Supporting information

The interface makes a difference: Lanthanide ion coated vesicles hydrolyze phosphodiesters

Michal Poznik[a], Uday Maitra[b] and Burkhard König[a]

[a.] Faculty of Chemistry and Pharmacy, University of Regensburg, 93040 Regensburg, Germany. E-Mail: burkhard.koenig@ur.de; Fax: +49 943 1717; Tel: +49 943 4576.
[b.] Department of Organic Chemistry, Indian Institute of Science, Bangalore, India. E-Mail: maitra@orgchem.iisc.ernet.in; Fax: +91-80-2360-0529; Tel: +91-80-2360-1968

Content

General methods and materials

General

Salts of metals were purchased from Acros or Sigma-Aldrich and used without any further purification. Phospholipids were purchased from Avanti Polar Lipids Inc. All solutions were prepared in miliQ deionised water or HEPES buffer (25 mM, 7.4 pH).

Dynamic Light Scattering

DLS measurements were performed on a Malvern Zetasizer Nano at 25 °C using 1 cm disposable polystyrene cuvettes (VWR).

Spectroscopy

Fluorescence of co-embedded carboxyfluorecein upon addition of metals was measured using FLUOstar Omega microtiter plate reader using excitation filter at 485 nm and emission filter 520 nm. Phosphorescence of Europium was recorded on Cary eclipse machine. Shimadzu UV-3600 Plus was used for all kinetic measurements.

Preparation and characterization of the vesicles

Vesicles were prepared by an established procedure.[1] Required amount of DOPC solution in chloroform (10 mM) was pipetted in a vial, evaporated in a stream of nitrogen and the residual solvent was removed in vacuo (30 min). Buffer was added to obtain the required concentration (1 mM) of DOPC and the mixture was sonicated for 15 min. The turbid emulsion was extruded through 100 nm polycarbonate membranes at room temperature to obtain uniform vesicles.

In case of different lipids, temperature of sonication and extrusion was always 10 °C above their transition temperature. In case of europium sensitisation, pyrene was added as chloroform solution in the first step of vesicle preparation.

Size and distribution of DOPC vesicles was determined by DLS before and after addition of europium chloride (Figure S1).

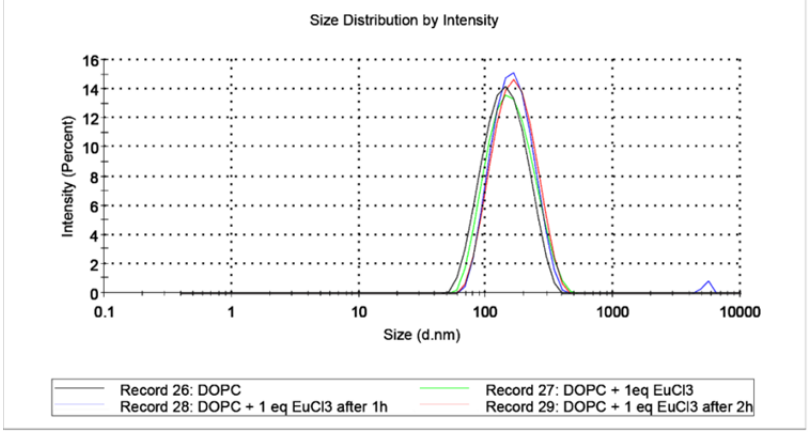

Figure S1: DLS measurement of vesicles before and after addition of equimolar amounts of europium chloride.

Detection of metal binding by carboxyfluorescein

A 96-well microtiter plate was used. In each well 90 µL of HEPES buffer, 10 µL of aqueous metal salt solution (100 µM) or miliQ water for the blank experiments and 25 µL of vesicular solution (0.25 mM) containing DOPC vesicles with 10 mol. % of amphiphilic carboxyfluorescein were pipetted.[2] The result of the measurements is the average of four experiments compared with the fluorescence intensity when no metal salt was added. Two salts of copper(II) were examined showing no difference in the fluorescent response due to different anions (Table S1).

