Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Electronic Supporting Information

Attenuation of neuroblastoma cell growth by nisin is mediated by modulation of phase behaviour and enhanced cell membrane fluidity

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Supporting Text

The dipole potential (Ψ_D) is an important physical property of biological membrane, which is related to the order in membrane. It is the potential difference that arises mainly due to the non-random arrangement of membrane molecular dipoles (water dipoles, carbonyl-groups and lipid head-groups) at the membrane interface. Gross et. al., in several of their papers on the development of this method have shown that depending on the electric field in the vicinity, potentiometric probe undergoes shift in fluorescence excitation spectrum.[1] The spectral shift $\Delta\nu$ is related to a corresponding change in the dipole potential:

$$\Delta\nu = \left(-\frac{1}{h}\right)\Delta\mu E - \left(\frac{1}{2h}\right)\Delta\alpha E^2$$

where $\Delta\mu$ is the change in the electric dipole moment of the probe upon electronic excitation, $\Delta\alpha$ is the change in polarizability of the probe upon excitation, E is the electric field vector at the location of the chromophore and *h* is the Planck's constant. The first term describes frequency changes that depend linearly on the electric field and is dominant contribution for the field strengths that pertain in biological membranes. The relationship between the spectral shift and potential is, therefore, linear. The most important point here is that the electric field E originates mainly from the dipole potential, as -

a) The transmembrane potential is smaller and decays over a larger area as a result this is much weaker and neglected.

b) The surface potential cannot affect the dye molecules as they are dissolved in the membrane and surface potential spans the surface and few layers of water attached to the surface.

The measurements for R are taken at the wings of the excitation spectrum where the total fluorescence is weak and changes steeply with wavelength. The fluorescence ratio (R), defined as the ratio of fluorescence intensity at an excitation wavelength of 420 nm to that at 510 nm (emission at 670 nm in both cases) was calculated.[2] The choice of the emission wavelength (670 nm) at the red edge of the spectrum has previously been shown to rule out membrane fluidity effects. Dipole potential (Ψ_D) was calculated from R using the linear relationship described in the Supplementary Table 1.

As for the question regarding the relation between lipid order and dipole potential. Membrane dipole potential is linearly related to dipole moment and inversely related to the Area per lipid molecule, according to the Helmholtz equation:

$$\Psi_D = \frac{\mu_\perp}{A\varepsilon_0\varepsilon}$$

where Ψ_D is the dipole potential, $\mu_\perp$ is the perpendicular component of the dipole moment along the bilayer normal, ε_0 is the permittivity in vacuum, ε is the dielectric constant, and A is the area/lipid molecule.

