Electronic Supplementary Material (ESI) for ChemComm.
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Electronic Supplementary Information

Low-toxicity Metallosomes for Biomedical Applications by Self-Assembly of Organometallic Metallosurfactants and Phospholipids

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This file includes:

Materials and Methods

ESI FIGURES S1-S4

ESI Table S1

Materials and Methods

1. General

The preparations and manipulation of metallosurfactants were performed under nitrogen using standard Schlenk tube techniques. All the solvents were dried and used freshly distilled. Tetrahydrofuran and methanol were distilled, respectively, over sodium/benzophenone and magnesium. Dichloromethane and acetonitrile were also distilled over sodium hydride, and all of them stored over 3Å molecular sieve. Infrared spectra were recorded with a Perkin-Elmer 2000 FT spectrometer. The NMR spectra were recorded in the *Servei de Ressonància Magnètica Nuclear de la Universitat Autònoma de Barcelona* on Bruker DPX-250, DPX-360 and AV400 instruments.

2. Metallosurfactants preparation

Metallosurfactants $Mo(CO)_5L_2$ (PCO) and $Mo(CO)_4L_2$ (TCO) were prepared using previously reported methods (L = $Ph_2PCH_2CH_2SO_3Na$).[1]

3. Preparation of Phophatidylcholine-Metallosurfactant mixed systems

The appropriate amounts of soybean phosphatidylcholine (SPC) and metallosurfactants were dissolved in chloroform/methanol (1:2 v/v) and solvent was eliminated by rotary-evaporation. The homogeneous dry film obtained was hydrated with water, vortexed and left at room temperature with magnetic stirring for at least 30 minutes before their use.

4. Dynamic Light Scattering and Z-potential Measurements

The particle size distribution was measured by dynamic light scattering (Ultrafine Particle Analyzer UPA150, USA) and results are expressed as volume distribution ($n \geq 3$).

The zeta potential of the aggregates was determined using a Zetasizer 4 (Malvern Instruments, Malvern, UK).

5. Cryo-TEM images

Micrographs of the samples were obtained at Microscopy Services of Universitat Autònoma de Barcelona using a Jeol JEM-1400 electron microscope

operating at 120 kV and equipped with a CCD multiscan camera (Gatan). During imaging samples were maintained at -177ºC with a Gatan cryoholder. 2 µL of the samples were blotted onto holey carbon grids (Quantifoil) previously glow discharged and subsequently they were plugged into liquid ethane at -180 ºC.

6. Analysis of MTS by FTIR spectroscopy

Samples contained in a plugged quartz cuvette were exposed to UVA light using a Spectroline lamp (maximum wavelength intensity at 365 nm) or illuminated with visible light from an halogen lamp (Seon) filtered using a Kodak Wraten 2B cut-off filter to eliminate any UV residual radiation. Aliquots of the samples (30 µL) were taken at different times and immediately frozen at -80ºC till they were analyzed. The corresponding spectra were obtained after putting the samples on IR windows and drying them in a desiccator connected to a vacuum pump. Spectra (50 scans) were acquired with a Varian 660-IR FTIR spectrometer at a resolution of 1 cm^{-1} at the Laboratory of Luminescence and Biomolecular Spectroscopy of Universitat Autònoma de Barcelona.

7. Direct detection of CO release by FTIR spectroscopy

1.3 mL of the samples were introduced into a 3.2-mL CaF_2 infrared cell with an optical path of 1 cm. In this way, when the cell was placed in vertical position, the infrared beam passed through the free space acquiring only the spectrum of the gas phase. The cell was submitted to radiation (UVA or visible, see 6. FTIR spectroscopy) and at given times placed into the spectrometer to monitor the composition of its gaseous free space.

8. FTIR Microscopy studies

Human dermal fibroblasts were directly cultured on CaF_2 windows into a 24 wells plate. After confluence they were treated for 12 hours with different formulations (metallosomes and small aggregates) previously diluted in cell medium (DMEM 1% SBF) at a final MTS concentration of 100 (PCO/SPC), 250 (TCO/SPC) and 1000 µM (PCO/SPC and TCO/SPC). Subsequently, the incubation medium was aspirated, cells rinsed with 0.5 mL of PBS, fixed with 0.5 mL of paraformaldehide 4%, and after extraction of the paraformaldehide, rinsed with water. CaF_2 windows were then taken from the wells and introduced into a Hyperion 3000 Microscope equipped with 36x magnification objective coupled to a Vertex 80 spectrometer (Brucker) at MIRAS beamline at Alba

synchrotron (Catalunya, Spain). Spectra were acquired selecting in an optical window of 8x8 µm.

