Anion transport across phospholipid membranes mediated by a diphosphine-Pd(II) complex.

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Electronic Supplementary Information (ESI)

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Materials and General Methods

General. All commercially available reagents were purchased from *Aldrich*, *Fluka* and *Strem Chemicals* and used without purification unless otherwise mentioned. L-α-phosphatidyl-DL-glycerol sodium salt (EYPG, 20 mg/mL chloroform solution) was purchased from *Avanti Polar Lipids*; egg yolk phosphatidylcholine (EYPC, 100 mg/mL chloroform solution) and 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS) were from *Sigma*; Triton® X-100 and HEPES buffer were from *Fluka*; all salts were of the best grade available from *Aldrich* and were used without further purification.

Ionophoric Activity. Liposomes were prepared by extrusion using a 10 mL *Lipex*^{TM} Thermobarrel EXTRUDER (*Northern Lipids Inc.*) connected to a thermostatic bath (25 °C if not otherwise indicated). The 100 nm polycarbonate membranes were *Nucleopore Track-Etch Membranes* from *Whatman*. Fluorescence spectra were recorded on a *Varian Cary Eclipse* fluorescence spectrophotometer. All fluorimetric measurements were performed at 25 °C. The

ionophores concentration is given in percent with respect to the total concentration of lipids. Mother solutions of ionophores were prepared in DMSO or in methanol. Control experiments showed that the amount of DMSO or methanol added to the vesicular suspension in the different experiments (maximum amount 1.0 % in volume) did not affect the permeability of the membrane.

Elemental Analysis. The analysis were performed at the Analytical Laboratories of the University of Udine (Italy).

Nuclear Magnetic Resonance (**NMR**)**.** Mono-dimensional NMR spectra (^{1}H (500 MHz), ^{31}P (202 MHz) and ^{19}F (470.12)) were recorded on a Varian 500 spectrometer at room temperature. ^{1}H chemical shifts were referenced to the peak of residual non-deuterated solvent (δ = 7.26 for $CDCl_3$, 5.32 for CD_2Cl_2, 1.96 for CD_3CN, and 2.08 for acetone-d_6); ^{31}P chemical shifts were referenced to the H_3PO_4 internal standard at 0.00 ppm; ^{19}F chemical shifts were referenced to the $CFCl_3$ internal standard at 0.00 ppm.

Infrared spectra (**IR**) were recorded on a *Perkin-Elmer FT-IR/Raman 2000* instrument in the transmission mode; samples were prepared in Nujol.

UV-Visible Spectroscopy (**UV-Vis**)**.** Spectra were recorded on a UV-Vis *Perkin Elmer Lamda 35* spectrophotometer.

Synthesis and Characterization

[Pd(dppp)Cl_2] (**2**).

101 mg (0.37 mmol) of [Pd(nbd)Cl_2] (nb = 2,5-norbornadiene) were suspended in 20mL of MeOH, and 106.4 mg (0.412 mmol) of dppp (dppp = 1,3-bis(diphenyl)propane) were added to the suspension under magnetic stirring. An abundant whitish solid starts to precipitate within half an hour. The system is kept under stirring at room temperature for 3 hours, the solid was then filtered, washed with cold methanol and vacuum dried. Yield 88%. M_W = 589.79; $C_{27}H_{26}P_2PdCl_2$, elemental analysis requires: C, 54.9; H, 4.3; N, 0.0, found: C, 54.9; H, 4.4; N, 0.0. ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.79 (d, 8H, Ph1), 7.45 (m, 12H, Ph2+Ph3), 2.39 (m, 4H, Hp), 2.04 (m, 2H, Hc); ^{31}P-NMR ($CDCl_3$) δ (ppm): 11.2 (s).

[Pd(dppp)(OTf)$_2$] (**1**).

118.4 mg (0.201 mmol) of **2** and 103.3 mg (0.402 mmol) of $AgSO_3CF_3$ were dissolved in 30 mL of anhydrous CH_2Cl_2 under argon, and kept under stirring at reflux for one day, protected from light. The AgCl precipitate was then filter over celite and the liquor concentrated under vacuum. The pale yellow product was precipitated with addition of diethyl ether and cooling at 4°C overnight, filtered, washed with diethyl ether and vacuum dried. Yield: 74%. M_W = 817.03; elemental analysis requires: C, 39.7; H, 3.3; N, 0.0, found: C, 40.0; H, 3.2; N, 0.0. IR (Nujol, cm^{-1}): 1281 (m), 1033 (st) (ν OTf); ^{1}H-NMR ($CDCl_3$), δ (ppm): 7.64 (dd, 8H, Ph1), 7.53 (t, 4H, Ph2), 7.45 (m, 8H, Ph3), 2.78 (m, 4H, Hp), 2.30 (m, 2H, Hc); ^{19}F-NMR ($CDCl_3$) (ppm): -78.5 (s, free OTf^-); ^{31}P-NMR ($CDCl_3$) δ (ppm): 15.9 (s); ^{1}H NMR (CD_2Cl_2) δ (ppm): 7.64 (dd, 8H, Ph1), 7.58 (t, 4H, Ph2), 7.46 (m, 8H, Ph3), 2.72 (m, 4H, Hp), 2.31 (m, Hc); ^{31}P-NMR (CD_2Cl_2) (ppm): 17.1 (s); ^{1}H-NMR (acetone-d_6) δ (ppm): 7.88 (dd, 8H, Ph1), 7.67 (t, 4H, Ph2), 7.56 (m, 8H, Ph3), 3.18 (m, 4H, Hp), 2.42 (m, 2H, Hc); ^{19}F-NMR (acetone-d_6) δ (ppm): -79.3 (s, free OTf^-); ^{31}P-NMR

(acetone-d_6) δ (ppm): 18.7 (s); ^{1}H-NMR (CD_3CN) δ (ppm): 7.64 (m, 12H, Ph1+Ph3), 7.54 (m, 8H, Ph2), 2.84 (m, 4H, Hp), 2.28 (m, 2H, Hc); ^{31}P-NMR (CD_3CN) δ (ppm): 11.2 (s).

