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Effects of guanidino modified aminoglycosides on mammalian membranes studied using a quartz crystal microbalance†‡

Torsten John,^{ab} Zhi Xiang Voo,^a Clemens Kubeil,^a Bernd Abel,^b Bim Graham,^c Leone Spiccia^a and Lisandra L. Martin^{id}*^a

The increase in bacterial and viral resistance to current therapeutics has led to intensive research for new antibacterial and antiviral agents. Among these, aminoglycosides and their guanidino derivatives are potent candidates targeting specific RNA sequences. It is necessary that these substances can pass across mammalian membranes in order to reach their intracellular targets. This study investigated the effects of the aminoglycosides kanamycin A and neomycin B and their guanidino derivatives on mammalian mimetic membranes using a quartz crystal microbalance with dissipation monitoring (QCM-D). Lipid bilayers as membrane models were deposited onto gold coated quartz crystals and aminoglycosides added afterwards. Notably, the guanidino derivatives exhibited an initial stiffening of the membrane layer indicating a quick insertion of the planar guanidino groups into the membrane. The guanidino derivatives also reached their maximum binding to the membrane at lower concentrations than the native compounds. Therefore, these modified aminoglycosides are promising agents for the development of new antimicrobial treatments.

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Introduction

Despite the availability of an arsenal of antibacterial and antiviral treatments and drug targets discovered to date,¹ there continues to be an increase in pathogen mutations leading to drug resistance.^{2–5} This reinforces the high demand for new classes or derivatives of active substances.⁶ The strategy of targeting RNA has the advantage of a low probability for point mutations within the RNA and is therefore less prone to resistance. RNA motifs are highly conserved among most bacterial and viral strains.^{7,8} Aminoglycosides act as polycationic antibiotics, mostly binding to the ribosomal 16S A-site RNA in the 30S subunit, thus reducing translation and subsequent protein biosynthesis.^{9–12} Hence, aminoglycoside antibiotics inhibit bacterial or viral replication.^{13–15} They have also shown high potency in inhibiting the HIV (human immuno-

deficiency virus) replication machinery by binding to, and cleavage of, the trans-activation response (TAR) element.^{16–18} Most aminoglycosides share a central 2-deoxystreptamine (2-DOS) ring to which different amino sugars are attached *via* glycosylation (see Fig. 1).^{17,19} Kanamycin A and neomycin B are widely investigated aminoglycoside representatives (see Fig. 2),¹³ but show low specificity towards RNA¹⁷ as well as reversible and irreversible toxic effects.^{20–23} Modifications of these aminoglycosides have been investigated and revealed enhanced selectivities and affinities of the guanidino and azido derivatives towards the target RNA sites.^{24,25} The guanidino group (see Fig. 1), protonated at physiological pH, is highly basic and possesses a delocalised electron system with planar structure. This planar functional group assists in the binding to specific RNA grooves.^{26,27} A disruptive effect of aminoglycosides towards bacterial mimetic membranes has already been demonstrated leading to its antibiotic activity.²⁸

^a School of Chemistry, Monash University, Wellington Rd, Clayton, VIC 3800, Australia. E-mail: Lisa.Martin@monash.edu^b Leibniz Institute of Surface Modification, and Wilhelm-Ostwald-Institute for Physical and Theoretical Chemistry, Leipzig University, Permoserstrasse 15, 04318 Leipzig, Germany^c Medicinal Chemistry, Monash Institute of Pharmaceutical Sciences, Monash University, 381 Royal Parade, Parkville, VIC 3052, Australia

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§ In memory of our friend, colleague and mentor Leone Spiccia, who passed away in December 2016.

Fig. 1 Molecular structures of 2-deoxystreptamine (2-DOS) ring (left) and guanidino group (right). The 2-DOS ring is the parent ring for the formation of aminoglycosides by addition of amino sugars to the hydroxy functional groups *via* glycosylation.

