SUPPLEMENTAL INFORMATION

for

Ex Vivo Analysis of Synergistic Anion Binding to FbpA in Gram-Negative Bacteria

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SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Chloroform extracts from *E. coli* expressing FbpA (pSBGL:JM109) or no FbpA (JM109). These preparations were run under standard SDS-PAGE conditions, transferred to a nitrocellulose filter, and visualized with either Coomassie blue for total protein (left) or affinity purified rabbit (right). The Coomassie blue stain shows the diversity of proteins expressed with molecular weights between 29 and 48 kDa. The * indicates the beta-lactamase band associated with the pSBGL plasmid construct. The FbpA-specific sera detects an FbpA band in the pSBGL sample and no band in the JM109 sample. These data confirm the presence of FbpA in the samples used for SUPREX analysis.

Figure S2. MALDI-TOF mass spectrum obtained on a periplasmic extract from the *E. coli* strain not expressing FbpA (JM109). The sample was submitted to MALDI-TOF analysis after it was desalted and concentrated using C_4 ZipTips as described in the experimental section. The arrows indicate the expected position of ion signals derived from FbpA (i.e. the $[M+H]^+$ and $[M+2H]^{+2}$ species), although, no such ion signals were detected.

Figure S3. Immunoprecipitation of FbpA from a periplasmic extract from *E. coli* overexpressing FbpA (pSBGL:JM109). A representative MALDI-TOF mass spectrum obtained on the extract prior to immunoprecipation is shown in (**A**). The immunoprecipitation reaction was initiated by incubating 5 µL of the periplasmic extract with 30 µL of rabbit affinity purified Anti-FbpA (1.2 mg/mL) in a total of 100 µL of PBS (10 mM phosphate buffer containing 150 mM NaCl, pH 7.4) for 30 min at room temperature. The solution, which contained the immune complex, was added to a spin cup fitted with a 0.45 µM cellulose acetate filter and containing approximately 0.1 mL crosslinked 6% agarose beads (settled) derivatized with protein G (Pierce, Inc.). The IgG binding capacity of the protein G coated beads as specified by the manufacturer

was 11-15 mg of human IgG/mL of settled beads. The solution in the spin cup was allowed to incubate for 15 min with gentle mixing. The spin cup was placed in a microcentrifuge tube and centrifuged for 45 s at 2000 × *g*. A mass spectrum of the flow-through (i.e., what did not interact with the protein G coated agarose beads) is shown in (**B**). The mass spectrum in (**B**) shows that FbpA was depleted from the lysate after the immune complex was captured on the protein G coated agarose beads and that there are no other ion signals at m/z 33,598 ± 30. The protein G resin containing the captured immune complex was washed with 0.5 mL PBS and centrifuged for 45 s at 2000 × *g*. The resin was washed and centrifuged two additional times. Ultimately, the FbpA was eluted off the protein G coated agarose resin by adding 0.1 mL of 0.1 M glycine (pH 2.0) and centrifuging the sample. A total of three elution fractions were collected. A mass spectrum obtained on the second elution fraction (most of the FbpA was present in this fraction) is shown in (**C**).

All the samples submitted to MALDI-TOF analysis (i.e., the initial sample, the flow-through, and elution fractions) were desalted and concentrated using C_4 ZipTips as described in the experimental section. The arrows indicate the expected position of ion signals derived from FbpA (i.e., the $[M+H]^+$ and $[M+2H]^{+2}$ species). The mass spectra were smoothed using the manufacturer's software. The smoothing parameters used were a Gaussian algorithm with a width of 20 m/z units.

Figure S4. Immunoprecipitation of FbpA from an *N. gonorrhoeae* periplasmic preparation. (**A**) Representative MALDI mass spectrum of the clinical isolate. The immunoprecipitation reaction was identical to that of the *E. coli* sample in Figure S3 except that 50 µL of the gonococcal clinical isolate and 50 µL of the antibody were used. Analogous to that shown in Figure S3 a mass spectrum of the flow-through from the immunoprecipitation reaction is shown in (**B**), and

no ion signals are detected at m/z 33,639 ± 30. (**C**) Mass spectrum of the second elution fraction from the immunoprecipitation reaction showing the FbpA ion signal.

