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COMMUNICATION

Immobilising Giant Unilamellar Vesicles with Zirconium Metal-Organic Framework Anchors

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Lipid bilayer vesicles have provided a window into the function and fundamental properties of cells. However, as is the case for most living and soft matter, vesicles do not remain still. This necessitates some microscopy experiments to include a preparatory immobilisation step. Here, we describe a straightforward method to immobilise giant unilamellar vesicles (GUVs) using zirconium-based metal-organic frameworks (MOFs) and demonstrate that GUVs bound in this way will stay in position on a timescale of minutes to hours.

As a result of their cell-like size (1 - 100 μm), spherical shape and single-bilayer composition, in addition to their straightforward and low-cost preparation, giant unilamellar vesicles (GUVs) represent a best-in-class biomimetic membrane model.¹ GUVs are typically prepared through electroformation, a procedure first described by Angelova and Dimitrov in 1986.² During electroformation, vesicles will peel away from indium-tin oxide (ITO)-coated glass slides and enter the surrounding solution as a low voltage is passed through the apparatus. Somewhat surprisingly, this protocol remains relatively unchanged as used in recent years.³⁻⁵ GUVs have been employed as models to provide insights into the presence and composition of membrane domains,^{6,7} membrane permeability^{8,9} and mechanical information such as elasticity.^{10,11} Many of these investigations rely on GUV imaging, or the manipulation of GUVs while under the microscope. Long-term observations or visualising an entire GUV through confocal z-stacking can be especially challenging with non-immobilised GUVs. The diffusive motion of GUVs in aqueous media can lead to misalignment of image slices, the introduction of artifacts, or simply preclude data measurement altogether. Moreover, several quantitative fluorescence-based techniques such as fluorescence recovery after photobleaching (FRAP),¹²

fluorescence correlation spectroscopy (FCS)¹³ and fluorescence-lifetime imaging microscopy (FLIM)¹⁴ require that the sample remain immobile during data acquisition.

At present, the most commonly used and well-established methods of immobilising GUVs entail specific biotin-avidin binding sites. A trace amount of biotinylated lipid¹⁵ or cholesterol¹⁶ is typically incorporated into a sample of vesicles, and immobilisation occurs over an avidin-exposing surface. A modified methodology has been developed, wherein a glass coverslip is treated with an APTES-glutaraldehyde composite.¹⁷ The authors employ amine-functionalised lipids/proteins in order to bind vesicles/cells to their APTES-glutaraldehyde anchor. While largely effective, these approaches can lead to changes in composition of the affixed area.^{18,19} Steric entrapment represents another general strategy, and has been achieved within silica matrices,²⁰ hydrogels,²¹ or, most recently, an agarose polymer gel.²² In such cases, as the vesicles are fully immobilised by the surrounding matrices, experimental methods that probe their morphological responses (such as electrodeformation²³ or line-tension-induced membrane curvature)²⁴ are no longer viable. It is also possible to trap vesicles with an electric field,²⁵ though this too will impart tension on the membrane. Finally, nanoparticles may be used for vesicle immobilisation. One approach sees encapsulated magnetic nanoparticles causing vesicles to settle when an external magnetic field is applied.²⁶ Charged nanoparticles adsorbed to the outer surface of vesicles have also been shown to affix them to surfaces with opposite charge.²⁷ While effective, these methods require sophisticated experimental setups that may not be universally available.

Zirconium ions have been shown to strongly bind to the phosphate groups of both phosphopeptides²⁸⁻³⁰ and phospholipid membranes.³¹ Recently, Li and co-workers reported a liposomes-zirconium-exosomes sandwich structure that effectively detects exosomes using zirconium-phosphate coordination chemistry.³² This affinity has also been used to decorate the surfaces of zirconium-based metal-organic framework (MOF) nanoparticles with phosphate-terminated oligonucleotides^{33,34} and lipids^{35,36} for biological applications.

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Zirconium MOFs are well-known for their high stability, particularly under physiological conditions.³⁷

Here, we immobilise GUVs using micron-sized zirconium MOF particles. GUVs of two different lipid compositions were successfully immobilised using a straightforward methodology with minimal changes to a typical electroformation protocol. We propose that the GUVs are bound by coordination interactions between their phospholipid head groups and exposed zirconium ions on the surface of the MOF.³⁶ This strategy allows for GUV immobilisation on a scale of minutes to hours with little to no influence on membrane properties.

