# Transmembrane transport by platinum-based metal-organic anionophores

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## Abstract

A series of Pt(II) metal complexes with urea-appended isoquinoline ligands act as efficient transmembrane chloride transporters and operate via classical hydrogen bonding interactions rather than ligand exchange. A number of the complexes exhibited potent transmembrane chloride transport activity.

## Introduction

Transmembrane movement of chloride is a particularly critical biological process and has been linked to several diseases.[1, 2] One prominent example is cystic fibrosis, caused by a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) channel, which prevents the natural movement of chloride across epithelial cell membranes.[3] More recently, chloride transport (as a component of HCl transport) has been shown to dissipate lysosomal pH gradients within cancer cells. Mechanistic studies have shown that this process disrupts autophagy and also induces apoptosis via a NaCl transport process within cancer cells, which presents another attractive potential application of synthetic anionophores.[4-7]

Much anion transport research has focused on using hydrogen bond donor motifs to bind to anions and facilitate their movement across cell membranes. In particular, urea and amide groups have been utilised extensively due to their polarised nature, resulting in stronger binding interactions,[8-11] as well as their relatively easy synthetic accessibility. These donor motifs can be exploited in combination by functionalising molecular scaffolds with multiple

hydrogen bond donor groups. Notable examples include *o*-phenylenediamines,[12] cholic acid derivatives,[13] and tetra-urea macrocycles.[14]

Metal-organic complexes contain a transition metal centre coordinated to one or more organic ligands. The geometry of the complex is pre-determined by the atomic orbitals of the metal centre, and the organic ligands can be used to tune properties such as overall lipophilicity, size, or functionality.[15] The attractiveness of the customisability of metal-organic complexes has resulted in these complexes being used as therapeutics and diagnostics.[16-18] In particular, Pt(II) has been used in some of the most effective anti-cancer therapeutics to date.[19-21] One notable example is cisplatin and, by extension, the platin family of platinum-based drugs. Cisplatin (*cis*-platinum(II)diamminodichloride) is a platinum-based drug that has found extensive use in the treatment of a wide variety of cancers.[22] The dichloro-diamine complex is activated in the cell cytoplasm by exchanging the labile chloride ligands with water molecules. The hydrated complex can enter the nucleus and bind to the purine bases of DNA, forming interstrand cross-links, which result in apoptosis.[23] However, cisplatin also has significant side effects, particularly nephrotoxicity, and is frequently paired with other drugs to minimise auxiliary damage.[24] Carboplatin and oxaliplatin are similar Pt(II) anticancer drugs with lower nephrotoxicity; however, they still damage other parts of the body, including bone marrow (carboplatin) and the nervous system (oxaliplatin).[25]

While the use of metal-organic complexes as therapeutics has been extensively investigated, their incorporation into the design of anionophores has not been explored to a significant extent. Current research into metal-organic anionophores is divided into two categories; complexes that can self-assemble into either ion channels or complexes that act as discrete transmembrane anion carriers. Complexes capable of performing as discrete carriers operate via two distinct mechanisms. A ligand exchange mechanism, similar to cisplatin, was reported for Pd(II) complexes in the HPTS assay.[26, 27] Alternatively, the ligands can remain on the metal centre and transport via traditional hydrogen bonding interactions between the ligand and anion. This mechanism was reported for an Ir(III) complex and a number of phosphazane-based complexes with various metal centres, such as Rh(I), Mo(0), and Au(I).[28, 29] The fixed-ligand complexes showed remarkable transport activity in both the $Cl^-/NO_3^-$ exchange and lucigenin assays.

The incorporation of Pt(II) molecules as metal centres in metal-organic complexes has been a fruitful endeavour in the search for anti-cancer therapeutics;[30-32] however, the transmembrane ionophoric activity of such complexes has been only briefly studied.[33] While Pt(II) complexes have been utilised in well-known cancer drugs, the use of Pt(II) transmembrane anionophores as anti-cancer therapeutics is unprecedented.

Earlier work in the Gale and Loeb groups explored using a Pt(II) centre as the scaffold for an anion receptor.[34] The complex utilised urea-functionalised isoquinolines and was demonstrated to bind to sulfate and various other halide anions. Using $^1$H-NMR binding studies, the complex displayed significant binding affinity towards sulfate in DMSO-$d_6$ ($K_a$ > $10^5$ $M^{-1}$) but also displayed a strong affinity for chloride ($K_{12}$ = 2223 $M^{-1}$). More recently, our group investigated a series of Pt(II) complexes capable of uphill $OH^-$ transport into vesicles in the HPTS assay.[35] The complex involved solvolysis of two labile triflate ligands, with the subsequent formation of a membrane-permeable neutral species after deprotonation of the new aqua-ligated complex. The ability for uphill $OH^-$ transport demonstrated a precedence for pH disruption by Pt(II) complexes. The results of these two studies inspired the development of a Pt(II) complex capable of inducing apoptosis.

