Pd(II)-mediated assembly of porphyrin channels in bilayer membranes

Usha Devi,[1] James R.D. Brown,[1] Andrew Almond[2] and Simon J. Webb[1]*

1. Manchester Interdisciplinary Biocentre and the School of Chemistry, University of Manchester, 131 Princess St, Manchester M1 7DN, United Kingdom. Fax: (+) 44-161-306-5201; Tel: (+) 44-161-306-4524.

2. Manchester Interdisciplinary Biocentre, University of Manchester, 131 Princess St, Manchester M1 7DN, United Kingdom.

S.1. Fluorescence and UV-visible titrations of 3-PyTPP with $PdCl_2(PhCN)_2$

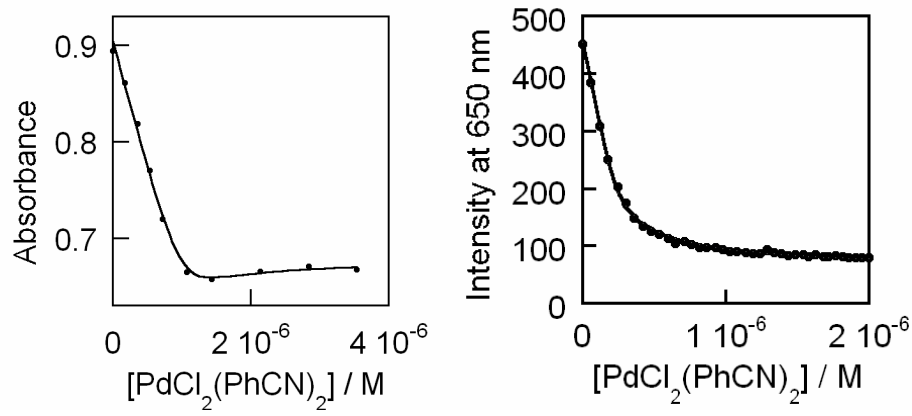


Figure S1: Fitted titration curves for **3-PyTPP** with **2**

Stock solutions of **3PyTPP** (6.25 mg, 1 mM, 10 mL anhydrous CH_2Cl_2) and $PdCl_2(PhCN)_2$ (3.84 mg, 0.4 mM, 25 mL anhydrous CH_2Cl_2) were prepared. To $PdCl_2(PhCN)_2$ stock solution (0.4 mM, 0.999 mL) was added **3PyTPP** (1 µL, 2 µM), which ensured **3PyTPP** concentration remained constant throughout the titration. The palladium-porphyrin solution was titrated to a stoppered quartz cuvette containing anhydrous CH_2Cl_2 (1.998 mL) and **3PyTPP** (2 µL, 2 µM). Changes in UV absorbance at 420 nm and changes in the fluorescence signal at 650 nm (excitation 420 nm) were monitored as $PdCl_2(PhCN)_2$ concentration was increased. For the fluorescence titration the procedure was repeated with 0.3 µM **3PyTPP** ensuring the concentration of **3PyTPP** remained constant.

S.2. Fluorimetric studies of the release of 5/6-carboxyfluorescein from 1-EYPC vesicles