$AlCl_3$	$Zn(ClO_4)_2$	$MgCl_2$	$MnCl_2$	$FeCl_3$	$PbCl_2$	$Cr(ClO_4)_3$	$CuSO_4$ $CuCl_2$	$LnCl_3$	$CeCl_3$	$EuCl_3$	$TbCl_3$	$YbTf_3$

Table S1: Table of salts used for metal sensing.

Experiment with fluorescein or non derivatized carboxyfluorescein under the same conditions show no significant difference when the different metal ions were added (Figure S2.

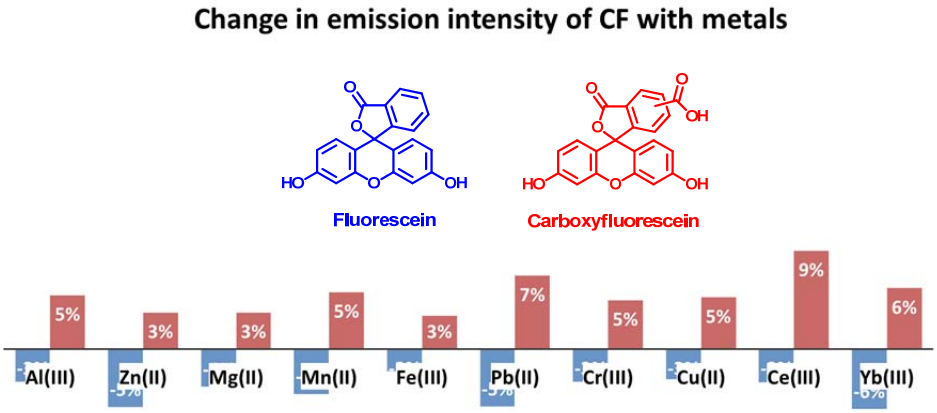

Figure S2: Fluorescence emission intensity changes of non-amphiphilic fluorescein derivatives upon addition of metal salts to a vesicular solution.

Europium phosphorescence in the presence of the DOPC membrane

The europium chloride concentration was always kept at 1 mM (either in MiliQ water or HEPES buffer) and the DOPC lipid concentration was varied from 0–5 mM. The emulsion was sonicated for 30 min to obtain a clear vesicular solution. Samples were excited at λ_{ex}= 394 nm and phosphorescence was measured after 100 µs.

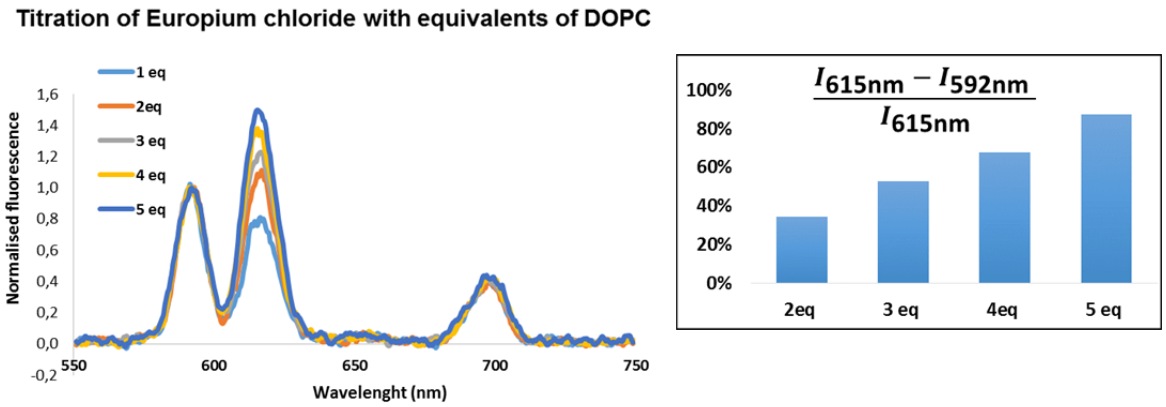

Figure S3: Europium ion emission depending on the amount of DOPC present.