Three samples of GUVs with differing constituent lipids were electroformed and imaged (ESI Fig. S1). First, a standard lipid mix of 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) sodium salt (POPG) and cholesterol in a 4 : 1 : 1 molar ratio (to an overall lipid concentration of 1 mg/mL) was made, and stained with 0.1 mol % of either 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) ammonium salt (Liss Rhod PE; ESI Fig. S2A, left) or Topfluor® cholesterol (BODIPY-labelled cholesterol; ESI Fig. S2A, centre). We were also interested in probing whether lipids with carboxylate functional groups would encourage GUV-MOF binding. As such, a third lipid mix was prepared with 1,2-dioleoyl-sn-glycero-3-[(N-(5-amino-1-carboxypentyl)iminodiacetic acid)succinyl] ammonium salt (DGS-NTA) in place of POPG (ESI Fig. S2A, right). Notable differences in the sizes of resulting GUVs were measured and this disparity is presented in ESI Fig. S2. In addition to producing the smallest GUVs, the Liss Rhod PE-containing mix led to GUVs with a fluorescent emission of a similar wavelength to that of our MOF immobiliser, making it difficult to visually separate MOF from GUV during confocal imaging. For these reasons, only the two Topfluor® cholesterol-containing lipid mixes were taken forward.

An appropriate MOF was selected to (i) immobilise GUVs through zirconium-phosphate/zirconium-carboxylate coordination chemistry and (ii) fluoresce in a region of the visible spectrum so as to be clearly identifiable when imaged alongside GUVs. We have previously reported a series of zirconium MOFs, one of which displays a large bathochromic shift (around 90 nm) when in aqueous suspension.³⁸ In this document, the MOF is termed **Zr-BTDZ**, as it comprises a benzothiadiazole-containing linker (synthesis is shown in Scheme 1). The substantial red shift in the presence of water puts the fluorescent emission of **Zr-BTDZ** ($\lambda_{\text{max}} \approx 590$ nm) comfortably far away from the emission of the Topfluor® cholesterol tag ($\lambda_{\text{max}} = 508$ nm), allowing for clear and distinct imaging of both materials.

With **Zr-BTDZ** in hand, the electroformation procedure was modified so as to incorporate the MOF. Immediately prior to sealing the setup and applying a low voltage across the lipid-deposited ITO-coated slides, a small sample (microspatula tip, approx. 5 mg) of **Zr-BTDZ** was sprinkled into the buffer solution and allowed to settle onto the slide (the full experimental procedure is provided in the ESI). GUVs were then electroformed, and naturally adhered to **Zr-BTDZ** particles. A

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Fig. 1: Confocal microscopy images of electroformed GUVs (false green colour) immobilised by **Zr-BTDZ** particles (false yellow colour). Images A and B show vesicles composed of the POPC : POPG : cholesterol lipid mix, while C and D show vesicles composed of the POPC : DGS:NTA : cholesterol mixture.

Fig. 2: Schematic depiction of the zirconium-phosphate binding (shown in red) that underpins GUV anchoring.

CGI animation is provided to illustrate the formation and anchoring processes (ESI-Video 1). We followed a typical procedure for GUV synthesis and imaging, by electroforming our GUVs in a sucrose-containing buffer and resuspending them in a glucose-containing buffer. This is routinely done in order to make the GUVs easier to image, as the heavier sugar inside the GUVs causes them to stay near the bottom of the imaging wells.³⁹ While GUVs that are formed this way are still mobile, we exploit the technique further here, in that MOF-GUV interactions are thereby encouraged. The protocol was carried out using the two previously-mentioned lipid mixes, and confocal microscopy images/videos of the resulting MOF-GUV adduct are shown in Fig. 1 and in ESI-Videos 2-4, respectively.