This work presents a series of eight metal-organic anionophores that incorporate four urea-functionalised isoquinoline ligands appended to a Pt(II) centre **(Figure 1)**. The ligands of the platinum complexes have varying *n*-alkyl chain lengths (from methyl to decyl), which were incorporated to examine to what extent the lipophilicity of the final complex would dictate chloride transport efficiency. The complexes were characterised extensively using spectroscopic and crystallographic methods before being subjected to a rigorous series of chloride transport assays. A number of compounds displayed strong transport activity in both the $Cl^-/NO_3^-$ exchange assay and the HPTS assay.

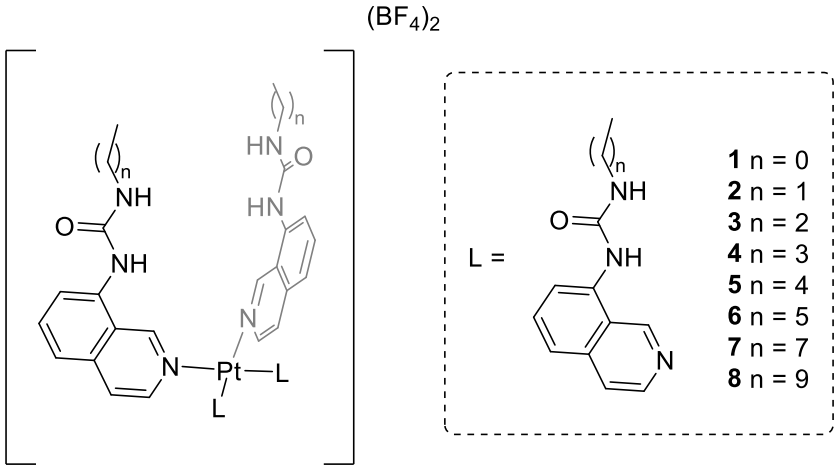
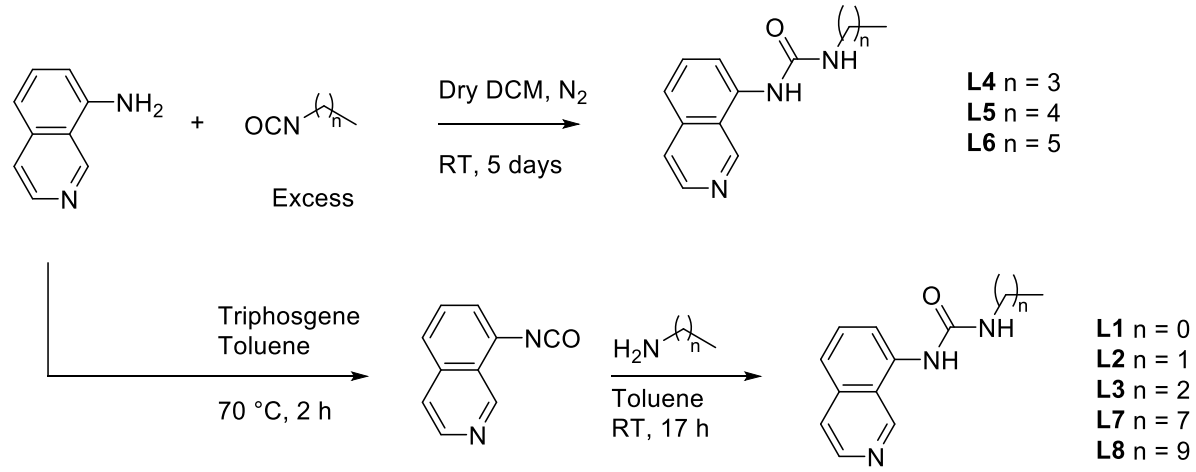



**Figure 1**. Structures of the Pt(II) complexes (**1**–**8**) studied in this work.

## Results and Discussion

Ligands **L1**–**L8** were synthesised following two distinct synthetic methods. For compounds **L4**, **L5**, and **L6**, 8-aminoisoquinoline was reacted with the isocyanate of the respective alkyl chain. (**Scheme 1**). For the remaining compounds, 8-aminoisoquinoline was converted to an isocyanate *in situ* using triphosgene before condensation with the relevant alkyl amine.

Complexes **1**–**8** were synthesised by treating a dichlorobis(propanenitrile)platinum(II) intermediate with the corresponding ligand **L1**–**L8**. The ligands were refluxed overnight in acetonitrile in the presence of silver tetrafluoroborate. The final complexes were purified by filtration of the cooled reaction mixture, followed by washing the precipitate with hot acetonitrile. The final complexes were recovered from the hot filtrate via evaporation.