Ionophoric Activity

HPTS assay.

A mixture of 150 µL of EYPC chloroform solution (100 mg/mL, 20 µmol) and 40 µL of EYPG chloroform solution (20 mg/mL, 1 µmol) was first evaporated with Ar-flux to form a thin film and then dried under high vacuum for 3 h. The lipid cake was hydrated in 1.5 mL of 0.1 mM HPTS solution (HEPES 25 mM, 100 mM NaCl, pH 7) for 30 min at 40 °C. The lipid suspension was submitted to 5 freeze-thaw cycles (–196 °C/40 °C) using liquid nitrogen and a thermostatic bath, and then extruded under nitrogen pressure (15 bar) at room temperature (10 extrusions through a 0.1 µm polycarbonate membrane).

The LUV suspension was separated from extravesicular dye by size exclusion chromatography (SEC) (stationary phase: pre-packed column *Sephadex*™ G-25, mobile phase: HEPES buffer) and diluted with HEPES buffer to give a stock solution with a lipid concentration of 5 mM (assuming 100% of lipids were incorporated into liposomes). 104 µL of the lipid suspension were placed in a fluorimetric cell, diluted to 3040 µL with the same buffer solution used for the liposome preparation and kept under gently stirring. The total lipid concentration in the fluorimetric cell was 0.17 mM. An aliquot of solution of the ionophore in DMSO (5-30 µL of the appropriate mother solution in order to obtain the desired $mol_{compound}/mol_{lipid}$ ratio) was then added to the lipid suspension and the cell was incubated at 25 °C for 10 min.

After incubation the time course of the fluorescence emission was recorded for 100 or 200 s (λ_{ex1} = 460 nm, λ_{ex2} = 403 nm, λ_{em}= 510 nm) and then 50 µL of 0.5 M NaOH were rapidly added through an injector port and the fluorescence emission was recorded for 600 or 1200 s. Maximal changes in dye emission were obtained by final lysis of the liposomes with detergent (40 µL of 5% aqueous Triton® X-100). The data set consists of emission intensities at 510 nm modulated by alternating excitation at 403 nm and 460 nm on a 0.5 + 0.5 s cycle. The concentration of the conjugate base form of HPTS is related to the emission intensity at 510 nm during the period when the dye is excited at 460 nm (E_{460}) while the concentration of the protonated form is related to the emission intensity at 510 nm during the period when the dye is excited at 403 nm (E_{403}). Fluorescence time course were normalized using the following equation, where the subscript 0, ∞ and *t* denote the emission ratio before the base pulse, after detergent lysis, and at an intermediate time, respectively.

$$FI = \frac{\left(\frac{E_{403}}{E_{460}}\right)_t - \left(\frac{E_{403}}{E_{460}}\right)_0}{\left(\frac{E_{403}}{E_{460}}\right)_\infty - \left(\frac{E_{403}}{E_{460}}\right)_0} \times 100$$

In Figure S1 the kinetic profiles obtained at different concentration of **1** are reported. The apparent first order rate constants for the transport process were obtained by non-linear regression analysis of the normalized fluorescence data *vs.* time of Figure S1 and are reported in Table S1.

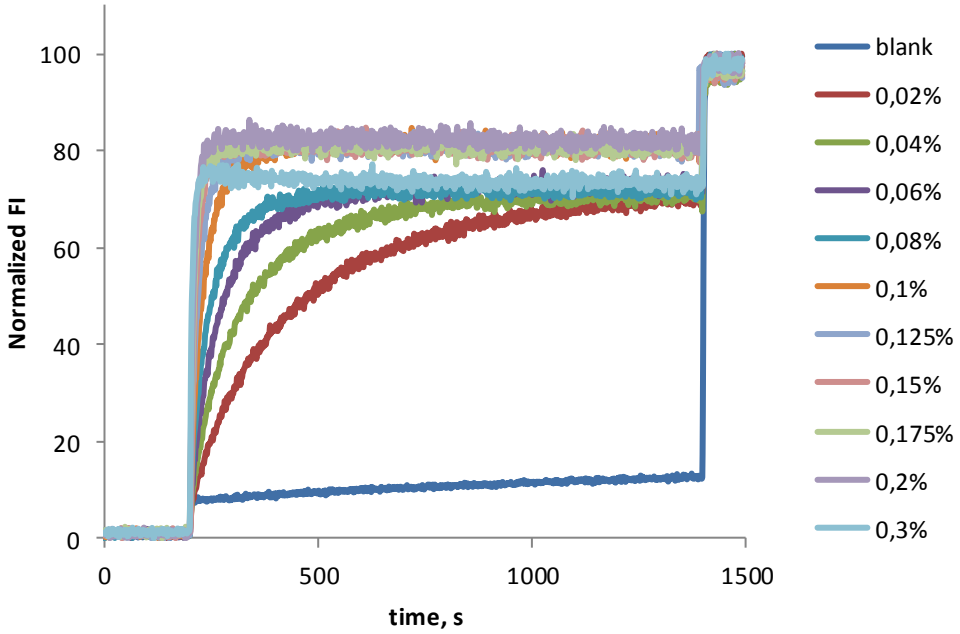

Figure S1. Normalized fluorescence change in HPTS emission as a function of time after addition of the base (50 μL of 0.5 M NaOH) to 95:5 PC/PG LUVs (100 nm diameter) loaded with HPTS (0.1 mM HPTS, 0.17 mM total lipid concentration, 25 mM HEPES, 100 mM NaCl, pH 7.0), in the presence of different concentration of complex **1**. The concentrations of ionophore, given in percent with respect to the total concentration of lipids, are indicated in the Figure.

Table S1. Apparent first order rate constants for the transport process (k_t, s^{-1}) at different concentration of complex **1** obtained from the fitting of the kinetic traces of Figure S1. The fitting error on the rate constants is below 2%. The concentration of **1** is expressed as percent with respect to the total concentration of lipids (0.17 mM).

