Plasticity of Lysteriolysin O Pores and its Regulation by pH and Unique Histidine

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SUPPLEMENTARY INFORMATION

Supplementary Movie: Formation of pore by LLO.

Supplementary Methods

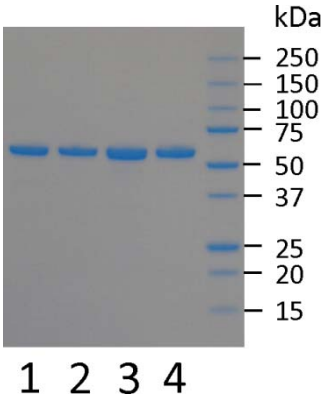
Circular Dichroism (CD) Spectroscopy

CD spectra were collected on Chirascan CD Spectrometer (Applied Photophysics, UK). Proteins were diluted in the respective buffer to a final concentration of 0.2 mg/ml. Spectra were taken at pH 5.7 (20 mM MES, 10 mM NaCl) and 7.5 (20 mM Tris-HCl, 10 mM NaCl), as well as at different temperatures with discrete temperature steps.

Viability of Caco-2 cells

Caco-2 cells were cultured on 96-well microtiter plates for 1 week in DMEM D5921 (pH 7.4) (Dulbecco's modified eagle medium) with 1 % glutamine (PAA – The cell culture company), 1 % Antibiotic-Antimycotic mixture (GIBCO) and 10 % FBS (fetal bovine serum). For experiments at pH 6.0, DMEM D5523 was used, upon addition of 1 % of the mixture 25 mM MES pH 6.0 and 0.23 g/ml NaCl. Prior to treatment, cells were washed with serum free medium of pH 7.4 or 6.0. Cells were then treated with different LLO concentrations and incubated for 3 hours at 37 °C in the buffer with respective pH. After 3-hour incubation cells were washed in the test medium described above (pH 7.4) and then incubated overnight at 37 °C. MTT cell viability reagent was then added to the cells followed by 2 hour incubation at 37 °C. The formed formazan crystals were then dissolved in DMSO and absorbance measured at 570 nm. Experiments were done twice for each pH and each time in two parallels.

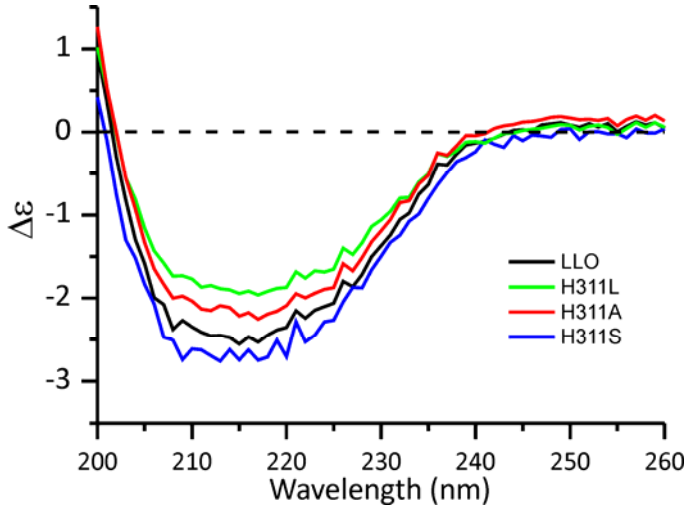
Supplementary Figure 1



Supplementary Figure 1. SDS-PAGE gel of LLO and its mutants

SDS-PAGE of purified proteins used in this study was stained with SimplyBlue™ SafeStain (Life Technologies, USA). 1, wild-type LLO; 2, H311L; 3, H311S; 4, H311A.

