Lipidic Cubic Phase-Induced Membrane Protein Crystallization: Interplay Between Lipid Molecular Structure, Mesophase Structure and Properties, and Crystallogenesis

Supporting Information

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Intimin expression and purification:

The plasmid containing the *E. coli* O157:H7 gene was transformed into BL21 (DE3) cells and plated onto Luria Broth plates containing kanamycin. Overexpression of the target protein was identified through pre-screening of single colonies using Western Blotting. Selected colonies were pre-cultured in 40 mL of Terrific Broth (TB) with 50 μg/mL kanamycin until reaching an OD value of 1. A 5 mL volume of the pre-culture was then transferred into 500 mL of TB-kanamycin media and allowed to grow at 20°C for 2-3 days, shaking at 220 rpm until a terminal OD of 15-20 was reached. Cells were harvested using centrifugation (5000 rpm, 10 min, 4°C) with a Beckman JA14 rotor and subsequently stored at -80°C.

After thawing on ice, the cells were resuspended in lysis buffer (50 mM TRIS pH 8.0, 200 mM NaCl, 5 mg/ml lysozyme, 1 tablet of protease inhibitor cocktail – EDTA free - Roche) and lysed using a probe sonicator (Misonix S4000), in 30 s interval at 60% amplitude. Cell debris and any unlysed *E. coli* were collected using centrifugation at 12000 g for 30 min at 4°C. Membranes containing the intimin protein were harvested from the supernatant via ultra-centrifugation (160,000 g, 60 min, 4°C). Membrane proteins were resuspended in solubilization buffer (50 mM TRIS pH 8.0, 200mM NaCl, 20 mM imidazole, 5% Elugent (Calbiochem)) by stirring overnight at 4°C. Insoluble material was subsequently removed from the sample by ultra-centrifugation (250,000 g, 120 min, 4°C). The resulting supernatant was loaded onto a Ni-NTA affinity column, pre-equilibrated with 50 mM TRIS pH 8.0, 200 mM NaCl, 10% glycerol, and 0.1% DDM (n-dodecyl-β-D-maltopyranoside) (Anatrace), and the protein eluted with 50 mM TRIS pH 8.0, 200 mM NaCl, 10% glycerol, 0.1% DDM, and 250 mM imidazole. The protein containing fractions were desalted into 50 mM TRIS pH 8.0 using a High Performance Desalting column (GE Healthcare), pre-equilibrated with Buffer A (50 mM TRIS pH 8.0, 0.1% DDM), and subsequently concentrated using a YM30 Amicon Ultra concentrator (Millipore).

LCP structural parameter calculation:

For bicontinuous cubic phases, the bilayer thickness is estimated to be double the value of L_{lip}, calculated following Mezzenga *et al*. (Ref. S1),

$$\phi = 2A_0\left(\frac{L_{lip}}{a}\right) + \frac{4}{3}\pi\chi\left(\frac{L_{lip}}{a}\right)^3 \qquad (1)$$

where Φ is the lipid volume fraction, a is the lattice parameter as measured by SAXS, and A_0 and χ have the following values, depending on the specific cubic phase symmetry:

$$A_0 = 3.091 \text{ and } \chi = -8 \text{ for Ia3d} \qquad (2)$$

$$A_0 = 1.919 \text{ and } \chi = -2 \text{ for Pn3m} \qquad (3)$$

The radius of the water channels, r, can then be calculated from:

$$r = 0.248a - L_{lip} \qquad (4)$$

$$r = 0.391a - L_{lip} \qquad (5)$$

which are valid for the Ia3d and Pn3m phases, respectively.

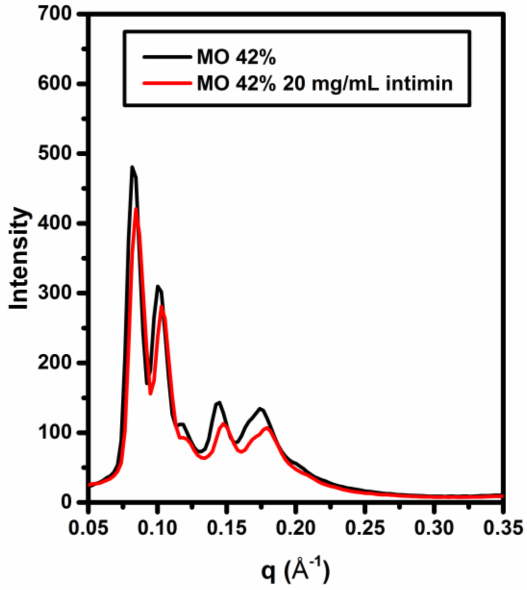

Figure S1. 1D SAXS spectra of scattering intensities versus scattering vector, q, for the obtained monoolein:water mesophases at 42% hydration with and without added intimin. The specific diffraction pattern and peak spacing ratios reveal a double diamond Pn3m cubic phase in both systems.