Figure S1

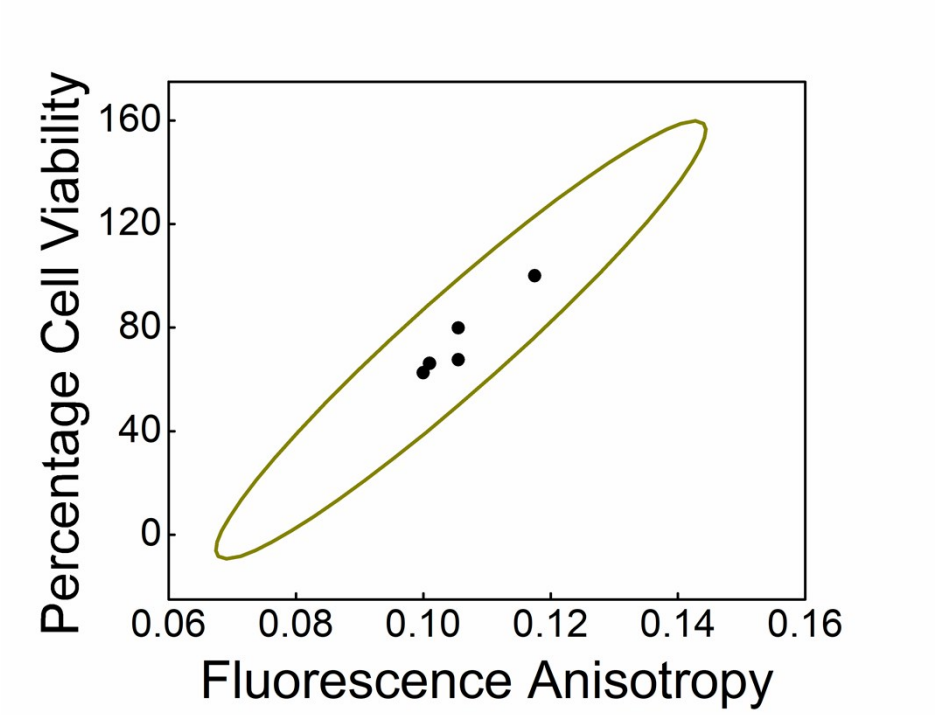

Figure S1. Correlation analysis between neuroblastoma cell viability and fluorescence anisotropy in the membrane (fluidity), (p-value is 0.01 obtained from 2- tailed test of significance).

Figure S2

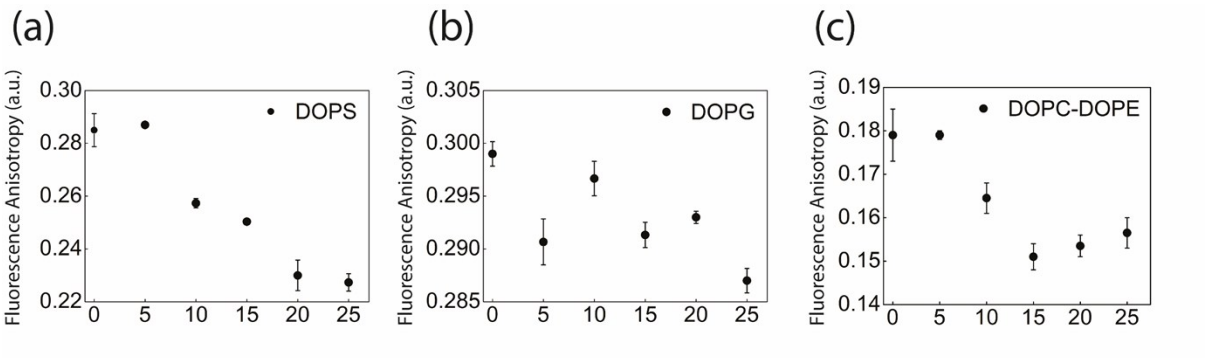

Figure S2. Variations in membrane fluidity of a lipid bilayer on interaction with nisin. Differential effect on fluorescence anisotropy of the dye in pure liposomal membranes due to increasing concentration of nisin, (a) DOPS membranes exhibit a maximum reduction in anisotropy. (b) DOPG exhibit very slight reduction in anisotropy due to nisin. However, in (c) DOPC-DOPE membrane, the uniform reduction is observed in fluorescence anisotropy. The data shown are mean ± S.E. from three independent experiments.

Figure S3.

