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Interaction of cisplatin and two potential antitumoral platinum(II) complexes with a model lipid membrane: a combined NMR and MD study

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Antitumoral properties of Pt(II) complexes

Antitumoral properties of complexes **1**, **2**, and **3** were evaluated in previous works[1,2] by measuring their GI_{50} values, i.e. the complex concentrations that induce 50% of maximal inhibition of cell proliferation with respect to the control culture. The GI_{50} values obtained for HeLa (cervix adenocarcinoma), A549 (non-small cell lung cancer) and H460 (large cell lung cancer) cell lines are reported in Table S1.

Table S1. Cell growth inhibition values (GI_{50}, μM) for the Pt complexes in different cell lines.

Complex	HeLa	H460	A549
1[1]	1.5 ± 0.6	0.76 ± 0.11	1.6 ± 0.7
2[1]	0.42 ± 0.06	1.1 ± 0.3	2.3 ± 0.7
3[2]	>20	>20	>20

(1) L. Dalla Via, A. N. García-Argáez, A. Adami, S. Grancara, P. Martinis, A. Toninello, D. Belli Dell'Amico, L. Labella and S. Samaritani, *Bioorg. Med. Chem*. 2013, **21**, 6965.
(2) V. Censi, Master's Thesis in Chemistry, University of Pisa, 2013, http://etd.adm.unipi.it/.

^{31}P NMR spectrum of DMPC/3 at 40 °C

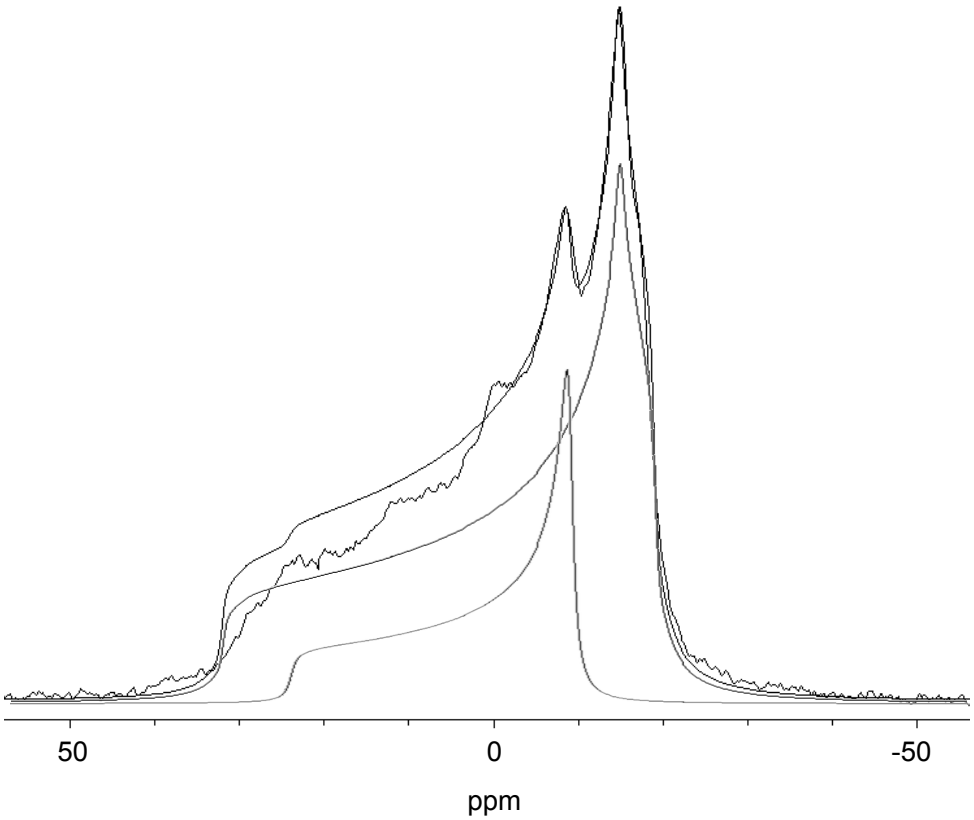

Figure S1. Experimental and fitted ^{31}P NMR spectra of **DMPC/3** at 40 °C; also the individual components are shown. The fitting was performed using the WSOLIDS1 software (version 1.20.21, Copyright (C) 1994, 2012 Klaus Eichele). The component due to the phosphine phosphorus of complex **3** was not considered in the fitting. The discrepancy in intensity at low fields between experimental and fitted spectra is possibly ascribable to an orientation dependence of the linewidth not considered in the procedure.