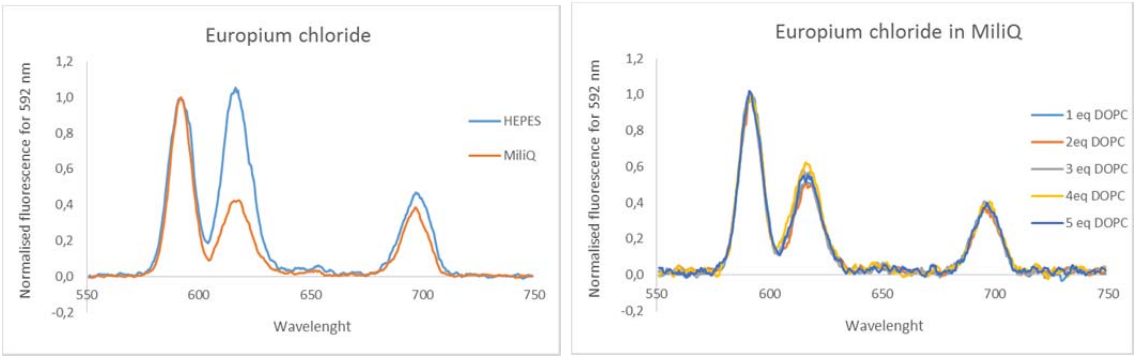

Figure S4: Europium ion emission in dest water (left) or HEPES (25 mmol/L) buffered aqueous solution.

Sensitisation of europium emission by pyrene

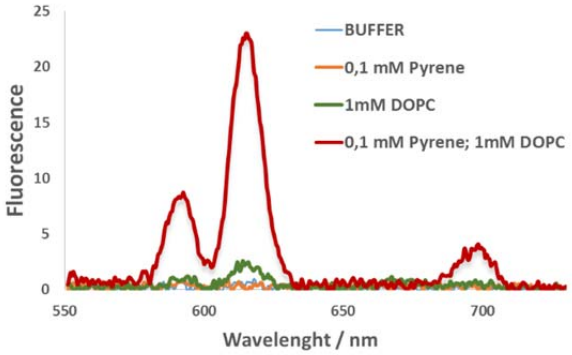

Figure S5: Full spectra of pyrene sensitisation.

In sensitisation experiments solutions of europium chloride (1mM) with vesicles containing 10 mol. % of pyrene in the DOPC membrane (1mM) were used. Pyrene was added as a stock solution in chloroform (with or without DOPC) and then evaporated and re-dissolved in buffer. Pyrene was excited at λ_{ex}= 336 nm and the phosphorescence of europium (after 100 µs) was recorded.

The effect of the substrate and products for hydrolysis on the emission intensity was examined by addition of 1 eq of the respective compound in buffer to obtain the same final concentration of the europium salt and vesicles as for the previous sensitisation

measurements. Phenyl phosphate and diethylphosphate were chosen for their slow or no hydrolysis during the course of the measurements (Figure S6).

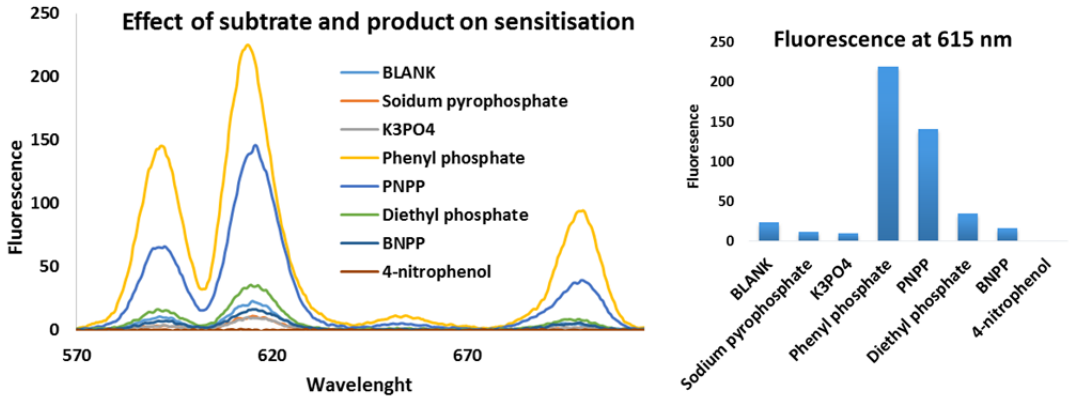

Figure S6: Effect of different phosphates on the europium ion emission intensity using pyrene sensitisation.

