conditions were observed for the other three bacterial strains, but with different sensitivities (Fig. 3c). A notable increase of $\Delta\log Z$ (%) was observed after incubation of the sensor with 10^2 CFU/mL bacteria. Therefore, this AMP based impedimetric biosensor can detect bacteria at concentrations as low as 10^2 CFU/mL.

Subsequently, we tested the ability of the sAMP biosensor to detect different gram-negative (*E. coli* and *P. aeruginosa)* and gram-positive (*S. aureus* and *S. epidermidis*) bacterial species. Fig. 3c illustrates the variation of the $\Delta\log Z$ (%) as a function of bacteria concentration for the four types of bacteria. It can be seen that all bacteria induced changes in the dielectric properties of the sAMP functionalized electrode but with different levels of sensitivity: the highest $\Delta\log Z$ (%) was observed with *P. aeruginosa*, followed by *S. aureus* and *S. epidermidis*, which showed almost the same response, and the least $\Delta\log Z$ (%) was observed with *E. coli*. The efficacy of the engineered sAMP to recognize the four bacteria and the different selectivity behavior is largely unknown, however it may be attributed to the different interactions between the sAMP and bacterial membrane which vary among bacterial types (Beveridge, 1999; Schneewind et al., 1995). The difference of membrane structure of gram-positive and gram-negative bacteria can also influence the recognition ability and the electron transfer process. For the gram-negative bacteria, the periplasmic space is

