Electronic Supplementary Material (ESI) for Nanoscale.

The Nanoscience Behind the Art of *in-meso* Crystallization of Membrane Proteins

Supporting Information

Alexandru Zabara[1,3], Thomas G. Meikle[2], Janet Newman[3], Thomas S. Peat[3], Charlotte E. Conn[1], Calum J. Drummond[1]*

Affiliations:

[1]RMIT University, School of Science, College of Science Engineering and Health 124 La Trobe Street, Melbourne, Victoria, 3000, Australia.

[2]School of Chemistry, Bio21 Institute, The University of Melbourne, Melbourne, Victoria 3010, Australia.

[3] Biomedical Manufacturing Program, Commonwealth Scientific and Industrial Research Organisation (CSIRO), 343 Royal Parade, Parkville, Victoria 3052, Australia.

*Correspondence to:

Professor Calum J. Drummond

DSc PhD FTSE FAICD FRACI FRSC CChem
Deputy Vice-Chancellor Research and Innovation and Vice-President

RMIT University, GPO Box 2476, Melbourne VIC 3001, Australia
Tel: +61 3 9925 4265

E-mail: calum.drummond@rmit.edu.au

Intimin expression and purification

The plasmid containing the E. coli O157:H7 gene was transformed into BL21 (DE3) cells and plated onto LB plates containing kanamycin. Single colonies were pre-screened by means of Western Blotting to identify overexpression of the target protein. Overexpressing colonies were pre-cultured into 40mL of TB containing 50µg/mL kanamycin until an OD value of 1. 5mL of pre-culture was then transferred into 500mL of TB-kanamycin media and allowed to grow at 20°C for 2-3 days while shaking at 220rpm until they reached a terminal OD of 15-20. Cells were harvested by means of centrifugation (5000rpm, 10 min, 4°C) using a Beckman JA14 rotor and then stored at -80°C.

Cells were thawed on ice and resuspended in lysis buffer (50mM TRIS pH 8.0, 200mM NaCl, 5 mg/ml Lysozyme, 1 tablet of protease inhibitor cocktail – EDTA free - Roche) and then lysed using a probe sonicator (Misonix S4000), in 30 seconds interval at 60% amplitude. Cell debris and unlysed E. coli were collected by centrifugation at 12000xg for 30 minutes at 4°C. Membranes containing the desired protein were harvested from the supernatant by ultra-centrifugation (160,000xg, 60 min, 4°C). Membrane proteins were solubilized by resuspension in solubilization buffer (50mM TRIS pH 8.0, 200mM NaCl, 20mM Imidazole, 5% Elugent (Calbiochem)) and left stirring O/N at 4°C. The next morning, the sample underwent ultra-centrifugation (250,000xg, 60 min, 4°C) to remove insoluble material. Supernatant was loaded onto a Ni-NTA affinity column that was pre-equilibrated with 50mM TRIS pH 8.0, 200mM NaCl, 10% glycerol, and 0.1% DDM (n-dodecyl-β-D-maltopyranoside)(Anatrace). The protein was eluted with 50mM TRIS pH 8.0, 200mM NaCl, 10% glycerol, 0.1% DDM, and 250mM imidazole. Fractions containing protein were desalted into 50mM TRIS pH 8.0 using a High Performance Desalting column (GE Healthcare) that was pre-equilibrated with Buffer A (50mM TRIS pH 8.0, 0.1 % DDM). Fractions containing protein were concentrated in a YM30 Amicon Ultra concentrator (Millipore) to prepare for crystallization experiments.

Table S1. 96 conditions randomized screen based for intimin crystallization. The screen was designed around previously found conditions: (1) 20% MPD; 0.1M NaCitrate/citric acid pH 4.5-5.5 (private communication, Dr. Susan K Buchanan) (2) 6% w/v PEG 6000; 0.1M NaCitrate/citric acid pH 4.5-5.5 (private communication; Dr. Susan K Buchanan) (3) 0.1M NaCitrate/citric acid pH 4.5-5.5, 0.05-0.1M NaCl, 0.1-0,15M MgCl2, 30-34 % v/v PEG 400 (39), in order to optimize growth and ensure reproducibility.