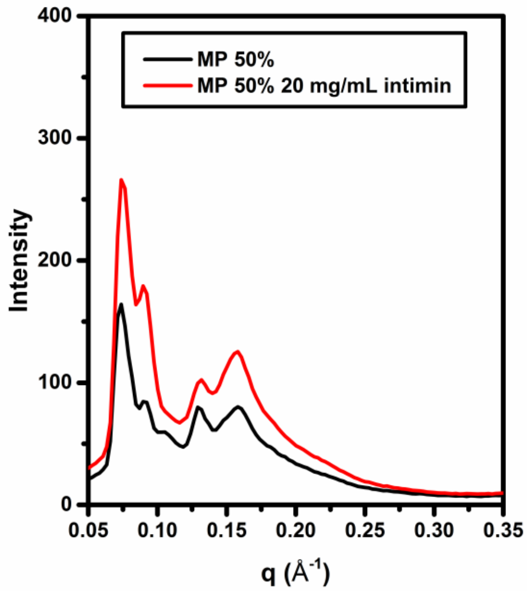

Figure S2. 1D SAXS spectra of scattering intensities versus scattering vector, q, for the obtained monopalmitolein:water mesophases at 50% hydration with and without added intimin. The specific diffraction pattern and peak spacing ratios reveal a double diamond Pn3m cubic phase in both systems.

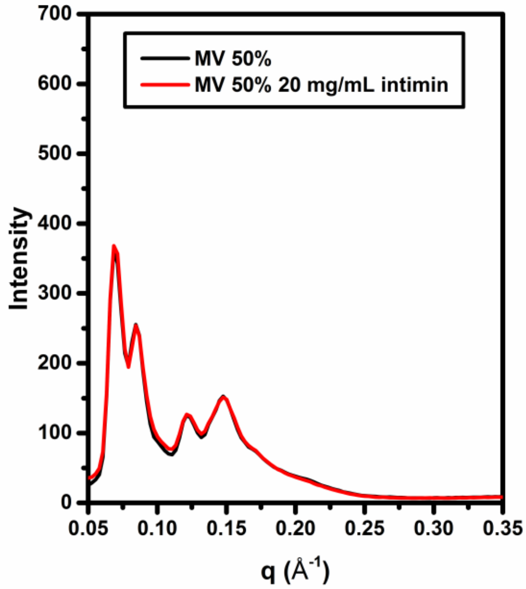

Figure S3. 1D SAXS spectra of scattering intensities versus scattering vector, q, for the obtained monovaccenin:water mesophases at 50% hydration with and without added intimin. The specific diffraction pattern and peak spacing ratios reveal a double diamond Pn3m cubic phase in both systems.

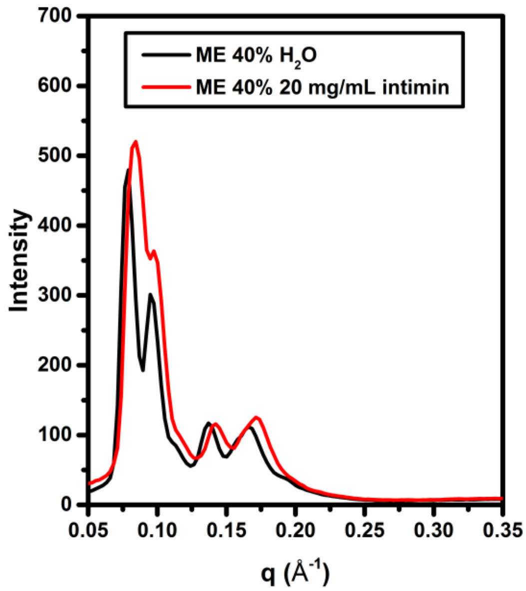

Figure S4. 1D SAXS spectra of scattering intensities versus scattering vector, q, for the obtained monoeicosenoin:water mesophases at 40% hydration with and without added intimin. The specific diffraction pattern and peak spacing ratios reveal a double diamond Pn3m cubic phase in both systems.

