Electronic Supplementary Information

Immobilising Giant Unilamellar Vesicles with Zirconium Metal-Organic Framework Anchors

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Materials and Methods

General Chemicals. All reagents were purchased from Sigma Aldrich, Fisher Scientific or Avanti Polar Lipids and used as received, unless otherwise stated.

Giant Unilamellar Vesicle Preparation. GUVs were grown using a literature-based electroformation technique.[S1] 1-Palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (sodium salt) (POPG) and cholesterol were purchased from Avanti Polar Lipids (dissolved in chloroform to a concentration of 10 mg/mL). Topfluor® cholesterol and Liss Rhod PE were also purchased from Avanti Polar Lipids and were made up to 1 mg/mL in chloroform. For a typical lipid mix: lipids POPC and POPG, and cholesterol were mixed in a 4 : 1 : 1 molar ratio, and this solution was made to and an overall concentration of 1 mg/mL. An additional 0.1 mol % of fluorescent tag (Topfluor® cholesterol or Liss Rhod PE) was added to the mix for confocal microscopy.

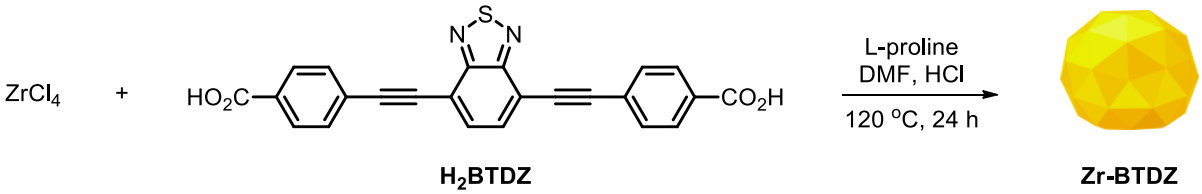
General Electroformation Procedure. The non-conductive sides of two indium−tin oxide (ITO)-coated glass plates were marked with a circle of 15 mm diameter in the center of each. An aliquot of 10 μL of lipid mixture was gently spread onto the electrically conductive sides of each of the ITO plates within the drawn circles using the tip of a microsyringe, while a gentle flow of air was passed over the solution until the chloroform had evaporated, leaving a thin lipid film. The ITO slides were dried in a vacuum desiccator for at least 30 mins to fully remove the organic solvent. During this time, the slides were loosely covered with aluminium foil to keep the samples in the dark. A 2 mm thick O-ring with a diameter of 15 mm had a small amount of silicon grease applied to it and was affixed onto one of the two slides. 350 μL of a premade electroformation buffer solution (0.1 M sucrose and 1 mM, pH 7.4 HEPES in water) was filled into the chamber. ***NOTE****: if MOF immobilisation is desired, a microspatula tip (approx. 5 mg) of MOF should be pre-mixed into the electroformation buffer sample by sonication, and when applied to the ITO slide this suspension should be left to settle for 10 to 15 minutes prior to the next stage.* The two ITO slides were then put together to form a closed chamber. For latex microparticle experiments, 40 μL of latex suspension was used in substitution of MOF particles. Using the NANION Vesicle Prep Pro, a sinusoidal AC electric field at 10 Hz was applied for electroswelling the lipid films. The sample was shielded from external light during this time. In the first phase of the electroswelling process, the amplitude of the applied field was linearly increased from 0.1 V (peak to peak) to 0.5 V (peak to peak) over 30 min, while the temperature was increased from 21 °C to 35 °C.

The voltage was then increased further over the next 15 minutes to 1.6 V. Thereafter, the amplitude of the AC field was kept constant at 1.6 V for 2 h to grow the vesicles. Finally, during the last 5 mins, the voltage was slowly lowered to 0 V. These details are outlined in Figure S7. Once electroformed, GUVs were made up to 5 mL in a resuspension buffer solution (0.1 M glucose and 1 mM, pH 7.4 HEPES in water). Both this step and transferring the resulting solution of GUVs into imaging chambers were done using a plastic pipette with the end cut off to leave an opening of at least 5 mm. This was done in order to prevent any pressure build up capable of lysing the GUVs during these transfer processes. At soonest notice, the GUV-containing solution is transferred to an appropriate imaging chamber and taken to be imaged by confocal microscopy.