As in Figure S3, all the *N. gonorrhoeae* samples submitted to MALDI-TOF analysis (i.e., the initial sample, the flow-through, and elution fractions) were desalted and concentrated using C_4 ZipTips as described in the experimental section. The arrows indicate the expected position of ion signals derived from FbpA (i.e. the $[M+H]^+$ and $[M+2H]^{+2}$ species). The mass spectra were also smoothed using the manufacturer's software. The smoothing parameters used were a Gaussian algorithm with a width of 20 m/z units.

Figure S1

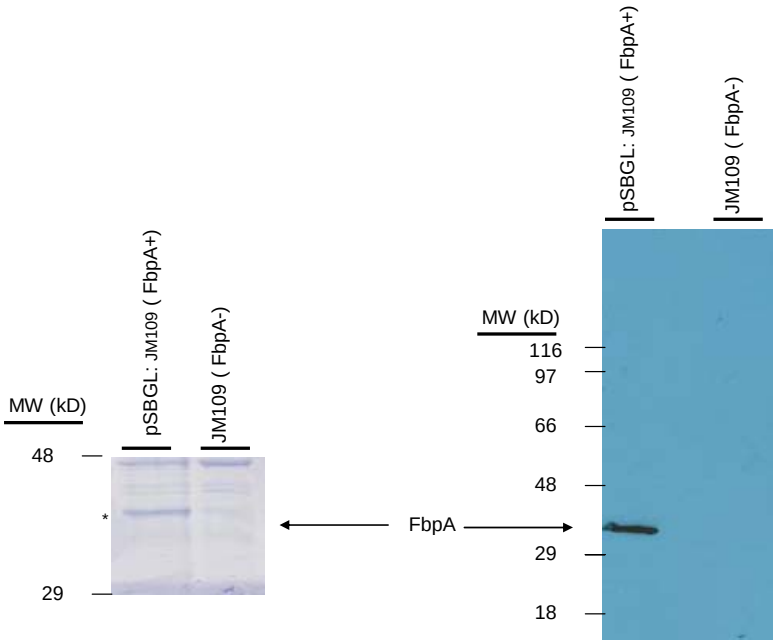
pSBGL: JM109 (FbpA+)
JM109 (FbpA-)
MW (kD)
48
29
*
FbpA
MW (kD)
116
97
66
48
29
18
pSBGL: JM109 (FbpA+)
JM109 (FbpA-)