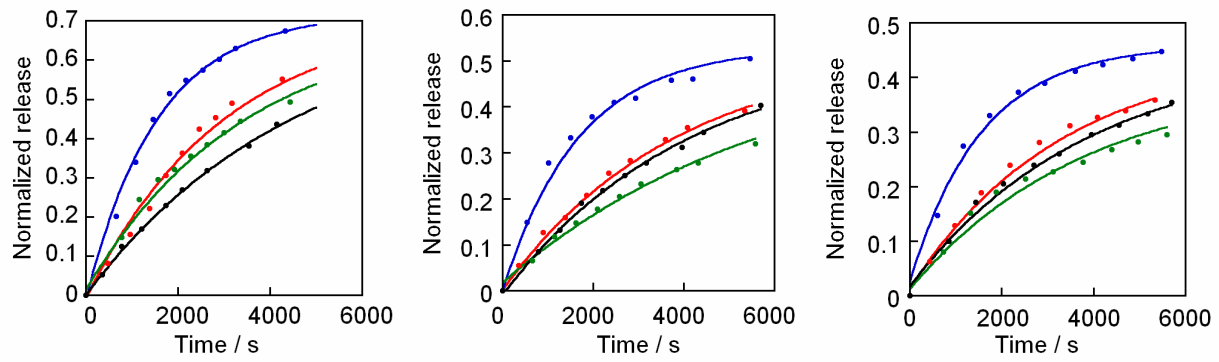


Figure S2: Repeats of 5/6-CF release profiles from 1-EYPC vesicles at different concentrations of 2 with first order curve fits (without blank subtracted): 1 µM $PdCl_2(PhCN)_2$ **2** (●) or 4 µM $PdCl_2(PhCN)_2$ **2** (■) or 20 µM $PdCl_2(PhCN)_2$ **2** (●) or 40 µM $PdCl_2(PhCN)_2$ **2** (●).

Porphyrin **1** (2.5 mg, 1 mM, 5% mol/mol) was added to EYPC (29 mg) in HPLC grade chloroform (2 mL)**,** then the solvent evaporated under reduced pressure to form a thin film on the

interior of the round bottom flask. Carboxyfluorescein solution was added (2 mL, 0.1 M in MOPS buffer), the mixture was agitated using a vortex mixer and then an aliquot of the sample (1 mL) was extruded through a polycarbonate membrane (800 nm pore size, 19×). To remove unencapsulated 5/6 carboxyfluorescein, an aliquot of the suspension (0.5 mL) was diluted with MOPS buffer (2 mL) and the suspension purified by gel permeation chromatography (GPC). This provided a purified suspension of dye-encapsulating vesicles (14.3 μM **1** at 0.5 % mol/mol total incorporation, EYPC 2.84 mM in 3.5 mL MOPS buffer).

Four aliquots (each 36 μL) of the sample were transferred to separate vials and diluted with 962 μL, 957 μL, 960 μL and 957 μL MOPS buffer respectively: to the first sample, 1.8 μL of $PdCl_2(PhCN)_2$ (0.57 mM in THF) to the second sample, 7.2 μL of $PdCl_2(PhCN)_2$ (0.57 mM in THF) to the third sample, 3.6 μL of $PdCl_2(PhCN)_2$ (5.7 mM in THF) and to the fourth sample, 7.2 μL of $PdCl_2(PhCN)_2$ (5.7 mM in THF). Each 1 mL sample contained 0.5 μM **1** and 1 μM (2 eq.), 4 μM (8 eq.), 20 μM (40 eq) and 40 μM (80 eq.) of $PdCl_2(PhCN)_2$ respectively. These samples are the active system (porphyrin **1** + $PdCl_2(PhCN)_2$ **2**). Aliquots (100 μL) were removed from each sample every ten minutes for 90 minutes and transferred to a fluorescence cell containing MOPS (1.90 mL). The release of 5/6 CF was measured using 5/6 CF fluorescence emission at 515 nm (excitation at 394 nm). To determine the effects of the addition of porphyrin **1** and THF upon the integrity of vesicles, release experiments (porphyrin **1** only) and (EYPC only) were also conducted. The same method as for the active species (porphyrin **1** + $PdCl_2(PhCN)_2$ **2**) was followed however aliquots of $PdCl_2(PhCN)_2$ were substituted for aliquots of THF (1.8 μL, 3.6 μL and 7.2 μL).

S.3. Fluorimetric studies of the release of 3-5 kDa FITC-dextran from [1-EYPC] vesicles

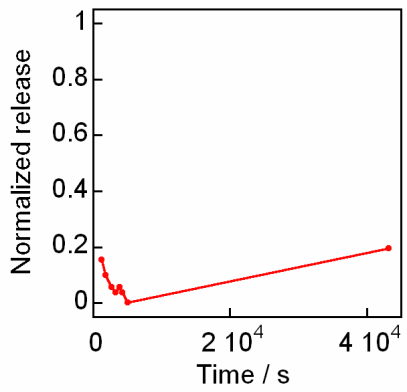


Figure S3: Release of FITC-dextran from **1**-EYPC vesicles upon the addition of 4 μM $PdCl_2(PhCN)_2$ **2** (●). Average plot taken from duplicate runs. The initial drop is due to quenching of adsorbed FITC-dextran by the added Pd(II) complex **2**.