0.1M trisodium citrate-citric acid pH 4.3, 7.890 w/v polyethylene glycol 6000	0.1M trisodium citrate-citric acid pH 4.5, 7.570 w/v polyethylene glycol 6000	0.1M trisodium citrate-citric acid pH 4.3, 6.580 w/v polyethylene glycol 6000	0.1M trisodium citrate-citric acid pH 4, 18% v/v 2-methyl-2,4-pentanediol	0.1M trisodium citrate-citric acid pH 4.1, 17.8% v/v 2-methyl-2,4-pentanediol	0.1M trisodium citrate-citric acid pH 4.3, 5.970 w/v polyethylene glycol 6000	0.1M trisodium citrate-citric acid pH 4.9, 37.1% v/v polyethylene glycol 400, 0.078 M magnesium chloride, 0.1M sodium chloride	0.1M trisodium citrate-citric acid pH 5.7, 36.3% v/v polyethylene glycol 400, 0.129 M magnesium chloride, 0.031 M sodium chloride	0.1M trisodium citrate-citric acid pH 4.3 30% v/v polyethylene glycol 400, 0.181 M magnesium chloride, 0.061 M sodium chloride	0.1 M trisodium citrate-citric acid pH 4.4, 34.7% v/v polyethylene glycol 400, 0.175 M magnesium chloride, 0.124 M sodium chloride	0.1 M trisodium citrate-citric acid pH 4.3, 39% v/v polyethylene glycol 400, 0.193 M magnesium chloride, 0.063 M sodium chloride	0.1 M trisodium citrate-citric acid pH 4.3, 31.7% v/v polyethylene glycol 400, 0.117 M magnesium chloride, 0.114 M sodium chloride
0.1 M trisodium citrate-citric acid pH 4.5, 22.1% v/v 2-methyl-2,4-pentanediol	0.1 M trisodium citrate-citric acid pH 4.4, 7.45% w/v polyethylene glycol 6000	0.1 M trisodium citrate-citric acid pH 4.3, 20.3% v/v 2-methyl-2,4-pentanediol	0.1 M trisodium citrate-citric acid pH 4.5, 18% v/v 2-methyl-2,4-pentanediol	0.1 M trisodium citrate-citric acid pH 4.1, 19.8% v/v 2-methyl-2,4-pentanediol	0.1 M trisodium citrate-citric acid pH 3.9, 16.5% v/v 2-methyl-2,4-pentanediol	0.1 M trisodium citrate-citric acid pH 4.5, 33.6% v/v polyethylene glycol 400, 0.068 ,M magnesium chloride, 0.041 ,M sodium chloride	0.1 M trisodium citrate-citric acid pH 5.3, 33.9% v/v polyethylene glycol 400, 0.102 M magnesium chloride, 0.106 M sodium chloride	0.1 M trisodium citrate-citric acid pH 4.2, 37.1% v/v polyethylene glycol 400, 0.194 M magnesium chloride, 0.085 M sodium chloride	0.1 M trisodium citrate-citric acid pH 4.7, 35.4% v/v polyethylene glycol 400, 0.153 M magnesium chloride, 0.080 M sodium chloride	0.1M trisodium citrate-citric acid pH 5.8, 25.8% v/v polyethylene glycol 400, 0.058 M magnesium chloride, 0.114 M sodium chloride	0.1 M trisodium citrate-citric acid pH 4.4, 33.5% v/v polyethylene glycol 400, 0.066 M magnesium chloride, 0.067 M sodium chloride