[**1**], %	k_t, s^{-1}
0	2.0E-04
0.02	0.0040
0.04	0.0074
0.06	0.012
0.08	0.019
0.1	0.027
0.125	0.042
0.15	0.069
0.175	0.081
0.2	0.099
0.3	0.198

The rate data of Table 1 were fitted with the equation proposed by S. Regen,[1] which is used to describe cooperative association phenomena between monomers forming higher order ionophoric active structures:

$$k_t = \frac{k_2}{K}[monomer]^n$$

In this equation, K is the dissociation constant of the higher order species formed, k_2 is the intrinsic rate constant for the transport process, and *n* is the number of monomers which self-assemble forming the higher order species. The fitting of the data is shown in Figure 1b of the manuscript and gives a *n* value of 1.7, suggesting that, at high concentration of **1**, dimeric Pd complexes, probably formed by μ-OH^- bridges, participate to the transport process.

Determination of cation selectivity with the HPTS assay.

The experiment was performed as described above except for the use of the appropriate chloride salt (MCl; M = Li^+, Na^+, K^+, Rb^+) in the liposome preparation. The base pulse is then obtained by addition of 50 μL of 0.5 M MOH (M = Li^+, Na^+, K^+, Rb^+). In this way only the alkaline cation under investigation is present inside and outside the liposomes.

Determination of anion selectivity with the HPTS assay.

The experiment was performed as described above except for the use of the appropriate Na^+ salt (NaX; X = Cl^-, Br^-, I^-, NO_3^-, ClO_4^-) in the liposome preparation. In this way only the anion under investigation is present inside and outside the liposomes. The rates of anions transport have been corrected for the membrane permeability of the different anions in the absence of ionophore determined in separate experiments.

Anion 'jump' experiment with the HPTS assay.

The vesicle stock solution was prepared as described for the standard HPTS assay, using HEPES buffer (25 mM, pH 7) without added salt for the swelling and for the size exclusion chromatography (SEC). 104 μL of the stock solution were placed in a fluorimetric cell, diluted up to 3040 μL with HEPES buffer (25 mM, pH 7) and kept under gently stirring. The total lipid concentration in the fluorimetric cell was 0.17 mM. The ionophore (0.1% concentration) was then added to the lipid suspension and the cell was incubated at 25 °C for 10 minutes. After incubation an anion gradient was externally applied by addition of 50 μL of 2 M NaX solution (X = Cl^-, Br^-, I^-, NO_3^-, ClO_4^-). The final external concentration of NaX was 33 mM. The kinetic process was then followed as described above. The data have been normalized on the maximum effect observed in the series (i.e. Br^-).

Chloride transport with lucigenin assay.

The lipid cake was prepared as previously described but using only EYPC (100 μL of EYPC chloroform solution (100 mg/mL, 14 μmol)). The lipid cake was hydrated in 1.0 mL of lucigenin solution 1 mM (25 mM HEPES, 100 mM $NaNO_3$, pH 7). The LUV suspension was separated from extravesicular dye by size exclusion chromatography (SEC) (stationary phase: pre-packed column *Sephadex*™ G-25, mobile phase: 25 mM HEPES, 100 mM $NaNO_3$, pH 7) and diluted with the same

HEPES buffer to give a stock solution with a lipid concentration of 5 mM (assuming 100% of lipids were incorporated into liposomes). The vesicle suspension (104 μL of stock solution) was placed in a fluorimetric cell and diluted to 3040 μL with 25 mM pH 7 HEPES buffer containing 100 mM $NaNO_3$. The total lipid concentration in the fluorimetric cell was 0.17 mM. An aliquot of a MeOH mother solution of the ionophore (10-30 μL in order to obtain the desired $mol_{compound}/mol_{lipid}$ ratio) was then added to the lipid suspension and the cell was incubated at 25 °C for 10 minutes. To the gently stirred mixture 50 μL of NaCl 2 M was added through an injector port to establish a chloride gradient. The final external concentration of NaCl was 33 mM. Maximal changes in dye emission were obtained by lysis of the liposomes with detergent (40 μL of 5% aqueous Triton® X-100). Lucigenin emission was monitored at 506 nm (excitation at 455 nm) as a function of time. The data were normalized as described above.

Calcein-Release Assay.[2]

The liposome suspension was prepared as previously described using a mixture of 72 μL EYPC chloroform solution (100 mg/mL, 9.5 μmol) and 19 μL EYPG chloroform solution (20 mg/mL, 0.5 μmol). The lipid cake was hydrated in 2.0 mL of 50 mM calcein solution (1 mM HEPES, pH 7.4). The LUV suspension was separated from extravesicular dye by size exclusion chromatography (SEC) (stationary phase: column ∅1x25cm Sephadex™ G-75, mobile phase: buffer 1 mM HEPES, 150 mM NaCl, pH 7.4) and diluted with the same HEPES buffer to give a stock solution with a lipid concentration of 0.4 mM (assuming 100% of lipid was incorporated into liposomes). The vesicle suspension (1292 μL stock solution) was placed in a fluorimetric cell and diluted to 3040 μL with the buffer solution used for preparation. The total lipid concentration in the fluorimetric cell was 0.17 mM. Calcein emission was monitored at 520 nm with excitation at 490 nm. During the experiment 10 μL of 0.52 mM solution of ionophore **1** in DMSO were added through an injector port every 100 s up to 50 μL. Maximal changes in dye emission were obtained by lysis of the liposomes with detergent (40 μL of 5% aqueous Triton® X-100).