Confocal Microscopy. Confocal imaging was performed at the UNB Microscopy and Microanalysis Facility with a Leica TCS-SP2 CLSM microscope using Leica confocal software package version 2.61 build 1537. Images were collected with the excitation wavelength of 488 nm and fluorescence was collected in the following bands: (i) 500-550 nm for Topfluor® cholesterol-stained vesicles, (ii) 550-600 nm for Liss Rhod PE-stained vesicles, and (iii) 600-650 nm for samples of **Zr-BTDZ**. False green, red and yellow colours were then assigned to each of these collections, respectively. Images of a **Zr-BTDZ**-immobilised GUV showing the separate channels are provided below (Fig. S8).

Synthetic Procedures

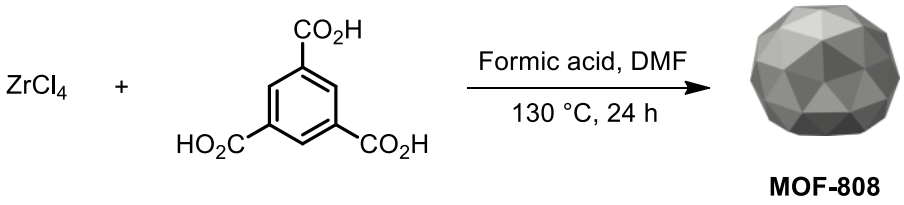
Zr-BTDZ



H_2BTDZ **Zr-BTDZ**

Zr-BTDZ was synthesised following a literature procedure.[S2] $ZrCl_4$ (0.026 g, 0.11 mmol, 1.0 eq), **H_2BTDZ** (0.048 g, 0.11 mmol, 1.0 eq), L-proline (0.052 g, 0.45 mmol, 4.0 eq), and DMF (5 mL) were added to a 50 ml PYREX reagent bottle and sonicated for 10 minutes. Concentrated HCl (0.01 mL) was added and the mixture was sonicated for a further 10 minutes before being placed in an oven at 120 °C for 24 hours. The bulk material was collected from the bottle upon completion, centrifuged once with fresh DMF (30 ml) and twice with acetone (2 x 30 ml), before being dried under vacuum to give **Zr-BTDZ** as a yellow powder.

MOF-808



MOF-808

MOF-808 was synthesised following a literature procedure.[S3] $ZrCl_4$ (0.233 g, 1.00 mmol, 3.0 eq.) and 1,3,5-benzenetricarboxylic acid (0.0701 g, 0.333 mmol, 1.0 eq.) were added to a WHEATON pressure plus bottle and dissolved in a mixture of DMF (15 mL) and formic acid (>98%, ACROS, 15 mL, 400 mmol). The mixture was sonicated until total dissolution, and then left in an oven at 130 °C for 24 h. A white powder evolved during this time, which was collected by vacuum filtration and washed with DMF (3 x 15 mL) and acetone (3 x 15 mL). The solid was dried under vacuum overnight to give MOF-808 as a white powder.

For both as-synthesised MOFs, measured powder X-ray diffraction patterns closely matched simulated patterns generated from existing crystallographic data (Figures S9 and S10, CIFs sourced from CSD).