**Scheme 1** Synthesis of ligands **L1**–**L8** from either an *in-situ* isocyanate or from the respective alkyl isocyanate.

## Binding Studies

Proton NMR titrations of the complexes with tetrabutylammonium chloride (TBACl) were completed in a competitive solvent mixture of DMSO-$d_6$/0.5 % $H_2O$. A downfield shift was observed for the resonances of the aromatic C–H in the 1 position and the two urea N–H peaks. The shift in the resonance of these protons agrees with previous titrations of complex **4**.[34] A shift in the resonance of the 1 position aromatic C-H suggested that the C-H protons pointing towards the urea were also involved in the binding interaction with chloride. This interaction was previously confirmed through X-ray structures of complex **4**.[34]

The changes in the chemical shifts (ppm) of the resonances attributed to the three interacting protons were plotted against the respective equiv. of chloride. The resulting data set was then fit to a 1:1 or 1:2 binding model using the BindFit v0.5 software.[36] As a 1:2 model naturally results in a better fit to the data, an analysis of the covariance of fit for both models was performed and the ratio between the 1:1 and 1:2 values is compared. A ratio greater than 5 is typically required to confirm that a 1:2 binding model is more favoured for a given compound.[37] The covariance of fit ratio was ambiguous for complexes **1**, **2**, **4**, **6**, and **10** as they were less than 5. However, there is literature precedence for a 1:2 stoichiometry with chloride for complex **4** and in similar Pt(II) complexes.[34, 38] The results of the $^1$H NMR titrations are displayed in **Table 1**.

**Table 1**. The $K_{11}$ and $K_{12}$ association constants ($M^{-1}$) at 298 K of complexes **1**–**8** with chloride in DMSO-$d_6$/0.5% $H_2O$. Chloride was added as TBACl. BindFit errors are <8 %. The data was fitted to a 1:2 model as determined by the covariance of fit analysis (**Table S1**) and through previous similar Pt(II) complexes in our group.

| $Cl^-$ Association constants | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Complex** | $K_{11}$ | $K_{12}$ | **α** | **Complex** | $K_{11}$ | $K_{12}$ | **α*** |
| **1** | 17,900 | 1,400 | 0.31 | **5** | 90,000 | 3,700 | 0.16 |
| **2** | 16,500 | 1,500 | 0.36 | **6** | 30,500 | 2,200 | 0.29 |
| **3** | 24,500 | 2,300 | 0.38 | **7** | 25,100 | 2,800 | 0.45 |
| **4** | 24,000 | 2,000 | 0.33 | **8** | 42,000 | 2,000 | 0.19 |

*The interaction parameter (α) was calculated by multiplying $K_{12}$ by four and dividing by $K_{11}$. An α value < 1 indicates negative cooperativity, a value > 1 indicates positive cooperativity, while a value of 1 describes non-cooperative binding.

The binding constants indicate that the complexes exhibit a strong affinity for chloride, with $K_{11}$ values exceeding $10^4$ $M^{-1}$ in all complexes and $K_{12}$ values exceeding $10^3$ $M^{-1}$. The lower $K_{12}$ value suggests a negative cooperativity in the second binding event. In hosts capable of binding two guest molecules, the first binding event often changes the binding properties of the compound.[37] The change in affinity for a second guest directly impacts the $K_{12}$ binding constant. Positive cooperativity causes the second binding event to be more favoured than the first. In contrast, negative cooperativity indicates a less favourable second binding event, while in non-cooperative binding, there is no change in affinity after the first binding.

This can be quantified through the interaction parameter α, where a value < 1 indicates negative cooperativity, a value > 1 indicates positive cooperativity, and a value of exactly 1 indicates non-cooperative binding. The value of α was < 1 for all the complexes, indicating negative chloride binding cooperativity (**Table 1**). The negative cooperativity for these host complexes results from the reduced electrostatic attraction and increased electrostatic repulsion of subsequent guests following the first binding event.

The $Cl^-$ binding affinities noticeably increased after complexes **1** and **2**, with an approximate plateau around 2000 – 3000 $M^{-1}$ for $K_{12}$. This was presumably due to the longer alkyl chains forming a more encapsulating coordination pocket for the anion. A similar effect was observed by Engberts and co-workers, where a general increase in binding affinity to trypsin was observed with successively longer *n*-alkyl chains.[39] Binding affinities began to decrease after $n$ = 5, suggesting the benefit of encapsulation at this length is only marginal. At longer chain lengths, there is potential that the alkyl chains will begin to sterically crowd the binding pocket, resulting in diminished chloride binding affinity.