Kinetic measurements

All the measurements were done at 25 °C with extruded vesicles (1mM of DOPC) in HEPES buffer (25 mM, 7.4 pH). All the stock solutions were prepared prior to use, BNPP in HEPES buffer (2 mM), europium salt solutions were prepared by diluting a 10 mM stock solution in miliQ water with buffer to obtain 1 mM solutions. Initially, the vesicular solution was added to the buffer to obtain the required concentration for measurements followed by the addition of europium salts. After 2 min of incubation the substrate (BNPP) was added and the increase of absorbance was monitored spectroscopically over 10 min at two wavelengths (500 nm and 400 nm). Increasing turbidity was excluded by subtracting absorbance at 500 nm (no absorption of product) from the value of the para-nitro-phenolate maximum absorption at 400 nm (Figure S7).

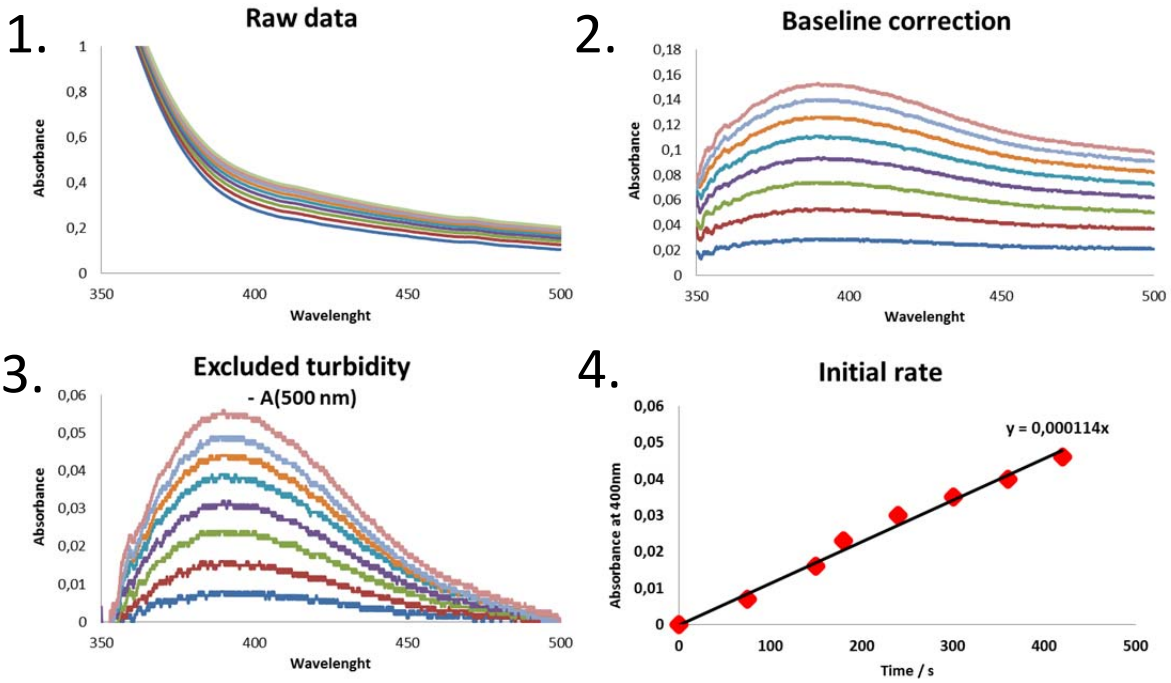

Figure S7: Example of the kinetic data evaluation.