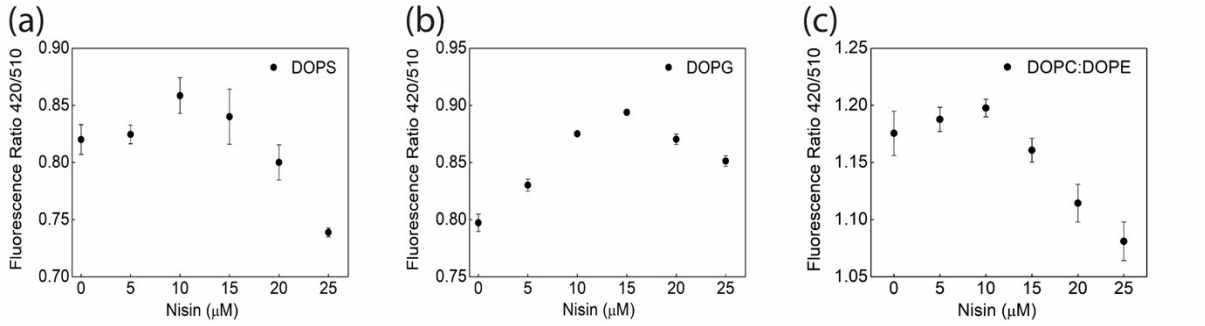

Figure S3. Effect of nisin on dipole potential of individual phospholipid membranes measured through a ratiometric method using potentiometric dye di-8-ANEPPS. (a) DOPS membranes exhibit a maximum reduction in dipole potential due to nisin, whereas (b)DOPG membranes exhibit a prominent increase, (c) DOPC-DOPE membrane dipole potential initially decreases up to 15 µM of nisin, above this concentration dipole potential value increased. Data points shown are the means ± S.E. of at least three independent measurements.

Figure S4.

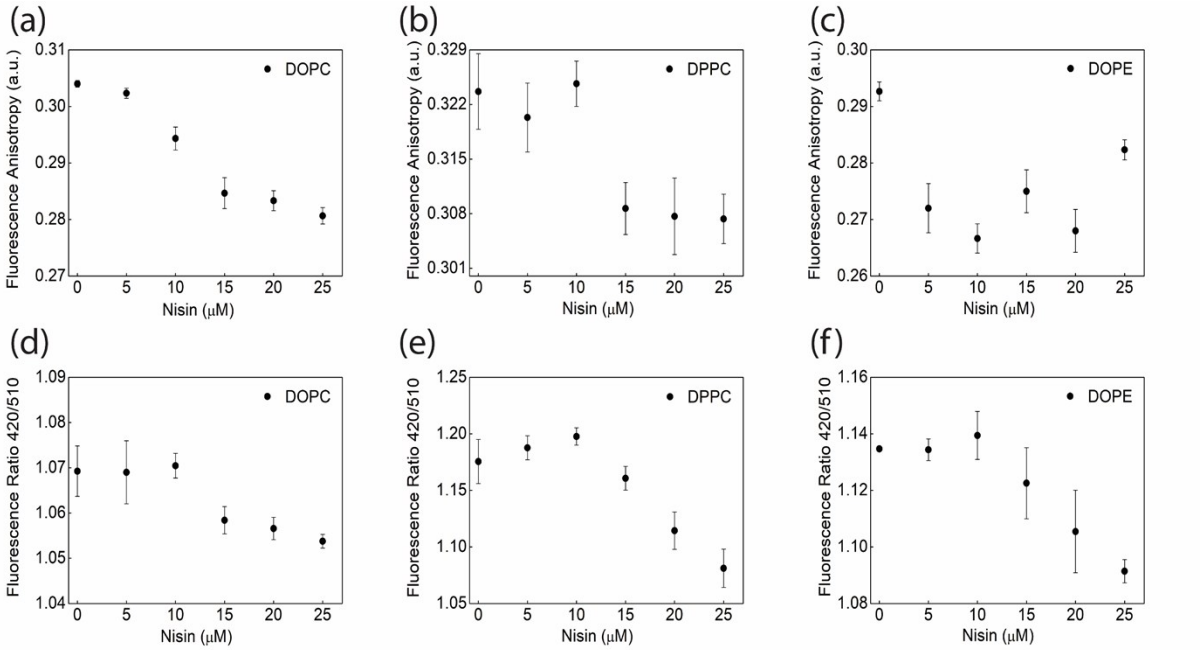

Figure S4. Changes in the fluorescence anisotropy of the dye (fluidity of the membrane) and dipole potential of DOPC, DPPC and DOPE membranes. (a-c) anisotropy of DOPC, DPPC and DOPE and (d-f) dipole potential of DOPC, DPPC and DOPE membrane respectively. Data points shown are the means ± S.E. of at least three independent measurements.