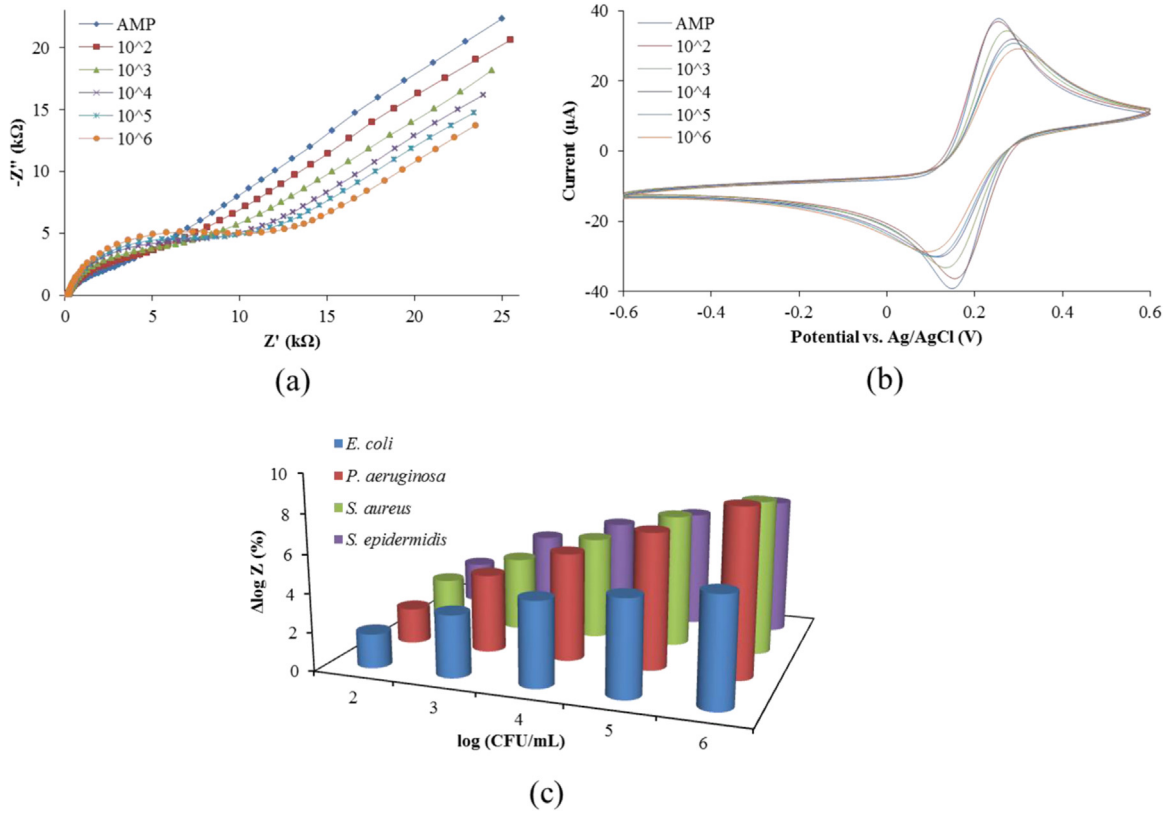

Fig. 3. (a) Nyquist plot and (b) CV of the sAMP impedimetric sensor incubated with sAMP and different *E. coli* concentrations in terms of CFU/mL, (c) impedimetric responses of the sAMP impedimetric sensor incubated with different bacteria at various concentrations in terms of impedance variation percentages ($\Delta\log Z$ (%)) registered at 12.12 Hz.

located between the inner surface of the outer membrane and the outer surface of the plasma membrane. In gram-positive bacteria, the periplasmic space is confined to a region between the lower surface of the peptidoglycan/teichoic acid structure and the outer surface of the plasma membrane (Beveridge, 1999). The periplasm of gram-positive and gram-negative bacteria contains enzymes and certain proteins which can be involved in the electron transfer. In gram-negative bacteria, the electron transfer could be facilitated through the periplasmic space by the naturally present electron carriers, including rusticyanin, soluble c-type cytochrome Cyc1 and cytochrome oxidase aa3. Such enzymes and carriers are not present in the periplasm of gram-positive cells (Ehrlich, 2008; Matias and Beveridge, 2005; Zuber et al., 2006). The amphiphilicity and molecular structures are the key parameters to tuning the membrane activity of sAMPs (Eckert et al., 2006; Zasloff, 2002). The ability to modularly design and engineer a library of sAMPs with tunable amphiphilicity and molecular secondary structures will offer opportunity to systematically investigate and understand the interaction between sAMPs and bacterial membranes. The different level of sensitivity observed could also be related to the size of bacteria. For example, the bulkier *P. aeruginosa* showed larger change in the impedimetric response as compared to *S. aureus* and *S. epidermidis* that are spherical and have a smaller size. To confirm the binding of bacteria, we have performed scanning electron microscopy (SEM) experiments to probe the presence of bacteria on a sAMP functionalized gold surface (Fig. 4). *P. aeruginosa*, which provided the higher impedance change in the EIS measurements formed high density bacterial "clusters" on the sAMP gold surface, while the other three bacteria showed lower coverage. This differential binding and the varying bacterial sizes may explain the sensitivity trend observed in the EIS measurements.

3.3. Differential responses to live and dead bacteria

One of the challenges in the development of bacteria detection is to discriminate between live and dead bacteria. Since the mechanism of antimicrobial activity of AMPs involves disruption of the bacterial cell envelope (Hartmann et al., 2010), we sought to investigate whether different binding of sAMPs to live/dead cell membranes can be used to differentiate between dead and live cells. Fig. 5 shows the impedimetric responses of the sAMP biosensor to the four bacteria in live and dead conditions. Bacteria grown to mid-log phase were used as live bacteria sample. Dead bacteria samples were prepared by incubating bacterial culture grown to mid-log phase at 100 °C for 1 h. The sensor shows significantly different responses to live and dead bacteria. Exposure to dead bacteria resulted in lower $\Delta \log Z$ (%) for all four bacteria species, as compared to sensors exposed to live bacteria. The results indicate a different recognition behavior of the dead bacteria for the immobilized sAMP layer. This phenomenon indicates that the sAMP impedimetric biosensor is not only able to detect bacteria but also to differentiate dead bacteria from live ones. The reason of the discriminative responses may be attributed to a different interaction and membrane permealizing action (e.g. differential changes in the lipid layer) between the sAMP and the dead bacteria, with an already compromised membrane, and therefore reduced recognition and surface binding ability. Additionally, SEM images of the sAMP modified gold exposed to dead bacteria show the presence of a significantly lower number of