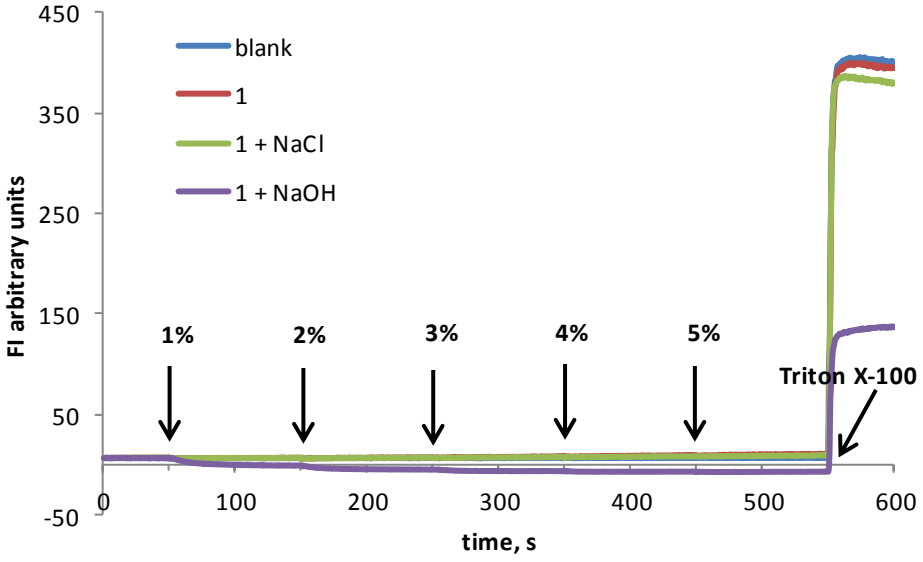

Figure S2. Fluorescence time course for calcein release from liposomes (λ_{ex} = 490 nm, λ_{em} = 520 nm). The arrows indicate the time of the addition of aliquots of solution of **1** (10 μL of a 0.52 mM solution). The total concentration of ionophore added to the liposome suspension is indicated above

the arrows. The final addition of detergent lysis the liposomes and the calcein is fully released. The Figure reports the experiment in the absence of Pd(II) complex (blank), with addition of increasing amount of **1** (**1**), with addition of increasing amount of **1** in the presence of externally added NaCl (**1** + NaCl, 50 µl of 2 M NaCl solution) or of externally added NaOH (**1** + NaOH, 50 µl of 0.5 M NaOH solution).

Figure S2 shows that the addition of increasing amount of **1** up to 5% concentration has no effect on calcein release from liposomes which is signaled by an increase of calcein fluorescence emission. The same inactivity is also observed when an 33 mM extra gradient of NaCl is applied by addition of 50 µl of a 0.5 M NaOH solution to the liposome suspension before starting the experiment (curve **1** + NaCl). Interestingly, the application of a pH gradient obtained by addition by 50 µl of 0.5 M NaOH solution to the liposome suspension before starting the experiment (curve **1** + NaOH) results in a more complex behavior. At higher pH the fluorescence emission of calcein is partially quenched as revealed by the lower emission intensity reached after lysis with Triton® X-100. Addition of the Pd(II) complex results in a small but clearly detectable decrease of fluorescence emission, which follows a kinetic process associated to the transport of OH^- from outside to inside the liposome driven by the applied pH gradient. The increase of pH inside the liposome partially quenches the fluorescence emission of calcein leading to the observed kinetic. However, no release of calcein, which should give an increase of fluorescence emission, is observed even at the highest concentration of ionophore tested (5%).

Release of calcein was also tested in liposomes pre-incubated with different concentrations of **1** (1% - 5%). In this experiment aliquots of a mother solution of **1** (0.52 mM in DMSO) were added to the liposome suspension and the fluorescence was recorded after 20 min of incubation time. The results reported in Figure S3 show that also in this case the effect of **1** on calcein release is negligible.

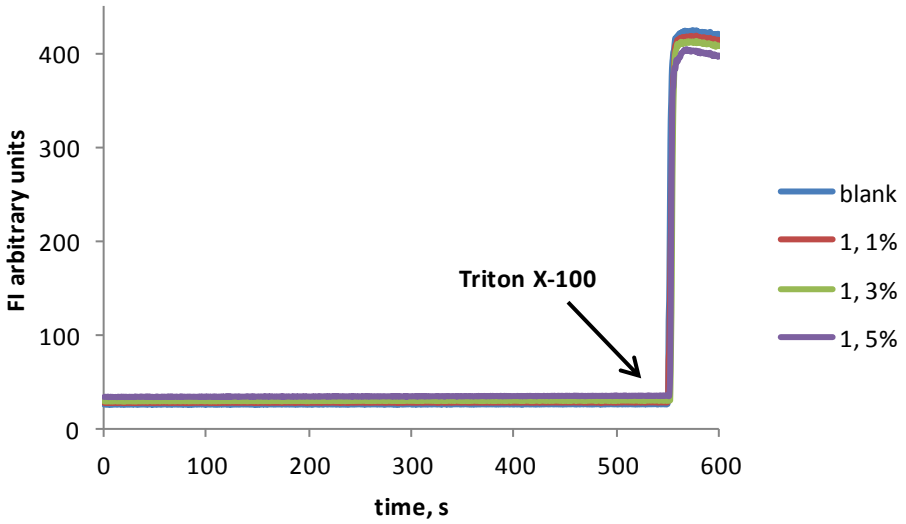

Figure S3. Fluorescence time course for calcein release from liposomes (λ_{ex} = 490 nm, λ_{em} = 520 nm) in the presence of different concentrations of **1**. In this experiments 10, 30 and 50 µL of a mother solution of **1** (0.52 mM in DMSO) were added to the liposome suspension 20 min before to start the experiment. The concentration of **1** tested are indicated in the Figure. The final addition of detergent lysis the liposomes and the calcein is fully released.

UV-Vis Titrations

pH Titration.

For the UV-Vis experiments at different pH values a series of 25 mM HEPES buffer solutions with pH ranging from 4.75 to 8.5 were prepared. These buffered solutions were mixed with DMSO in 1:1 ratio. 800 μL of each of these mixtures were placed in a cuvette and an aliquot of a $[Pd(dppp)(OTf)_2]$ (**1**) solution in DMSO was added in order to give a 5.0×10^{-5} M final concentration of metal complex. Similar solutions in pure DMSO and in DMSO/H_2O at pH 4 (HNO_3) were prepared. The UV-Vis spectra of these solutions were recorded with a Perkin Elmer Lamda 35 spectrophotometer and are reported in Figure S4.

