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# Reconstitution of OmpF Membrane Protein on Bended Lipid Bilayers: Perforated Hexagonal Mesophases

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## Supporting Information

*Materials*

Dimodan U/J, was a gift of Danisco (Denmark) and was used as received. This commercial-grade form of monolinolein contains more than 98 wt % monoglyceride. The same batch of Dimodan was used throughout the whole work. Tetradecane (for sample preparation), caffeine (for the release studies) and all the necessary salts for the different used buffers were purchased from Sigma Aldrich-Chemie (Schnelldorf, Germany).

*OmpF expression and purification*

The OmpF wildtype protein was expressed and purified using a previously described protocol (Ref S1). Briefly, OmpF was expressed into the host E coli BE strain BL21 (DE) opm8 (F_ hsdSB (rB_ mB) gal ompT dcm(DE3) DlamB ompF::Tn5 DompA DompC).
10 mL of overnight culture were used to inoculate 1L LB medium (250 rpm, 37˚C). The OmpF expression was induced at an OD600 of 0.6 by addition of isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 1 mM. Cells were further grown until an OD600 2.0 when they were harvested by centrifugation (20 min, 6000rpm, 4˚C).
Cells were resuspended in 10 ml 20 mM Tris-Cl pH 8.0, 2% SDS buffer/ 1g cell pellet and the mixture was passed three times through a high-pressure homogenizer at 2000 bar. The membranes were separated by centrifugation (60 min, 22000 rpm, 4˚C).
A pre-extraction step followed in 0.125% octyl-POE in 20mM phosphate buffer pH 7.4 with an incubation time of 1 h (37°C, 250 rpm). The membrane fractions were removed by centrifugation (40 min, 4°C, 40000 rpm) and further treated with 3% octyl-POE in 10mM phosphate buffer pH 7.4 for OmpF extraction. The solubilised OmpF was separated from the membranes by centrifugation (20 min, 40000 rpm, 4˚C).
The purity of the extracted protein was verified by SDS-acrylamide gel electrophoresis (12%) (see S.I. Fig S6) and the protein concentration was determined spectrophotometrically (260 nm).

*Sample preparation*

The composition of each LLC system (empty and loaded) was identical with the sole difference of the LLC loaded with protein, where 0.24 or 0.5 wt% OmpF dispersed in protein buffer (10mM phosphate buffer with 3 wt% octyl-POE) was incorporated into the LC mesophases. The reverse columnar hexagonal phase ($H_{II}$) contained 72 wt% MLO, 8 wt% tetradecane and 20 wt% buffer (protein solution). For the samples used in the release studies, 2 wt% of caffeine was dispersed into the protein buffer (protein solution for samples with OmpF) used to prepare the hexagonal phase.
A stock solution consisting of MLO and tetradecane in a ratio of 90:10 (w:w) was initially prepared by mixing weighed amounts of the two components and vortexing until a homogenous mixture was obtained. The same stock solution was used consistently throughout the study. The liquid crystalline mesophases were then prepared by mixing weighed quantities of MLO:tetradecane mixture and protein buffer (protein solution for samples with OmpF) inside sealed Pyrex glass tubes followed by heating to 45 °C and vortexing until a homogenous mixture was obtained. The prepared mesophase was then allowed to cool down and equilibrate over a period of 48h at 37°C in an incubator.
All the buffers and protein solution were prepared using ultra-pure water and the pH was adjusted using a 1M solution of HCl.
For the ion conductivity measurements the LLC systems were preloaded with 600mM NaCl by dissolving the appropriate amount of salt in the protein buffer (protein solution for the systems containing OmpF) used to prepare the liquid crystalline phases. The prepared mesophases were then allowed to equilibrate for a period of 48h at 37°C in an incubator.

*Small Angle X-Ray Scattering measurements*

SAXS measurements were used to identify the symmetry of the mesophases at the different conditions. Experiments were performed on a MicroMax-002+ microfocused beam, operating at voltage and filament current of 45 kV and 0.88mA, respectively. The Ni-filtered Cu Kα radiation (λCu Kα = 1.5418 Å) was collimated by three pinhole (0.4, 0.3 and 0.8 mm) collimators and the data was collected by a two dimensional argon-filled Triton detector. An effective scattering-vector range of $0.03\ Å^{-1} < q < 0.45\ Å^{-1}$ was probed, where q is the scattering wave-vector defined as $q = 4\pi \sin(\theta)/ \lambda$ Cu Kα, with a scattering angle of 2θ, calibrated using silver behenate. For all measurements the samples were placed inside a Linkam HFS91 stage, between two thin mica sheets and sealed by an O-ring, with a sample thickness of ca. 1 mm. Measurements were performed at 37°C, and samples were equilibrated for 30 min prior to measurements, while scattered intensity was collected over 30 min.

