SUPPLEMENTAL INFORMATION FOR:

DIRECT MEASUREMENTS OF THE OUTER MEMBRANE STAGE OF FERRIC ENTEROBACTIN TRANSPORT: POST-UPTAKE BINDING

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Running head: PUB determinations of OM transport
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Figure S1. Graphic representation of normal, blocked and post-uptake binding assays of FepA-mediated FeEnt transport. In this cartoon format, the relative sizes of the various molecules are not drawn to scale: FepA is drawn from PDB entry 1FEP, FepB is modeled based on the structure of *Haemophilus influenzae* Fbp (PDB entry 1D9V), and FepCDG is modeled based on the structure of the *H. influenzae* ABC-transporter (PDB entry 2N2Q). **(Left)** In normal binding assays the bacteria are grown in iron-free media and chilled to 0 °C; all FepA proteins in the OM are free to adsorb ^{59}FeEnt (cyan). This determination establishes the ^{59}FeEnt binding capacity. **(Center)** In blocked binding assays aliquots of the same bacterial culture are chilled on ice and exposed to excess ^{56}FeEnt (CPK colors), which saturates their FepA proteins. However, when the cells are pelleted by centrifugation and resuspended in fresh media at 0 °C the binding interaction re-equilibrates, and a fraction of the FepA-FeEnt complexes dissociate. The amount of ^{59}FeEnt that the cells bind, relative to the normal binding results, reveals the number of ligand-free (and remaining ligand-bound) FepA proteins. **(Right)** In post-uptake binding assays the bacteria are prepared as in blocked binding, but they are allowed to transport bound ^{56}FeEnt by incubation at 37 °C for 1 min, prior to re-cooling. The amount of ^{59}FeEnt that the cells subsequently bind, relative to the blocked binding results, reveals the number of FepA proteins that internalized bound ligand during the incubation period.

Figure S2. Immunoprecipitation of FepA in the presence of FeEnt and FepB. Affinity-purified FepA (42), alone (Panels A and B) or pre-incubated with 10 nM FeEnt (C and D), was mixed with purified anti-FepA MAb 45 (40), in the absence or presence of purified FepB (20), and incubated overnight prior to the addition of protein A-agarose (Pierce #22810). After an additional 5-hr incubation the mixtures were spun at 4000 x g for 5 min, and the supernatants (lanes 1-7) and re-suspended pellets (lanes 8-14) were analyzed by SDS-PAGE. Lanes 1, 8: FepA; lanes 2, 9: FepA + PAA; lanes 3, 10: FepA + PAA + MAb 45; lanes 4, 11: FepB; lanes 5, 12: FepB + PAA; lanes 6, 13: FepB + PAA + MAb 45; lanes 7, 14: FepB + PAA + MAb 45 + FepA.

Figure S3. Precipitation of OM fragments containing FepA in the presence of FeEnt and FepB. MOPS-grown BN1071 was saturated with FeEnt, pelleted by centrifugation, and its OM fractions were purified by sucrose gradient fractionation (44). We tested both the material that pelleted through the sucrose gradient (lanes 2, 4, 7, 9), and the OM band suspended in the gradient (lanes 3, 5, 8, 10) for their ability to adsorb purified FepB (20); lanes 1, 6). OM fragments were mixed with FepB, incubated on ice for 2 hr, collected by centrifugation (100,000 x g for 45 min), and the pellets (lanes 1-5) and supernatants (lanes 6-10) were analyzed by non-denaturing SDS-PAGE (i.e., without boiling the samples, which allowed better visualization of FepB). Most FepB remained in the supernatant during the ultracentrifugation (a smaller amount pelleted), and this distribution was not affected by the presence of the FepA-FeEnt complex in the incubation mixture.

