Supplementary Information

for the manuscript

Effects of guanidino modified aminoglycosides on mammalian membranes studied by a quartz crystal microbalance

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Characterisation of a typical QCM-D experiment

Aminoglycoside-membrane interactions were analysed using QCM-D. Each experiment consisted of two main steps: 1) lipid bilayer deposition and 2) introduction of the aminoglycoside. Typical results for a QCM-D experiment are shown in Fig. S1.

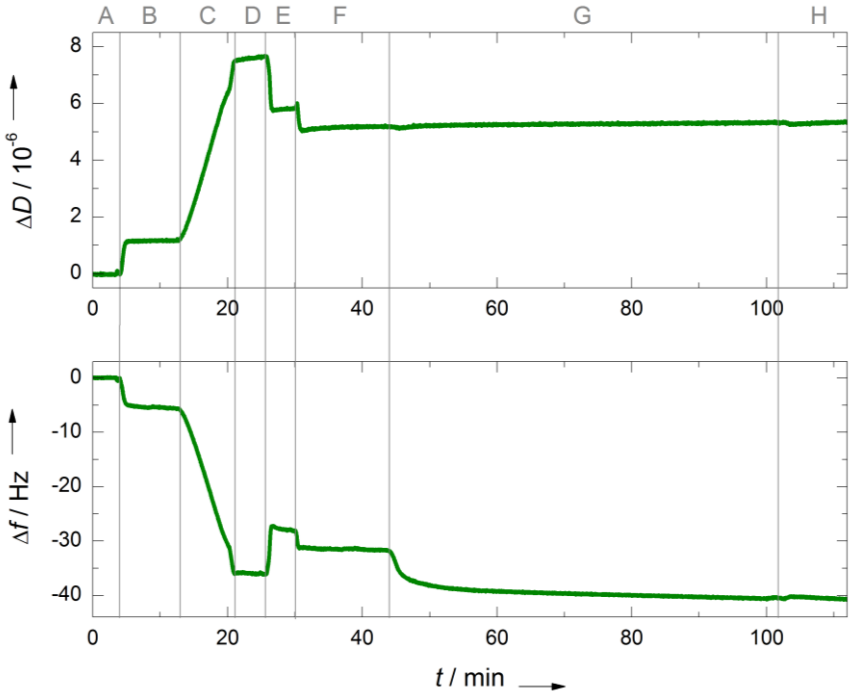

Fig. S1 Δ*f(t)* and Δ*D(t)* plots for a typical QCM-D experiment. A: Initial water baseline (no flow), B/D/F/H: High salt buffer baselines (300 µL/min), C: Liposome introduction (50 µL/min), E: Low salt buffer for liposome bursting (300 µL/min), G: Aminoglycoside introduction (50 µL/min).

High salt PBS (phosphate buffered saline) solution (**B** in Fig. S1) was introduced into the measurement chamber to obtain a stable baseline before addition of the liposomes. Changes in density and viscosity of the liquid led to a "bulk shift" of Δf and ΔD, e.g. between water (**A**) and buffer solution (**B**). Liposomes were pumped into the chamber (**C**) and adsorbed onto the MPA-SAM (3-mercaptopropionic acid self-assembled monolayer) modified gold sensor surfaces (increase in mass and dissipation, $\Delta f \propto -\Delta m$). The liposome introduction was stopped and high salt PBS buffer was introduced to remove weakly bound liposomes or lipid residues from the tubing and chamber and a stable PBS buffer baseline was obtained (**D**). The formation of a lipid bilayer, without embedded liposomes, was achieved by reducing the salt concentration to a low salt buffer to encourage the rupture of any liposomes, due to osmotic pressure (**E**). The more rigid lipid bilayer structure caused a decrease in dissipation. High salt PBS solution was pumped into the system to achieve a stable baseline before addition of the aminoglycoside solution (**F**). The buffer baseline difference between (**B**) and (**F**) is characteristic for a lipid bilayer structure (Δf = -26±5 Hz, ΔD = (4±1) x 10^{-6}). Note that the change in dissipation is higher for SAM-modified gold sensors than for SiO_2 sensors. Lipid bilayers deposited onto SAM-modified gold sensors represent more viscous and dynamic membrane structures. The aminoglycoside was introduced into the chamber in different concentrations to investigate its membrane effect over about 60 minutes each (**G**). The experiment was completed by washing with high salt PBS solution and achieving a baseline (**H**). Small drifts in frequency (2 Hz hr^{-1}) and dissipation (0.2 x 10^{-6} hr^{-1}) are within the accuracy of the method.

Study of azido-guanidino-neomycin B

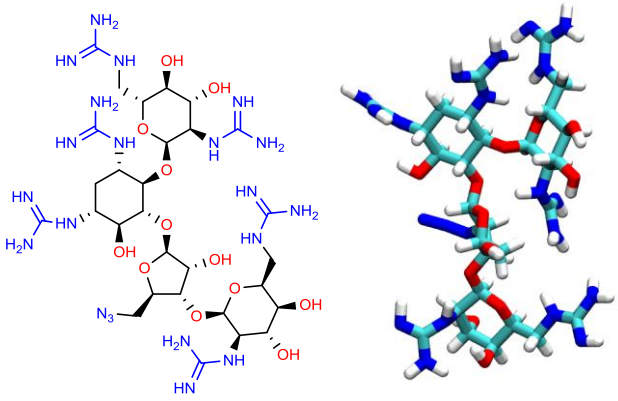

Fig. S2 Molecular structure (left) and 3D representation (right) of azido-guanidino-neomycin B.