The initial rate was calculated as a slope of the increasing absorbance. Pseudo first order rate constants were determined using the absorption coefficient obtained from the 4-nitrophenol calibration curve. The estimated error of the kinetic measurement is smaller than 5 %.

Calibration curve for 4-nitrophenol in HEPES buffer

Initial rates of reaction were determined from the increase of the absorbance of 4-nitorphenol. 4-Nitrophenol can be present in solution in protonated and deprotonated form, therefore we determined its absorption coefficient under our reaction conditions of 7.4 pH in 25 mM HEPES buffer via calibration curve (ε = 9.8 $mM^{-1}cm^{-1}$, Figure S8).

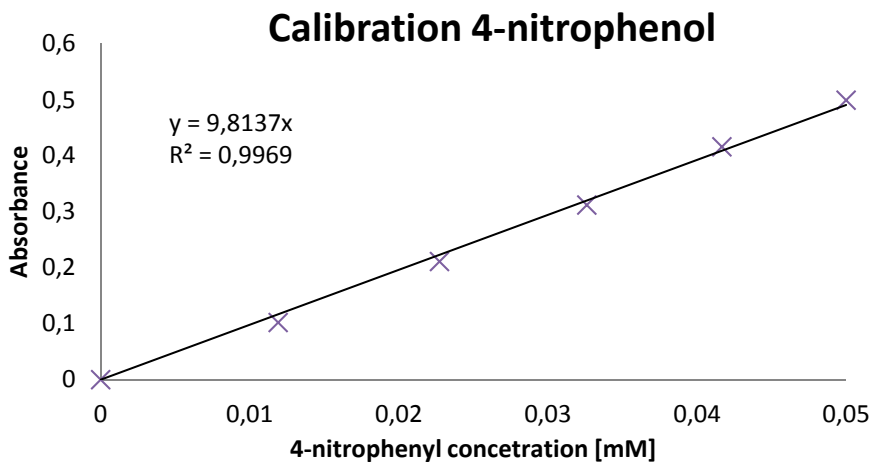

Figure S8: 4-Nitrophenol absorption calibration curve in HEPES buffer.

Screening of metals

In a 1 cm quartz cuvette was added 300 µL of buffer followed with 50 µL of DOPC vesicles. 50 µL of metal salt solution or buffer was added (1mM) and incubated for 2 minutes. Measurement started after the addition 100 µL of BNPP stock solution.

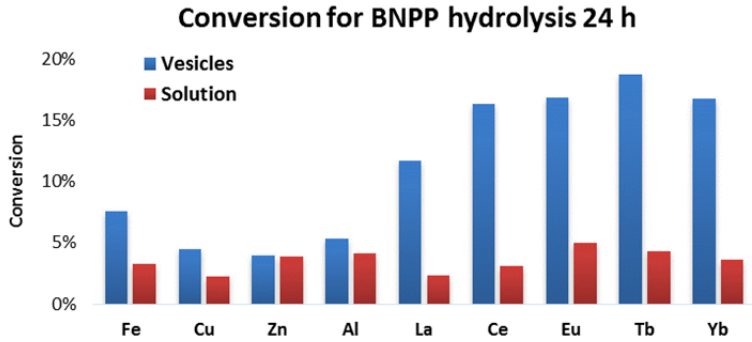

Figure S9: Conversion of substrate after 24 h for different metals.

$FeCl_3$	$CuSO_4$	$ZnCl_2$	$Al(NO_3)_3$	$La(NO_3)_3$	$CeCl_3$	$EuCl_3$	$TbCl_3$	$YbAc_3$

Table S2: Table of metal salts used for screening.

Screening of lipids

Same measurement conditions were used for the screening of lipids using only europium chloride and varying lipids

Lipid	DOPC	DMPC	SMPC	DPPC	DSPC
Transition temperature (°C)	-20	23	30	41	55

Table S3: Transition temperatures of lipids.