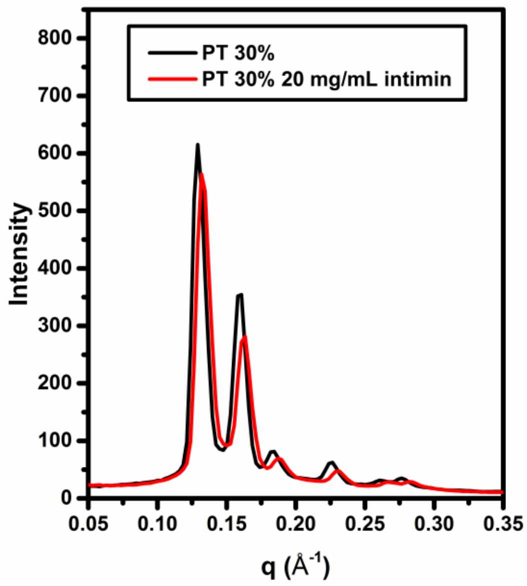

Figure S5. 1D SAXS spectra of scattering intensities versus scattering vector, q, for the obtained phytantriol:water mesophases at 30% hydration with and without added intimin. The specific diffraction pattern and peak spacing ratios reveal a double diamond Pn3m cubic phase in both systems.

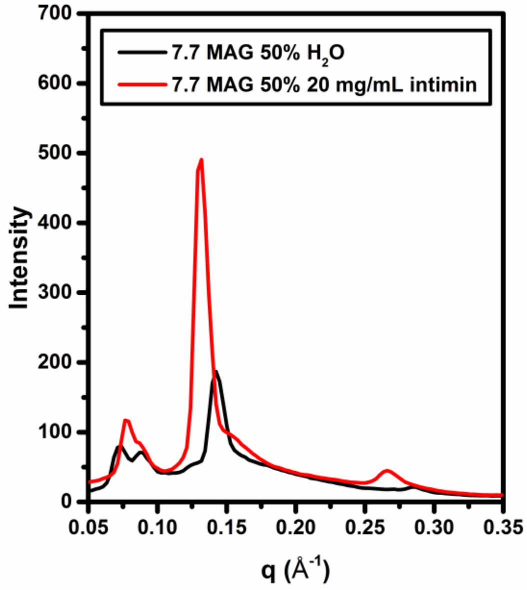

Figure S6. 1D SAXS spectra of scattering intensities versus scattering vector, q, for the obtained MAG7.7:water mesophases at 50% hydration with and without added intimin. The specific diffraction pattern and peak spacing ratios reveal a coexistence of phases with a prominent lamellar phase and more importantly the absence of the double diamond Pn3m cubic phase.

Table S1. Randomized, 96 condition screen designed for intimin crystallization. The screen was designed using previously found conditions: (1) 20% MPD; 0.1 M NaCitrate/citric acid pH 4.5-5.5 (private communication; Dr Susan K. Buchanan) (2) 6% w/v PEG 6000; 0.1 M NaCitrate/citric acid pH 4.5-5.5 (private communication; Dr Susan K. Buchanan) (3) 0.1 M NaCitrate/citric acid pH 4.5-5.5, 0.05-0.1 M NaCl, 0.1-0.15 M $MgCl_2$, 30-34% v/v PEG 400, 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