## Chloride/Nitrate Exchange

Following confirmation of the binding affinity of the complexes towards chloride, their chloride transport properties were tested initially in the ion-selective electrode (ISE) $Cl^-/NO_3^-$ exchange assay using POPC vesicles (**Figure 2a**). This assay was performed using a chloride ISE and experimental methods well established in the literature.[40] Vesicles were prepared using POPC lipids and loaded with a NaCl (487 mM) internal solution, buffered to pH 7.2 in sodium phosphate salts (5 mM). The vesicles were then suspended in a $NaNO_3$ (487 mM) solution, similarly buffered to pH 7.2 in sodium phosphate salts. Chloride efflux was induced with the addition of the anionophore as a DMSO solution, and the efflux of chloride into the

extracellular solution was recorded using the ISE over 300 s. A dose-response curve was collected by testing the chloride efflux facilitated by the anionophore at different loading concentrations. This curve was fit to the Hill equation to calculate an $EC_{50}$ value, the concentration of transporter (in mol% relative to the lipid concentration) needed for 50% efflux.[40]

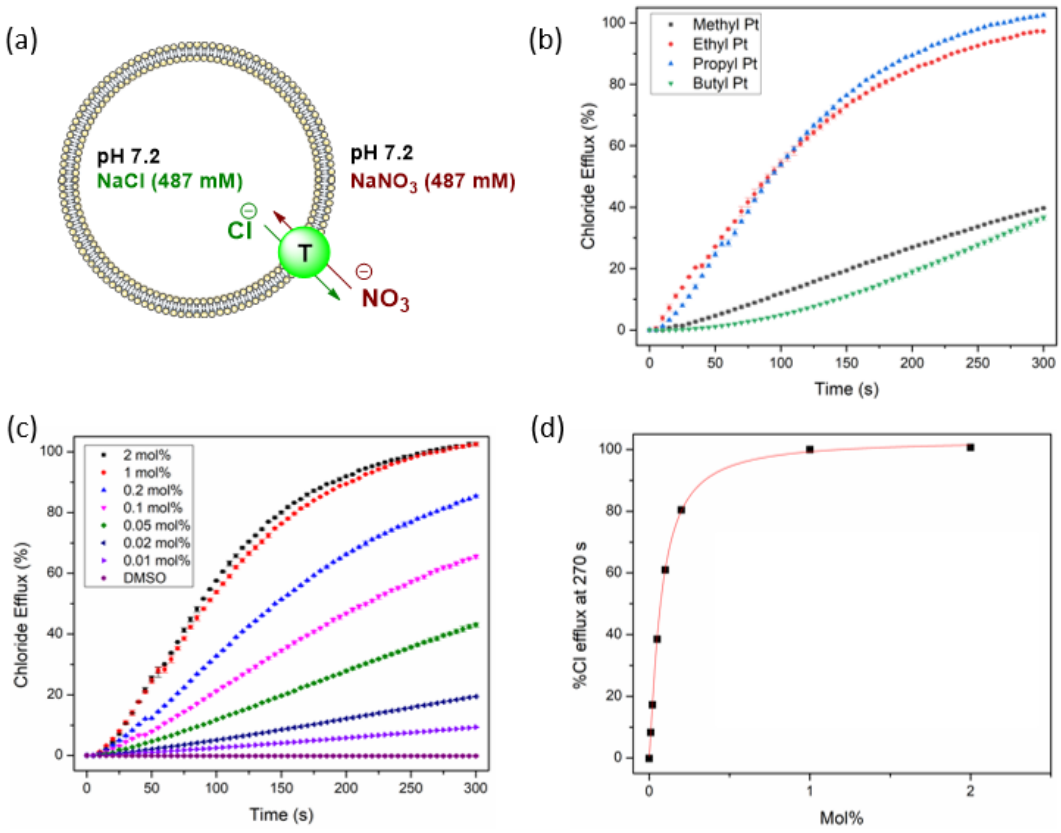



**Figure 2.** Overview of ISE $Cl^-/NO_3^-$ exchange assay in 200 nm POPC vesicles loaded with NaCl (487 mM) and suspended in $NaNO_3$ (487 mM) at pH 7.20: (a) schematic diagram of the assay showing $Cl^-/NO_3^-$ exchange mediated by the complex; (b) transport activity of complexes **1**–**4** at 1 mol%; (c) dose-response curve of complex **3**; (d) transport activity at 270 s of complex **3**, used to calculate an $EC_{50}$ value using the Hill equation.