Supplementary Figures

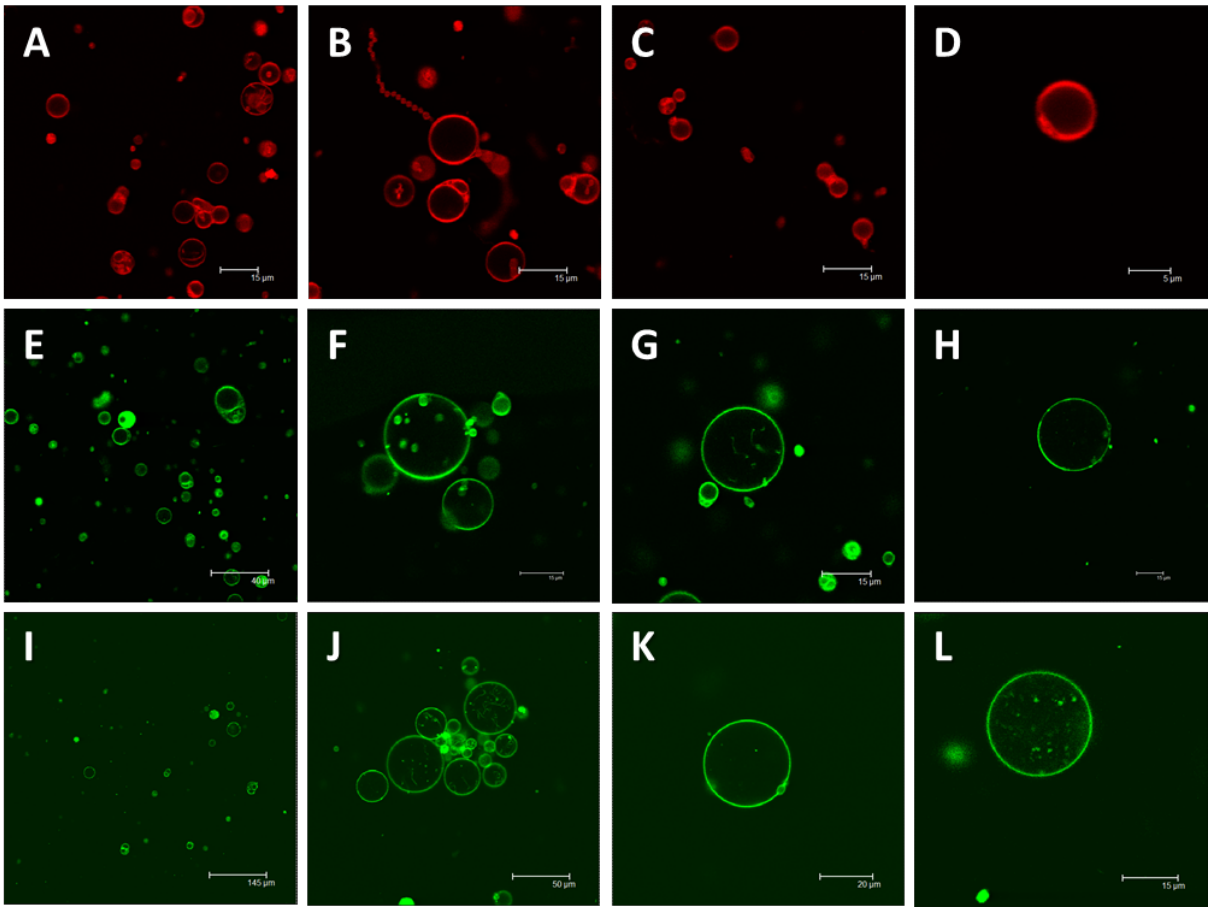


Figure S1. Confocal microscope images of GUVs. Images A-D depict GUVs formed from the POPC : POPG : cholesterol lipid mix stained with Liss Rhod PE (false red colour), E-H depict GUVs formed from the POPC : POPG : cholesterol mix stained with Topfluor® cholesterol (false green colour) and I-L depict GUVs formed from the POPC : DGS:NTA : cholesterol mix stained with Topfluor® cholesterol (also false green colour).