Figure S5

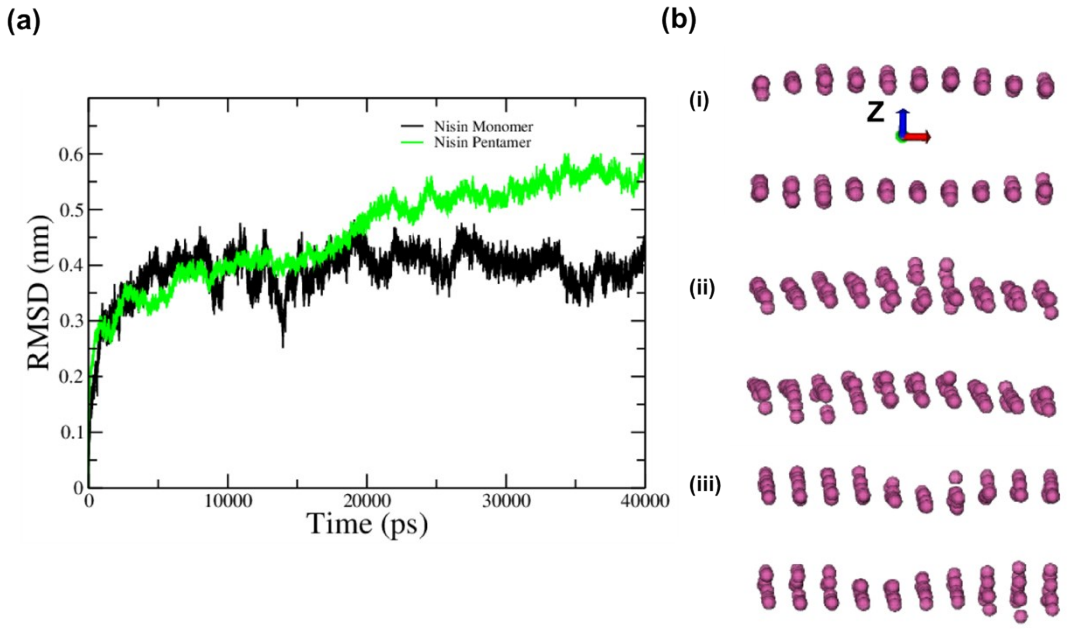

Figure S5. **Nisin induced changes in DOPG-DOPE-DPPC-DOPS-DOPC lipid bilayer:** (a) RMSD of nisin peptide monomer and pentamer backbone along the smulation trajectory of 40 ns. (b) membrane curvature of lipid bilayer was analysed from the simulation trajectories of 30-40ns. Lipid headgroups were shown as mauve coloured beads. (i) Membrane curvature of lipid bilayer in absence of nisin peptide, (ii) membrane curvature of lipid bilayer on interaction with nisin peptide monomer (iii) membrane curvature of lipid bilayer on interaction with nisin peptide pentamer.