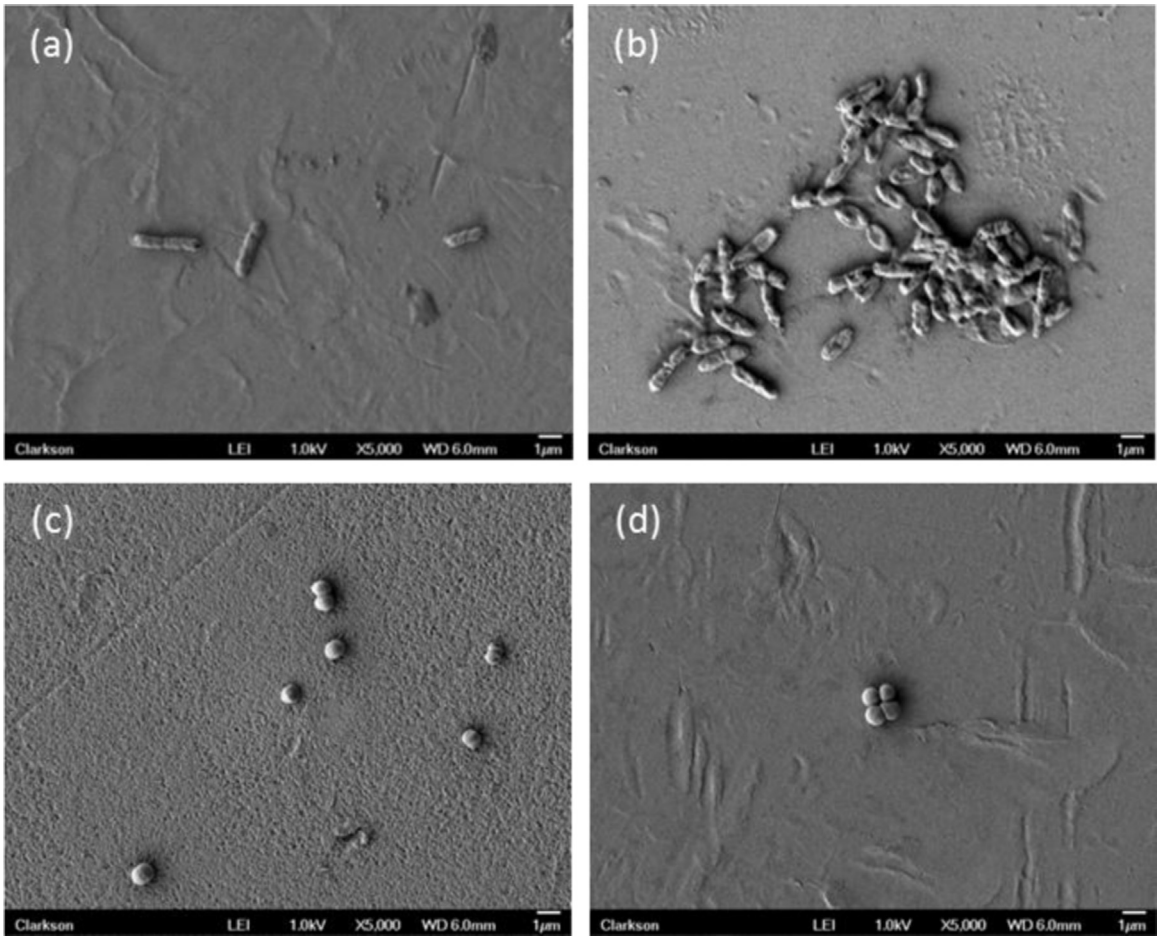

Fig. 4. SEM graphs of sAMP modified gold surface after incubation with (a) *E. coli*, (b) *P. aeruginosa*, (c) *S. aureus* and (d) *S. epidermidis* at 5000 × magnification.