Porphyrin **1** (0.625 mg, 1 mM, 5% mol/mol) was added to EYPC (7.25 mg) in HPLC grade chloroform (1 mL), then the solvent evaporated under reduced pressure to form a thin film on the interior of the round bottom flask. FITC-dextran solution was added (3 to 5 kDa, 0.5 mL, 20 mM in

MOPS buffer), the mixture was agitated using a vortex mixer, then the sample (0.5 mL) was extruded through a polycarbonate membrane (800 nm pore size, 19×). Centrifuge separation was used to remove unecapsulated FITC-dextran. Extruded suspension (0.1 mL) was diluted 7-fold with MOPS buffer and purified by successive centrifugation and removal of the supernatant (removed supernatant was replaced by an equivalent volume of MOPS buffer). This provided a purified suspension of dye-encapsulating vesicles (14.3 μM **1** at 0.5 % mol/mol total incorporation, EYPC 2.84 mM in 0.7 mL MOPS buffer).

An aliquot (36 μL) of the sample was transferred to a separate vial and diluted with 957 μL MOPS buffer and $PdCl_2(PhCN)_2$ (7.2 μL, 0.57 mM in THF) was added. Aliquots (100 μL) containing 0.5 μM **1** and 2 μM **2** were removed every ten minutes for 90 minutes and transferred to a fluorescence cell containing MOPS (1.90 mL). The release of FITC-dextran was measured using FITC fluorescence emission at 515 nm (excitation at 394 nm). After 12 hours the sample was additionally checked for release of FITC-dextran by separating the vesicles in the sample from non-encapsulated material by GPC.

S.4. Fluorimetric studies of the release of 5/6-carboxyfluorescein from 3PyTPP-vesicles

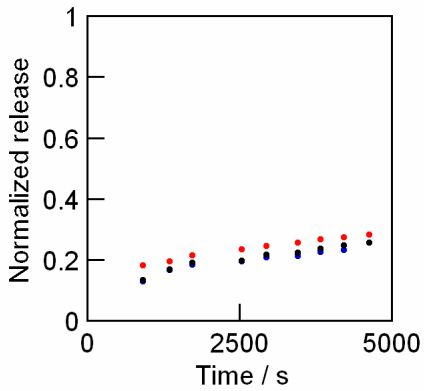


Figure S4: (●) Release of 5/6-CF from 3PyTPP-EYPC vesicles upon the addition of 4 μM $PdCl_2(PhCN)_2$ **2**; (●) Release of 5/6-CF from EYPC vesicles upon the addition of 4 μM $PdCl_2(PhCN)_2$ **2**; (●) Release of 5/6-CF from EYPC vesicles upon the addition of 7.2 μL THF.

3PyTPP (1.23 mg, 1 mM, 5% mol/mol) was added to EYPC (29 mg) in HPLC grade chloroform (2 mL), then the solvent evaporated under reduced pressure to form a thin film on the interior of the round bottom flask. Carboxyfluorescein solution was added (2 mL, 0.1 M in MOPS buffer), the mixture was agitated using a vortex mixer and then an aliquot of the sample (1 mL) was extruded through a polycarbonate membrane (800 nm pore size, 19×). To remove unencapsulated 5/6 carboxyfluorescein, an aliquot of the suspension (0.5 mL) was diluted with MOPS buffer (2 mL) and the suspension purified by gel permeation chromatography (GPC). This provided a purified suspension of dye-encapsulating vesicles (8.57 μM **3PyTPP** at 0.3 % mol/mol total incorporation, EYPC 2.85 mM in 3.5 mL MOPS buffer).