Kanamycin A

Guanidino-kanamycin A

Neomycin B

Guanidino-neomycin B

Fig. 2 Molecular structures (left) and 3D representations (right) of kanamycin A, neomycin B and its guanidino derivatives based on their crystal structures (PDB entries KAN (1L8T) and NMY (2B0Q)).³⁰

Several multidrug-resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA), invade mammalian cells.²⁹ Hence, potential drugs need to be able to penetrate mammalian membranes to target intracellular bacterial infections.²⁹ Thus, knowledge on the interaction mechanism of aminoglycosides with mammalian membranes is essential for the development of potential therapeutics with improved de-

livery properties.^{31,32} The cationic character of aminoglycosides significantly contributes to electrostatic interactions between drug and membrane lipid molecules.²²

To achieve a better understanding of the aminoglycoside-membrane interaction mechanism, the quartz crystal microbalance with dissipation monitoring (QCM-D) technique is powerful. QCM-D monitors binding events to a sensor target with nanogram sensitivity *in vitro*.^{33–37} Mass changes due to drug binding to the surface cause frequency changes of the QCM sensor.^{38–41} The technique is based upon the piezoelectric effect of single quartz crystals. The fundamental relationship between the added mass Δm and the change in resonance frequency Δf_n was described for rigid, thin and evenly distributed adsorbed layers by Sauerbrey in 1959.^{42,43} Viscoelastic factors are included for biological and fluid systems.^{44–46}

In this study, the well-known aminoglycosides kanamycin A and neomycin B and their guanidino derivative are investigated (see Fig. 2). The mammalian membrane interface is modelled using its characteristic lipid components disregarding minor constituents.^{33,47,48} An enhanced membrane interaction and potential membrane-permeability of the guanidino derivatives was found that enable the potential application of these derivatives as drugs. Our results on aminoglycoside membrane interaction can support the design of future antibacterial therapeutics with improved delivery properties.

Materials and methods

Preparation of buffer solutions

Sodium chloride ($\geq 99.5\%$), potassium phosphate monobasic ($\geq 99.0\%$) and potassium phosphate dibasic ($\geq 98.0\%$) were obtained from Sigma-Aldrich (USA). Ultrapure water (18.2 M Ω cm) was used for all experiments (Sartorius, Germany). Phosphate buffered saline (PBS) solutions with 20 mM phosphate were prepared both as high-salt (100 mM sodium chloride) and low-salt (30 mM sodium chloride) PBS solution at pH 6.9 ± 0.05 . The solutions were filtered through hydrophobic polypropylene membrane filters (GH Polypro, 0.2 μ m, PALL Life Sciences, USA).

Synthesis and preparation of aminoglycosides

Kanamycin A sulfate and neomycin B trisulfate were both purchased from Sigma-Aldrich (USA). The derivatives guanidino-kanamycin A and guanidino-neomycin B were synthesised as TFA (trifluoroacetic acid) salts.⁴⁹ Sample solutions (1 μ M, 10 μ M, 15 μ M and 30 μ M) were prepared in high salt PBS buffer and filtered through 0.45 μ m membrane filters (Acrodisc Supor, PALL Life Sciences, USA).

Guanidino-kanamycin A. Kanamycin A sulfate (0.100 g, 0.172 mmol) was converted with *N,N'*-di-Boc-1-pyrazole-1-carboxamide (0.283 g, 0.912 mmol) in methanol (5 mL) and triethylamine (0.35 mL, 2.43 mmol) to guanidino(Boc)₂-kanamycin A (1). TLC analysis confirmed the completion of the reaction after stirring for four days at room temperature.

Normal phase column chromatography (silica gel, methanol/dichloromethane 97:3 v/v) was used to give a colourless crystalline solid (1). Compound (1) (0.079 g, 0.054 mmol) was deprotected by stirring in TFA/CHCl₃/toluene (1:1:3) at room temperature for four hours. Removal of the solvent *in vacuo* afforded the guanidino-kanamycin A TFA salt (2) (*M* = 652.33, ESI MS *m/z*: 653.1 [M + H]⁺). The product was used without further treatment.