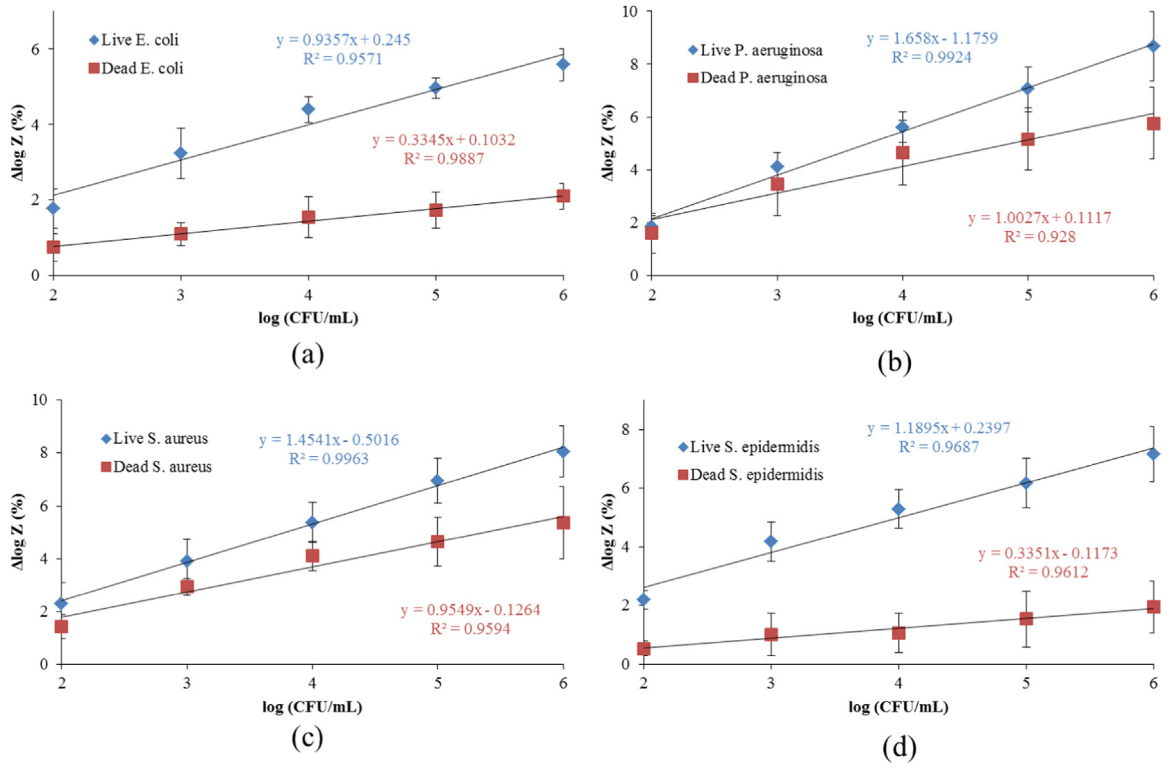

Fig. 5. Impedimetric responses (represented in terms of Δlog *Z* (%) registered at 12.12 Hz) of the sAMP biosensor incubated with live and dead (a) *E. coli*, (b) *P. aeruginosa*, (c) *S. aureus* and (d) *S. epidermidis* at various concentrations.

bacteria onto the gold surface, compared to that exposed to live bacteria under identical exposure conditions (Fig. S2 in SI).