Analysis of ^{2}H NMR spectra

1. De-Pake-ing procedure

A de-Pake-ing procedure was applied to ^{2}H NMR spectra of **DMPC**, **DMPC/1**, and **DMPC/2** using the NMR Depaker 1.0rc1 software (Copyright (C) 2009 Sébastien Buchoux);[3] an example is shown in Figure S2.

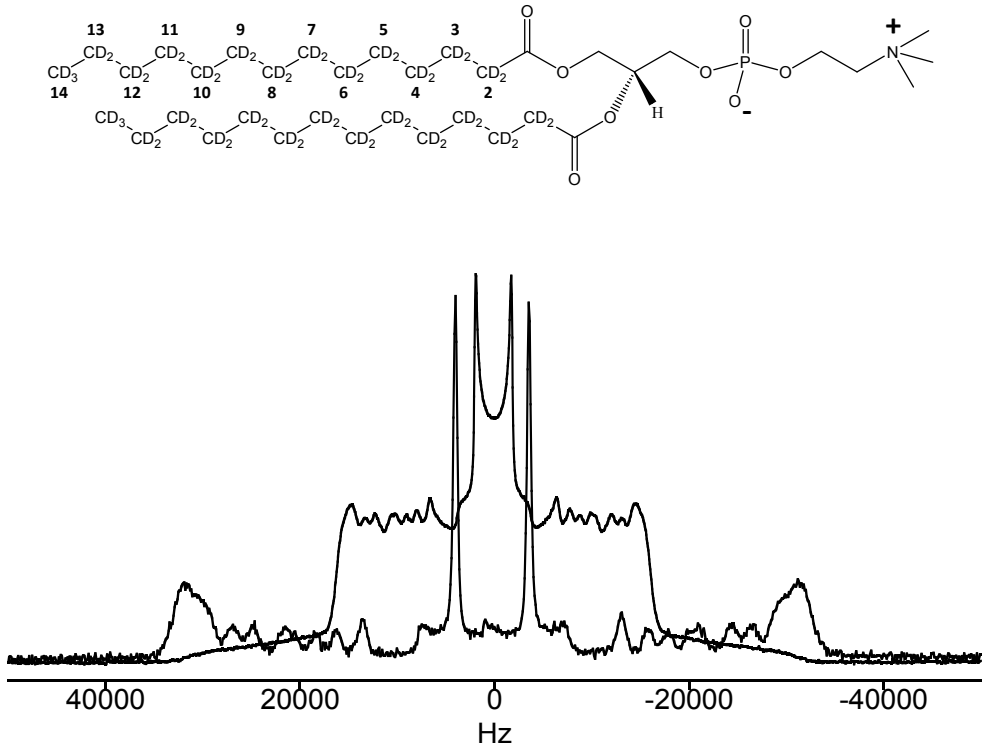

Figure S2. (a) Experimental and de-Paked ^{2}H NMR spectrum of **DMPC/2** at 35 °C; (b) DMPC-d_{54} structure with carbon position numbering.

(3) E. Sternin, M. Bloom and A. MacKay, *J. Magn. Reson.* 1983, **55**, 274.

2. Quadrupolar splittings

Thanks to the higher resolution of the de-Paked spectra, it was possible to determine the quadrupolar splitting ($\Delta\nu_{q,i}$) of the different doublets. As an example, the quadrupolar splittings obtained from the de-Paked spectra at 35 °C for **DMPC**, **DMPC/1**, and **DMPC/2** are shown in Table S2. The assignment was made by comparison to ^{2}H NMR results for the corresponding specifically deuterated phospholipids.[4]

Table S2. ^{2}H quadrupolar splittings obtained from the de-Paked spectra at 35 °C for **DMPC**, **DMPC/1**, and **DMPC/2**. Acyl chain carbons are numbered as in Figure S2.

	$\Delta\nu_{q,i}$ (kHz)		
Acyl chain carbon	**DMPC**	**DMPC/1**	**DMPC/2**
2	54.4	72.2	63.0
3	54.4	72.2	63.0
4	54.4	72.2	59.7
5	50.5	69.0	59.6
6	45.8	66.4	53.4
7	42.1	65.0	49.0
8	36.6	61.3	42.6
9	34.9	55.7	40.8
10	31.2	50.1	36.2
11	27.6	43.8	32.1
12	23.2	35.4	26.7
13	18.2	27.8	21.2
14	6.8	10.6	7.6

3. Determination of bilayer thickness and area per lipid

The bilayer thickness d was estimated from the equation:[5]

$$d = 2d_0\left(0.5 + \langle|S_{CD}|\rangle\right) \quad \text{(S1)}$$

(4)H. I. Petrache, S. W. Dodd and M. F. Brown, *Biophys. J.* 2000, **79**, 3172.
(5) J. Baber, J. F. Ellena and D. S. Cafiso, *Biochemistry* 1995, **34**, 6533.

where d_0 is the maximum bilayer thickness, corresponding to the acyl chains in the all trans conformation, and $\langle|SCD|\rangle$ is the average order parameter value determined as:

$$\langle|S_{CD}|\rangle = \frac{1}{N-1}\sum_{i=2}^{N}|S_{CD_i}| \quad \text{(S2)}$$

For DMPC, $d_0 = n \times 1.27$ Å, with n the number of chain carbons, is equal to 16.51 Å.