By changing the solvent from pure DMSO to DMSO/H_2O at pH 4 a strong decrease in the band at 275 nm and a smaller increase in the shoulder at 350 nm is observed. These spectral changes are reasonably associated to the exchange of the OTf^- ligands with water forming the $[Pd(dppp)(H_2O)_2]$ complex. Subsequent increase of the pH causes the decrease of the bands at 275 and 345 nm and the appearance of a new band at 260 nm and a shoulder at 315 nm, with one isosbetic point at 267 nm. Figure S5 shows the dependence of the absorbance at 275 and 347 nm in function of pH which follows the typical profile associated with the deprotonation of an acidic function, most probably a water molecule coordinated to the metal ion. Fitting of the two profiles of Figure S5 gives a averaged pKa of 6.1, which is compatible with the 5.6 value reported for the dissociation of a water molecule in the $[Pd(en)(H_2O)_2]$.[3] On this ground the observed spectral changed are compatible with the following scheme in which one water molecule of the $[Pd(dppp)(H_2O)_2]$ is deprotonated with an apparent pKa of 6.1.

$$[Pd(dppp)(OTf)_2] + 2H_2O \rightleftharpoons [Pd(dppp)(H_2O)_2]^{2+} + 2OTf^-$$

$$[Pd(dppp)(H_2O)_2]^{2+} + OH^- \overset{Ka}{\rightleftharpoons} [Pd(dppp)(H_2O)OH]^+ + H_2O$$

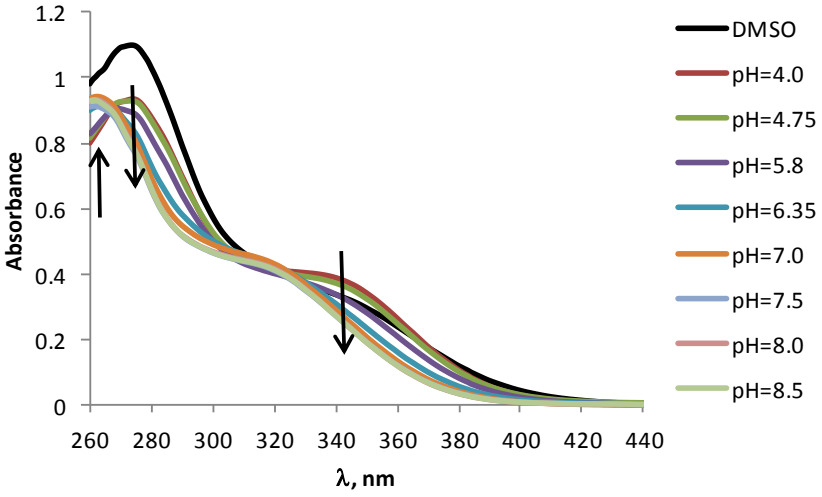

Figure S4. UV-Vis spectra of 5×10^{-5} M **1** recorded in DMSO/HEPES (25 mM) at different pH values. The trace at pH 4 is recorded in DMSO/H_2O made acidic with HNO_3. The black trace is recorded in pure DMSO. The pH values are measured in the water solution before mixing with DMSO.

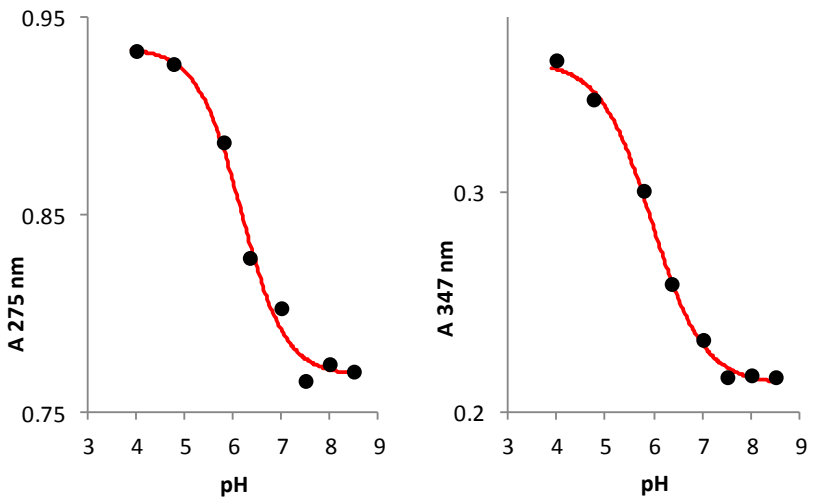

Figure S5. Dependence of the absorbance at 275 and 347 nm with pH. Data from Figure S4. The red curve is the calculated profile with pKa = 6.1.

Anions titration.

For the UV-Vis experiments a solution 6.0×10^{-5} M of [Pd(dppp)$(OTf)_2$] (**1**) was prepared in a 1:1 DMSO/HEPES (0.025 M, pH 7) mixture. 800 μL of this solution was placed in a cuvette and the UV-Vis spectra were recorded with a Perkin Elmer Lamda 35 spectrophotometer before and after the addition of 10 μL of 2 M NaX (X = Cl^-, Br^-, I^-, NO_3^-, ClO_4^-) solution in water in order to obtain a 0.025 M final concentration of NaX. In the case of $NaNO_3$ the spectra were corrected by subtraction of those of $NaNO_3$ alone recorded in the same experimental conditions. The results are shown in Figure 4 in the main text.

In the case of halides UV-Vis titrations were performed using 2 mL of **1** solution (6.0×10^{-5} M) in 1:1 DMSO/buffer and adding aliquots of NaX (X = Cl^-, Br^-, I^-) solutions in water (0.02 M, 0.1 M, 0.6 M, 2 M concentration), in order to explore a halide concentration range between 0 and 0.025 M. The maximum total volume of salt solution added was 100 μL, which corresponds to a 5% dilution. The absorbance/concentration data were fitted with a stepwise 1:1 and 1:2 stoichiometry complex formation model using the Scientist package from MicroMath.

Titration of [Pd(dppp)$(OTf)_2$] (1) with NaCl.