Guanidino-neomycin B. Neomycin B trisulfate (0.100 g, 0.110 mmol) was converted with *N,N'*-di-Boc-1-pyrazole-1-carboxamide (0.273 g, 0.880 mmol) in methanol (5 mL) and triethylamine (0.35 mL, 2.43 mmol) to guanidino(Boc)₁₂-neomycin B (3). TLC analysis confirmed the completion of the reaction after stirring for five days at room temperature. Normal phase column chromatography (silica gel, ethyl acetate/*n*-hexane 1:1 v/v to 2:1 v/v) was used to give a colourless crystalline solid (3). Compound (3) (0.092 g, 0.106 mmol) was deprotected by stirring in TFA/CHCl₃ (1:1) at room temperature for four hours. Removal of the solvent *in vacuo* afforded the guanidino-neomycin B TFA salt (4) (*M* = 866.44, ESI MS *m/z*: 289.9 [M + 3H]³⁺). The product was used without further treatment.

Liposome preparation

DMPC (1,2-dimyristoyl-*sn*-glycero-3-phosphocholine) (Avanti Polar Lipids, USA) and cholesterol (Steraloids, USA) were each dissolved in pure ethanol-free chloroform (Sigma-Aldrich, USA) to prepare 5 mM stock solutions. Aliquots of 100 µL of the desired lipid composition of DMPC and cholesterol (v/v, 4:1) were transferred into test tubes. The solvent was evaporated under a gentle stream of nitrogen gas and dried under vacuum. The lipid mixtures were resuspended in 1 mL high salt PBS buffer (0.5 mM lipid), incubated at 37 °C for at least 30 minutes, vortexed and sonicated for 20 minutes in a sonication bath (Unisonics FXP8, Australia) at room temperature to obtain a cloudy solution of dissolved liposomes. The liposome mixture was filtered through 0.45 µm membrane filters (Acrodisc Supor, PALL Life Sciences, USA) and diluted with PBS buffer to 0.1 mM lipid.^{34,50,51} DMPC/cholesterol liposomes had a radius of 70 to 90 nm determined by dynamic light scattering (DLS) (Malvern Zetasizer Nano ZS, UK).

QCM-D sensor preparation and experiments

Hydrogen peroxide (30%), ethanol and isopropanol were obtained from Merck (Germany). Ammonium hydroxide (28%) was purchased from Ajax Finechem (New Zealand) and concentrated sulfuric acid was purchased from Sigma-Aldrich (USA). MPA (3-mercaptopropionic acid, ≥99.0%) was obtained from Fluka BioChimica (Switzerland).

The QCM-D sensors were polished, gold-coated, AT-cut quartz crystals with a fundamental frequency of about 5 MHz (Q-Sense, Sweden). After immersing in a solution of hydrogen peroxide, ammonium hydroxide and water (v/v, 1:1:3) at about 70 °C for 20 minutes, the sensors were cleaned in piranha solution (sulfuric acid and hydrogen peroxide, v/v, 3:1)

for one minute.^{52,53} After rinsing with ultrapure water and isopropanol, the sensors surface was modified with a self-assembled carboxylate monolayer by immersing the sensors into a 1 mM solution of MPA in isopropanol for at least one hour.^{50,54–56} Excess MPA was removed by rinsing with isopropanol. The sensors were dried under a gentle stream of nitrogen gas.