The area per lipid A was determined according to:[6]

$$A = \frac{nV_{CH_2}}{d_n} \quad \text{(S3)}$$

where V_{CH_2} = 27.6 Å^3 is the volume of a liquid crystalline methylene segment[7] and d_n is determined from Eq. S1 considering the first n methylene groups. The values of d and A determined for **DMPC**, **DMPC/1** and **DMPC/2** samples at 35 °C taking n = 6 are reported in Table S3.

Table S3. Bilayer thickness (d) and area per lipid molecule (A) for **DMPC**, **DMPC/1** and **DMPC/2** at 35 °C.

Sample	d (Å)	A (Å^2)
DMPC	21.7	61.0
DMPC/1	23.6	55.9
DMPC/2	22.1	59.4

(6) J. F. Nagle, *Biophys. J.* 1993, **64**, 1476.
(7) J. F. Nagle and M. C. Wiener, *Biochim. Biophys. Acta* 1998, **942**, 1.

Convergence of the free energy profiles

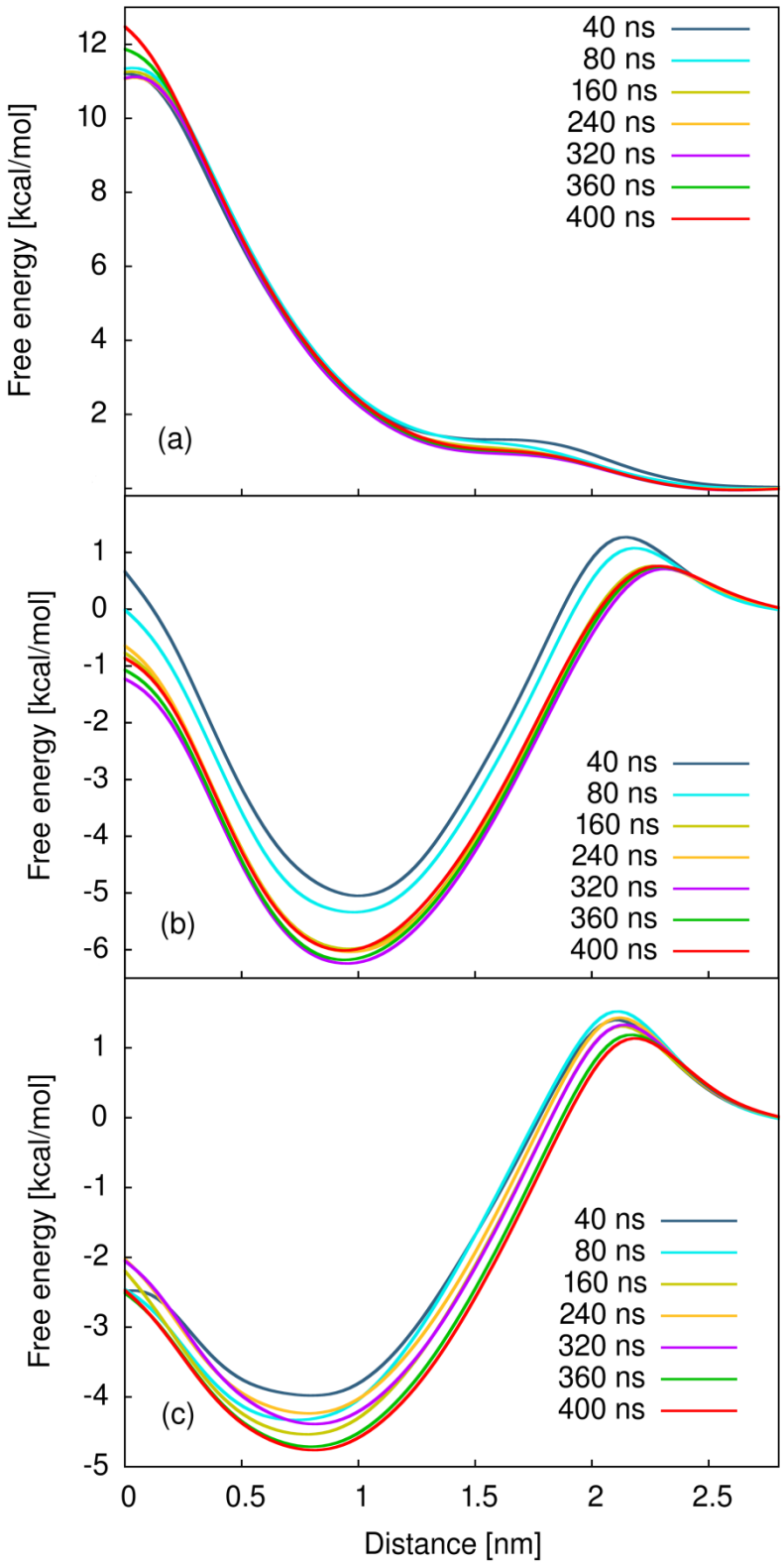

Figure S3. Convergence of the free energy profiles for the transfer of complex **1** (a), complex **2** (b) and complex **3** (c) from the aqueous phase to the DMPC bilayer.

Calculation of partition and permeability coefficients

The partition coefficients for the bilayer/water system were obtained by the following formula:

$$P = \frac{2}{z'} \int_0^{z'} e^{-G(z)/k_B T} dz \tag{S4}$$

with *z'* half bilayer thickness, and *G(z)* the Gibbs free energy.

The permeability coefficient *p* for the Pt complexes through the DMPC bilayer was determined using the following formula:[8]

$$\frac{1}{p} = 2 \int_0^{z'} \frac{\exp\left(\frac{G(z)}{k_B T}\right)}{D(z)} dz \tag{S5}$$

where *D(z)* is the diffusion coefficient. For each of the complexes the value of *G(z)* = 0 was set for the global minimum of the corresponding free energy profile. The value of *D(z)* was calculated from the local mean square displacement analysis of the Pt complexes.