Complexes **1**–**3** displayed enough transport activity to calculate an $EC_{50}$ value, but the longer chain complexes (**4**–**8**) precipitated upon addition to the aqueous experimental solution at concentrations required for suitable analysis (**Figure 2b**). An increase in transport activity was observed for complexes **1**–**3** as an extra carbon was added to the alkyl chain of the urea units (**Table 2**). One rationale for these findings is the higher binding constants observed in **Table 1**

which indicate a better ability to bind chloride within the vesicles and facilitate anion transport. Furthermore, the extra carbons increase the overall lipophilicity of the complexes, which enhances their transport activity. Lipophilicity has been previously shown to be a significant determinant of the activity of anion transporters, as it directly impacts both the aqueous solubility and the ability to cross the phospholipid bilayer.[27, 41-44] Due to the presence of four identical isoquinoline ligands, each additional carbon greatly enhances lipophilicity. Highly lipophilic compounds typically display diminished aqueous solubility, which is likely responsible for the observed precipitation of complexes **4**–**8** during the ISE experiments.

The complexes most likely transport $Cl^-$ via hydrogen bonds instead of a ligand exchange mechanism. Due to the strength of the isoquinoline group as a metal ligand, it is unlikely that they will be displaced even in the presence of another competing ligand, such as DMSO. Complex **4** showed no signs of decomposition when left in a DMSO solution for up to 21 days (**Figure S1**). In addition, previous work has indicated the viability of DMSO as a ligand for Pt(II) complexes in cytotoxic applications.[45] However, they are easily displaced by other ligands containing a pyridine group.[46]

**Table 2.** Calculated $EC_{50}$ values and Hill coefficients for complexes **1–3** in the $Cl^-/NO_3^-$ exchange assay. The calculated partition coefficient (CLogP) values were calculated using the VCC Labs Pt(II) drug library tool.

| ISE $Cl^-/NO_3^-$ Transport Activities | | | |
|---|---|---|---|
| **Complex** | **$EC_{50}$ (mol %)** | **Hill coefficient (n)** | **cLogP** |
| 1 | 1.66 ± 0.17 | 1.66 ± 0.13 | 2.70 ± 0.54 |
| 2 | 0.150 ± 0.014 | 1.32 ± 0.12 | 3.14 ± 0.55 |
| 3 | 0.0712 ± 0.0019 | 1.26 ± 0.03 | 3.47 ± 0.57 |
| 4 | –* | – | 3.85 ± 0.60 |

*An $EC_{50}$ could not be calculated for complex **4** due to solubility issues.

The CLogP values of the complexes were obtained using the VCC Labs Pt(II) drug library reference tool and are displayed in **Table 2**.[47] The values support the hypothesis that introducing additional carbons increases lipophilicity, as each additional carbon increases the lipophilicity by approx. 3 times. Interestingly, the Hill coefficients (a measure of the transport stoichiometry with chloride) indicate that complex **1** can transport two chloride anions simultaneously, whereas **2** and **3** only transport one. This may be due to the shorter methyl groups allowing better access to the binding site compared to longer chains. These $EC_{50}$ values represent modest transport compared to some of the best organic chloride

transporters in the literature.[12, 48-50] However, the complexes show comparable $EC_{50}$ values to those reported by Mao and Wright,[28, 29] which are among the lowest reported to date for inorganic complexes.

## Cationophore Coupled Assay

We further investigated the mechanism of chloride transport using a modified version of the $Cl^-/NO_3^-$ exchange assay. This assay uses potassium salts instead of sodium salts, and the external solution contains gluconate. Gluconate is a large, hydrophilic anion that cannot be transported or freely diffuse across the phospholipid bilayer. Under these conditions, chloride transport occurs in the presence of a cationophore, either valinomycin or monensin. Valinomycin strictly uniports $K^+$; thus, coupling to the cationophore requires the ability to uniport chloride, known as electrogenic transport. In contrast, monensin facilitates $H^+/K^+$ exchange, meaning that coupling an anionophore to this species requires the co-transport of $H^+/Cl^-$, known as electroneutral transport.

The results indicate that complexes **1**–**3** faciliate electroneutral transport via coupling to monensin (**Figure S2**). This was not unexpected due to the difficulty in achieving electrogenic transport. The mechanism of electrogenic transport would require the complex to diffuse across the phospholipid bilayer as a neutral species. However, this process can easily be disrupted by phosphate headgroup interactions.[51] Our previous work on complex **4** indicated strong phosphate binding affinities, likely explaining the inability of **4** to facilitate electrogenic transport.

On the other hand, electroneutral transport does not require the complex to diffuse across the bilayer as a neutral species. Instead, the mechanism involves a deprotonation step, and the transporter diffuses as an anionic species. This is possible because the negative charge is sufficiently distributed across the entire complex structure. As the species is anionic, this also minimises interactions with phosphate head groups. The most likely deprotonation site is one of the four urea groups, with the negative charge distributed throughout the isoquinoline group or onto the Pt(II) centre.