An aliquot (60 μL) of the sample was transferred to a separate vial and diluted with 933 μL MOPS buffer and $PdCl_2(PhCN)_2$ (7.2 μL, 0.57 mM in THF) was added. Aliquots (100 μL) containing 0.5 μM **1** and 4 μM **2** were removed every ten minutes for 90 minutes and transferred to a fluorescence cell containing MOPS (1.90 mL). The release of 5/6 CF was measured using 5/6 CF fluorescence emission at 515 nm (excitation at 394 nm).

S.5. Procedure for U-Tube carrier transport experiments

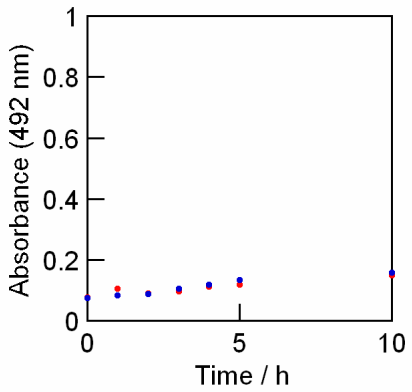


Figure S5: Blank control (●) or transport of 5/6-CF by **1** + **2** complexes (●)**.**

A glass U-tube (14 mm i.d.) was incubated in a water bath at 25 ºC. A chloroform solution of **1** and **2** (1 mM **1** and 2 mM **2**, 10 mL) was placed in the U-tube to form the bottom layer. To one side of the U-tube was added a receiving phase of distilled water (5 mL), and to the other side was added a source phase of carboxyfluorescein solution (0.05 M in MOPS buffer, 5 mL). Addition of the source phase marked the start of the experiment and an aliquot (2 mL), was taken from the receiving phase and analysed via UV spectroscopy for the presence of 5/6-CF (Abs. 394 nm). After the measurement, the sample was immediately replaced back to the U-tube. Measurements were taken every hour for five hours. The chloroform phase was stirred at 300 rpm during the entirety of the experiment to ensure efficient diffusion of any transporting complexes to the receiving phase. A control experiment was also conducted following the same procedure however the **1** + **2** chloroform phase was substituted for spectroscopic grade chloroform only (10 mL).

S.6. Procedure for FRET between membrane-embedded porphyrin 1 and perylene

Porphyrin **1** (1.25 mg, 1 mM, 5% mol/mol) and/or perylene (20 μL of 1 mM chloroform solution, 0.2 mM, 0.1% mol/mol) was added as appropriate to EYPC (14.5 mg) in HPLC grade chloroform (1 mL)**,** then the solvent evaporated under reduced pressure to form a thin film on the interior of the round bottom flask. The lipid film was detached with MOPS buffer (1 mL) and agitated using a vortex mixer and an aliquot of the sample (1 mL) was extruded through a polycarbonate membrane

(800 nm pore size, 19x). Unincorporated **1** and perylene was removed by diluting an aliquot of the suspension (0.5 mL) into MOPS buffer (2 mL) and loaded onto an equilibrated PD10 desalting column. The vesicle suspension was eluted with MOPS buffer (3.5 mL) to provide a purified vesicle suspension. The eluant was once again purified by GPC chromatography.

The resulting vesicle suspensions were diluted in a fluorescence cuvette containing MOPS buffer to give suspension that were 0.1 mM in total lipid. Emission spectra were recorded for each sample after perylene excitation at 411 nm (this wavelength was shorter than λ_{max} for the membrane-embedded porphyrin **1**).

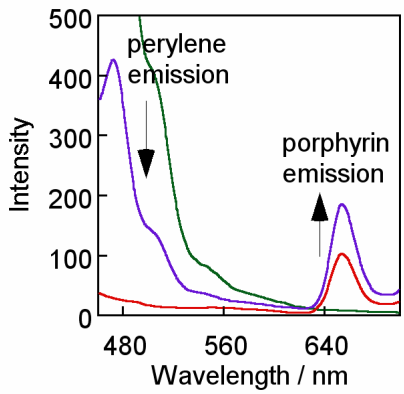


Figure S6: Emission spectra for EYPC vesicles containing perylene only (—), porphyrin **1** only (—) and both perylene and porphyrin **1** (—).