9. Toxicity studies

Human dermal fibroblast cell viability upon incubation with MTS samples was monitored by the XTT assay. Cells were seeded at a concentration of $7.5 \cdot 10^4$ cells/mL in DMEM 10% SBF. After confluence, cells were maintained in DMEM 1% SBF for 24 h. Subsequently they were incubated for 24 hours with increasing concentrations of MTS suspensions diluted in cell medium (DMEM 1% SBF). After the incubation the medium was absorbed, cells rinsed with PBS (0.1 mL) and the cell medium and the reagent were added. Absorbance at 450 nm was read after incubation for 4 h at 37ºC.

References

[1] a) E. Parera, F. Comelles, R. Barnadas, J. Suades, *Chem. Commun*. **2014**, *50*, 3644–3660; b) E. Parera, M. Marín-García, R. Pons, F. Comelles, J. Suades, R. Barnadas-Rodríguez, *Organometallics* **2016**, *35*, 484–493.

ESI Figures S1-S4

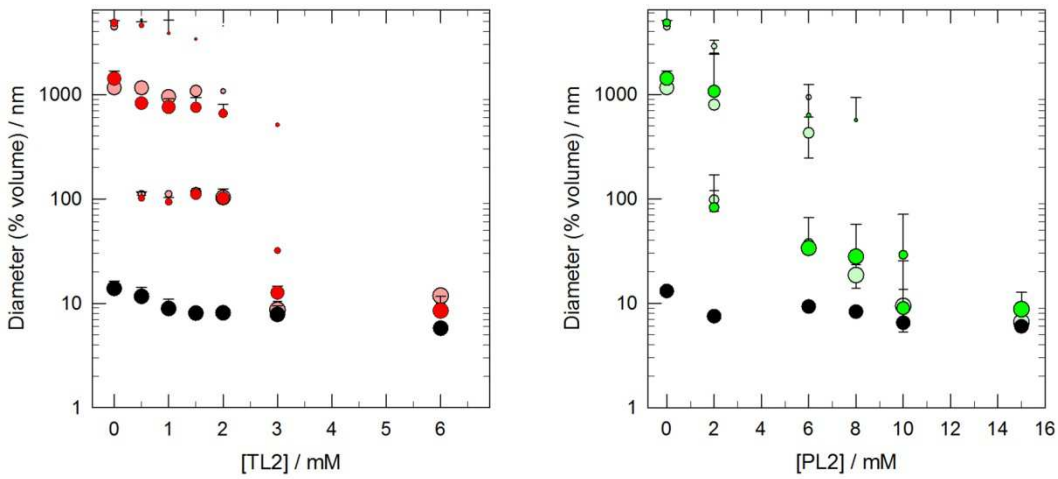

Figure S1. Size distribution of (A) TCO/SPC (red circles) and (B) PCO/SPC (green circles) aggregates as function of the concentration of MTS obtained by DLS (n≥3). Light symbols correspond to samples kept at 4ºC during 15 days. Molar concentration of SPC was kept constant at 3 mM. After addition of Triton X-100 (final concentration 10 mM) all the aggregates were disrupted and formed small mixed micelles (black circles). Size of the symbols is proportional to the amount of aggregates of a given diameter.