0.1 M trisodium citrate-citric acid pH 4.7, 5.64% w/v polyethylene glycol 6000	0.1 M trisodium citrate-citric acid pH 5.6, 6.24% w/v polyethylene glycol 6000	0.1 M trisodium citrate-citric acid pH 5.2, 19.3% v/v 2-methyl-2,4-pentanediol	0.1 M trisodium citrate-citric acid pH 4.3, 5.71% w/v polyethylene glycol 6000	0.1 M trisodium citrate-citric acid pH 5.5, 17.2% v/v 2-methyl-2,4-pentanediol	0.1 M trisodium citrate-citric acid pH 4.2, 7.56% w/v polyethylene glycol 6000	0.1 M trisodium citrate-citric acid pH 3.8, 27.4% v/v polyethylene glycol 400, 0.138 M magnesium chloride, 0.086 M sodium chloride	0.1 M trisodium citrate-citric acid pH 5, 25.4% v/v polyethylene glycol 400, 0.143 M magnesium chloride, 0.065 M sodium chloride	0.1 M trisodium citrate-citric acid pH 4.2, 37.4% v/v polyethylene glycol 400, 0.074 M magnesium chloride, 0.035 M sodium chloride	0.1 M trisodium citrate-citric acid pH 4.9, 26.2% v/v polyethylene glycol 400, 0.084 M magnesium chloride, 0.099 M sodium chloride	0.1 M trisodium citrate-citric acid pH 4.1, 25.1% v/v polyethylene glycol 400, 0.167 M magnesium chloride, 0.041 M sodium chloride	0.1 M trisodium citrate-citric acid pH 4, 26.7% v/v polyethylene glycol 400, 0.168 M magnesium chloride, 0.046 M sodium chloride
0.1 M trisodium citrate-citric acid pH 4.1, 15.9% v/v 2-methyl-2,4-pentanediol	0.1 M trisodium citrate-citric acid pH 4.3, 6.84% w/v polyethylene glycol 6000	0.1 M trisodium citrate-citric acid pH 4, 7.91% w/v polyethylene glycol 6000	0.1 M trisodium citrate-citric acid pH 4.4, 16.6% v/v 2-methyl-2,4-pentanediol	0.1 M trisodium citrate-citric acid pH 4.6, 4.92% w/v polyethylene glycol 6000	0.1 M trisodium citrate-citric acid pH 4.4, 21.5% v/v 2-methyl-2,4-pentanediol	0.1 M trisodium citrate-citric acid pH 3.9, 39.2% v/v polyethylene glycol 400, 0.140 M magnesium chloride, 0.110 M sodium chloride	0.1 M trisodium citrate-citric acid pH 5.6, 34.7% v/v polyethylene glycol 400, 0.142 M magnesium chloride, 0.092 M sodium chloride	0.1 M trisodium citrate-citric acid pH 4.3 36.5% v/v polyethylene glycol 400, 0.159 M magnesium chloride, 0.113 M sodium chloride	0.1 M trisodium citrate-citric acid pH 5.6 28.5% v/v polyethylene glycol 400, 0.078 M magnesium chloride, 0.03 M sodium chloride	0.1 M trisodium citrate-citric acid pH 5.6 31.1% v/v polyethylene glycol 400, 0.104 M magnesium chloride, 0.067 M sodium chloride	0.1 M trisodium citrate-citric acid pH 5.5, 32.2% v/v polyethylene glycol 400, 0.195 M magnesium chloride, 0.117 M sodium chloride