S.7. UV-Visible absorption band of 1 embedded in the membranes of EYPC vesicles

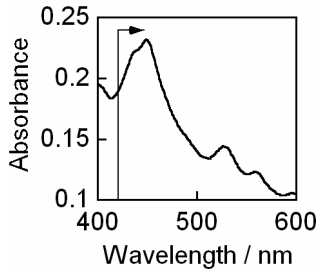


Figure S7: UV-Visible absorption band of **1** embedded in the membranes of EYPC vesicles at 0.5 % mol/mol showing the shift in the Soret band from the solution spectrum.

S.8. Curve fits for the UV-visible titration of 1 with 2 with different K_{12} values

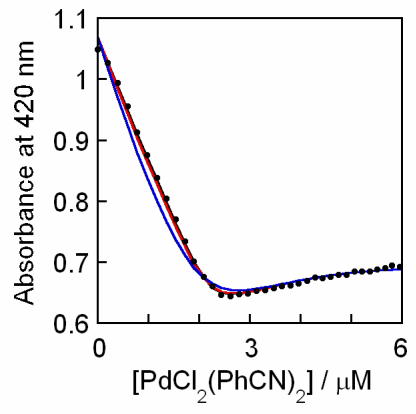


Figure S8: UV-visible titration of **1** with $PdCl_2(PhCN)_2$ in CH_2Cl_2 (●) with different calculated curve fits for K_{12} = 10^4 M^{-1} (—), K_{12} = 10^5 M^{-1} (—), K_{12} = 10^6 M^{-1} (—).

S.9. Fluorimetric studies of the release of 5/6-carboxyfluorescein from 1-DPPC vesicles and from EYPC vesicles with externally added 1 and 2