## HPTS Assay for HCl Transport

As the complexes exhibited electroneutral HCl co-transport (or the equivalent $OH^-/Cl^-$ exchange), their activity was further investigated using the HPTS assay.[52] POPC vesicles were

loaded with a solution of KCl (100 mM) and HPTS (1 mM) dye, adjusted to pH 7.0 in HEPES buffer (10 mm). HPTS is a water-soluble fluorescent dye that is sensitive to pH changes and changes its excitation wavelength in response to deprotonation at the hydroxyl group.

After adding a base pulse of NaOH (0.5 M), raising the external pH to approx. 8, the change in fluorescence emission of HPTS was recorded and analysed to give insights into the direction and magnitude of HCl transport. Due to the lower vesicle concentrations and ionic strength in this assay, transport activity could be detected for complexes **1**–**5**, unlike in the ISE assay. These results are summarised in **Table 3**.

**Table 3.** Calculated $EC_{50}$ values and Hill coefficients for complexes **1**–**5** in the HPTS assay.

| HPTS Transport Activities | | | |
|---|---|---|---|
| **Complex** | **$EC_{50}$ (mol %)** | **Hill coefficient (n)** | **CLogP** |
| 1 | 0.092 ± 0.004 | 1.21 ± 0.03 | 2.70 ± 0.54 |
| 2 | 0.0085 ± 0.0008 | 2.2 ± 0.3 | 3.14 ± 0.55 |
| 3 | 0.005 ± 0.003 | 2.1 ± 0.2 | 3.47 ± 0.57 |
| 4 | 0.0111 ± 0.0016 | 1.2 ± 0.2 | 3.85 ± 0.60 |
| 5 | –* | – | 4.32 ± 0.64 |

*An $EC_{50}$ could not be calculated for complex **5** as the activity plateaued at around 60 %.

Overall, the $EC_{50}$ values were noticeably lower in the HPTS assay compared to the ISE assay. This could be attributed to improved solubility conditions in the HPTS assay as $EC_{50}$s could be calculated for up to complex **4**, which precipitated out of solution in the ISE assay. The trend of increasing transport activity between complexes **1**–**3** in the ISE assay was also observed in the HPTS assay, with complex **3** having an $EC_{50}$ of 0.005 mol%. The Hill coefficients in the HPTS assay for complexes **1**–**3** are inverted compared to the ISE assay coefficients. Complex **1** prefers a 1:1 binding stoichiometry with chloride, whereas **2** and **3** prefer a 1:2 binding mode. This is likely a result of the lower concentrations of transporter required for the conditions of this assay compared to the ISE.

Interestingly, the transport activity decreased for complexes **4** and **5**, resulting in a 'bell curve' of activity relative to alkyl chain length, peaking at three carbons. The bell curve can be rationalised when looking at the lipophilicity of the complexes, quantified by the cLogP values in **Table 3**. We have previously shown that for anion transporters, there is a bell curve relationship between transport $EC_{50}$ and ClogP. Below an optimal ClogP, the transporters are too hydrophilic to pass through the phospholipid bilayer, whereas too high of a ClogP means the transporter is likely to be embedded within the bilayer and cannot act as a carrier.[41, 44] A

similar bell curve was also recently observed by Tecilla and co-workers in a series of Pd(II) diphosphine complexes. By varying the lengths of a bidentate aliphatic linker, the complex displayed varying levels of chloride transport. It was determined that the lipophilicity of the complexes was responsible for the changes in transport activity detected.[27] Based on the precipitation of complexes **4**–**8** in the ISE assay, it is also likely that the longer chain complexes are too lipophilic and end up buried inside the phospholipid bilayer, inhibiting chloride transport.

The $H^+/Cl^-$ selectivity of the complexes can also be investigated in the HPTS assay by adding an ionophore, either valinomycin or carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP), an $H^+$ uniporter. Increased activity with valinomycin indicates $H^+$ selectivity over $Cl^-$, while increased activity for CCCP means the complex is more selective for $Cl^-$ over $H^+$. Complexes **1** and **5** showed coupling to valinomycin and CCCP, indicating they are capable of uniporting either ionic species. While introducing both ionophores resulted in a drop in activity, both complexes had lower activity in the presence of CCCP, suggesting a higher $H^+$ selectivity. For complexes **2**–**4**, there was no coupling to the ionophores and no transport activity was detected.