0.1 M trisodium citrate-citric acid pH 4, 23.6% v/v 2-methyl-2,4-pentanediol	0.1 M trisodium citrate-citric acid pH 4.7, 5.72% w/v polyethylene glycol 6000	0.1 M trisodium citrate-citric acid pH 4.2, 7.14% w/v polyethylene glycol 6000	0.1 M trisodium citrate-citric acid pH 4.5, 24.3% v/v 2-methyl-2,4-pentanediol	0.1 M trisodium citrate-citric acid pH 4.4, 5.91% w/v polyethylene glycol 6000	0.1 M trisodium citrate-citric acid pH 4.4, 16.5% v/v 2-methyl-2,4-pentanediol	0.1 M trisodium citrate-citric acid pH 5, 29.5% v/v polyethylene glycol 400, 0.189 M magnesium chloride, 0.09 M sodium chloride	0.1 M trisodium citrate-citric acid pH 4.6, 30.3% v/v polyethylene glycol 400, 0.052 M magnesium chloride, 0.039 M sodium chloride	0.1 M trisodium citrate-citric acid pH 4.9, 35.9% v/v polyethylene glycol 400, 0.07 M magnesium chloride, 0.068 M sodium chloride	0.1 M trisodium citrate-citric acid pH 5.2, 36.5% v/v polyethylene glycol 400, 0.069 M magnesium chloride, 0.042 M sodium chloride	0.1 M trisodium citrate-citric acid pH 4.8, 31.5% v/v polyethylene glycol 400, 0.067 M magnesium chloride, 0.037 M sodium chloride	0.1 M trisodium citrate-citric acid pH 4.7, 27.5% v/v polyethylene glycol 400, 0.062 M magnesium chloride, 0.09 M sodium chloride
0.1 M trisodium citrate-citric acid pH 4.1, 16.2% v/v 2-methyl-2,4-pentanediol	0.1 M trisodium citrate-citric acid pH 4.4, 16.3% v/v 2-methyl-2,4-pentanediol	0.1 M trisodium citrate-citric acid pH 4.1, 15% v/v 2-methyl-2,4-pentanediol	0.1 M trisodium citrate-citric acid pH 4.7, 22.5% v/v 2-methyl-2,4-pentanediol	0.1 M trisodium citrate-citric acid pH 5.6, 5.92% w/v polyethylene glycol 6000	0.1 M trisodium citrate-citric acid pH 3.8, 6.35% w/v polyethylene glycol 6000	0.1 M trisodium citrate-citric acid pH 5.1, 28.9% v/v polyethylene glycol 400, 0.107 M magnesium chloride, 0.049 M sodium chloride	0.1 M trisodium citrate-citric acid pH 5, 25.7% v/v polyethylene glycol 400, 0.12 M magnesium chloride, 0.122 M sodium chloride	0.1 M trisodium citrate-citric acid pH 4.3, 27% v/v polyethylene glycol 400, 0.078 M magnesium chloride, 0.113 M sodium chloride	0.1 M trisodium citrate-citric acid pH 4.7, 27.2% v/v polyethylene glycol 400, 0.077 M magnesium chloride, 0.059 M sodium chloride	0.1 M trisodium citrate-citric acid pH 4, 32.6% v/v polyethylene glycol 400, 0.116 M magnesium chloride, 0.122 M sodium chloride	0.1 M trisodium citrate-citric acid pH 4.1, 26.3% v/v polyethylene glycol 400, 0.166 M magnesium chloride, 0.061 M sodium chloride