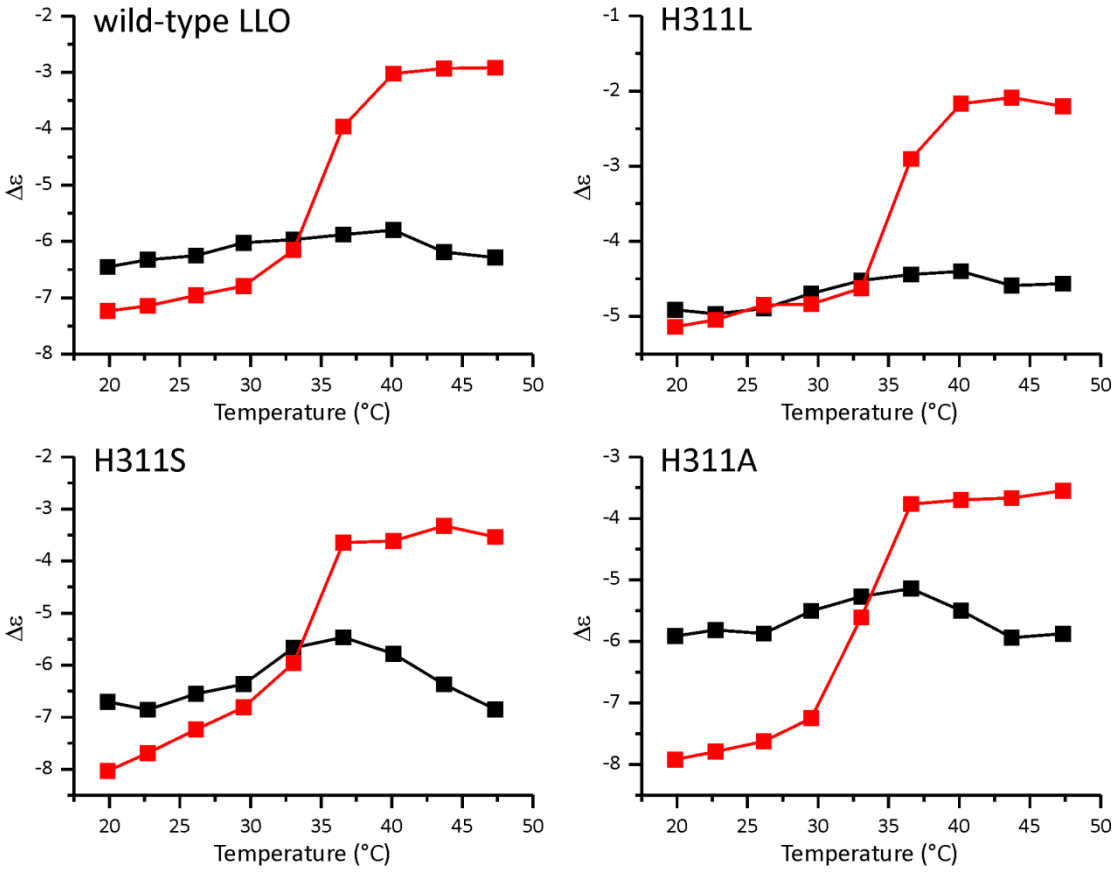
Supplementary Figure 2



Supplementary Figure 2. Far-UV circular dichroism spectra of LLO and its mutants

Proteins were diluted in 20 mM MES, 10 mM NaCl, pH 5.7 at a final concentration of 0.2 mg/ml. Temperature was 20 °C.

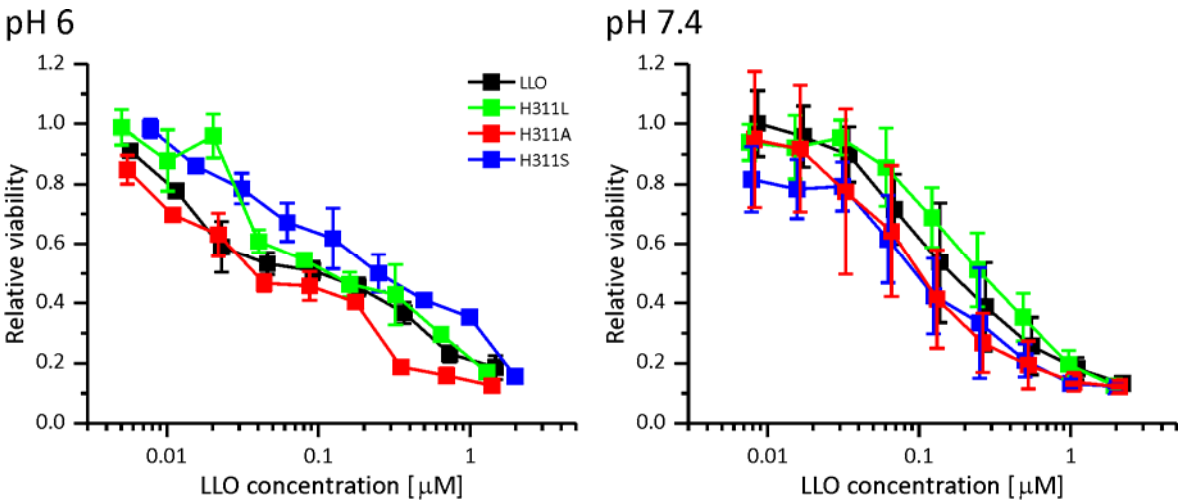
Supplementary Figure 3



Supplementary Figure 3. Unfolding of proteins as measured by circular dichroism spectroscopy

Spectra were taken at pH 5.7 (black) or 7.5 (red). Protein concentration was 0.2 mg/ml.

Supplementary Figure 4



Supplementary Figure 4. Toxicity of proteins used in the study towards Caco-2 cells

Proteins were added to Caco-2 cells at respective pH, incubated for 3 h at 37 °C, washed with fresh medium, incubated overnight and then assayed with MTT reagent. The averages ± S.D. of three to four parallels of two different independent experiments are presented.