*Caffeine release studies*

For the release studies the LLCs (with or without OmpF) were pre-loaded with 2wt% caffeine (see sample preparation). After a 48h equilibration period in sealed Pyrex tubes at 37°C, a respective amount of 10mM phosphate, 150mM NaCl, pH 7.4 buffer solution (usable pH range 6.2-8.2) was added on top of the mesophase in order to ensure a sufficient excess ratio of 9:1 mesophase:excess buffer.
To simulate perfect sink conditions, the buffer solution was then periodically replaced by an identical amount of fresh buffer and the amount of caffeine released was measured by means of UV spectroscopy at a fixed wavelength of 273nm. The variations in UV absorbance signal for different concentrations of caffeine with respect to changes in wavelength were previously recorded and a perfect linearity between solute concentration and emitted signal was found for the fixed wavelength of 273nm (see S.I. Fig. S5). At the end of the release experiments, the LC mesophase was removed and measured by small-angle X-ray diffraction to rule out possible structural changes during the experiments.

 

*Ion conductivity measurements*

Ion conductivity measurements were performed on reverse hexagonal bulk mesophases containing 20wt% protein solution (buffer solution for the blank control experiments) using a conductivity meter type EL-30 (Metler Toledo, Switzerland) and a In-Lab 751-4mm micro-probe (Metler Toledo, Switzerland). The samples were prepared as previously described and contained 600mM NaCl, in order to generate a sufficient charge without any observable changes in the symmetry of the mesophase. The specific conductivity was measured by immersing the micro-electrode within the bulk cubic phase at a fixed temperature of 25 °C. A total of four measurements were performed on each of the tested LLCs, and for each condition studied (with or without the OmpF protein) the conductivity was measured on four different samples in order to ensure reproducibility.

*Structural parameters calculations*

Calculating the structural parameters of reverse hexagonal phases requires a mathematical model that takes into account that the length of the lipid chains is not constant in the repeat cell due to packing frustrations in the interstitial spaces. By determining the lattice parameter, *a*, using SAXS and considering both the fixed lipid volume fraction, $\phi$ and the hexagonal geometry of the system one can calculate the radius of the water channels by following Mezzenga et al. (Ref. S2):

$$r = a(1-\phi)^{1/2}\left(\frac{\sqrt{3}}{2\pi}\right)^{1/2} \qquad (1)$$

Moreover, along a center to center edge of the repeat cell we have:

$$r = \frac{a - 2L_{lip.,U}}{2} \qquad (2)$$

Where $L_{lip.,U}$ represents the length of the unfrustrated lipid chain and can be readily obtained by equalizing the two equations.