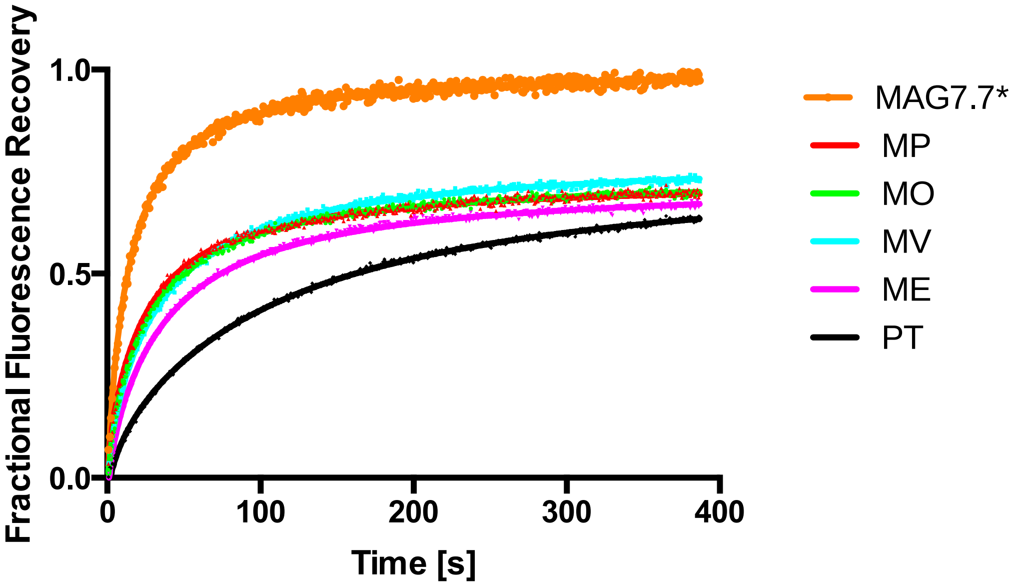

Figure S7. Diffusion characteristics of intimin-Cy3 in various lipidic mesophases measured by Fluorescence Recovery After Photobleaching (FRAP). *MAG7.7 was not cubic and the obtained fluorescence recovery curve represents lipid fluidity rather than protein diffusion.

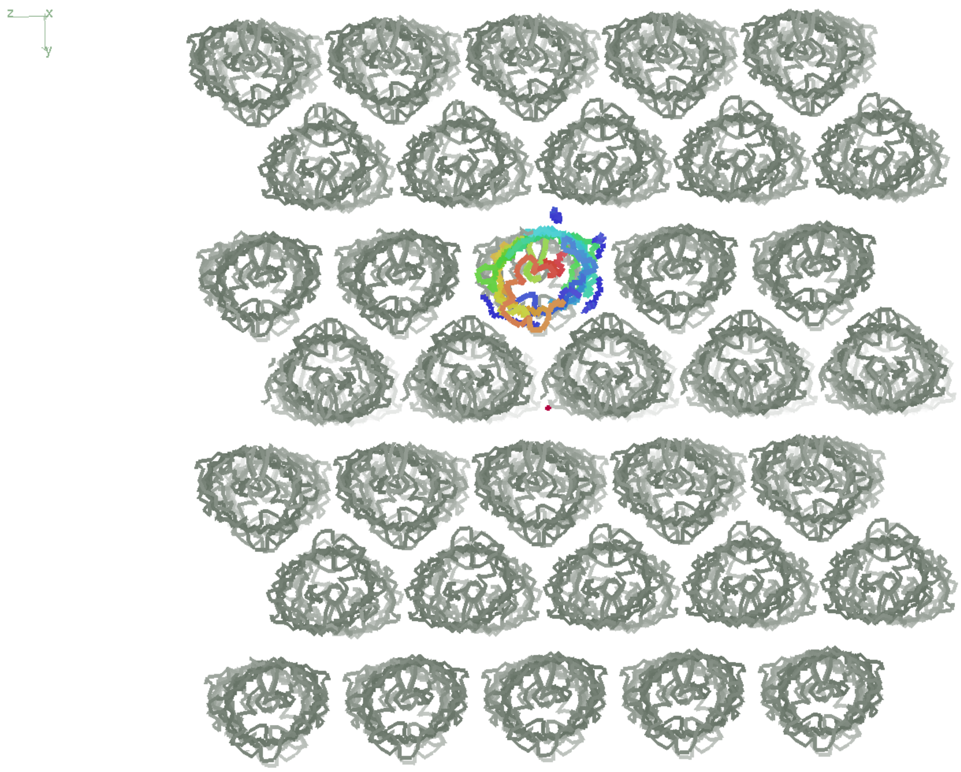

Figure S8. Packing of intimin molecules within the protein crystal obtained via the LCP-based crystallization. Regardless of the choice of cubic phase forming lipid, the space group of the formed protein crystal (C2221) and the packing of the protein molecules was identical.

References:

S1. R. Mezzenga, M. Grigorov, Z. Zhang, C. Servais, L. Sagalowicz, A. I. Romoscanu, V. Khanna, C. Meyer, *Langmuir* 2005, **21**, 6165-6169.