QCM-D measurements were performed using a Q-Sense E4 instrument (Q-Sense, Sweden) consisting of four flow cells at 22 ± 0.05 °C at least in triplicate. Experiments were conducted as previously published.^{34,51} In brief, liposomes were deposited onto the gold sensors and ruptured to form a lipid bilayer using a low salt PBS buffer. The formation of a consistent supported lipid bilayer is essential to study and compare the aminoglycoside interactions. The lipid bilayer formation is verified by a characteristic change in frequency ($\Delta f = -26 \pm 5$ Hz) and dissipation ($\Delta D = (4 \pm 1) \times 10^{-6}$) of the supported lipid bilayer.³⁸ Note that the change in dissipation is higher for SAM-modified gold sensors than for SiO₂ sensors.^{38–40} After a supported lipid bilayer was formed, the aminoglycoside samples were continuously introduced at 50 µL min⁻¹ for about one hour. The system was finally rinsed with high salt PBS buffer. A typical QCM-D experiment is outlined in the ESI† (see Fig. S1).

QCM-D data analysis

Raw data were processed and analysed by QTools (Q-Sense, Sweden) and OriginPro 8G (OriginLab Corporation, USA). The fundamental frequency ($f_0 = 5$ MHz) was not analysed, as it is too sensitive to flow effects within the chamber and thus unreliable.^{41,57} Δf and ΔD values for the 3rd, 5th, 7th, 9th and 11th harmonic were analysed for depth profiling, whereas only the 7th harmonic was used for all other plots in this study.

Data for each compound at each concentration were averaged and normalised to consistent Δf and ΔD values for the lipid bilayer deposition to account for any variation in bilayer thickness and density.⁵⁰ This step enables a reliable comparison of all data sets. The membrane coated sensors before aminoglycoside addition were set to zero to obtain overlapping baselines. All diagrams show the progression of frequency and dissipation between three minutes before addition of the aminoglycoside molecules up until the high salt buffer wash at the end of the experiments.

Molecular structures of the aminoglycosides were visualised and edited using ChemDraw Professional 15.1 and Maestro.⁵⁸ 3D representations of the aminoglycosides were visualised using the VMD 1.9.2 software package.⁵⁹

Results and discussion

The interaction of aminoglycosides with mammalian mimetic membranes was investigated by employing QCM-D.⁵⁰ The mammalian membrane was mimicked by a supported lipid bilayer (SLB)⁶⁰ consisting of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and cholesterol (4:1 molar ratio) (see Fig. 3).⁵⁰ This approach is widely used by numerous

Fig. 3 Molecular structures of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and cholesterol.

groups to study membrane interactions^{33,47,48,61} and complements our previously studied effects of aminoglycosides on bacterial membranes.²⁸ The QCM-D technique measures changes in frequency (Δf) and dissipation (ΔD) of a quartz

crystal sensor. A decrease in frequency (Δf) is correlated with an increase in mass (Δm) while an increase in dissipation (ΔD) refers to a softer and less rigid surface structure.^{42,43} The frequency f is inversely correlated with the quartz sensor's mass while the dissipation factor D is derived from the rate of energy lost into the system after the voltage is switched off.⁴³

The effect of aminoglycosides on the mammalian membrane interface was followed by measuring the concentration dependent behaviour, *i.e.* $\Delta f(t)$ and $\Delta D(t)$ data as shown in Fig. 4. The data display a total frequency decrease and dissipation increase for all compounds which relates to an aminoglycoside binding and concomitant softening of the structure of the composite aminoglycoside-membrane layer. The binding profiles are illustrated for four concentrations between 1 μM and 30 μM . An increased and faster aminoglycoside binding is observed with increasing concentration approaching a saturation level for most compounds. Neomycin B shows the highest mass binding while kanamycin A features the lowest. The guanidino derivatives have an intermediate effect but reached their maximum effects already at lower concentrations than the native compounds, thus are more potent.

Fig. 4 $\Delta f(t)$ and $\Delta D(t)$ traces from QCM-D monitoring of the interaction of (a) kanamycin A, (b) guanidino-kanamycin A, (c) neomycin B and (d) guanidino-neomycin B at different concentrations (1 μM black, 10 μM red, 15 μM blue, 30 μM green) with a DMPC/cholesterol membrane. Arrows mark the moments of aminoglycoside addition (up) and final buffer wash (down).