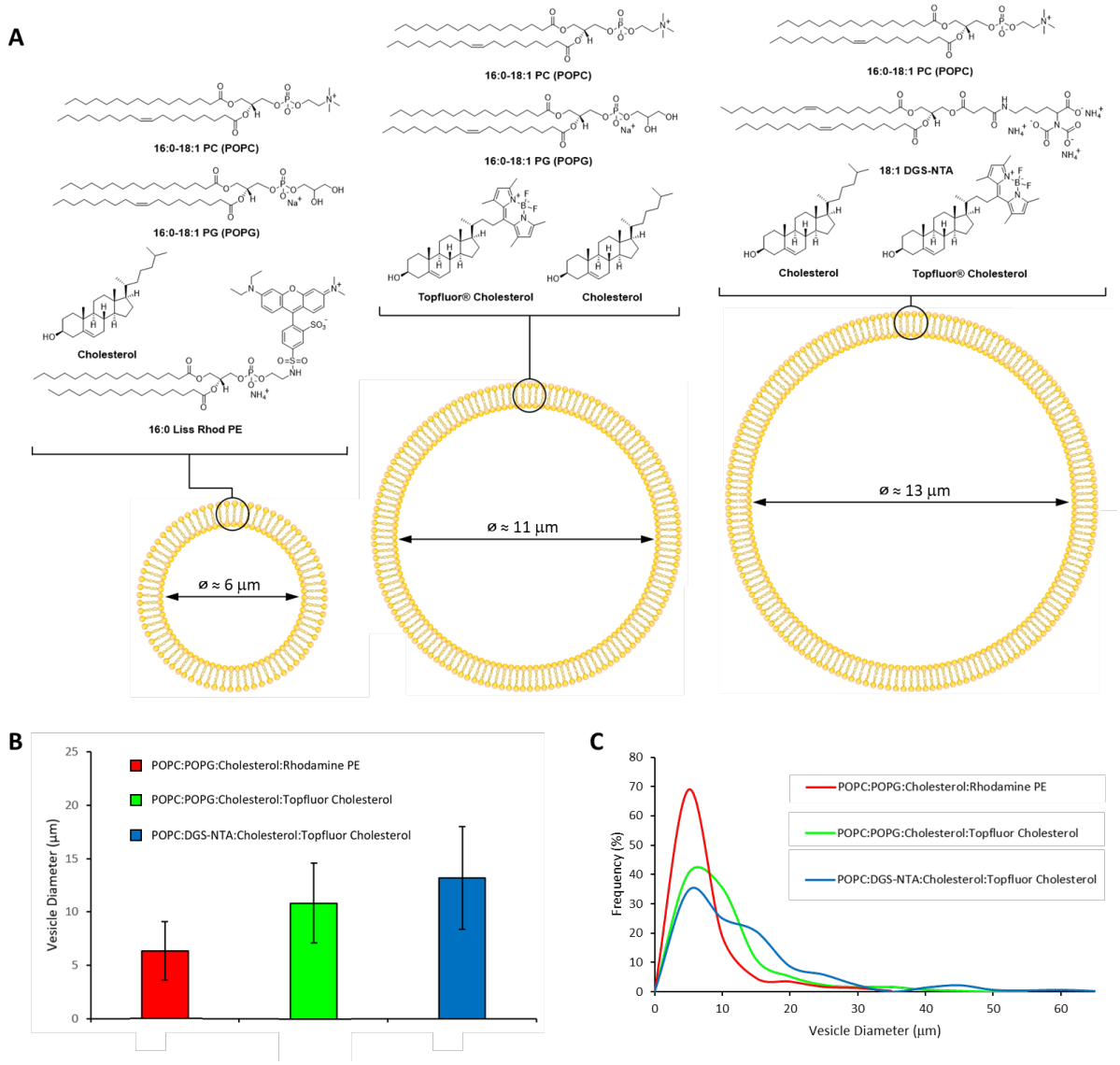


Figure S2. (A) Schematic representation of the disparate sizes of GUVs formed from different lipid compositions; (B) Mean diameters of GUVs with differing compositions (error bars reflect one standard deviation); and (C) Size distribution of GUVs based on their lipidic makeup (diameters rounded to nearest 5 µm). Graphs B and C were produced from a data set of 1,761 vesicles in total, which were sampled from nine separate runs (three per lipid mix).