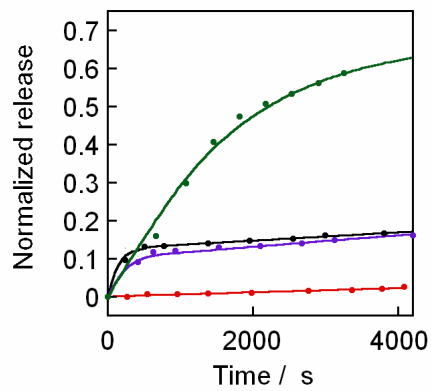


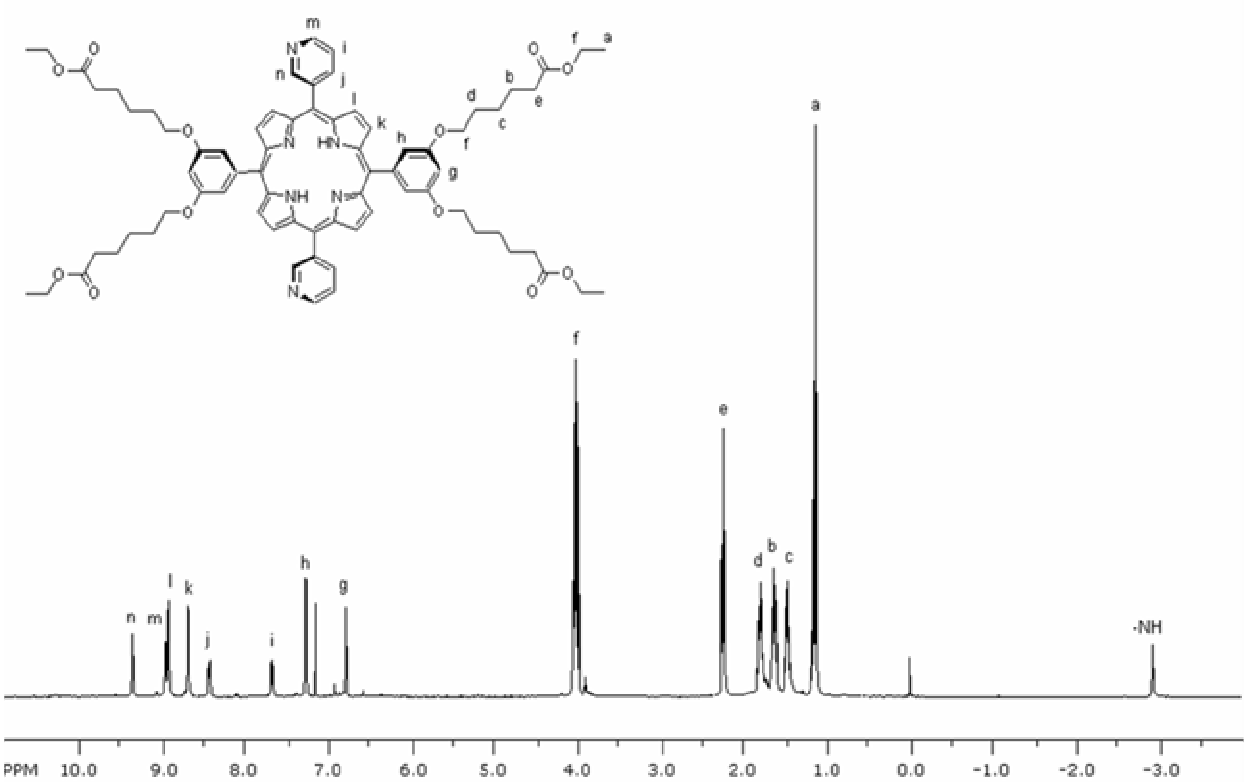
Figure S9: 5/6-CF release profile from **1**-EYPC vesicles and 4 μM $PdCl_2(PhCN)_2$ **2** (—); 5/6-CF release profile from **1**-DPPC vesicles and 2 μM $PdCl_2(PhCN)_2$ **2** (—);5/6-CF release profile from EYPC vesicles to which porphyrin **1** has been added (—); 5/6-CF release profile from EYPC vesicles to which **1** and 4 μM $PdCl_2(PhCN)_2$ **2** have been added (—).