Figure S6

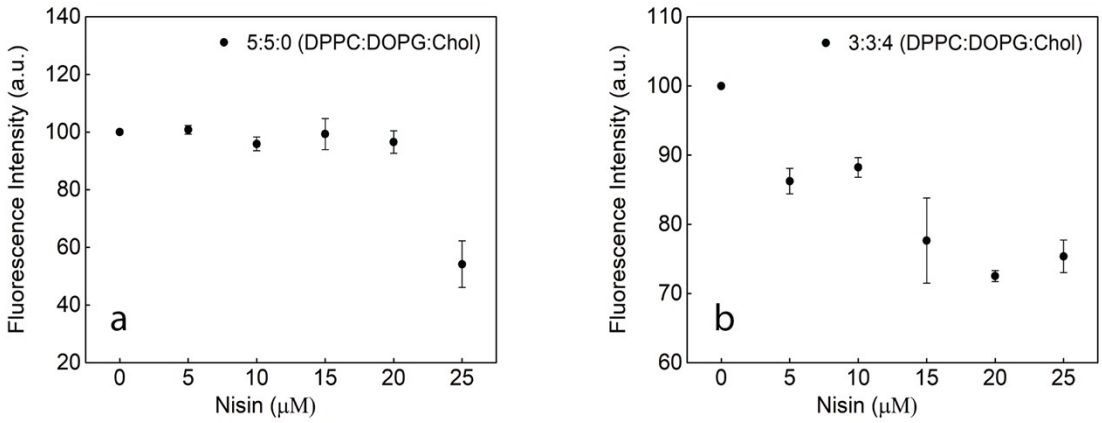

Figure S6. Emission intensities change of Di-8-ANEPPS in DPPC:DOPG:Cholesterol membrane with increasing concentration of nisin where (a) is the 5:5:0 molar ratio membrane and (b) 3:3:4 membrane. Data points shown are the means ± S.E. of at least three independent measurements.

Figure S7

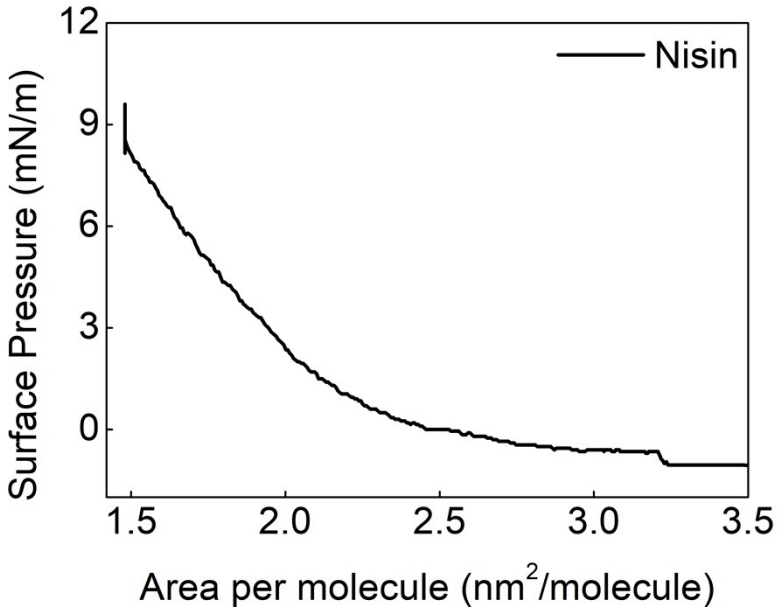

Figure S7. Surface pressure-area per molecule isotherm of nisin peptide.