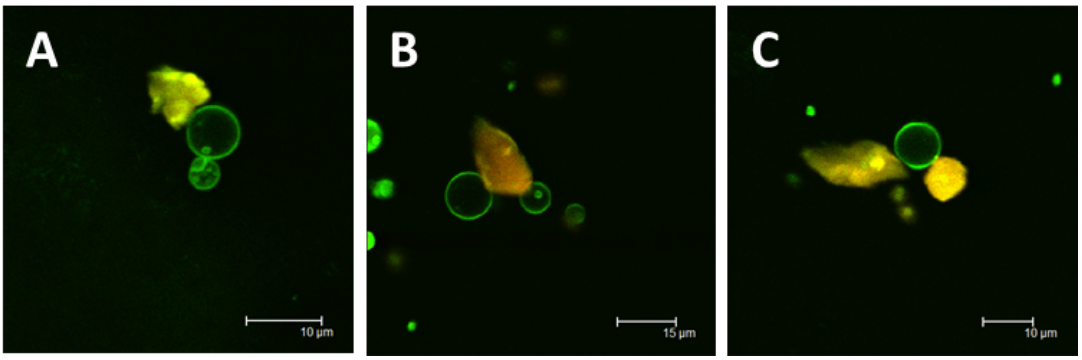


Figure S3. Confocal microscope images of (A) **Zr-BTDZ**/GUV/GUV, (B) GUV/**Zr-BTDZ**/GUV and (C) **Zr-BTDZ**/GUV/**Zr-BTDZ** adducts.

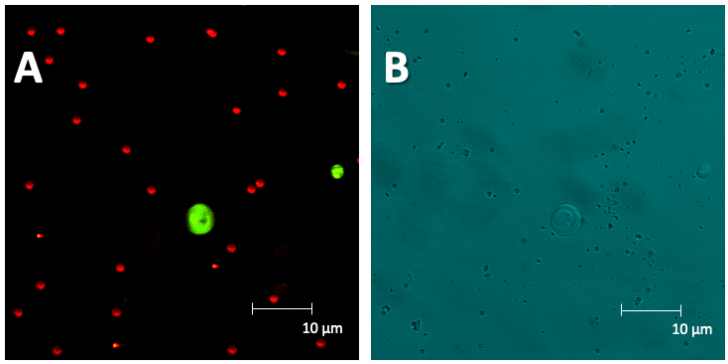


Figure S4. Confocal microscope images of GUVs (false green colour) in the presence of carboxylate functionalised red-fluorescent latex microparticles (2 µm diameter). Image A is two channel overlay illustrating positions of GUVs (green) and microparticles (red) and B is the corresponding bright-field image.

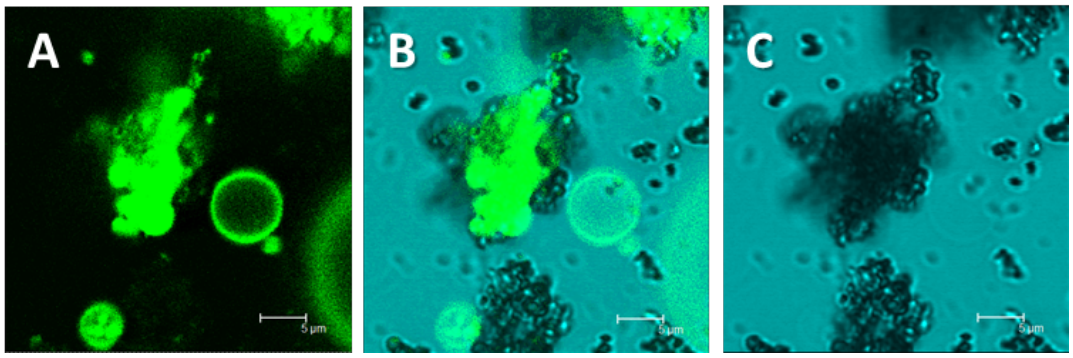


Figure S5. Confocal microscope images of GUVs (false green colour) immobilised with **MOF-808**. Images A and C are corresponding fluorescence and bright-field images, respectively, and image B is an overlay of the two.