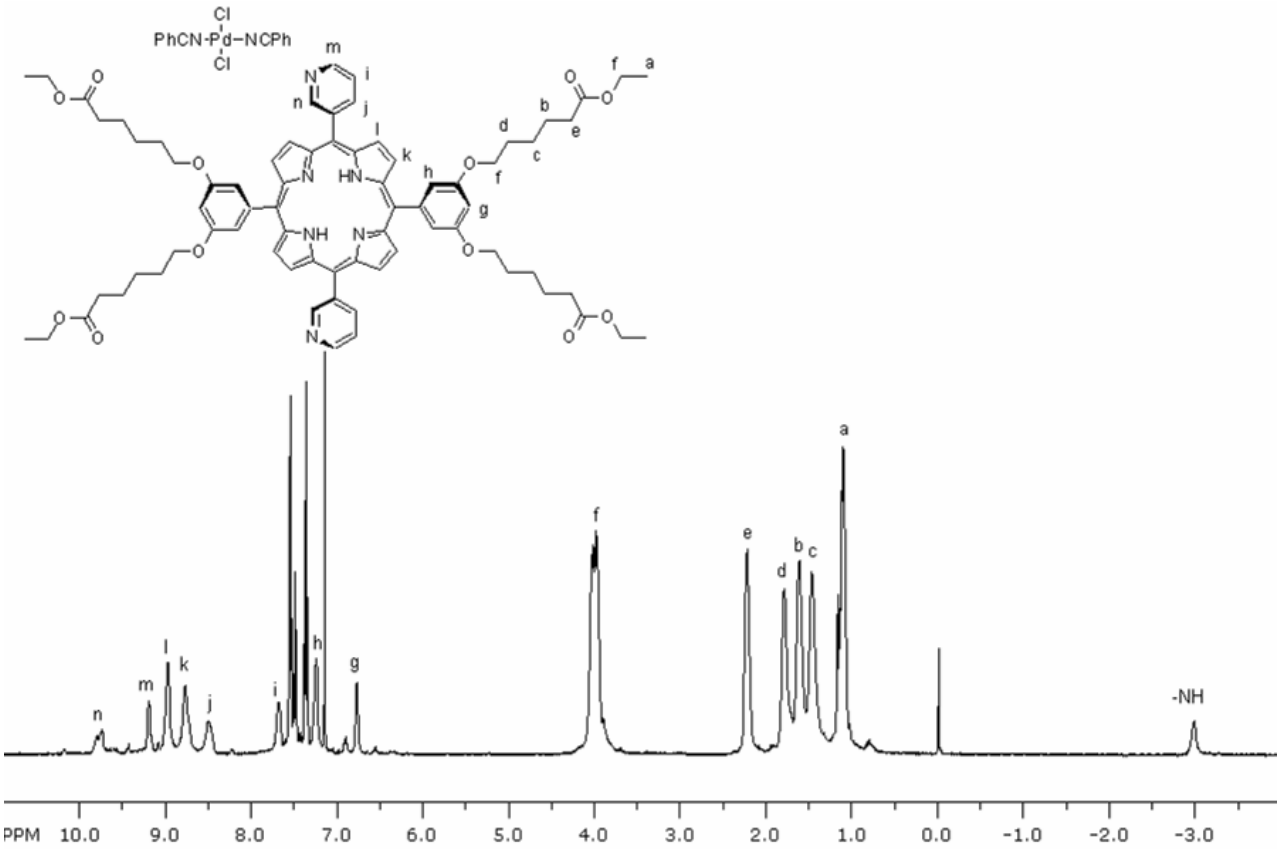
Membrane incorporation of **1** in DPPC vesicles was determined by UV-spectroscopy as detailed for EYPC vesicles and found to be 0.25 % mol/mol. Consequently 2 μM of **2** was used instead of 4 μM in order to maintain the ratio of **1** to **2** at eight equivalents of **2** as for **1**-EYPC experiments. **1**-DPPC vesicles containing 5/6-CF were created by extrusion as detailed previously, but with heating to maintain T > T_m for DPPC bilayers (~50 ℃). Unencapsulated 5/6-CF was removed by size exclusion chromatography as detailed previously. An aliquot (36 μL) of the sample was diluted with MOPS buffer (0.96 mL) and **2** (3.6 μL, 0.57 mM) was added with mixing. This 1 mL sample contained the active system (porphyrin **1** + $PdCl_2(PhCN)_2$ **2**) with the