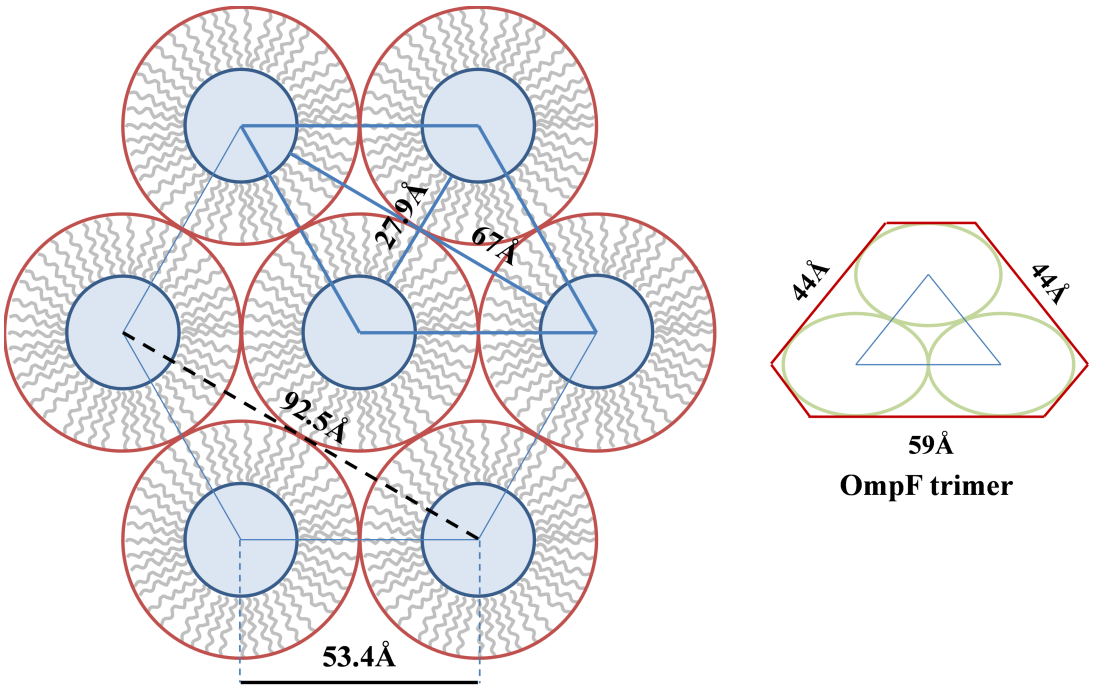


Figure S1. Schematic representation (left) of an unperturbed reverse hexagonal phase showing the calculated structural parameters using the equations described above. Simplified model of the OmpF trimeric state (right) as viewed from above, showing the estimated space needed for the protein to reconstitute within the hydrophobic bilayer of the host hexagonal phase

*Higuchi equation:*

In order to determine the apparent diffusion coefficient [cm2/s], D, of the caffeine across the matrix, the quantity expressing the moles of drug released per unit area [mol/cm2], Q, has been plotted against the square root of time [s^0.5] to determine D from the Q vs t^0.5 slope applying the Higuchi equation (Ref. S3):

$$Q = 2 \cdot C_0 \cdot \sqrt{\frac{D \cdot t}{\pi}} \qquad (3)$$

where $C_0$ is the initial molar concentration [mol/cm$^3$] of drug in the matrix.

This equation, which is based on Fickian diffusion, allows direct comparison between the different release rates of the same drug from mesophases with different structures.

 

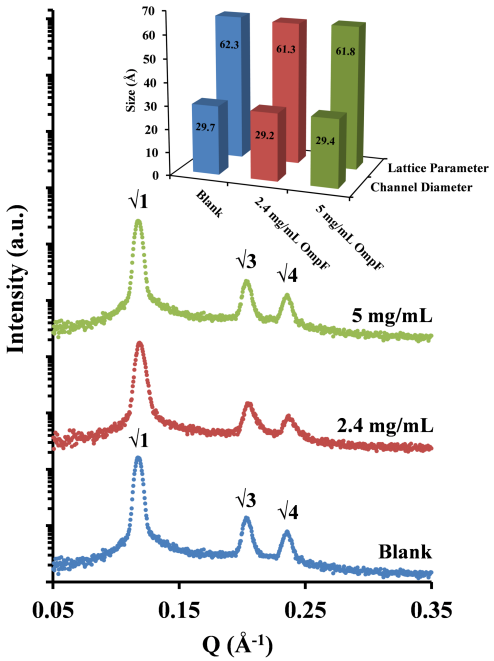


Figure S2. 1D SAXS spectra and calculated structural parameters (inset) for the systems without (blue), with 0.24wt% Ompf (red) and respectively with 0.5wt% OmpF (green) after the full release of caffeine. In all cases the host mesophase maintained its hexagonal symmetry and moreover, no significant topological differences (i.e. nearly identical structural parameters) were observed between the OmpF-containing mesophases and the unperturbed phase.