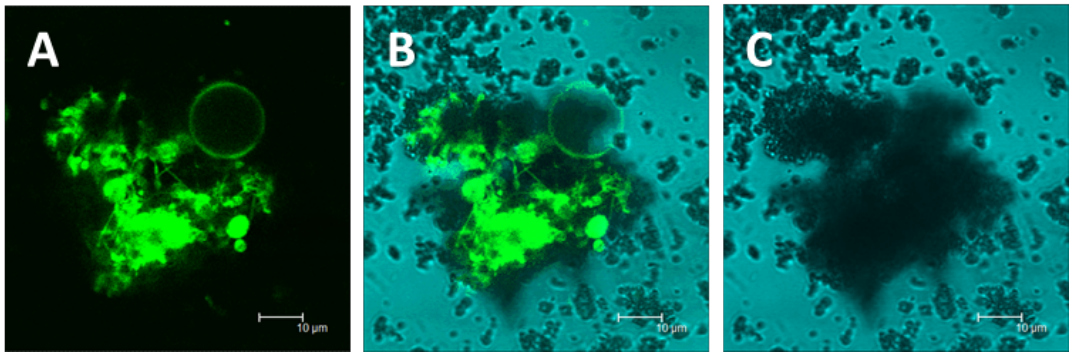


Figure S6. Additional confocal microscope images of GUVs (false green colour) immobilised with **MOF-808**. Images A and C are corresponding fluorescence and bright-field images, respectively, and image B is an overlay of the two.