3.4. Peptides specificity

To demonstrate that the binding between the bacteria and the synthetic peptide is due to the antimicrobial activity, a peptide with a similar sequence but without significant antimicrobial activity, $WK_2(QL)_6K_2G_3C$, was tested against *P. aeruginosa* (Fig. 6). Design of peptides with different antimicrobial activity has been realized by synthetic engineering. The lack of antimicrobial activity of this sequence was determined previously (Xu et al., 2015). An active peptide without cysteine, $WK_3(QL)_6K_2$, was also used in this experiment to probe the importance of orientation on the gold surface (Di Felice et al., 2003; Vallee et al., 2010). The sensors modified with $WK_2(QL)_6K_2G_3C$ showed lower impedance variation upon exposure to bacteria as compared to the antimicrobial active $WK_3(QL)_6K_2G_3C$ peptide. This trend agrees with the potency of the antimicrobial activity of these two peptides, $WK_3(QL)_6K_2G_3C$ and $WK_2(QL)_6K_2G_3C$, and further confirms that their antimicrobial activity is a dominant factor in the bacteria-sAMP recognition. The impedance change recorded with $WK_3(QL)_6K_2$ is smaller than that of the $WK_3(QL)_6K_2G_3C$ demonstrating lower binding ability of bacteria to peptides attached non-specifically through physical interactions. In the absence of cysteine, carboxyl or amino groups of the peptide can attach to the gold surface in a less controlled pattern (Vallee et al., 2010). The lack of orientation might restrict the interaction between the immobilized peptides and bacteria, limiting the binding of bacteria. The response of a bare gold electrode exposed to the same amount of bacteria was also tested to assess the effect of nonspecific attachment of bacteria (Fig. 6). No significant change in impedance was observed, indicating negligible attachment of bacteria in the absence of sAMP.

SEM images of the gold surface functionalized with the four sAMPs show significantly higher numbers of *P. aeruginosa* obtained with the active cysteine modified $WK_3(QL)_6K_2G_3C$ peptide

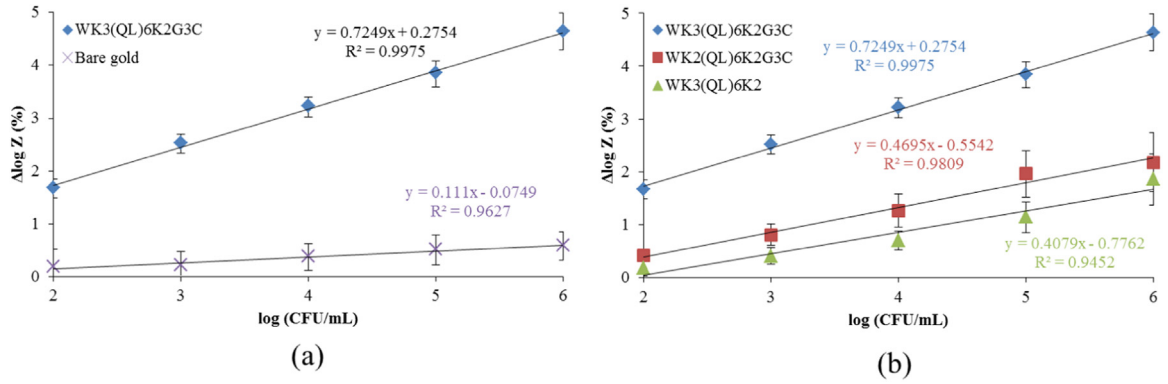

Fig. 6. (a) Impedimetric responses of sAMP modified impedimetric sensor comparing to bare gold electrode and (b) different peptides modified gold electrode incubated with *P. aeruginosa* at various concentrations in terms of impedance variation percentages (Δlog *Z* (%)) registered at 12.12 Hz.

as compared to the corresponding control peptides, $WK_3(QL)_6K_2$, $WK_2(QL)_6K_2$ and $CE_2(QL)_6E_2$ (Fig. S3). Additionally, a sensor modified with a different control peptide lacking both cysteine and antimicrobial activity, CE2(QL)6E2 showed no impedance change upon exposure to bacteria (Fig. S4). The binding of bacteria to bare gold was negligible. These results demonstrate that both the cysteine residue and the antimicrobial activity are essential features of the engineered sAMPs for providing sufficient binding and detection sensitivity for impedimetric detection of bacterial pathogens.