(8) S. J. Marrink and H. J. C. Berendsen, *J. Phys. Chem.* 1994, **98**, 4155.

Calculation of solvent accessible surface area

The solvent accessible surface area (SASA) is the effective area of all complex molecules within the system that is accessible to a solvent-sized probe, which is expected to decrease upon aggregation. In order to account for differences in shape and size, the SASA values for the three complexes were normalized by dividing by the SASA of 5 isolated molecules. The results are shown in Figure S3.

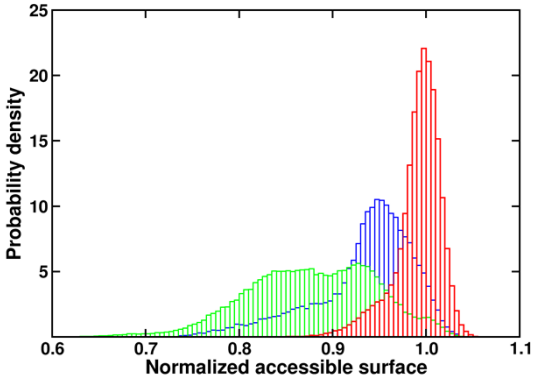

Figure S4. Histograms of normalized SASA for complex **1** (red), complex **2** (blue), and complex **3** (green).

Calculation of phospholipid domain size

In the case of the coexistence of L_o and L_α domains, distinct ^2H NMR subspectra are observed if $k << \Delta(\Delta\nu_{qo}-\Delta\nu_{q\alpha})$, with k the exchange rate of phospholipid molecules between the two environments, and $\Delta\nu_{qo}$ and $\Delta\nu_{q\alpha}$ the quadrupolar splittings in the L_o and L_α phase, respectively. the lifetime τ of a lipid molecule in each domain is k^{-1} and the minimum domain diameter d_o (= d_α) can be obtained from the following equation:

$$d_o = 2\sqrt{4D\tau} \qquad \text{(S6)}$$

with D the lateral diffusion rate of the lipid molecules, here taken equal to 1×10^{-11} m^2s^{-1}, as reported in the literature for a phospholipid in multilamellar liposomes.[9] Considering the high temperature ^2H NMR spectrum, $\Delta\nu_{qo}$ was here taken as the average quadrupolar splitting from the two distinguishable methyl groups in the L_o subspectrum and $\Delta\nu_{q\alpha}$ from the quadrupolar splitting of the intense methyl doublet in the L_α subspectrum.

(9) H. C. Gaede and K. Gawrisch, *Biophys. J.* 2003, **85**, 1734.