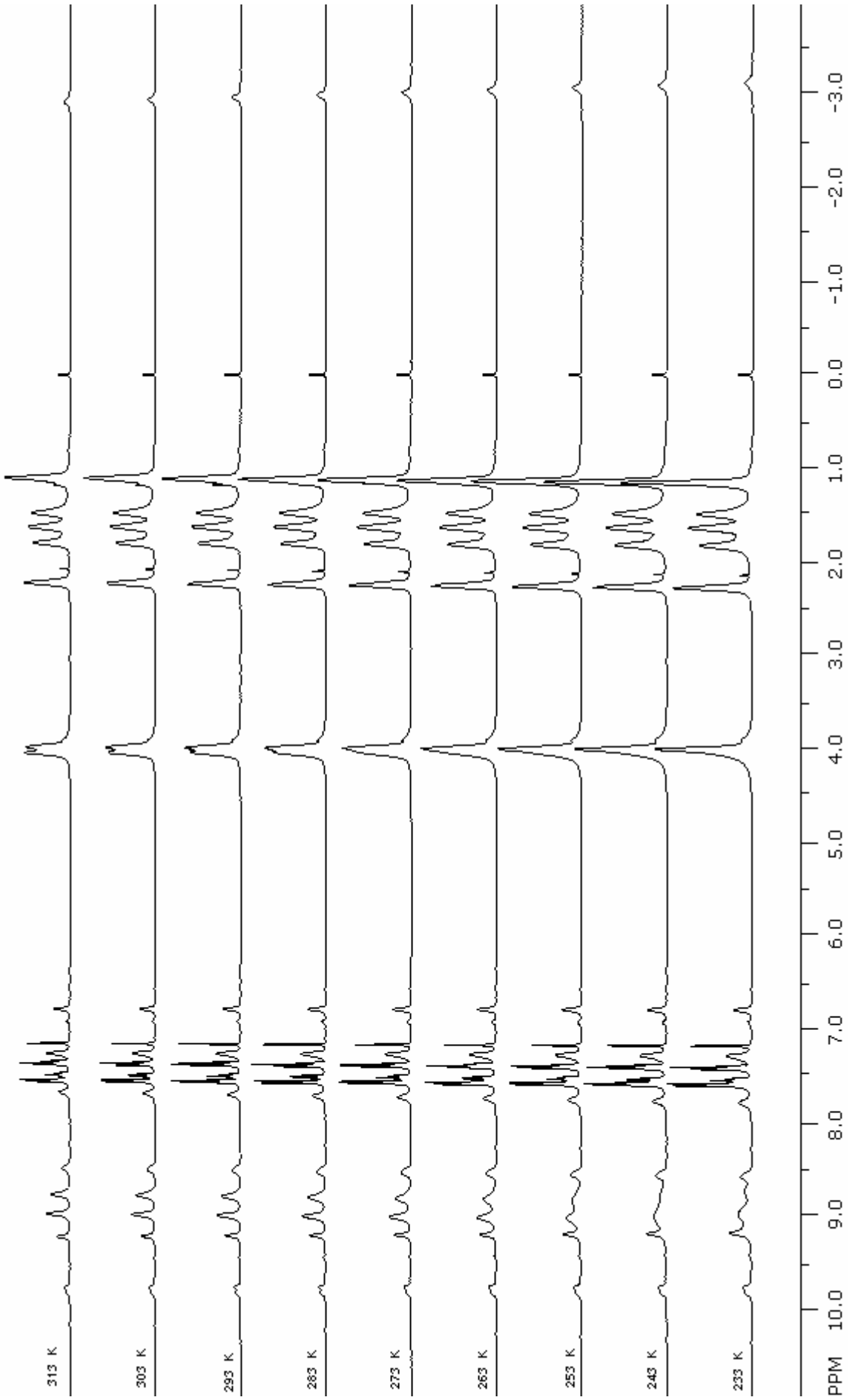
composition 0.25 μM **1** and 2 μM (8 eq.) **2**. Aliquots (100 μL) were removed every ten minutes for 90 minutes and transferred to a fluorescence cell containing MOPS (1.90 mL). The release of 5/6 CF was measured using 5/6 CF fluorescence emission at 515 nm (excitation at 394 nm).

EYPC vesicles containing 5/6-CF were created by extrusion and unencapsulated 5/6-CF was removed by size exclusion chromatography as detailed previously. Two aliquots (each 36 μL) of the vesicle suspension were transferred to separate vials and diluted with 995 μL MOPS buffer. To the first sample was added 5 μL of **1** (0.1 mM in THF) and to the second sample a 5 μL mixture of **1** and **2** (0.1 mM **1** and 0.8 mM **2** in THF) was added. Each 1 mL sample contained 0.5 μM **1** with the second sample also containing 4 μM **2**. Aliquots (100 μL) were removed from each sample every ten minutes for 90 minutes and transferred to a fluorescence cell containing MOPS (1.90 mL). The release of 5/6 CF was measured using 5/6 CF fluorescence emission at 515 nm (excitation at 394 nm).

S.10. VT-NMR studies of 1 + 2 mixtures in $CDCl_3$




PhCN-Pd-NCPh
Cl
Cl
HN
NH
N
-NH
PPM 10.0 9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0.0 -1.0 -2.0 -3.0


313 K
303 K
293 K
283 K
273 K
263 K
253 K
243 K
233 K
PPM
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
-1.0
-2.0
-3.0

S.11. Procedure for GUV electroformation

GUV electroformation was performed according to published protocols.[S1] Vesicles were electroformed in glucose solution (~300 mOsm) in a chamber constructed from ITO slides. Aliquots of the electroformed GUV suspension (20 µL) were gently mixed with sucrose (20 µL, 300 mOsm).

References

(S1). Angelova M.; Dimitrov D., *Faraday Discuss*., **1986**, *81*, 303–311