4. Discussion

This study demonstrates the utility of synthetic sAMP peptides as a biorecognition element for label free quantitative detection of bacterial pathogens using EIS impedance spectroscopy. We used a site specific immobilization of sAMPs, which provided enhanced detection sensitivity of the EIS sensor. This was achieved by engineering a cysteine anchor at the C-terminus of the peptide chain. The peptide will then attach to a gold electrode surface by virtue of the cysteine affinity for gold, providing flexibility for binding and an enhanced recognition behavior for bacterial pathogens. The sensor showed response to both gram-negative and gram-positive bacteria, with higher sensitivity for *P. aeruginosa,* followed by *S. epidermis, S. aureus* and *E. coli*. The difference of the bacterial size and the membrane structure might contribute to these different responses. The sAMP modified electrode before and after exposure to bacterial pathogens showed a mixed electron transfer resistance and a diffusion resistance process in EIS. Attachment of bacteria to gold through the cysteine site was also demonstrated by SEM, which showed preferential attachment of bacteria on the cysteine-sAMP functionalized gold surface. By comparison, the sAMPs without cysteine and/or antimicrobial activity showed significantly lower number of attached bacteria and lower impedimetric responses. Oriented sAMP might provide a favorable interaction between peptide assemblies and the bacterial cell membrane on the electrode surface. The experiments demonstrate that the bacteria bind to the gold surface via the immobilized cysteine containing sAMP. The results show different binding profile of the four bacteria and the capability of the sAMP impedimetric sensor to differentiate live/dead bacteria. In addition to the detection capability, such measurements can also facilitate study and fundamental understanding of the mechanism of action of antimicrobial peptides. For example, the method could be useful for screening large libraries of sAMPs with varying antimicrobial activities before such peptides are being used in more advanced applications including antimicrobial coatings and sensing. With further development of peptide engineering, it may be possible to engineer peptides that have selective binding abilities against particular bacteria and enable differentiation between bacterial types.

Current methods for monitoring bacterial pathogens include plating and colony counting assays, immunological techniques such as enzyme-linked immunosorbent assay (ELISA) and PCR methods. Electrochemical immunosensors, genosensors, phagosensors and AMP sensors have also been used for pathogen detection (Li et al., 2014; Liebana et al., 2014). As compared to previous methods, the method described here has a number of advantages including: i) label-free detection, ii) low cost and high production capabilities of sAMPs, and iii) detection limit down to 10^2 CFU/mL. Table S2 shows a comparison of the performance of this sensor versus other bacteria detection methods reported in literature. The customized sequence of the sAMP enables a straightforward one-step fabrication procedure through the cysteine affinity tag. The total fabrication and analysis time is short, with 1 h for sensor fabrication and 0.5 h for sample testing. Sample analysis requires no further concentration, amplification, labeling or multistep procedures, providing additional advantages of this design. An attractive feature of the sAMP sensor is the ability to differentiate between live and dead bacteria. Viable bacteria are the direct cause of bacterial infections. The culturing and colony forming assay is the main technique able to discriminate live from dead bacteria, without sample pretreatment but the method requires several days. Live/dead assays using fluorescence labels use costly fluorescence dyes and involve extensive sample preparation and data processing time, which limit the broad applicability of this method for screening purposes. Other methods, such as PCR, although sensitive, are unable to differentiate live and dead bacteria. The method described in this work provides a new pathway for the development of devices for detecting viable bacterial pathogens. The preparation and assay time of the sAMP sensor are significantly lower than current bacteria detection methods. Furthermore, the method could be extended to measure other species such as fungus and viruses, based on the engineered sequence targeting the characteristic components of these microbes.