Figure S6 shows the UV-Vis spectra of [Pd(dppp)$(OTf)_2$] (**1**) recorded increasing NaCl concentration from 0 to 0.025 M. Addition of the Cl^- anion produces distinct but complex changes in the spectra with increase and batochromic shift of the peaks at 265, decrease and hypsochromic shift of the band at 300 nm and the appearance of a new band at 340 nm. Interestingly at 290 nm the absorbance first increases and then, at higher concentration of NaCl, decreases. Moreover an isosbestic point is observed at 278 nm but only for concentration of NaCl above 5×10^{-4} M, while the spectra recorded at lover concentrations do not show isosbestic points.

This complex behavior clearly indicates the formation of more than one specie in solution. Based on the work of Van Eldik and Hohman, who investigated the kinetic and thermodynamic of chloride complexation by [Pd(en)$(H_2O)_2$],[3] we applied to the spectroscopic data a model in which two chloride ions are stepwise coordinated by the metal ion:

$$[Pd(dppp)(OTf)_2] + Cl^- \underset{}{\overset{K_1}{\rightleftharpoons}} [Pd(dppp)(OTf)Cl] + OTf^-$$

$$[Pd(dppp)(OTf)Cl] + Cl^- \underset{}{\overset{K_2}{\rightleftharpoons}} [Pd(dppp)Cl_2] + OTf^-$$

The fit of the absorbance/concentration data at 290 nm with this model is shown in Figure S7 and the value of K_1 and K_2 obtained are reported in Table S2. Fitting of the data at different wavelengths gives very similar association constant values.

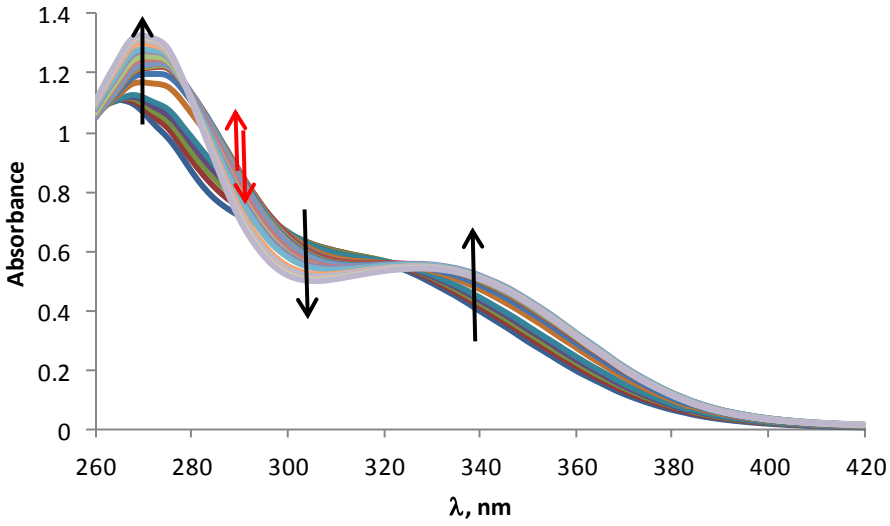

Figure S6. UV-Vis spectra recorded in DMSO/HEPES (0.025 M, pH 7) of the $[Pd(dppp)(OTf)_2]$ (**1**) complex with concentration of NaCl increasing from 0 to 0.025 M. The arrows show the evolution of the spectra on increasing the concentration of Cl^-. At 290 nm (red arrows) the absorbance first increases and then, at higher concentration of NaCl, decreases.

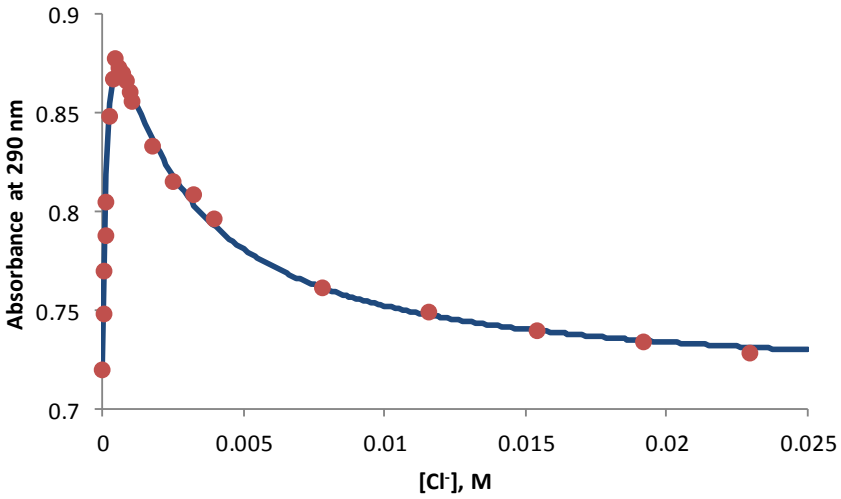

Figure S7. Absorbance data from Figure S6 at 290 nm with increasing concentration of Cl^-. The blue line is the calculated titration curve on the basis of the stepwise 1:1 and 1:2 model described above. The values of the association constants are reported in Table S2.

Titration of [Pd(dppp)(OTf)$_2$] (1) with NaBr.

Figure S8 shows the UV-Vis spectra of [Pd(dppp)(OTf)$_2$] (**1**) recorded increasing NaBr concentration from 0 to 0.023 M. Addition of the Br^- anion produces a distinct change in the spectra with the formation of new bands at 282 and 353 nm and a decrease of the bands at 265 and 320 nm. At 270, 302, 330 nm three isosbestic points are observed. However, as in the case of the NaCl titration, the isosbestic points are not followed during the entire titration and a wavelength drift is observed by increasing the NaBr concentration suggesting that also in this case the stepwise coordination of two Br^- anions occurs.

The fit of the absorbance/concentration data at 282 nm with the model described above is shown in Figure S9 and the value of K_1 and K_2 obtained are reported in Table S2. Attempts to apply a 1:1 binding model to the same data gave a much lower quality fitting.

