Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office via the User Portal:

https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Reports supporting requests for additional beam time

Reports can be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** The dynamic structure of swollen cubosomes	**Experiment number**: SC 4258
Beamline: ID02	**Date of experiment**: from: 22/4/2016 to: 25/4/2016	**Date of report**: 10/10/2016
Shifts: 9	**Local contact(s)**: Rajeev Dattani	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

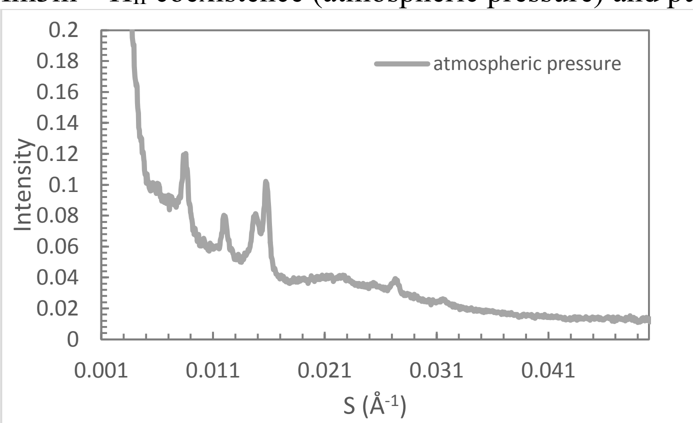
Nicholas Brooks, Oscar Ces, Robert Law, John Seddon, Hanna Barriga

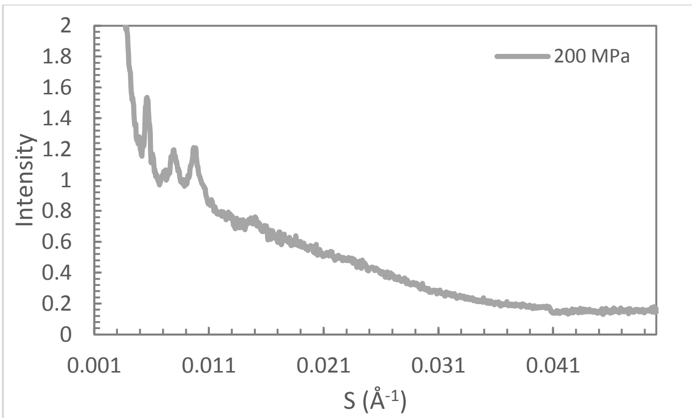
Imperial College London Department of Chemistry South Kensington Campus Exhibition Road GB - SW7 2AX LONDON

Report:

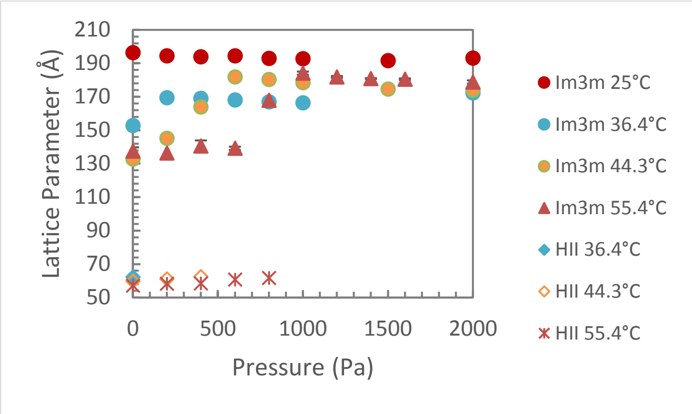
We have engineered lipid nanoparticles with an internal bicontinuous cubic structure with large pore structures and controllable internal connectivity. To use them for applications such as drug encapsulation and synthetic self-contained enzymatic microreactors it was essential to first understand their structural response to temperature, pressure and physiological conditions. In addition to this we have used salts with high X-ray contrast to probe the connectivity of the internal aqueous networks to their external environment.

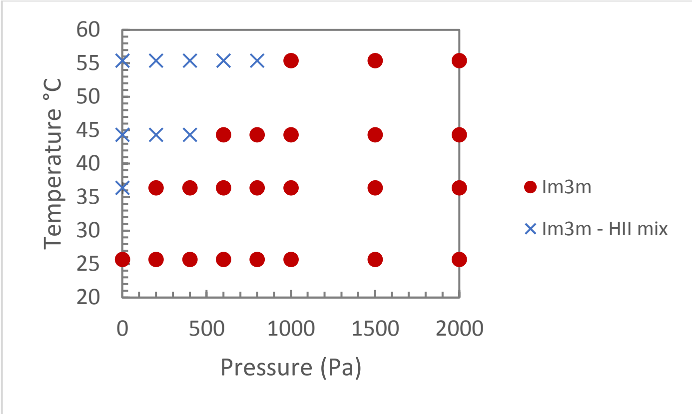
Lipid cubic nanoparticle dispersions were formed via sonication using ternary lipid mixtures of monoolein, cholesterol and charged lipids DOTAP, DOPA, DOPS or DOPG in the presence of the pluronic block copolymer F-127. To charactise their structural response to temperature and presssure we investigated the structural changes between 25°C and 55°C in 10°C steps, performing pressure scans at each temperature. Analysis is currently ongoing, however we have summarised the results of three samples containing 5mol% of charged lipid. For each composition, changes in lattice parameter have been shown as a function of temperature (T) and pressure (P) and preliminary P-T phase diagrams have been constructed. Notably all changes in lattice parameter and phase coexistences were reversible. Sample diffraction patterns showing Im3m – H_{II} coexistence (atmospheric pressure) and pure Im3m at 37.6°C at 200 MPa are shown below.