Figure S8

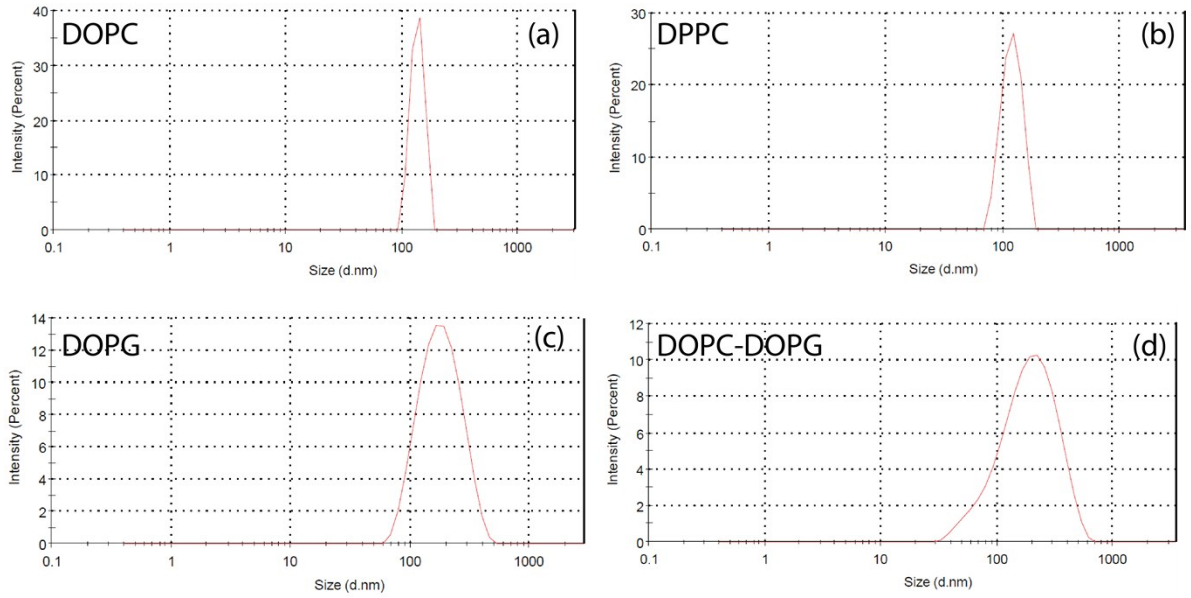

Figure S8. Size distribution of large unilamellar vesicles (LUVs) recorded by the intensity of the dynamic light scattering (DLS) where, (a) is for DOPC, (b) for DPPC, (c) DOPG and (d) DOPC-DOPG large unilamellar vesicle, respectively.

Supplementary Table S1. Membrane dipole potential of individual lipids incubated with increasing concentration of nisin.

	Dipole Potential (mV)				
Nisin (µM)	**DOPS**	**DOPG**	**DOPE**	**DPPC**	**DOPC**
0	266.13 ± 2.59	253.47 ± 0.86	333.65 ± 0.11	354.40 ± 6.76	319.73 ± 0.04
5	303.86 ± 40.4	261.79 ± 1.02	333.93 ± 1.42	345.64 ± 4.23	319.63 ± 1.75
10	242.79 ± 9.38	273.02 ± 0.66	334.51 ± 3.39	347.20 ± 2.44	319.73 ± 0.75
15	223.86 ± 7.25	277.79 ± 0.91	328.07 ± 1.69	339.01 ± 4.05	316.60 ± 0.19
20	224.36 ± 3.85	273.05 ± 0.99	330.23 ± 0.47	327.4 ± 6.11	315.88 ± 0.72
25	238.61 ± 3.9	268.72 ± 0.82	324.52 ± 0.34	317.68 ± 3.08	314.68 ± 0.56

Dipole potential for each test were calculated using the following formula:

$$Dipole\ Potential, \Psi_d = \frac{R + 0.3}{4.3\ X\ 10^{-3}}$$

Where R is the ratio of fluorescence intensities of excitation spectra at 420 nm and 510 nm respectively.

Supplementary Table S2. Values of thermodynamic parameters- Gibbs excess energy (ΔG_{mix}), interaction parameter (α) and enthalpy values (ΔH) of cholesterol, non-raft like liquid disordered (L_d) 5:5:0 (BSM:DOPC:Chol) and raft-like liquid ordered (L_o) 3:3:4 (BSM:DOPC:Chol) monolayers in presence of nisin at different surface pressures.

Surface Pressure π, (mNm^{-1})	**π = 5 mNm^{-1}**			**π = 9 mNm^{-1}**		
	ΔG_{mix} ($Jmol^{-1}$)	α	ΔH ($Jmol^{-1}$)	ΔG_{mix} ($Jmol^{-1}$)	α	ΔH ($Jmol^{-1}$)
(1) Cholesterol	162.51	0.35	140.77	321.48	0.68	278.48
(2) 5:5:0	424.60	0.72	884.58	780.30	1.33	1625.63
(3) 3:3:4	1665.51	2.87	3507.13	2791.50	4.81	5876.97

References:

1. E. Gross, R. S. Bedlack Jr and L. M. Loew, *Biophysical journal*, 1994, 67, 208-216.
2. T. Starke-Peterkovic, N. Turner, M. F. Vitha, M. P. Waller, D. E. Hibbs and R. J. Clarke, *Biophysical journal*, 2006, 90, 4060-4070.