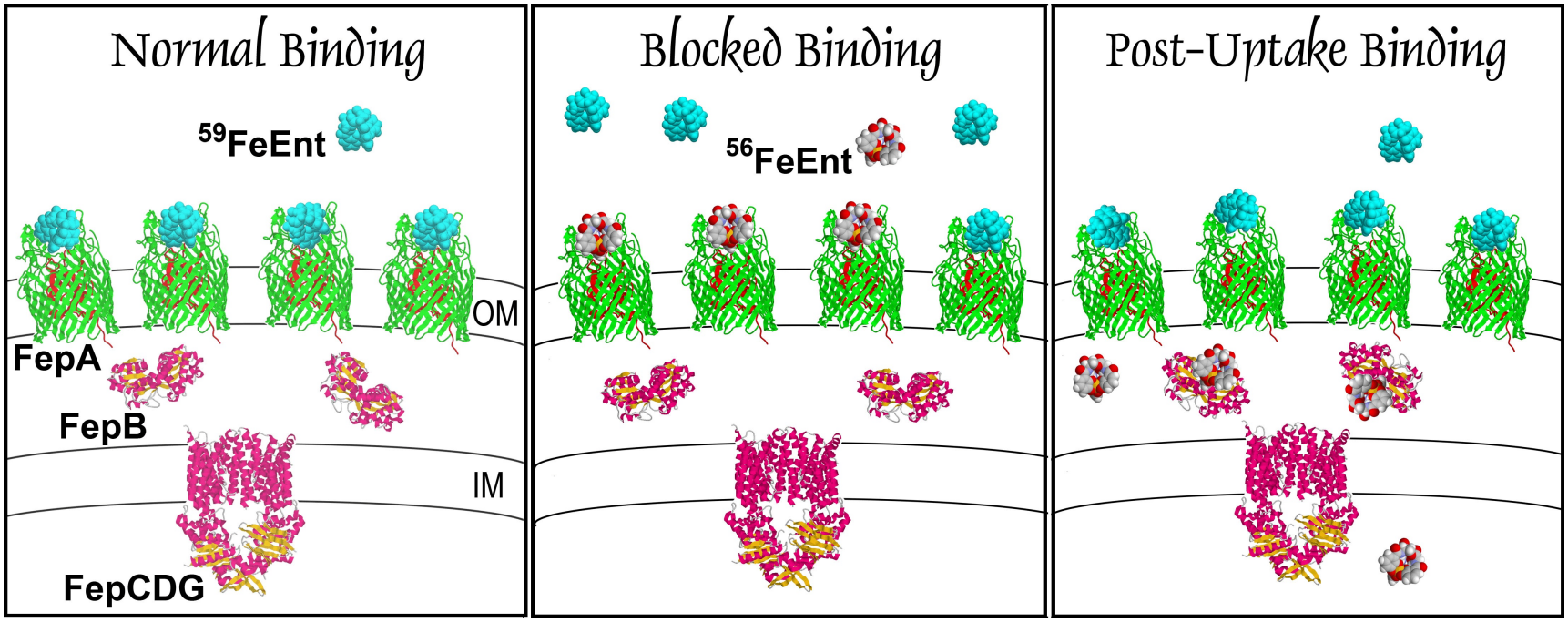
Normal Binding
59FeEnt
OM
FepA
FepB
IM
FepCDG
Blocked Binding
56FeEnt
Post-Uptake Binding

Figure S1.

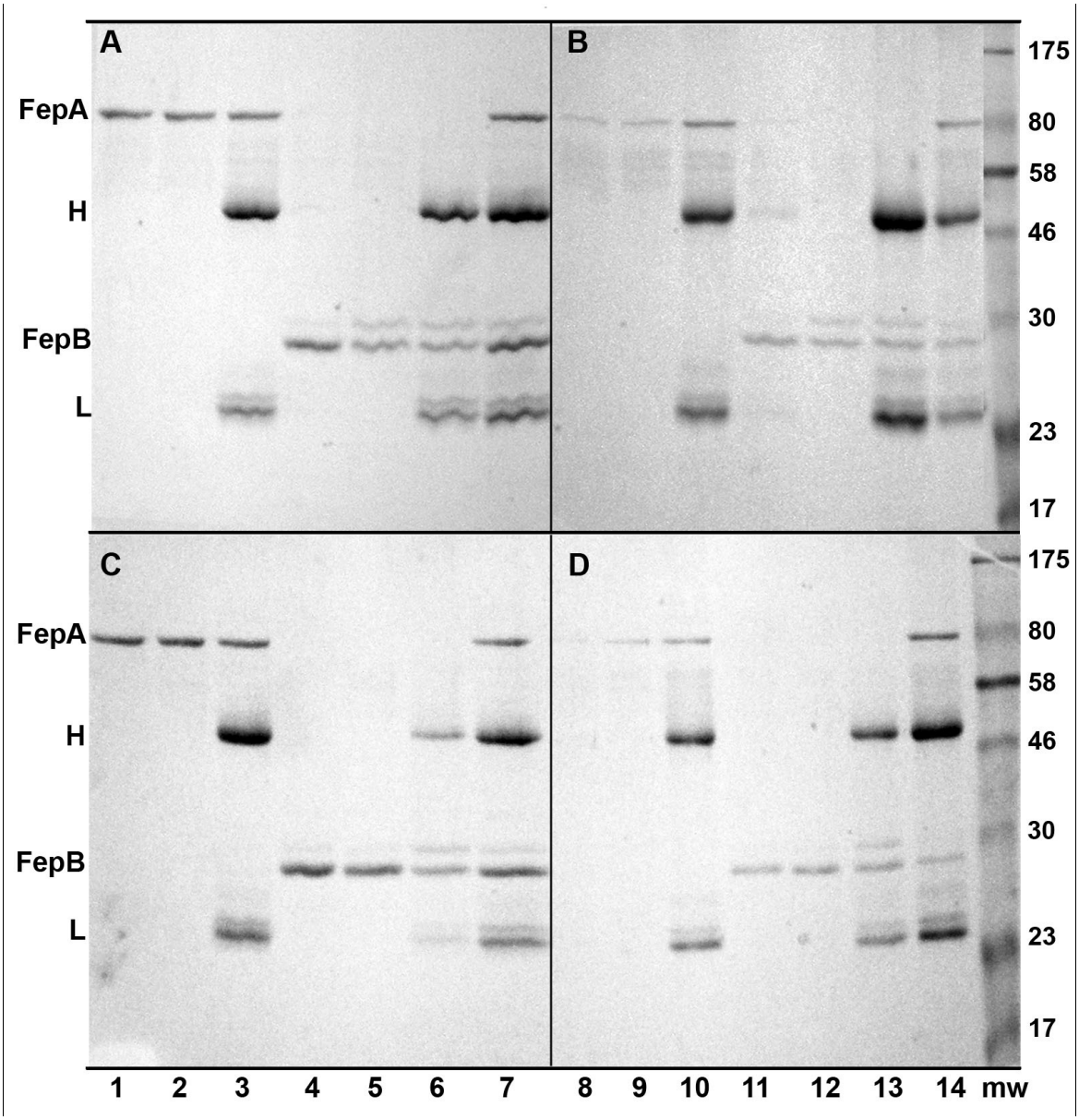
A
B
C
D
FepA
H
FepB
L
175
80
58
46
30
23
17
1 2 3 4 5 6 7 8 9 10 11 12 13 14 mw