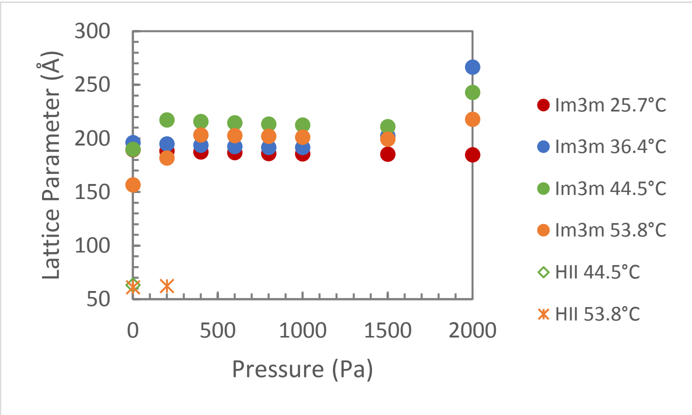


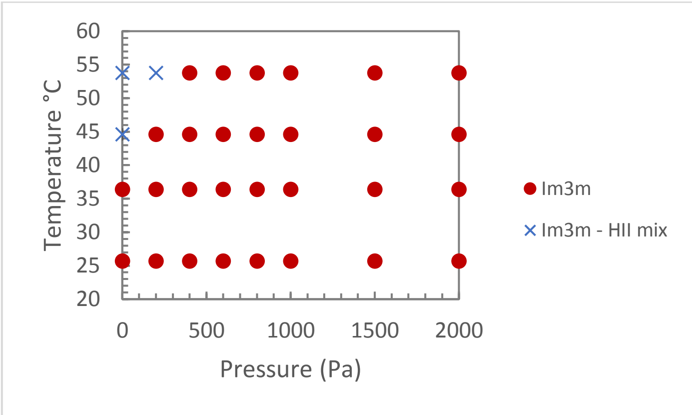
MO:CHOL:TAP 65:30:5



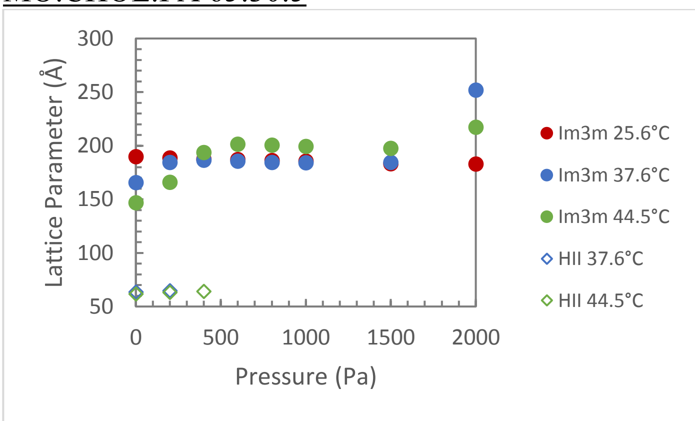


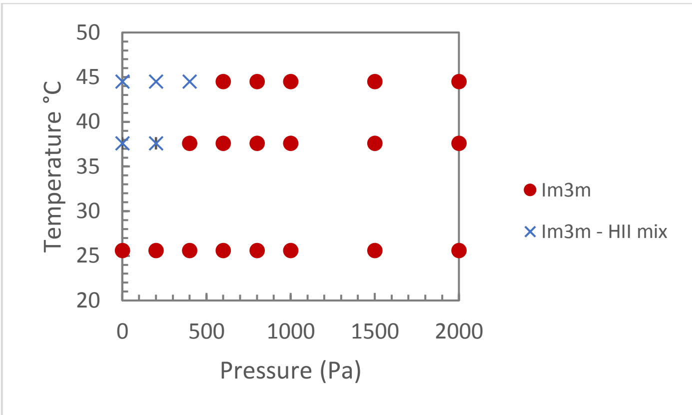
MO:CHOL:PS 65:30:5





MO:CHOL:PA 65:30:5





In addition to characterisation with temperature and pressure, we also investigated the effects of different salts with high X-ray contrast to probe the connectivity of the water channels at 25°C. Cubosome samples were prepared in concentrated KI, KCl, CsCl or NaCl and diluted into a different salt to study the structural changes due to the ionic interactions. Analysis is still ongoing for this data.

Summary of Results

At 25°C and atmospheric pressure, all three ternary lipid cubosome dispersion mixtures exhibit pure Im3m phase morphology with little change upon a pressure increase of 200 MPa. As the temperature increases all three samples exhibit an Im3m – H_{II} mixture, which upon increasing pressure converts to a pure Im3m phase at pressures and temperatures specific to the composition. The reduction of the proportion of H_{II} phase, leads to a significant swelling of the Im3m phase. In addition to the swelling of the cubic phase due to a reduction in the H_{II} phase, a pressure increase of 200 MPa appears to swell the cubic phase at temperatures above 25°C. We are currently investigating this behaviour in the data taken for other mixtures. This is the first study of the effects of temperture and pressure in highly swollen dispersed lipid nanoparticles. The observed swelling of the cubic phase and changes in phase morphology are in good agreement with the behaviour of swollen mixtures of bulk cubic phase. These studies have enabled us to systematically study the kinetics of swelling of these cubosome systems we will enable ourselves and other researchers to manufacture cubosomes for targeted drug encapsulation and delivery, significantly accelerating this field of research.