0.1 M trisodium citrate-citric acid pH 5.2, 4.91% w/v polyethylene glycol 6000	0.1 M trisodium citrate-citric acid pH 4.4, 22.2% v/v 2-methyl-2,4-pentanediol	0.1 M trisodium citrate-citric acid pH 4.2, 15.9% v/v 2-methyl-2,4-pentanediol	0.1 M trisodium citrate-citric acid pH 4.4, 4.23% w/v polyethylene glycol 6000	0.1 M trisodium citrate-citric acid pH 4.2, 19.4% v/v 2-methyl-2,4-pentanediol	0.1 M trisodium citrate-citric acid pH 4.8, 6.9% w/v polyethylene glycol 6000	0.1 M trisodium citrate-citric acid pH 4.3, 35% v/v polyethylene glycol 400, 0.098 M magnesium chloride, 0.063 M sodium chloride	0.1 M trisodium citrate-citric acid pH 4.7, 26.6% v/v polyethylene glycol 400, 0.064 M magnesium chloride, 0.091 M sodium chloride	0.1 M trisodium citrate-citric acid pH 5.2, 30% v/v polyethylene glycol 400, 0.108 M magnesium chloride, 0.032 M sodium chloride	0.1 M trisodium citrate-citric acid pH 5.4, 38.4% v/v polyethylene glycol 400, 0.193 M magnesium chloride, 0.084 M sodium chloride	0.1 M trisodium citrate-citric acid pH 5.5, 29.2% v/v polyethylene glycol 400, 0.136 M magnesium chloride, 0.059 M sodium chloride	0.1 M trisodium citrate-citric acid pH 4.9, 34.7% v/v polyethylene glycol 400, 0.116 M magnesium chloride, 0.095 M sodium chloride
0.1 M trisodium citrate-citric acid pH 3.9, 5.96% w/v polyethylene glycol 6000	0.1 M trisodium citrate-citric acid pH 4.3, 22.5% v/v 2-methyl-2,4-pentanediol	0.1 M trisodium citrate-citric acid pH 4.1, 4.57% w/v polyethylene glycol 6000	0.1 M trisodium citrate-citric acid pH 5.6, 4.83% w/v polyethylene glycol 6000	0.1 M trisodium citrate-citric acid pH 4.3, 18.1% v/v 2-methyl-2,4-pentanediol	0.1 M trisodium citrate-citric acid pH 4.7, 15.1% v/v 2-methyl-2,4-pentanediol	0.1 M trisodium citrate-citric acid pH 4, 33.5% v/v polyethylene glycol 400, 0.196 M magnesium chloride, 0.076 M sodium chloride	0.1 M trisodium citrate-citric acid pH 5.2, 36.4% v/v polyethylene glycol 400, 0.131 M magnesium chloride, 0.114 M sodium chloride	0.1 M trisodium citrate-citric acid pH 4.9, 32.8% v/v polyethylene glycol 400, 0.168 M magnesium chloride, 0.046 M sodium chloride	0.1 M trisodium citrate-citric acid pH 4, 34.2% v/v polyethylene glycol 400, 0.157 M magnesium chloride, 0.09 M sodium chloride	0.1 M trisodium citrate-citric acid pH 4.6, 33.7% v/v polyethylene glycol 400, 0.079 M magnesium chloride, 0.1 M sodium chloride	0.1 M trisodium citrate-citric acid pH 5, 32.3% v/v polyethylene glycol 400, 0.127 M magnesium chloride, 0.124 M sodium chloride