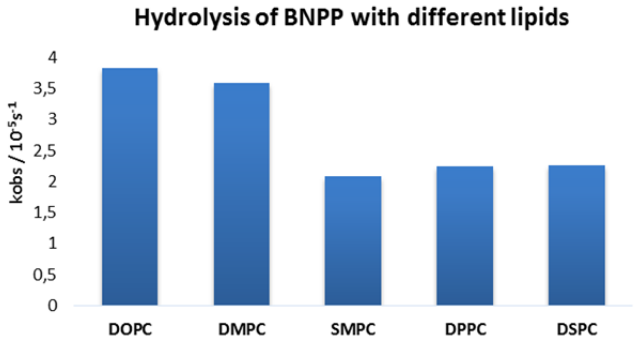

Figure S10: Pseudo first order rate constants for different lipids.

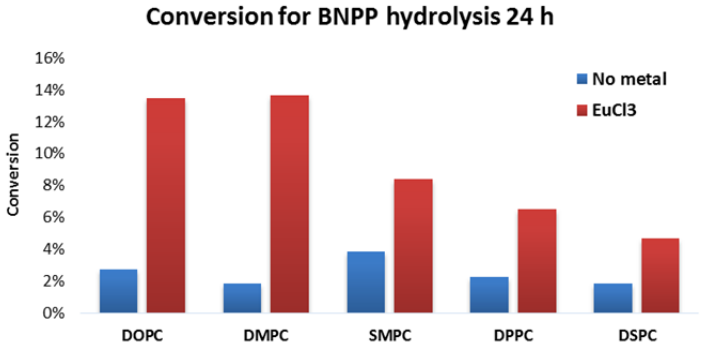

Figure S11: Conversion of BNPP (c = $4 \cdot 10^{-4}$ mol/L) using different lipids (c = 10^{-4} mol/L) with and without the addition of europium chloride (c = 10^{-4} mol/L) in HEPES buffer (c = $25 \cdot 10^{-3}$ mol/L, 7.4 pH).

Optimisation of the Eu:DOPC ratio

Same conditions were used for the measurements as in the previous investigations.

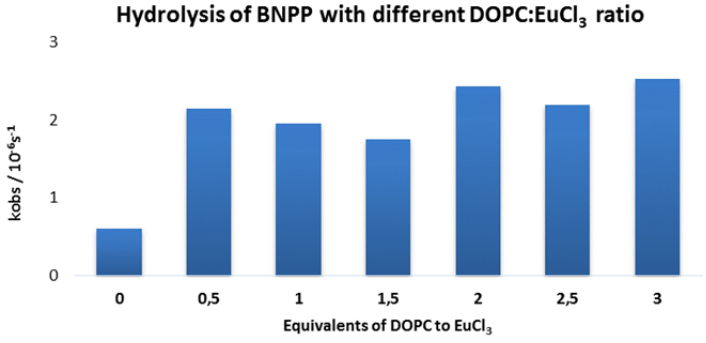

Figure S12: Pseudo first order rate constants for BNPP hydrolysis with increasing amounts of DOPC. The oncentration of europium salts is kept constant (0.1 mM).

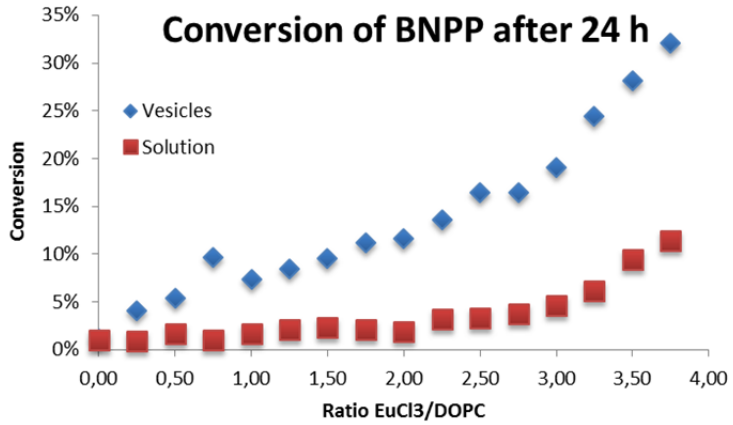

Figure S13: Conversion of BNPP after 24 h for increasing amount of europium. The DOPC concentration is constant (0.1 mM), only the europium ion concentration is increased (0.1–0.4 mM).