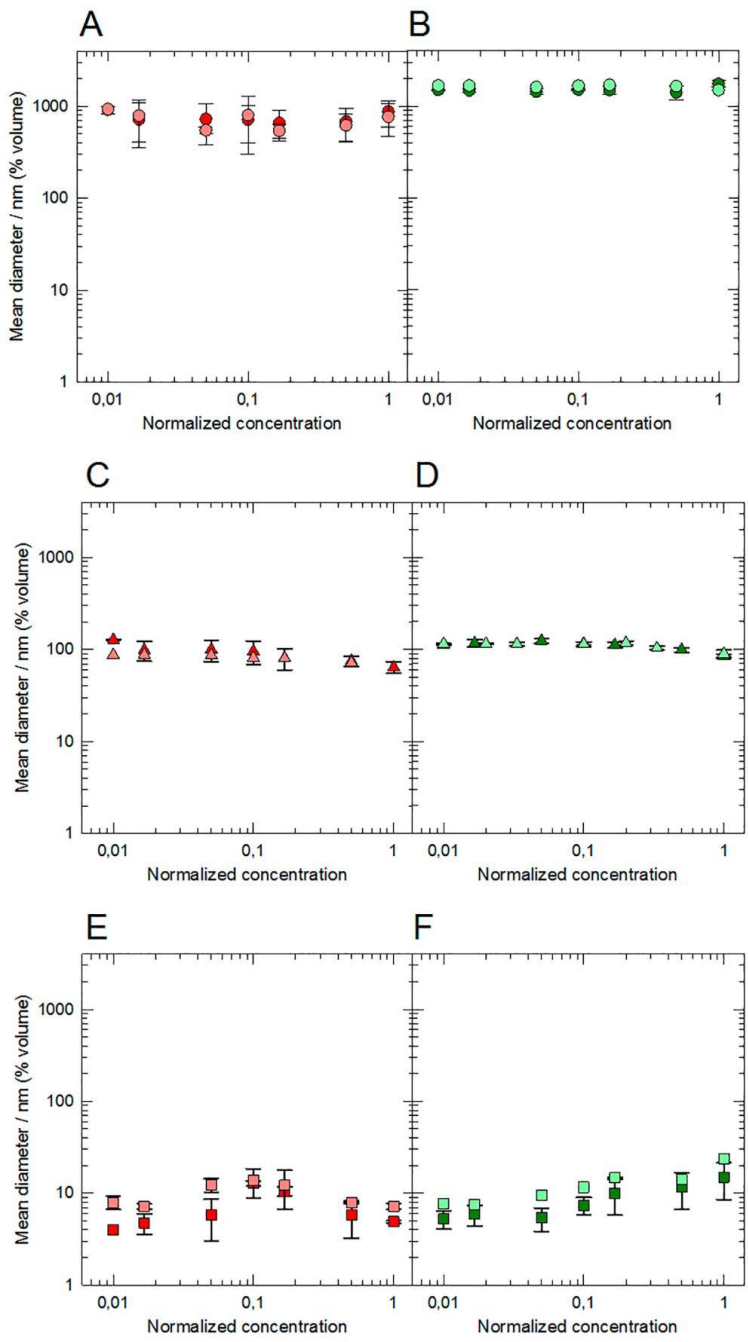

Figure S2. Size of the MTS/SPC aggregates as function of the relative concentration upon dilution and time of mixed MTS/SPC systems constituted by: A) non-extruded vesicles of TCO/SPC 6:18 mM/mM (red circles); B) non-extruded vesicles of PCO/SPC 6:18 mM/mM (green circles); C) 100 nm-extruded vesicles of TCO/SPC 6:18 mM/mM (red triangles); D) 100 nm-extruded vesicles of PCO/SPC 6:18 mM/mM; E) small aggregates of TCO/SPC 6:3 mM/mM (red squares); and F) small aggregates of PCO/SPC 6:1.8 mM/mM (green squares). The given concentrations correspond to the initial concentrations (normalized concentration=1). Dark colors correspond to the initial value of size (t=0 h) and light colors to results obtained after 24 h.