5. Conclusion

The current study demonstrated the utilization of sAMPs in the development of a biosensing platform for label-free detection of bacterial pathogens. Integration of an engineered cysteine modified sAMP in an EIS sensor design provided a pathway to ultrasensitive detection of bacterial pathogens with a simple, single step and inexpensive procedure, with a detection limit of 10^2 CFU/mL for *E. coli*, *P. aeruginosa*, *S. aureus* and *S. epidermidis*. Since various sAMPs with different bacterial selectivity can be produced by engineered synthesis, this method can be expanded to fabricate automated multiarray sensors for the detection and classification of pathogens, based on different recognition patterns to the immobilized sAMPs. Another discovery reported here is that synthetic sAMPs can differentiate live and dead bacteria. This discovery opens up the door for multiple applications of sAMPs in the diagnostics, food monitoring and clinical fields as receptors for viable bacteria detection. The system can be implemented in the design of low cost screening devices of a large number of samples that require increased detection sensitivity and a rapid response time.

Acknowledgements

MM gratefully acknowledges the Fulbright Foundation for a research fellowship at Clarkson University.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2016.01.041.

References

Bayoudh, S., Othmane, A., Ponsonnet, L., Ben Ouada, H., 2008. Electrical detection and characterization of bacterial adhesion using electrochemical impedance spectroscopy-based flow chamber. Colloids Surfaces A: Physicochem. Eng. Asp. 318 (1–3), 291–300.

Beumer, R.R., Brinkman, E., 1989. Detection of Listeria spp. with a monoclonal antibody-based enzyme-linked immunosorbent assay (ELISA). Food Microbiol. 6 (3), 171–177.

Beveridge, T.J., 1999. Structures of gram-negative cell walls and their derived membrane vesicles. J. Bacteriol. 181 (16), 4725–4733.

Cai, H.Y., Caswell, J.L., Prescott, J.F., 2014. Nonculture molecular techniques for diagnosis of bacterial disease in animals: a diagnostic laboratory perspective. Vet. Pathol. Online 51 (2), 341–350.