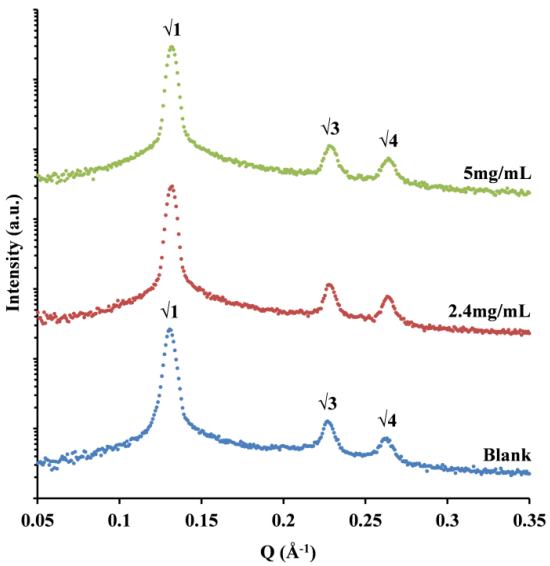


Figure S3. 1D SAXS spectra of scattered intensities *vs.* scattering vector *q* for the monolinolein:tetradecane:water reverse hexagonal phases systems used for the determination of OmpF-enhanced transport properties via ionic conductivity measurements. The different spectra represent the blank mesophase (no OmpF – blue) and the two phases containing progressively larger OmpF amounts, 0.24wt% (red) and 0.5wt% (green) after being preloaded with 750mM NaCl. All the three systems maintained their hexagonal symmetry throughout the experiments as indicated by the SAXS diffraction peaks in the ratio: √1: √3: √4.

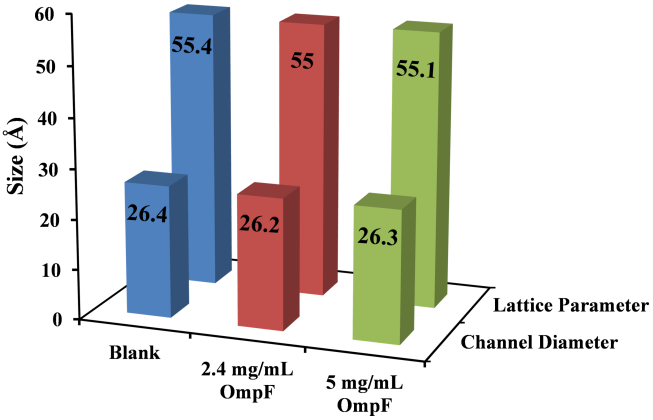


Figure S4. Corresponding analysis of mesophase structural parameters (lattice parameter and water channel radii) for the three corresponding systems depicted in Figure S2. The analysis clearly reveals that encapsulation of OmpF within the lipidic bilayer of the reverse hexagonal phase had no significant effect on the mesophase topology apart from the interconnectivity of the distinct aqueous domains.

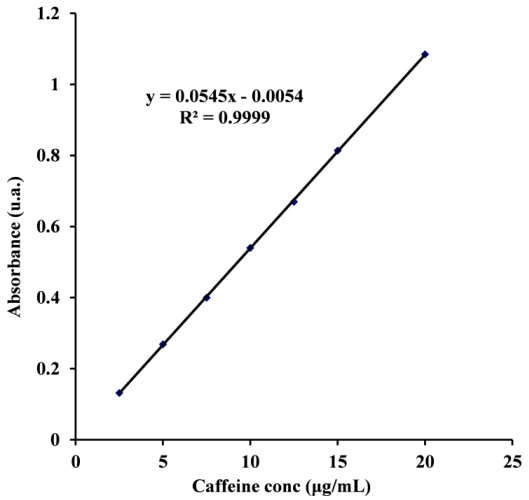


Figure S5. Caffeine calibration curve plotted as absolute concentration vs. specific UV absorbance signal at 273nm.

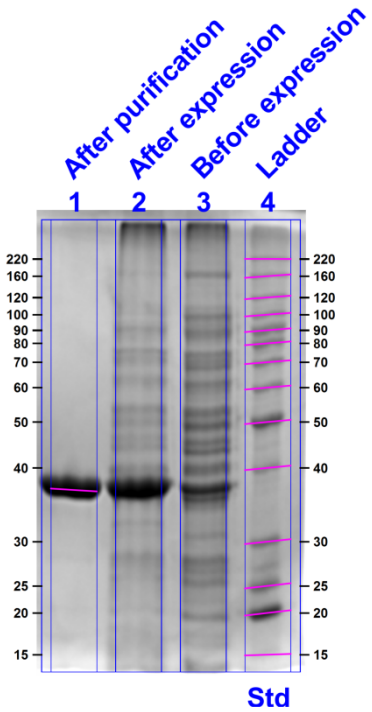


Figure S6. SDS-acrylamide electrophoresis gel (12%) showing the different steps of OmpF expression and purification.

**References:**

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