Figure S2

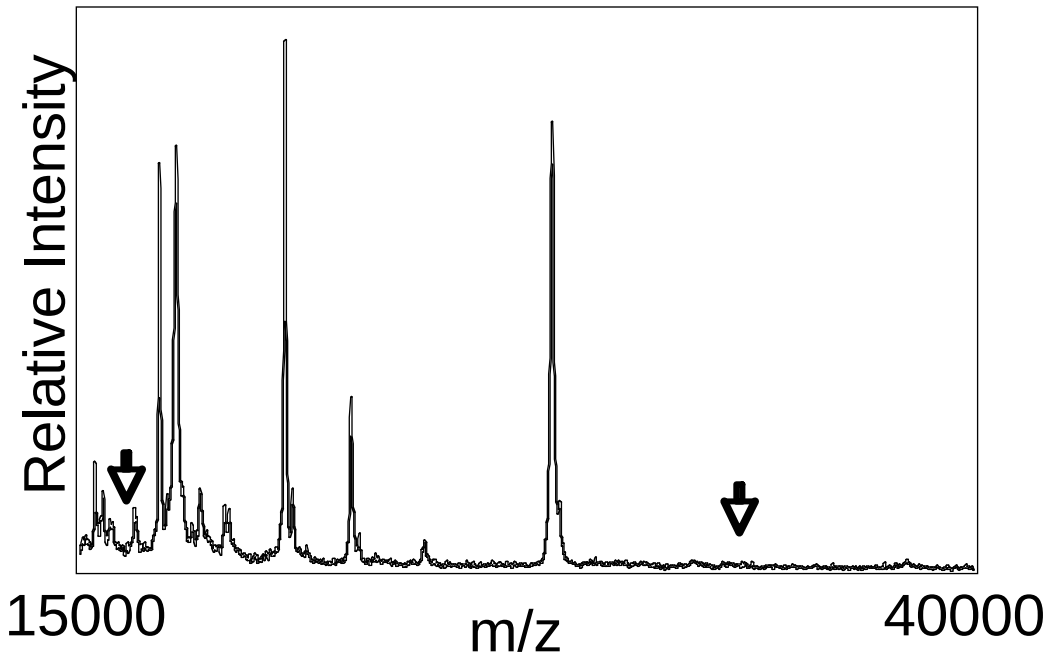

Figure S3

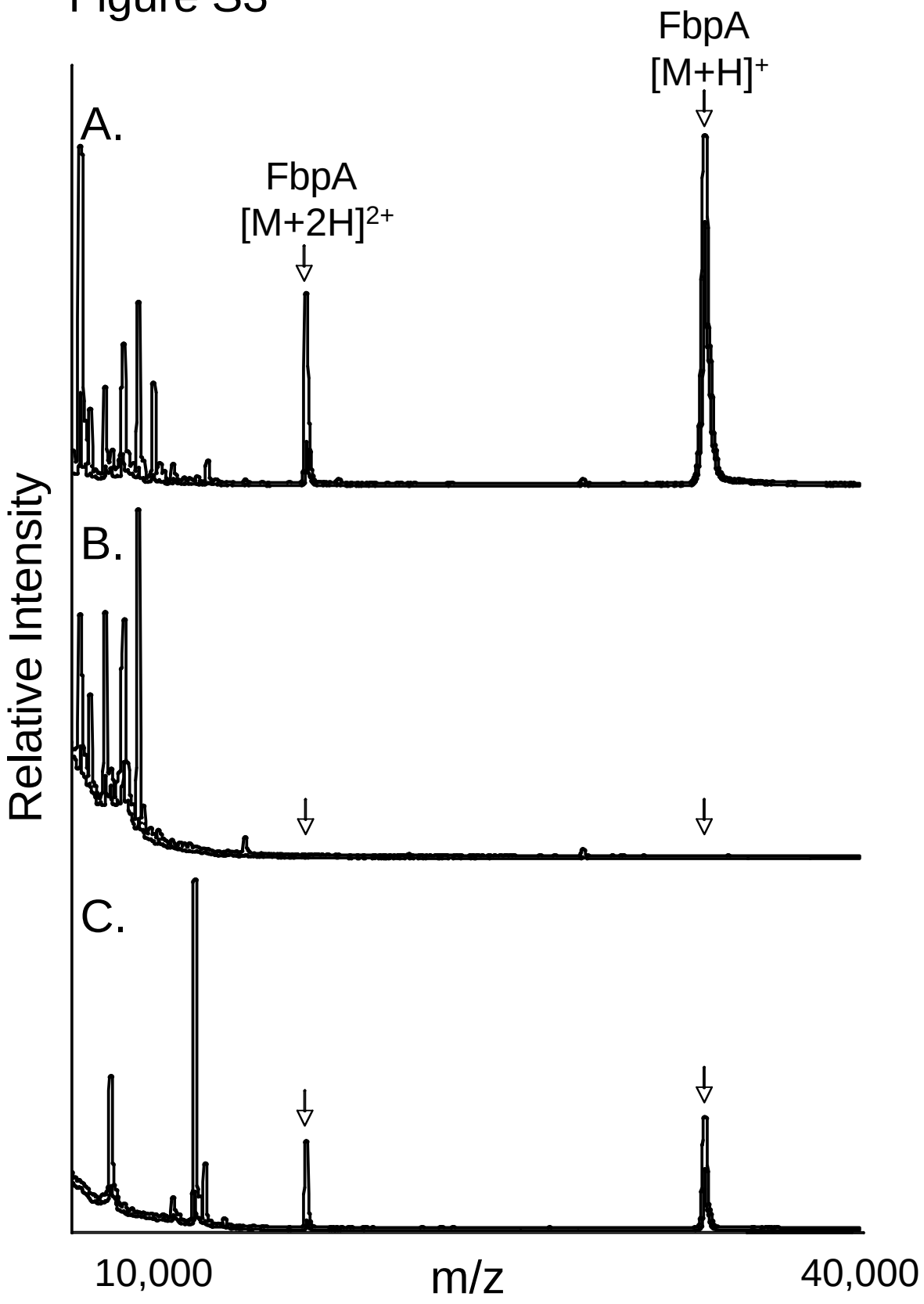

Figure S4