Urea-based anion transporters are known to facilitate proton transport via a transporter deprotonation mechanism or a fatty acid flip-flop mechanism that involves the transporter carrying the deprotonated form of fatty acids across the membrane.[51] Previous HPTS assays were conducted with POPC lipids from Corden Pharma that contain a high fatty acid content (up to 2 mol%). In order to investigate whether the fatty acid flip-flop mechanism contributes to the proton transport activity, the HPTS assay was performed using lipids containing a low fatty acid content (purchased from Avanti Polar Lipids) before and after the addition of oleic acid (OA) at 2 mol% and 4 mol%. Complex **1** showed the most dramatic increase of the transport rate with OA addition (**Figure 3**), with the activity comparable to the $EC_{50}$ level obtained when Corden lipids were used. This confirms that fatty acid flip-flop was the dominant proton transport pathway for complex **1**.

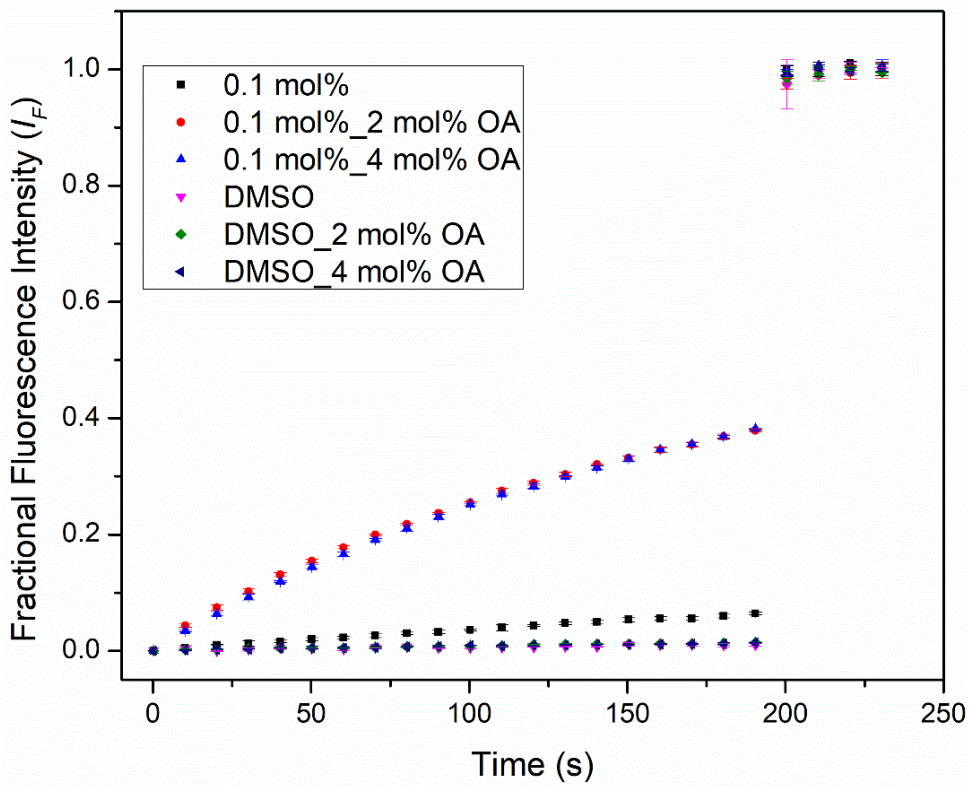



**Figure 3**. Investigation of fatty acid flip-flop by the addition of oleic acid (2–4 mol%) to complex **1** (0.1 mol%) in the HPTS assay with Avanti Polar Lipids.

Complexes **2** and **3** showed similar increases in activity after adding 2 mol% OA, indicating the reliance on fatty acid flip-flop. However, a further addition to 4 mol% OA resulted in a decrease, suggesting competitive binding to the complex between oleate and chloride. In contrast, for complexes **4** and **5** the addition of OA did not further increase activity for either complex. In particular, complex **5** showed a significant drop after the addition of OA (**Figure 4**). These results indicate that the short chain Pt complexes require fatty acids to transport protons, but the longer chain complexes can facilitate proton transport by themselves, presumably via a transporter deprotonation mechanism. It is likely that the deprotonated forms of the short chain Pt complexes cannot effectively cross the membrane due to poor lipophilicity and thus binding of a fatty acid anion to the short chain Pt complexes is required to form a sufficient lipophilic species that carries an $OH^-$ equivalent across the membrane. For the long chain complexes, fatty acids are not involved in proton transport but instead competes with chloride binding leading to attenuated activity.