MOF-immobilised GUVs would typically be monitored across the duration of a 3- or 4-hour imaging session, establishing that this approach is suitable for long-term observations. This proposition is supported by a 1 hour 45-minute time-lapse video of an immobilised GUV (ESI-Video 5). Further, in addition to two-component adducts, GUVs bound to more than one MOF particle and a single MOF particle binding two distinct GUVs were also observed (ESI Fig. S3 and Videos 6-9). These unexpected multi-component structures, and the fact that incorporating carboxylate-functionalised lipid molecules into the membrane did not appear to further promote MOF-GUV interactions, are a testament to the robustness of the zirconium-phosphate coordination that underpins this work. These zirconium-phosphate bonds are illustrated in a schematic shown above in Fig. 2. Concomitantly, we performed a control experiment substituting **Zr-BTDZ** with carboxylate-functionalised red fluorescent labelled latex microparticles (2 μm ; ESI Figs S4), suggesting this interaction on the surface of the MOF, where free carboxylate may be found, is of lesser importance in anchoring GUVs.

The practical nature of this method was further established by immobilising GUVs with a second Zr-based MOF, **MOF-808**.⁴⁰ Although **MOF-808** does not fluoresce within the

Fig. 3: Confocal microscopy images of GUVs with Lucifer yellow dye in the surrounding solution (false green/ yellow-green colour). Images A and B show free GUVs and images C and D show GUVs affixed to **Zr-BTDZ** MOF particles.

visible spectrum and our as-synthesised **MOF-808** particles were approximately 25 times smaller than those of **Zr-BTDZ**, we were able to show that GUVs could be readily affixed with the same general procedure (ESI Figs S5-6 and Videos 10 and 11).⁴¹ The fact that this immobilisation technique remained effective when a MOF with contrasting organic ligands, intrinsic network topology and particle size was used highlights the broad applicability this approach offers for future studies involving GUVs.⁴²

Finally, experiments to probe membrane stability post-affixation were carried out. For this, we followed a standard approach for validating membrane integrity.⁴³ GUVs were electroformed and then resuspended in a buffer solution containing Lucifer yellow dye (LYD). It was then confirmed by confocal microscopy that the vesicle lumen remained dye-free, as molecules of LYD cannot freely diffuse across the GUVs' membranes. This was then repeated for MOF-immobilised GUVs, and no dye was seen within the GUVs in this case either. If GUV anchoring were to disrupt the membrane in any way, GUVs would not be discernible by confocal microscopy during these experiments as LYD would permeate into the vesicles and lead to the whole sample fluorescing equally. The images in Fig. 3 show that this did not occur, broadly validating that anchoring GUVs with our method does not lead to diminished membrane stability.

In this work, we have developed a straightforward method to efficiently immobilise GUVs using micron-sized zirconium MOF particles. Two lipid mixtures were used, though the incorporation of carboxylate-functionalised lipid molecules did not further promote GUV-MOF interactions, which we attribute to the sufficiently strong nature of zirconium-phosphate bonds. The immobilisation protocol defined here is facile and

reproducible - simply involving the addition of a small sample of MOF to the lipid-coated ITO slide prior to electroformation. GUVs will then autonomously affix to the surface of MOF particles through zirconium-phosphate binding. Under these conditions, immobilised GUVs were observed on a scale of minutes- to hours, and we have shown that immobilisation in this way does not cause any notable damage to the membrane. On account of both its simplicity and efficacy, this strategy may find application across long term imaging techniques, e.g. high-quality or 3D imaging, the collection of data via fluorescence-based techniques, single-vesicle drug loading, etc. With these prospects in mind, the fact that GUVs are immobilised over an extended period without the structural integrity of the membrane being overly compromised is a standout aspect of this method.

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- 41 We note that our MOF-808 immobilisation experiments show fluorescence on the surface of the MOF, which we attribute to the lysis of anchored GUVs. We are currently investigating this further. Nonetheless, we have demonstrated here the effective use of MOF-808 to successfully immobilise GUVs.
- 42 These results, in addition to corroborating the scientific foundation of our GUV-immobilising protocol, show that costly materials that require specific and sophisticated chemistry - such as **Zr-BTDZ** - need not be employed. **MOF-808** can be readily synthesised from inexpensive ZrCl_4 and trimesic acid starting materials, and we posit that this approach is accessible to any modern laboratory.
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