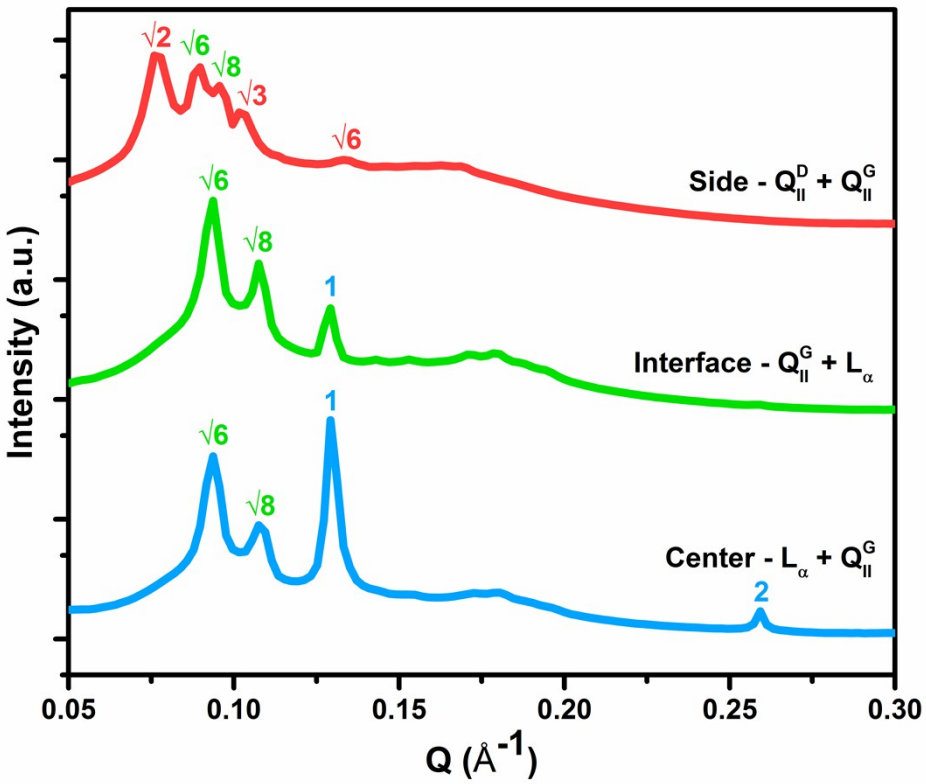

Figure S1. 1D spectra of scattered intensities versus scattering vector, *q*, showing the distinct symmetries adopted by the lipidic mesophase in three different regions of a LCP drop: (1) The crystal growth region in the center of the LCP bolus where crystal growth invariably occurs; (2) The interface region found towards the edge of the protein concentrated region of the LCP bolus; (3) The LCP region found towards the edge of the well where the LCP is depleted in protein and crystal growth does not occur.

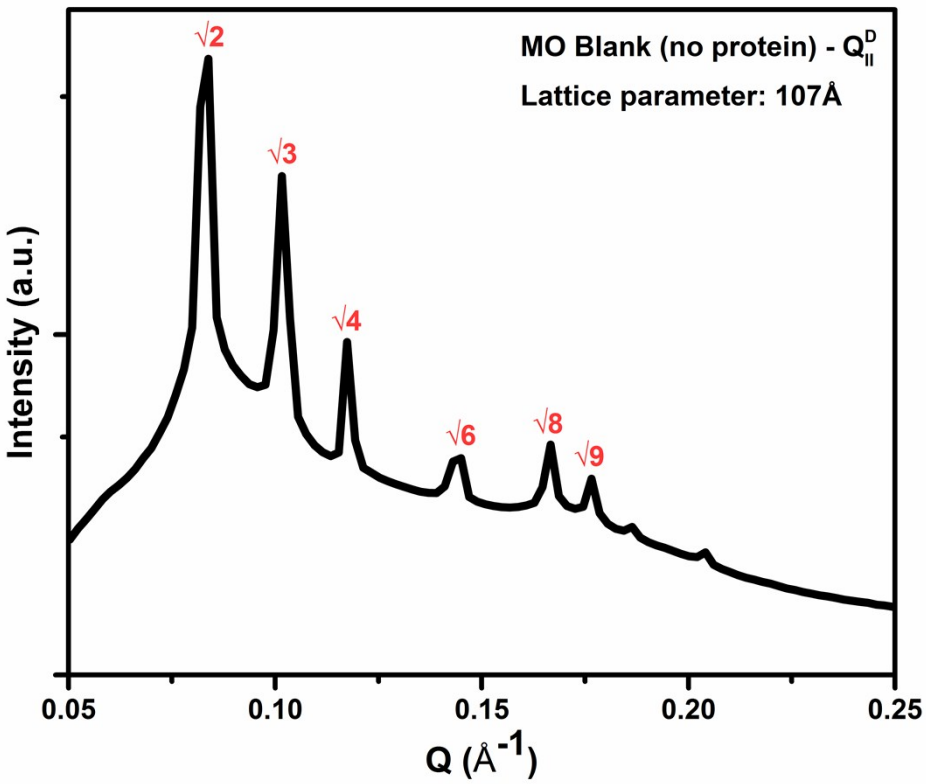

Figure S2. 1D SAXS spectra showing a control crystallization experiment for the MO system comprising of an identical LCP drop and crystallization buffer but without added protein.