Figure S2.

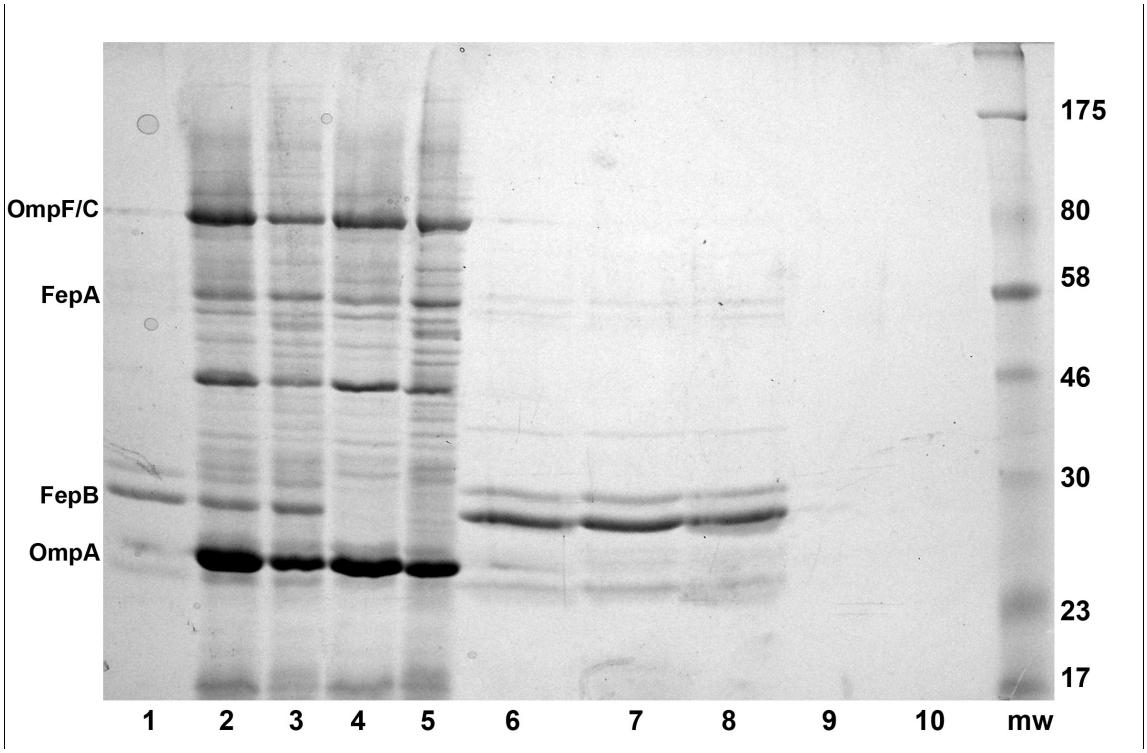

Figure S3.