Synthesis: Azido-neomycin B TFA salt[S1] **(5)** (1.02 g, 0.771 mmol) was converted with *N*,*N*"-di-Boc-1-pyrazole-1-carboxamidine (1.90 g, 6.17 mmol) in methanol (35 mL) and triethylamine (2.32 mL, 17.7 mmol) to 5"-azido-guanidino$(Boc)_{12}$-neomycin B **(6)**. 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*-pentane 3:7 v/v) was used to give a colourless crystalline solid **(6)**. Compound **(6)** (0.080 g, 0.038 mmol) was deprotected by

stirring in TFA/$CHCl_3$ (1:1) at room temperature for four hours. Removal of the solvent *in vacuo* afforded the 5”-azido-guanidino-neomycin B TFA salt **(7)** (M=891.45, ESI MS *m/z*: 223.2 $[M+4H]^{4+}$). The product was used without further treatment.

S1 J. L. Childs-Disney, M. Wu, A. Pushechnikov, O. Aminova and M. D. Disney, *ACS Chem. Biol.*, 2007, **2**, 745–754.

QCM-D results

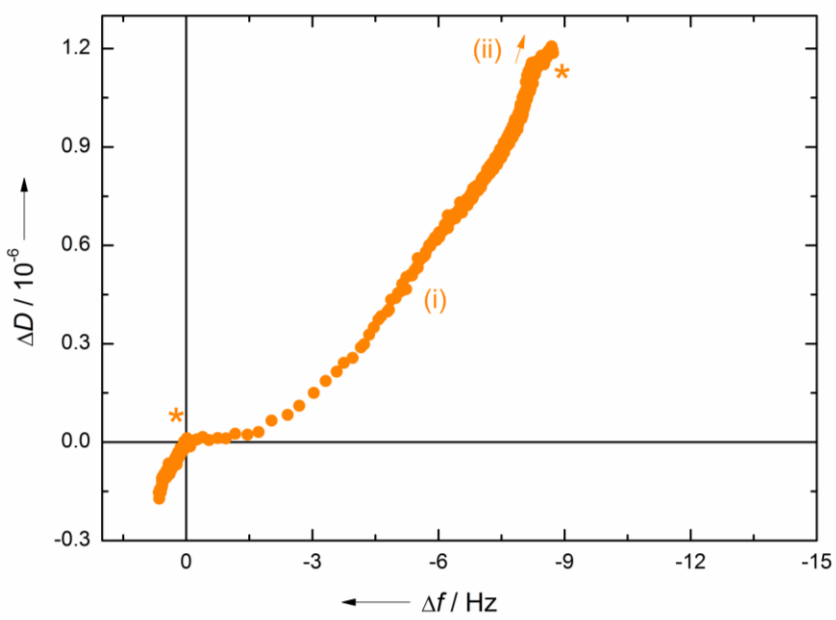

Fig. S3 Δ*D* vs. Δ*f* plot for the interaction of 30 µM azido-guanidino-neomycin B with the DMPC/cholesterol membrane. The point of origin corresponds to the time of aminoglycoside addition. Asterisks indicate the start of aminoglycoside addition and the final buffer rinse. Stages: (i) aminoglycoside addition (continuous flow) and (ii) final buffer wash where weakly bound aminoglycosides can be removed under flow.

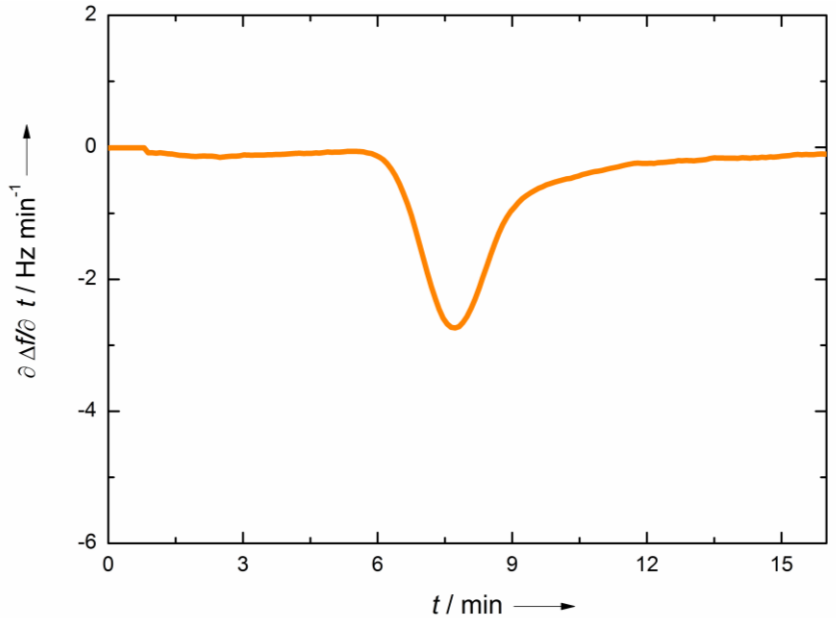

Fig. S4 First-order derivative of Δ*f(t)* trace vs. time for the interaction of 30 µM azido-guanidino-neomycin B with the DMPC/cholesterol membrane during first aminoglycoside binding. The graphs are smoothed using the Savitzky-Golay method (polynomial order: 2, points of window: 20). Please note that the graph shows three minutes before aminoglycoside addition into the system and the response time of the QCM-D instrument within the first minutes.