Kanamycin A, guanidino-kanamycin A and especially guanidino-neomycin B present a dip with a transient decrease in dissipation immediately after introduction of the aminoglycoside. This indicates that the aminoglycoside molecules inserted into the lipid bilayer stiffening its structure before softening due to subsequent adsorption on top of the membrane or the bound aminoglycoside molecules. After the initial aminoglycoside addition, at around ten minutes, both the frequency and dissipation present a continuous binding or adsorption of aminoglycoside molecules onto the lipid bilayer surface. This binding is especially prevalent for the unmodified aminoglycosides indicating that these continue to bind and soften the membrane. The dissipation changes are quite small for all compounds.

The final buffer rinse led to an increase in frequency and decrease in dissipation according with a mass removal and stiffening of the membrane for all compounds except neomycin B. Neomycin B presented no significant change in mass and a small softening of the membrane upon the final buffer rinse. The mass removal for kanamycin A was the biggest, confirming a weak binding of the kanamycin A molecules onto the bilayer surface after the initial aminoglycoside interaction. Both frequency and dissipation continue to progress during the washing step until reaching a stable state. The aminoglycoside-lipid bilayer system restructures its dynamic configuration, especially for the cases in which weakly attached aminoglycosides are rinsed off.

Another way to analyse QCM-D data are ΔD vs. Δf plots as shown in Fig. 5.^{33,34,62} These ‘fingerprint’ representations illustrate changes in the structural organisation of the lipid bilayer during aminoglycoside addition. Both frequency and dissipation can be interpreted in context using these plots. A negative change in frequency and positive change in dissipa-

tion are characteristic for drug binding (top right quadrant in plot) whereas a positive change in frequency and negative change in dissipation represent membrane disruption (bottom left quadrant in plot). In our study, all aminoglycosides and derivatives studied showed a binding to the membrane. The transient decrease in dissipation for kanamycin A and the two guanidino derivatives is shown as a dip in Fig. 5. The differences in binding, as well as the greater mass removal, observed for kanamycin A, upon the final buffer rinse, are clearly visible from the ΔD vs. Δf representation.

Qualitative kinetic information about the aminoglycoside-membrane interaction can be obtained by evaluating the first order derivatives of the time dependent frequency changes $\delta\Delta f/\delta t(t)$.^{51,63} The kinetic profiles of the aminoglycosides present only one major binding event (see Fig. 6). The aminoglycosides started binding quickly for all compounds and decreased to a baseline with very low and constant binding rate for all aminoglycosides. Neomycin B shows the highest binding rate which is about four times as high as for kanamycin A (3.2 vs. 0.8 Hz min⁻¹). The derivatives lay in between and, in contrast to the unmodified compounds, the guanidino derivatives show a distinct tailing of the major binding peak. This means that these derivatives undergo a subsequent slower process. This might indicate a reorganisation of the aminoglycosides within the lipid bilayer. The binding rate for the guanidino derivatives decreases at a slower rate than the native kanamycin A or neomycin B.

The aminoglycoside-membrane effect is examined at varying distances from the sensor surface by studying the harmonics of the crystal fundamental frequency.^{33,37,41,51,64} The $\Delta f(t)$ and $\Delta D(t)$ plots for the aminoglycosides investigated at 30 μ M are presented in Fig. 7. Representative experiments

Fig. 5 ΔD vs. Δf plot for the interaction of 30 μ M kanamycin A (black), neomycin B (red), guanidino-kanamycin A (blue) and guanidino-neomycin B (green) with the DMPC/cholesterol membrane. The point of origin corresponds to the time of aminoglycoside addition. Asterisks indicate the start of aminoglycoside addition and the final buffer rinse. Stages: (i) aminoglycoside addition (continuous flow) and (ii) final buffer wash where weakly bound aminoglycosides can be removed under flow.