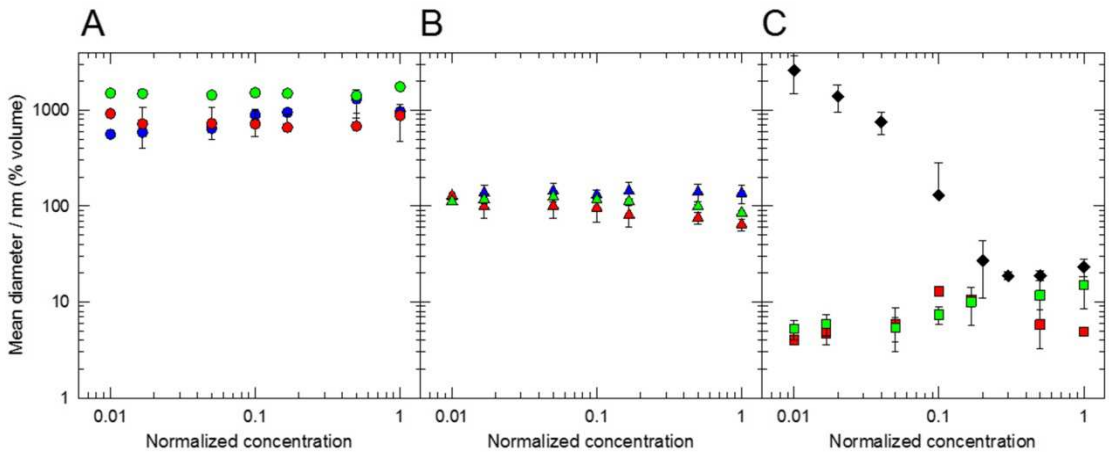

Figure S3. Size of the MTS/SPC aggregates as function of their relative concentration upon dilution of mixed systems constituted by: A) non-extruded vesicles of TCO/SPC 6:18 mM/mM (red circles) and PCO/SPC 6:18 mM/mM (green circles), compared to pure SPC liposomes 18 mM (blue circles); B) 100 nm-extruded vesicles of TCO/SPC 6:18 mM/mM (red triangles) and PCO/SPC 6:18 mM/mM (green triangles) compared to pure SPC 100 nm-extruded liposomes 18 mM (blue triangles); and C) small aggregates of TCO/SPC 6:3 mM/mM (red squares) and PCO/SPC 6:1.8 mM/mM (green squares), compared to an initial bicellar suspension constituted by dimyristoyl PC/dihexanoyl PC (302:100 mM/mM) (black diamonds). The given concentrations correspond to the initial concentrations (normalized concentration = 1).

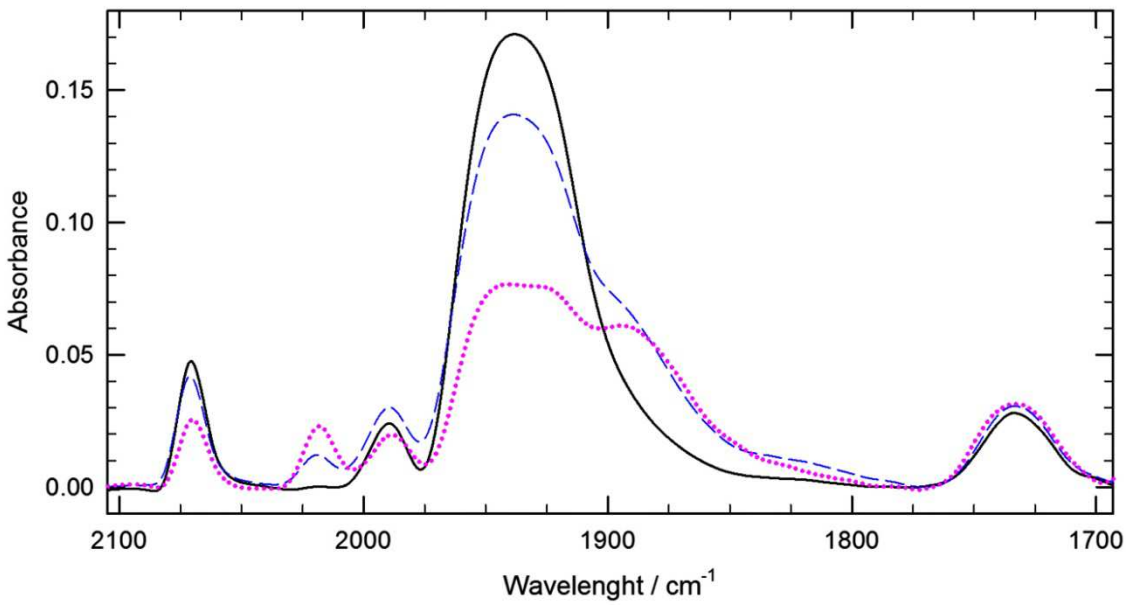

Figure S4. Infrared spectra of PCO/SPC 10:3 mol/mol under irradiation with UVA (365 nm) at 0 (black), 85 (blue) and 300 min (magenta).

ESI Table S1

SPC	TL2/SPC		PL2/SPC	
	1:3 mol/mol	6:3 mol/mol	2:3 mol/mol	10:3 mol/mol
-17.1 ± 1.0 mV	-50.3 ± 2.1 mV	-34.5 ± 1.4 mV	-71.6 ± 3.5 mV	-34.8 ± 6.3 mV

Table 1. Z-potential of SPC liposomes and mixed aggregates as function of their composition.