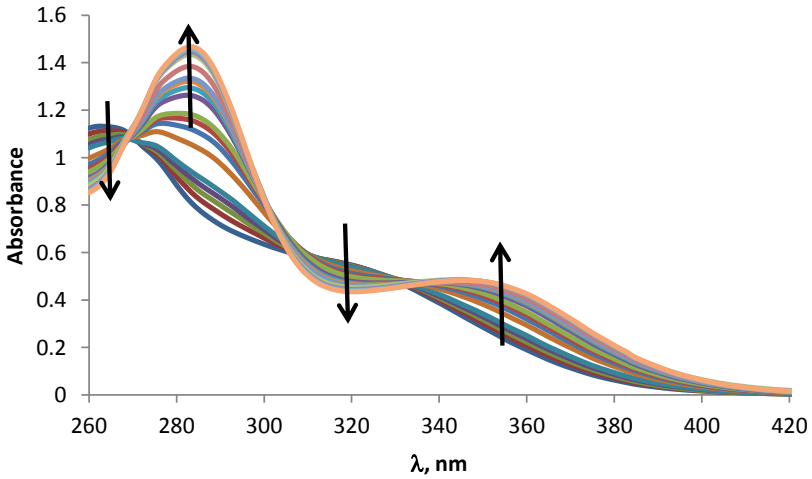

Figure S8. UV-Vis spectra recorded in DMSO/HEPES (0.025 M, pH 7) of the [Pd(dppp)(OTf)$_2$] (**1**) complex with concentration of NaBr increasing from 0 to 0.023 M. The arrows show the evolution of the spectra on increasing the concentration of Br^-.

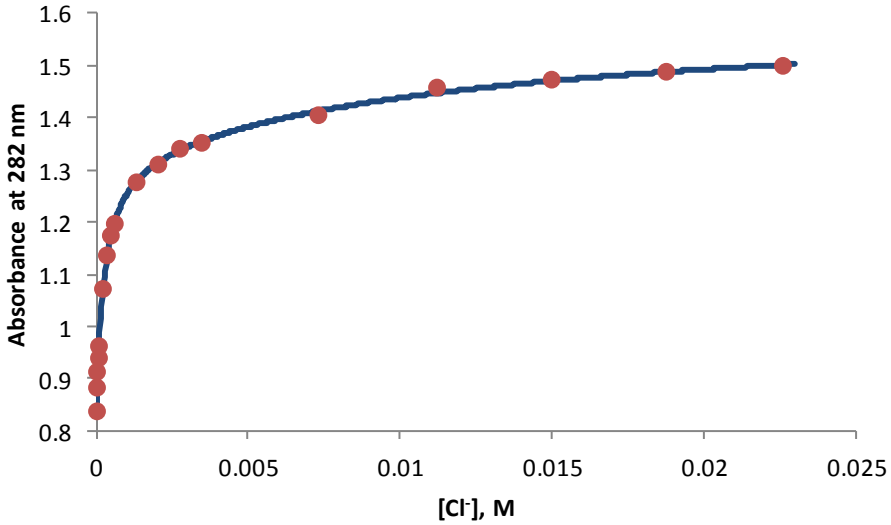

Figure S9. Absorbance data from Figure S8 at 282 nm with increasing concentration of Br^-. The blue line is the calculated titration curve on the basis of the stepwise 1:1 and 1:2 model described above. The values of association constants are reported in Table S2.

Titration of [Pd(dppp)(OTf)$_2$] (1) with NaI.

Figure S10 shows the UV-Vis spectra of [Pd(dppp)(OTf)$_2$] (**1**) recorded increasing NaI concentration from 0 to 0.023 M. Addition of the I^- anion produces a distinct but complex change in the spectra with formation of new bands at 303 and 400 nm. Similarly to the case of NaCl at 338 nm the absorbance first increases and then, at higher concentration of NaI, decreases. In comparison with Cl^- the position of the maximum at 338 nm is shifted to lower concentration of anion suggesting a stronger association constant. Moreover, there are no clear isosbestic points followed during the entire titration.

In general the behavior is similar to that observed with Cl^- and the same binding model has been applied. However in this case fitting of the data at 303 nm and at 338 nm gives satisfactory interpolation but substantially different association constant. We therefore fitted simultaneously the data at the two wavelengths in order to obtain averaged value of the association constants. The simultaneous fit of the absorbance/concentration data at 303 and 338 nm with the model described above is shown in Figure S11 and the value of K_1 and K_2 obtained are reported in Table S2.

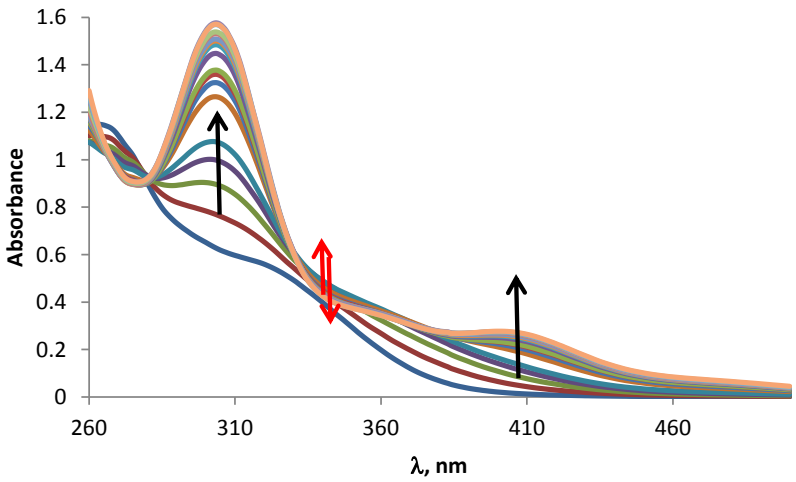

Figure S10. UV-Vis spectra recorded in DMSO/HEPES (0.025 M, pH 7) of the [Pd(dppp)(OTf)$_2$] (**1**) complex with concentration of NaI increasing from 0 to 0.023 M. The arrows show the evolution of the spectra on increasing the concentration of I^-. At 338 nm (red arrows) the absorbance first increases and then, at higher concentration of NaI, decreases.