Fig. 6 First-order derivatives of $\Delta f(t)$ traces ($\delta\Delta f/\delta t(t)$) vs. time for the interaction of 30 μ M kanamycin A (black), neomycin B (red), guanidino-kanamycin A (blue) and guanidino-neomycin B (green) with the DMPC/cholesterol membrane during first aminoglycoside interaction. The graphs are smoothed using the Savitzky-Golay method (polynomial order: 2, points of window: 20). Please note that the graphs show three minutes before aminoglycoside addition into the system and the response time of the QCM-D instrument within the first minutes. Arrow marks the moment of aminoglycoside addition.

Fig. 7 QCM-D monitoring of interaction of 30 μ M (a) kanamycin A, (b) guanidino-kanamycin A, (c) neomycin B and (d) guanidino-neomycin B at different harmonics (3rd harmonic black, 5th harmonic red, 7th harmonic blue, 9th harmonic green, 11th harmonic orange) with a DMPC/cholesterol membrane. Arrows mark the moments of aminoglycoside addition (up) and final buffer wash (down).

(not averaged repetitions) for each compound are shown. Data were smoothed using the adjacent-averaging method (moving average) with 15 points of window. The 3rd harmonic probes the furthest distance from the sensor surface and above the lipid bilayer whereas the 11th harmonic probes interactions close to the gold sensor surface and within the lipid bilayer. The aminoglycosides investigated all bound to the lipid membrane over all harmonics.

The first interactions of kanamycin A with the mammalian model membrane led to a slightly higher mass addition for the higher harmonics. Together with a transient decrease in dissipation indicating stiffening of the membrane bilayer, this confirms a strong binding within the polar head groups of the lipid bilayer. Neomycin B presents an initial strong mass binding and structure softening similar at all distances. The membrane effects for the guanidino derivatives are similar to kanamycin A but guanidino-neomycin B presented a much stronger aminoglycoside insertion and membrane stiffening (seen as a dip in the dissipation). The guanidino-neomycin B molecules inserted well within the head groups of the lipid bilayer. The rapid initial membrane binding caused the biggest change in dissipation for the harmonics

close to the sensor surface. This means that the membrane structure was influenced across the bilayer prior to being stabilised.

Subsequent surface adsorption of the aminoglycosides and membrane softening was similar at all harmonics, *i.e.* distances across the membrane from the quartz sensor, for kanamycin A and the guanidino derivatives, whereas it was primarily at the membrane surface for neomycin B.

To emphasize the role of the guanidino groups, we modified a hydroxy group close to several guanidino groups to a more bulky azido group. The azido-guanidino-neomycin B derivative (see Fig. 8 and S2 to S4†) reached its maximum membrane interaction at a lower concentration than the unmodified neomycin B as also found for the guanidino-derivative. In contrast to guanidino-neomycin B, the azido-guanidino-derivative presented no transient decrease and a stronger effect in dissipation, *i.e.* a softening of the membrane layer. This indicates that the aminoglycoside did not insert but bound to the membrane surface (see Fig. 8). This mechanism was confirmed by analysing the different harmonics. It presents the greatest effects in both frequency and dissipation for the lower harmonics indicating that surface-

Fig. 8 QCM-D monitoring of the interaction of azido-guanidino-neomycin B with a DMPC/cholesterol membrane. (a) $\Delta f(t)$ and $\Delta D(t)$ traces at different concentrations (1 μM black, 10 μM red, 15 μM blue, 30 μM green). (b) $\Delta f(t)$ and $\Delta D(t)$ at different harmonics (3rd harmonic black, 5th harmonic red, 7th harmonic blue, 9th harmonic green, 11th harmonic orange). Arrows mark the moments of aminoglycoside addition (up) and final buffer wash (down).

binding is more prevalent than membrane insertion. The binding is similar to the unmodified neomycin B but weaker.