Centers for Disease Control and Prevention, 2013. Surveillance for Foodborne Disease Outbreaks, 2009–2010: Morbidity and Mortality Weekly Report. MMWR, United States. 62(3), 41.
Centers for Disease Control and Prevention, C., 2014a. Surveillance for Foodborne Disease Outbreaks, United States, 2011, Annual Report. In: US Department of Health and Human Services, C. (Ed.), Atlanta, Georgia.
Centers for Disease Control and Prevention, C., 2014b. Surveillance for Foodborne Disease Outbreaks, United States, 2012, Annual Report. In: US Department of Health and Human Services, C. (Ed.), Atlanta, Georgia.
Centers for Disease Control and Prevention, C., 2015. Surveillance for Foodborne Disease Outbreaks, United States, 2013, Annual Report. In: US Department of Health and Human Services, C. (Ed.), Atlanta, Georgia.
de Boer, E., Beumer, R.R., 1999. Methodology for detection and typing of foodborne microorganisms. Int. J. Food Microbiol. 50 (1–2), 119–130.
Di Felice, R., Selloni, A., Molinari, E., 2003. DFT study of cysteine adsorption on Au (111). J. Phys. Chem. B 107 (5), 1151–1156.
Dong, H., Paramonov, S.E., Aulisa, L., Bakota, E.L., Hartgerink, J.D., 2007. Self-assembly of multidomain peptides: Balancing molecular frustration controls conformation and nanostructure. J. Am. Chem. Soc. 129 (41), 12468–12472.
Eckert, R., Qi, F.X., Yarbrough, D.K., He, J., Anderson, M.H., Shi, W.Y., 2006. Adding selectivity to antimicrobial peptides: Rational design of a multidomain peptide against Pseudomonas spp. Antimicrob. Agents Chemother. 50 (4), 1480–1488.
Ehrlich, H.L., 2008. Are gram-positive bacteria capable of electron transfer across their cell wall without an externally available electron shuttle? Geobiology 6 (3), 220–224.
Friedrich, C., Scott, M.G., Karunaratne, N., Yan, H., Hancock, R.E.W., 1999. Salt-resistant alpha-helical cationic antimicrobial peptides. Antimicrob. Agents Chemother. 43 (7), 1542–1548.
Hartmann, M., Berditsch, M., Hawecker, J., Ardakani, M.F., Gerthsen, D., Ulrich, A.S., 2010. Damage of the bacterial cell envelope by antimicrobial peptides gramicidin S and PGLa as revealed by transmission and scanning electron microscopy. Antimicrob. Agents Chemother. 54 (8), 3132–3142.
Li, Y., Afrasiabi, R., Fathi, F., Wang, N., Xiang, C., Love, R., She, Z., Kraatz, H.-B., 2014. Impedance based detection of pathogenic E. coli O157:H7 using a ferrocene-antimicrobial peptide modified biosensor. Biosens. Bioelectron. 58, 193–199.
Liebana, S., Brandao, D., Alegret, S., Pividori, M.I., 2014. Electrocehmical immunosensors, genosensors and phagosensors for Salmonela detection. Anal. Methods 6, 8858–8873.
Matias, V.R.F., Beveridge, T.J., 2005. Cryo-electron microscopy reveals native polymeric cell wall structure in Bacillus subtilis 168 and the existence of a periplasmic space. Mol. Microbiol. 56 (1), 240–251.
Mejri, M.B., Baccar, H., Baldrich, E., Del Campo, F.J., Helali, S., Ktari, T., Simonian, A., Aouni, M., Abdelghani, A., 2010. Impedance biosensing using phages for bacteria detection: Generation of dual signals as the clue for in-chip assay confirmation. Biosens. Bioelectron. 26 (4), 1261–1267.
Palumbo, J.D., Borucki, M.K., Mandrell, R.E., Gorski, L., 2003. Serotyping of listeria monocytogenes by enzyme-linked immunosorbent assay and identification of mixed-serotype cultures by colony immunoblotting. J. Clin. Microbiol. 41 (2), 564–571.
Rydlo, T., Rotem, S., Mor, A., 2006. Antibacterial properties of dermaseptin S4 derivatives under extreme incubation conditions. Antimicrob. Agents Chemother. 50 (2), 490–497.
Schneewind, O., Fowler, A., Faull, K.F., 1995. Structure of the cell-wall anchor of surface-proteins in Staphylococcus-Aureus. Science 268 (5207), 103–106.
Vallee, A., Humblot, V., Pradier, C.-M., 2010. Peptide interactions with metal and oxide surfaces. Accounts Chem. Res. 43 (10), 1297–1306.
Wilson, I.G., 1997. Inhibition and facilitation of nucleic acid amplification. Appl. Environ. Microbiol. 63 (10), 3741–3751.
Xu, D., Jiang, L., Singh, A., Dustin, D., Yang, M., Liu, L., Lund, R., Sellati, T.J., Dong, H., 2015. Designed supramolecular filamentous peptides: balance of nanostructure, cytotoxicity and antimicrobial activity. Chem. Commun. 51 (7), 1289–1292.
Yang, M., Xu, D., Jiang, L., Zhang, L., Dustin, D., Lund, R., Liu, L., Dong, H., 2014. Filamentous supramolecular peptide-drug conjugates as highly efficient drug delivery vehicles. Chem. Commun. 50 (37), 4827–4830.
Zasloff, M., 2002. Antimicrobial peptides of multicellular organisms. Nature 415 (6870), 389–395.
Zuber, B., Haenni, M., Ribeiro, T., Minnig, K., Lopes, F., Moreillon, P., Dubochet, J., 2006. Granular layer in the periplasmic space of gram-positive bacteria and fine structures of enterococcus gallinarum and streptococcus gordonii septa revealed by cryo-electron microscopy of vitreous sections. J. Bacteriol. 188 (18), 6652–6660.