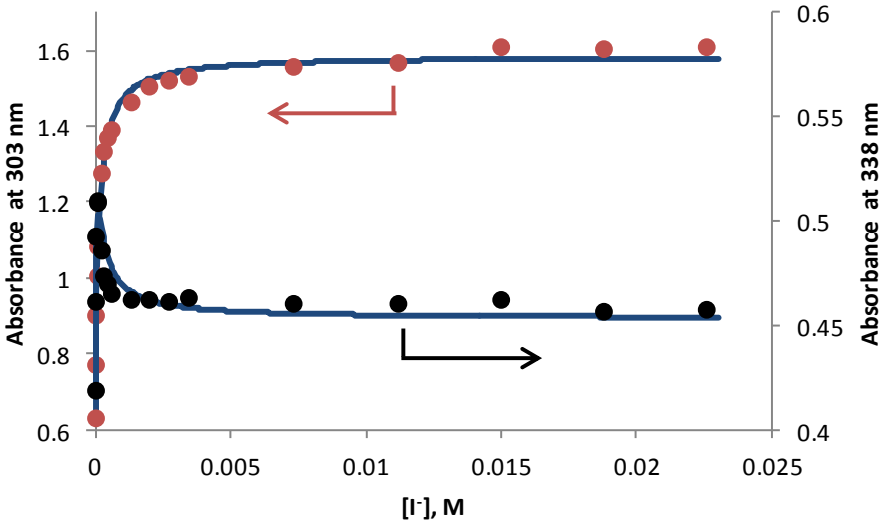

Figure S11. Absorbance data from Figure S10 at 303 nm (red dots, left vertical axis) and 338 nm (black dots, right vertical axis) with increasing concentration of I^-. The blue lines are the titration curves simultaneously calculated on the basis of the stepwise 1:1 and 1:2 model described above. The values of the association constants are reported in Table S2.

Table S2. Formation constants for the mono- and di-halide complex formed by $[Pd(dppp)]^{2+}$ and $[Pd(en)]^{2+}$.

	$[Pd(dppp)]^{2+,a}$		$[Pd(en)]^{2+,b}$	
	$logK_1$ (sd)	$logK_2$ (sd)	$logK_1$ (sd)	$LogK_2$ (sd)
Cl^-	3.93 (0.03)	2.68 (0.02)	3.60 (0.01)	2.13 (0.01)
Br^-	3.67 (0.03)	1.88 (0.15)	-	-
I^-	5.1 (0.4)	3.5 (0.5)	-	-

Conditions: a) 1:1 DMSO/HEPES (0.025 M, pH 7), 25 °C; b) H_2O, 0.1 M $NaClO_4$, 25 °C, data from ref. 3.

Chloride Transport Across a Bulk Liquid Membrane

The ability of $[Pd(dppp)(OTf)_2]$ (**1**) to act as an anion carrier was investigated using the classical U-tube set-up. In this experiment a U-shaped tube is charged on the bottom with chloroform which separates an aqueous donor phase from an aqueous receiving phase confined in the two arms of the U-tube (Figure S12a). The donor phase was buffered at pH 7.0 with HEPES 0.025 M and loaded with the anion (NaCl = 100 mM) while the receiving phase contained only HEPES 0.025 M buffered at pH = 7.0. **1** was dissolved in the organic phase at 1 mM concentration and all the three phases were kept under magnetic stirring. Thus, if complex **1** is able to transport

the anion across the bulk chloroform membrane, from the source to the receiving phase, the concentration of anion in the receiving phase should increase. This was monitored by analyzing aliquots of the receiving phase at different times using as chloride sensor the same [Pd(dppp)$(OTf)_2$] which by complexation of the anion give a distinct UV-Vis spectra change (see Figure S6). The concentration of chloride in the receiving phase was, therefore, calculated by UV-Vis measuring the spectral change at 273 nm of a solution of **1** in 1:1 DMSO/HEPES (0.025 M, pH =7) upon addition of aliquots of the receiving phase. The absorbance data were transformed in concentration data using a calibration curve obtained by titration of **1** in the same conditions with solution of known concentration of NaCl. Figure S12b reports the increase of concentration of chloride in the receiving phase as a function of time showing clearly anion transport. On the contrary control experiments in the presence of NaCl in the source phase but in the absence of **1** in the chloroform, or in the absence of NaCl in the source phase but in the presence of **1** in chloroform, did not show any transport of chloride anion from the source to the receiving phase.

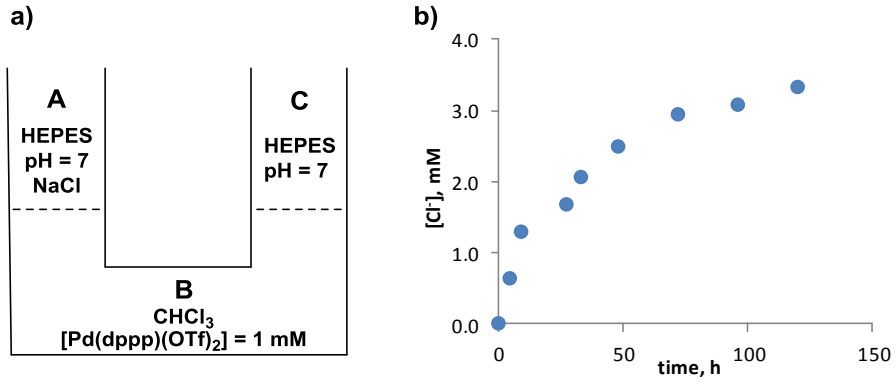

Figure S12. a) Schematic representation of the U-tube transport experiment. A chloroform phase (B) containing **1** (1 mM) separates two water phases. The source water phase (A) is buffered with HEPES at pH = 7 and contains NaCl 100 mM. The receiving water phase (C) contains only HEPES at pH 7. b) Variation of the chloride concentration in the receiving phase with time.

References

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