Structural consequences. All aminoglycosides studied here bound to the mammalian mimetic membrane interface. The guanidino derivatives displayed their maximum binding effects at lower concentrations in comparison to the unmodified aminoglycosides and thus were more potent. A proposed model for aminoglycoside–membrane interaction is shown in Fig. 9. It is assumed that the aminoglycoside molecules inserted and bound within the polar head groups of the lipid bilayer, since interactions with the hydrophobic tail regions are less attractive. The insertion and binding within the head groups influenced the membrane across all distances from the quartz sensor and within both layers of the lipid bilayer structure. In all cases, an initial insertion was observed with neomycin B having the strongest effect. This could be caused by its overall higher positive charge (five of the six amines protonated),⁶⁵ favouring an electrostatic interaction, compared to kanamycin A (three of the four amines protonated).⁶⁶ The initial insertion is followed by a subsequent adsorption of aminoglycoside molecules to the membrane surface. The final buffer rinse resulted in some desorption of weakly bound aminoglycoside molecules, especially notable for kanamycin A.

The aminoglycosides kanamycin A, guanidino-kanamycin A and especially guanidino-neomycin B show a dip in the dissipation-time dependence upon initial aminoglycoside binding (see Fig. 7). This temporary decrease in dissipation represents a stiffening of the lipid bilayer. This can be caused by a rapid initial aminoglycoside insertion. Relaxation and membrane softening occurs in a subsequent slower process. Guanidino groups and arginine-rich peptides are known to be relevant for the translocation through cell membranes.⁶⁷

The different behaviour of the kanamycin A and neomycin B guanidino derivatives might be caused by its varying molecular structure (see Fig. 2). In kanamycin A, the amino and almost all hydroxy functional groups are arranged so that three (of the four) amines lie on one side, creating a positively charged domain, and the opposite surface is dominated by polar hydroxy functional groups. The modification of the amino to guanidino functional groups has no significant

Fig. 9 Proposed model for membrane interaction of kanamycin A (red), neomycin B (blue), guanidino-kanamycin A (magenta) and guanidino-neomycin B (green) with initial aminoglycoside insertion and subsequent insertion or surface adsorption. Neomycin B has shown the fastest and highest mass binding due to its high cationic charge. The guanidino derivatives bound to the mammalian membrane mimetic at lower concentrations than the unmodified compounds and its planar guanidino groups quickly inserted within the lipid bilayer head groups. Subsequent additional surface adsorption softened the membrane layer for neomycin B. A final buffer wash eliminated some of the aminoglycoside molecules for kanamycin A and the guanidino derivatives.

steric effect on the side with the hydroxy groups. In contrast, this guanidino modification strongly influences neomycin B since the hydroxy and amino functional groups are broadly distributed within the molecule. The dip in dissipation could be caused by quickly inserting planar guanidino groups within the membrane layer. Addition of an azido group to the guanidino derivative did not show such behaviour (see ESI† Fig. S7) and thus a binding configuration without insertion of the guanidino groups into the membrane can be assumed.

Conclusions

In summary, the QCM-D studies on aminoglycoside-membrane interactions presented a rapid insertion and binding of all compounds to the artificial mammalian membrane interface. Neomycin B has shown the fastest and highest mass binding due to its high cationic charge and electrostatic interactions with the membrane surface. Aminoglycoside binding displayed a general trend towards a softening of the membrane layer. The guanidino derivatives, especially guanidino-neomycin B, stiffened the membrane upon first aminoglycoside binding indicating a quick insertion of the planar guanidino groups within the lipid bilayer within the first few minutes. The role of guanidino groups is known to be relevant for drug-membrane permeation. The guanidino derivatives presented strong interactions with the mammalian membrane mimetic at lower concentrations in comparison to the native compounds. The enhanced membrane interaction suggests they could be promising therapeutics in the design of new antimicrobial and antiviral treatments. Further studies on the role of individual functional groups of the aminoglycosides need to be conducted to understand aminoglycoside-membrane interactions at the molecular level.

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