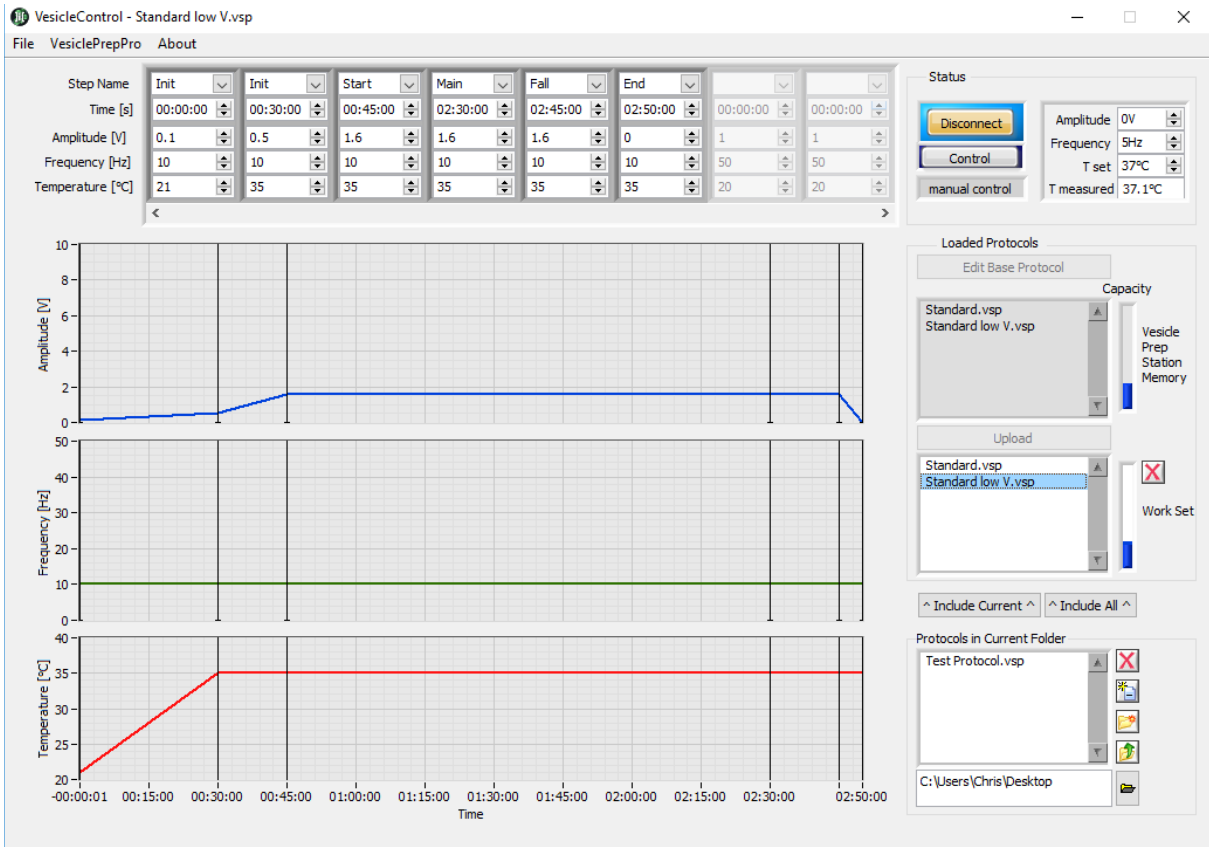


Figure S7. Electroformation protocol parameters displayed on the Nanion Vesicle Prep Pro *VesicleControl* software.

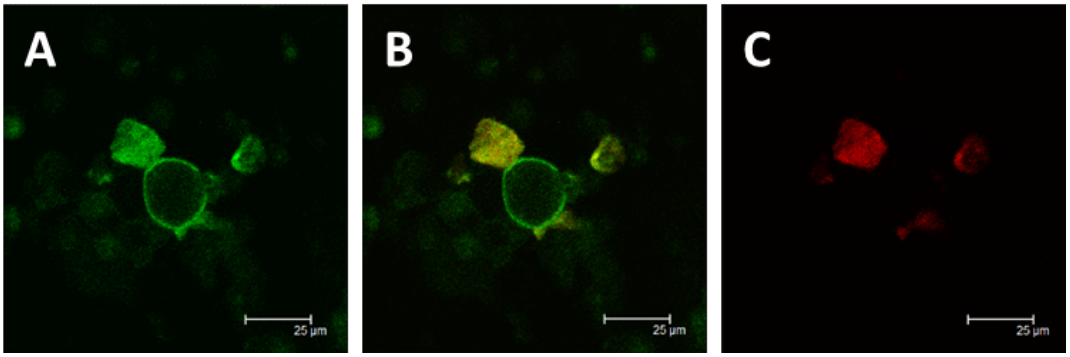


Figure S8. Additional confocal microscope images of a GUV immobilised by a **Zr-BTDZ** particle. Images A and C show the collections from 500-550 nm (both MOF and GUV fluorescence observed) and 550-600 nm (only MOF fluorescence observed), respectively and B shows these two channels combined.

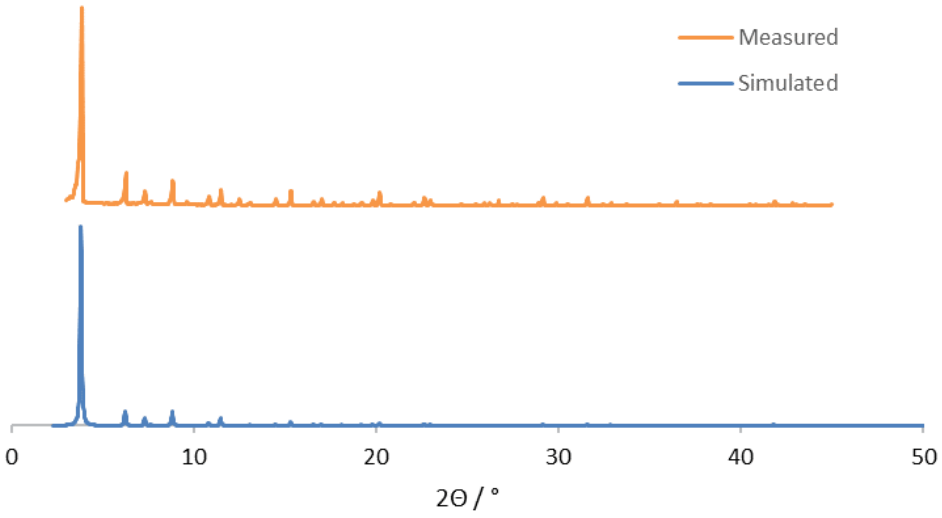


Figure S9. PXRD patterns (measured and simulated) for **Zr-BTDZ**.

