Supplementary Information.

Crystal structure of JlpA, a surface-exposed lipoprotein adhesin of *Campylobacter jejuni*

Materials and Methods

Materials - Restriction enzymes were purchased from New England Biolabs. *Pfu* DNA polymerase and T4 DNA ligase were purchased from Stratagene and Promega, respectively. Primers used for PCR were synthesized by IDT. Unless indicated otherwise, chemicals were purchased from Sigma.

Cloning, expression, and purification of JlpA - The DNA fragment encoding the mature form of JlpA (without the signal peptide) corresponding to amino acids residues 18 to 372 ($JlpA_{18\text{-}372}$) was amplified by PCR using *C. jejuni* strain 81-176 genomic DNA and was inserted into NdeI / BamHI sites of pET19b (Novagen) to express an N-terminal His-tagged protein. For the phasing purpose, $JlpA_{18\text{-}372}$ variants ($JlpA_{I45M/I160M}$, $JlpA_{I45M/L284M}$, $JlpA_{I160M/I284M}$, and $JlpA_{I45M/I160M/L284M}$) were produced by using the plasmid pET19b::$JlpA_{18\text{-}372}$ as template and QuikChange® II XL Site-Directed Mutagenesis Kit (Stratagene) according to the manufacturer's instructions. Primers used to create the mutants are listed in Table S1. To confirm the correct sequences in all clones, DNA sequencing was performed by SeqWright (Houston, TX).

To purify His-tagged JlpA proteins, the pET-based plasmids containing the genes encoding $JlpA_{18\text{-}372}$ and its variants were expressed in *E. coli* BL21(DE3) or B384(DE3) (Stratagene) using liquid Luria-Bertani (LB) or SelenoMet (Molecular Dimensions Ltd.) media supplemented with 100 μg/ml ampicillin. Bacteria (in 1 L culture) were grown to an A_{600} 0.8, and expression was induced with 0.2 mM IPTG at room temperature for 3 h. Bacterial cells were

harvested by centrifugation at 4,000*g* (Sorvall) for 20 min. Subsequently, cells were resuspended in 80 ml of buffer-A (20 mM Tris-HCl, pH 8.0, 250 mM NaCl, 5 mM β-mercaptoethanol) containing 10 mM imidazole, 0.1 % Triton X-100 and EDTA-free Protease Inhibitor Cocktail Tablets (Roche, Cat# 04693132001), and disrupted using sonication at 30% cycles for 3 min (3 time) on ice. After centrifugation at 40,000*g* (Sorvall) for 30 min, supernatants were subjected to a binding reaction with 4 ml of Ni^{2+}-NTA agarose (Qiagen) for 30 min in a batch purification procedure. Protein-resin complexes were then packed onto a column and washed with buffer-A containing 20 mM imidazole. The proteins were eluted using a step gradient method including 50, 100, 250, and 500 mM imidazole in buffer-A. Following SDS-PAGE analyses, fractions containing target proteins were diluted five times with buffer-B (20 mM Tris-HCl, pH 8.0), loaded onto a HiTrap-Q column (GE Healthcare), and eluted with a linear gradient of 50 mM to 1M NaCl in buffer-B using an AKTA Purifier system (GE Healthcare). The peak fractions were combined, concentrated, and further purified using a HiPrep 16/60 Sephacryl S-100 HR gel filtration column (GE Healthcare) equilibrated with buffer-C (20 mM Tris-HCl, pH 8.0, 150 mM NaCl). The protein concentration was determined by UV spectroscopy or by Bradford assay (Bio-Rad).

Table S1: Primers used in this Study

Name	Primer Sequence
I45M_forward	5'- GTT AAA CAA GAA ATT GCA AGC ATG TCT CAG GAT TCT GGA ATA AAG - 3'
I45M_reverse	5'- CTT TAT TCC AGA ATC CTG AGA CAT GCT TGC AAT TTC TTG TTT AAC - 3'
I160M_forward	5'- GAT CCA AAA ATC AGC TCT TTT ATG AAT AAA TTA AGC TCG GAT TCT - 3'
I160M_ reverse	5'- AGA ATC CGA GCT TAA TTT ATT CAT AAA AGA GCT GAT TTT TGG ATC - 3'
L284M_forward	5'-ATA GCA ACT GCT AAG GAA AAT ATG CAA ACC TTA AAA GCT CAA AGT - 3'
L284M_reverse	5'-ACT TTG AGC TTT TAA GGT TTG CAT ATT TTC CTT AGC AGT TGC TAT - 3'

Crystallization and data collection – For crystallization, purified JlpA and its variants were concentrated to ~7 mg/ml. Crystals of both native and SeMet-labeled JlpA were obtained at 16°C using the hanging drop vapor diffusion method with a reservoir solution containing 32-35% (v/v) 2-methyl-2,4-pentanediol (MPD), 0.03M $CaCl_2$ and 100 mM Na-acetate (pH 5.6). Various shapes of crystals were observed within a week and grew for several weeks. Crystals were extremely unstable and impossible to treat with a cryo-protectant. Therefore, for the data collection purposes, single crystals were swiftly collected from crystallization drops, and directly flash-cooled in liquid nitrogen, without additional cryo-protectant. Data sets were collected at the wavelength of 0.97942 Å for native crystals and at three wavelengths (peak=0.97940, inflection=0.97954, and remote=0.97172 Å) for SeMet-derivatized crystals at 100K on Advanced Photon Source (APS) Beamline 19ID, Argonne. Data sets were processed with HKL3000 (Minor et al., 2006). Crystals were in space group $P4_12_12$ with unit cell dimensions $a=b$=111.8 Å, c=171.4 Å, and contain 2 molecules per asymmetric unit.