Second order rate constants

Second order rate constants were derived from pseudo first order rate constants recorded at different europium salt concentrations.

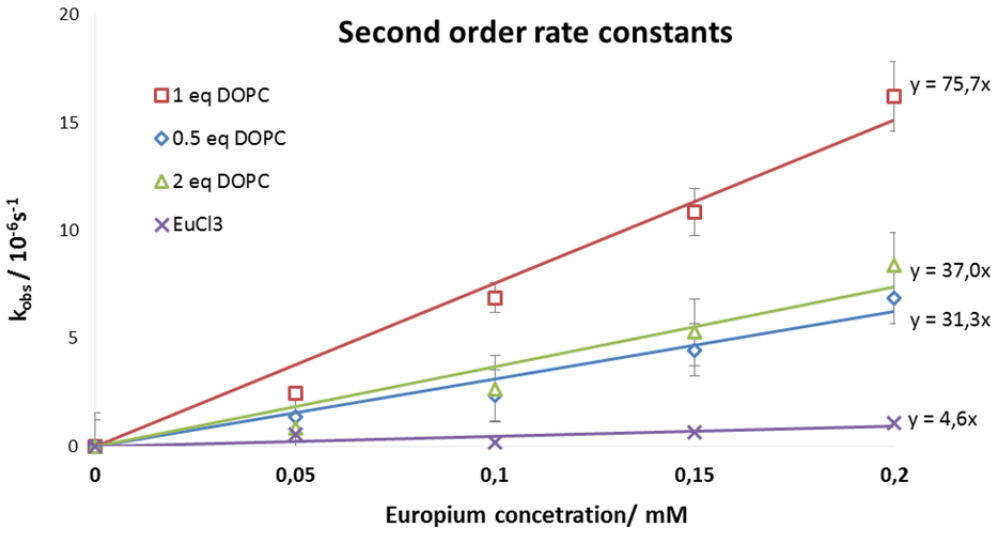

Figure S14: Determination of second order rate constant for $EuCl_3$:DOPC in ratio 1:1 (red), 1:2 (green), 2:1 (blue).

Monophosphate hydrolysis

Attempts to use monophosphate esters as substrates for hydrolysis resulted in rapid precipitation in the presence of DOPC vesicles. Therefore in most cases no hydrolytic rates could be measured. The hydrolysis of 4-nitrophenylphosphate (PNPP) by europium

chloride measured after 24 hours is comparable in the presence or absence of lipids (Figure S15).

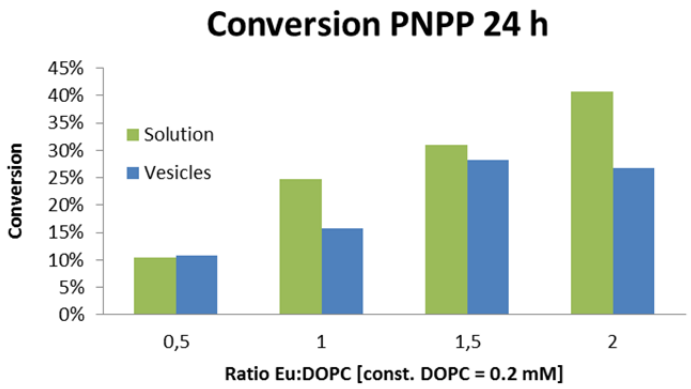

Figure S15: Conversion of PNPP (0.4 mM) after 24h using europium chloride in the presence and absence of DOPC membranes.

References

1. B. Gruber, S. Stadlbauer, K. Woinaroschy and B. Konig, *Organic & Biomolecular Chemistry*, 2010, **8**, 3704-3714.
2. B. Gruber, S. Stadlbauer, A. Späth, S. Weiss, M. Kalinina and B. König, *Angewandte Chemie International Edition*, 2010, **49**, 7125-7128.