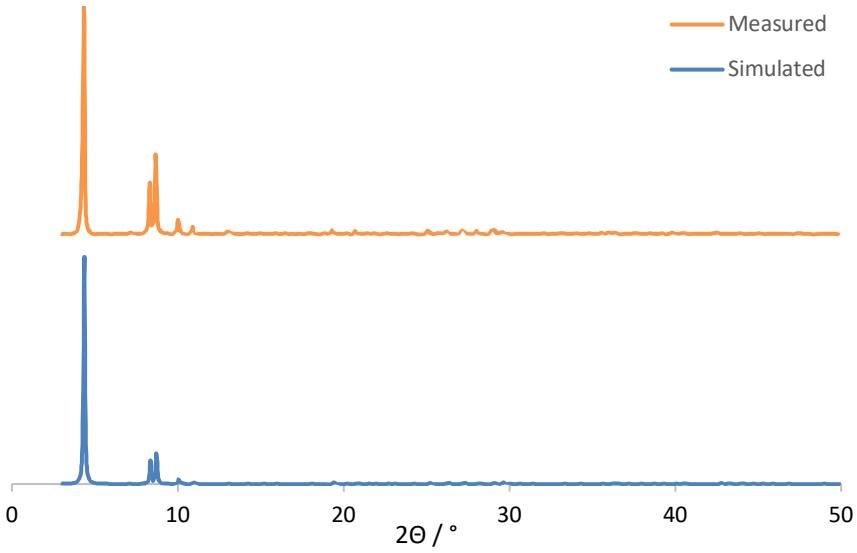


Figure S10. PXRD patterns (measured and simulated) for **MOF-808**.

Supplementary Videos

Supplementary Video 1 - A computer generated animation depicting GUV electroformation and adhesion to **Zr-BTDZ** particle. This video was created and rendered using Blender version 2.82.

Supplementary Videos 2 and 3 - Time-lapse videos showing GUVs anchored by MOF **Zr-BTDZ**. These videos each comprise 100 frames and span a real-time duration of 2 minutes 42 seconds.

Supplementary Video 4 - Z-stack video of **Zr-BTDZ**/GUV adduct. This video comprises 41 frames and spans a total length in the z direction of 91.3 µm.

Supplementary Video 5 - Long time-lapse video of **Zr-BTDZ**-anchored vesicle. This video comprises 420 frames and spans a real-time duration of 1 hour 45 minutes. A timer is superimposed in the top left of this video.

Supplementary Video 6 - Time-lapse video depicting **Zr-BTDZ**/GUV/GUV sandwich. This video comprises 100 frames and spans a real-time duration of 2 minutes 42 seconds.

Supplementary Video 7 - Z-stack video of GUV/**Zr-BTDZ**/GUV sandwich. This video comprises 36 frames and spans a total length in the z direction of 80.1 µm.

Supplementary Video 8 - Time-lapse video of **Zr-BTDZ**/GUV/**Zr-BTDZ** sandwich. This video comprises 100 frames and spans a real-time duration of 2 minutes 42 seconds.

Supplementary Video 9 - Z-stack video of **Zr-BTDZ**/GUV/**Zr-BTDZ** sandwich. This video comprises 33 frames and spans a total length in the z direction of 73.5 µm.

Supplementary Video 10 - Time-lapse video depicting **MOF-808**/GUV stability. This video comprises 100 frames and spans a real-time duration of 2 minutes 42 seconds.

Supplementary Video 11 - Z-stack video of **MOF-808**/GUV adduct. This video comprises 77 frames and spans a total length in the z direction of 171.5 µm.

References

S1. M. I. Angelova and D. S. Dimitrov, *Faraday Discuss. Chem. Soc.*, 1986, **81**, 303.

S2. R. J. Marshall, Y. Kalinovskyy, S. L. Griffin, C. Wilson, B. A. Blight and R. S. Forgan, *J. Am. Chem. Soc*., 2017, **139**, 6253.

S3 W. Liang, H. Chevreau, F. Ragon, P. D. Southon, V. K. Peterson and D. M. D'Alessandro, *CrystEngComm*, 2014, **16**, 6530.