Flow cytometry based JlpA binding to INT-407 cells - INT-407 cells were seeded in 75cm^2 tissue culture flasks (Corning) in DMEM and grown at 37ºC 5% CO_2 until approximately 80% confluent. After washing twice with PBS, cells were harvested in 10 ml of dissociation buffer (Gibco) and pelleted at 500*g*. Cells were washed twice with PBS to remove the dissociation buffer, resuspended in PBS, and counted to determine concentration. 7.5x10^4 cells were aliquoted in 1.5 ml eppendorf tubes and pelleted. The cells were immediately resuspended in 200 μl ice cold PBS + 2% BSA.

JlpA was labeled with Alexa Fluor 488 Protein Labeling Kit (Invitrogen) following the manufacturer's directions. Briefly, 500 μl of 2.5 mg/ml of purified JlpA was reacted with 50 μg of

dye at room temperature for 1 h. Unbound dye was quenched by the addition of hydroxylamine. To remove unbound dye, the labeled protein was dialyzed extensively against PBS, pH 7.4. Final protein concentration was determined utilizing the Pierce BCA Kit. As a control JlpA was mock labeled as described above without the addition of dye.

For saturation binding experiment, 0.1, 0.3, 1, 3, 10, 30, 60, 100, or 300 μg of labeled protein was added to an eppendorf tube and the final volume adjusted to 500 μl with PBS + 2% BSA. The previously aliquoted cells were then added to the protein, resulting in a final volume of 700 μl. The tubes were rocked on ice for 2 h to allow for binding.

To investigate the specificity of JlpA binding, cells were prepared as described above. In separate tubes 1 μg of Alexa Fluor 488 labeled JlpA was mixed with either 0.03, 0.1, 0.3, 1, 3,10, 30, 100, 300, or 1000 μg of unlabeled JlpA in 400 μl of PBS + 2% BSA. The previously aliquoted cells were then added to the protein, resulting in a final volume of 600 μl. Following binding on ice for 2 h, cells were diluted by the addition of PBS to a final volume of approximately 1.2 ml. Cells were analyzed on a Becton Dickinson FACScan Flow Cytometer.

Supplementary Figure S1

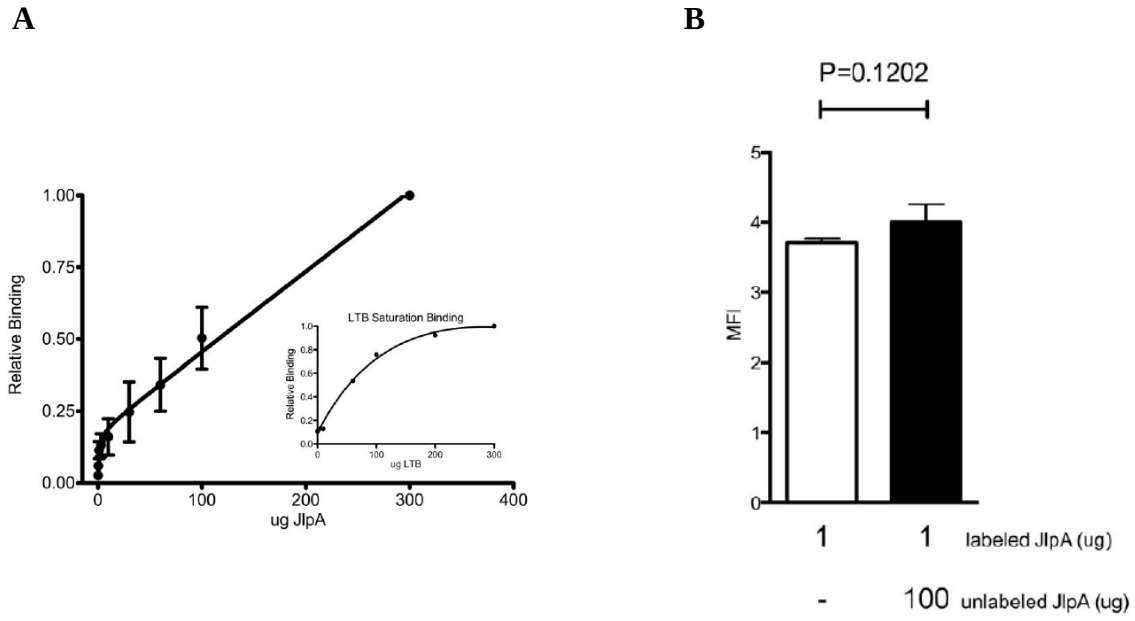

Figure S1. JlpA binding to INT407 cells. (**A**) Relative binding of JlpA to INT407 cells as a function of protein concentration (0.1-300 μg). Experiments were conducted in triplicate, normalized and expressed as a function of relative maximal binding. Inset, relative binding of control protein (LTB) demonstrating saturation binding. (**B**) Competition assay. Relative binding of fluorescently labeled JlpA in the absence (white bar) or presence (black bar) of 100-fold excess of unlabeled JlpA. Experiments were conducted in triplicate and represented as mean fluorescence intensity (MFI). Error bars are standard deviations. Statistical significance was calculated using unpaired *t*-test.

Reference

Minor, W., Cymborowski, M., Otwinowski, Z., Chruszcz, M., 2006. HKL-3000: the integration of data reduction and structure solution - from diffraction images to an initial model in minutes. Acta Crystallogr. D Biol. Crystallogr. 62, 859-866.