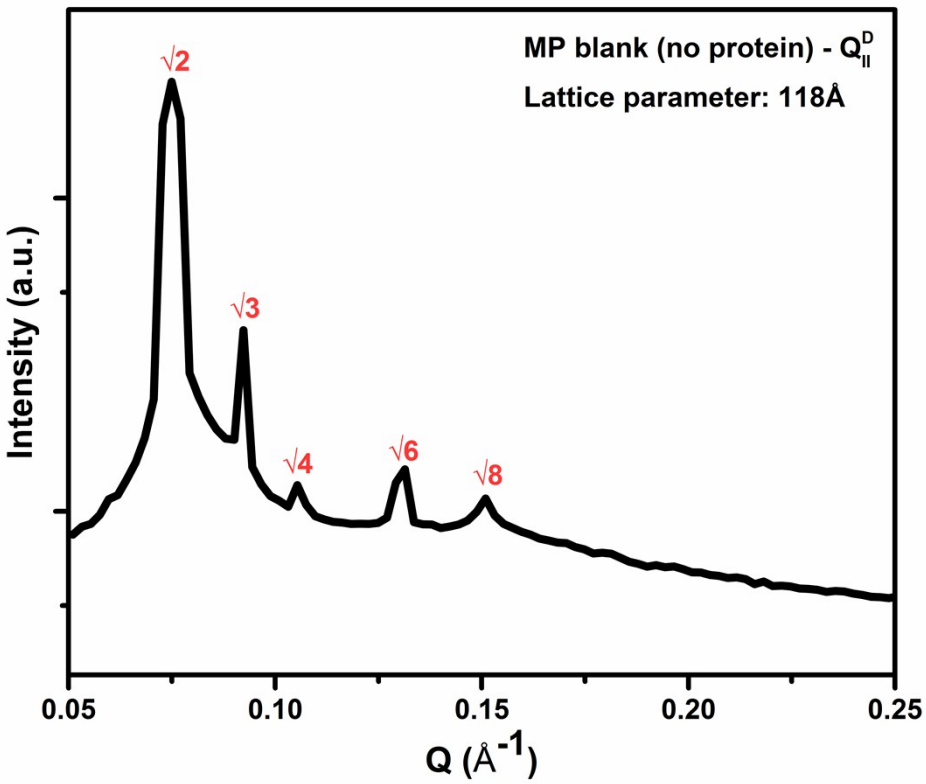

Figure S3. 1D SAXS spectra showing a control crystallization experiment for the MP system comprising of an identical LCP drop and crystallization buffer but without added protein.

PDB	5G26
Space group	$C222_1$
Cell dimensions	
a, *b*, *c* (Å)	115.6, 119.7, 39.0
a, b, g (°)	90, 90, 90
Resolution (Å)	41.6-2.42 (2.51-2.42)
R_{merge}	0.143 (0.822)
R_{pim}	0.046 (0.266)
CC1/2	0.998 (0.888)
I / sI	12.3 (2.9)
Completeness (%)	99.1 (91.8)
Redundancy	10.2 (10.1)
Refinement	
Resolution (Å)	41.5 - 2.42
Unique reflections	10,728
R_{work} / R_{free} (%)	22.2 / 27.8
No. atoms	2,089
Protein	1,964
Lipid	92
Water	33
B-factors (Å^2)	40.7
Protein	40.5
Lipid	55.0
Water	36.0
R.m.s. deviations	
Bond lengths (Å)	0.012
Bond angles (°)	1.527

Table S2. Data collection and refinement statistic for the native intimin protein crystallized from an MP-based cubic phase.