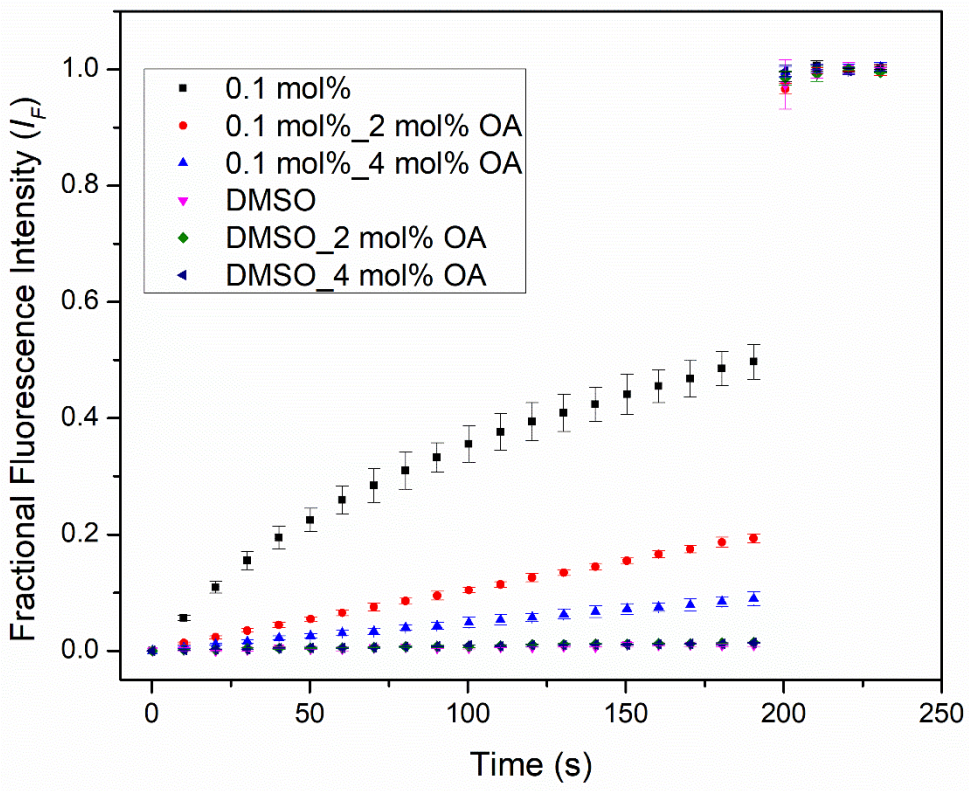



**Figure 4**. Investigation of fatty acid flip-flop by the addition of oleic acid (2–4 mol%) to complex **5** (0.5 mol%) in the HPTS assay with Avanti Polar Lipids.

## HPTS Anion Selectivity Assay

The anion selectivity of the complexes was also investigated in a modified HPTS assay using a recent method reported within our group.[53] POPC vesicles were loaded with the same buffered solution as typical HPTS vesicles, with the exception of using NaCl (100 mM) instead. The vesicles were suspended in an isotonic external buffer with $Cl^-$, $Br^-$, $NO_3^-$, $I^-$, or $ClO_4^-$ anions (100 mM, as the respective NaX salts) instead. None of the complexes **1–5** showed preferential $Cl^-$ selectivity **(Figure S3)**. This was surprising, as we expected that the large binding constants for $Cl^-$ compared to other halides reported in previous work would have resulted in higher $Cl^-$ selectivity.[34] However, the higher solvation energy of $Cl^-$ would reasonably require a high level of receptor pre-organisation to overcome compared to $Br^-$ and $I^-$. Interestingly, the complexes generally showed higher selectivity for halides compared to $ClO_4^-$, which is relatively hydrophobic and would be expected to be easier to transport. We previously observed that complex **4** displayed slow exchange on the NMR timescale when binding to $SO_4^{2-}$ with an exceedingly high binding constant observed. This strong binding could potentially explain why $ClO_4^-$ was more difficult to transport than $I^-$, as it is

geometrically similar to $SO_4^{2-}$ despite being singly charged. To further explore this difference, a modified version of the ISE $Cl^-/NO_3^-$ assay was performed with $Cl^-/SO_4^{2-}$ antiport instead. Complexes **3–6** were tested at 1 mol%, but no transport activity was detected **(Figure S5)**.

## Conclusions

We present a series of complexes based on a Pt(II) centre and have demonstrated, for the first time, their ability to transport the biologically relevant chloride anion across a phospholipid bilayer in synthetic vesicles. Their transport activities are among the best out of all discrete metal-organic anionophores. We have shown that varying the lipophilicity of the complexes plays a significant role in their transport efficiency, with a propyl chain length being the most effective for chloride transport. Biological studies are in progress to test their efficacy in cancer cells. Additional modifications of the ligands are being undertaken to study the effect of aromatic side groups compared to the alkyl ones.

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