

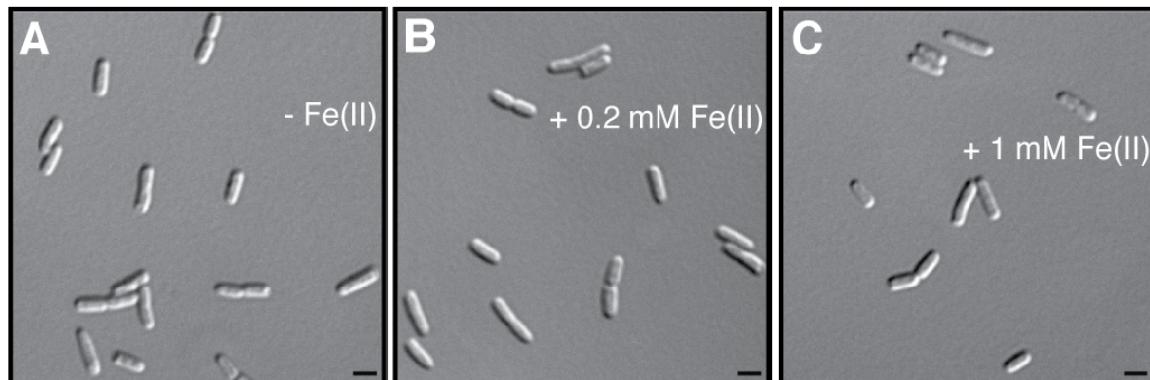
Supplemental Information

A Ferritin-Based Label

for Cellular Electron Cryotomography

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Figure S1. Effect of Fe(II) on *E. coli* growth and morphology



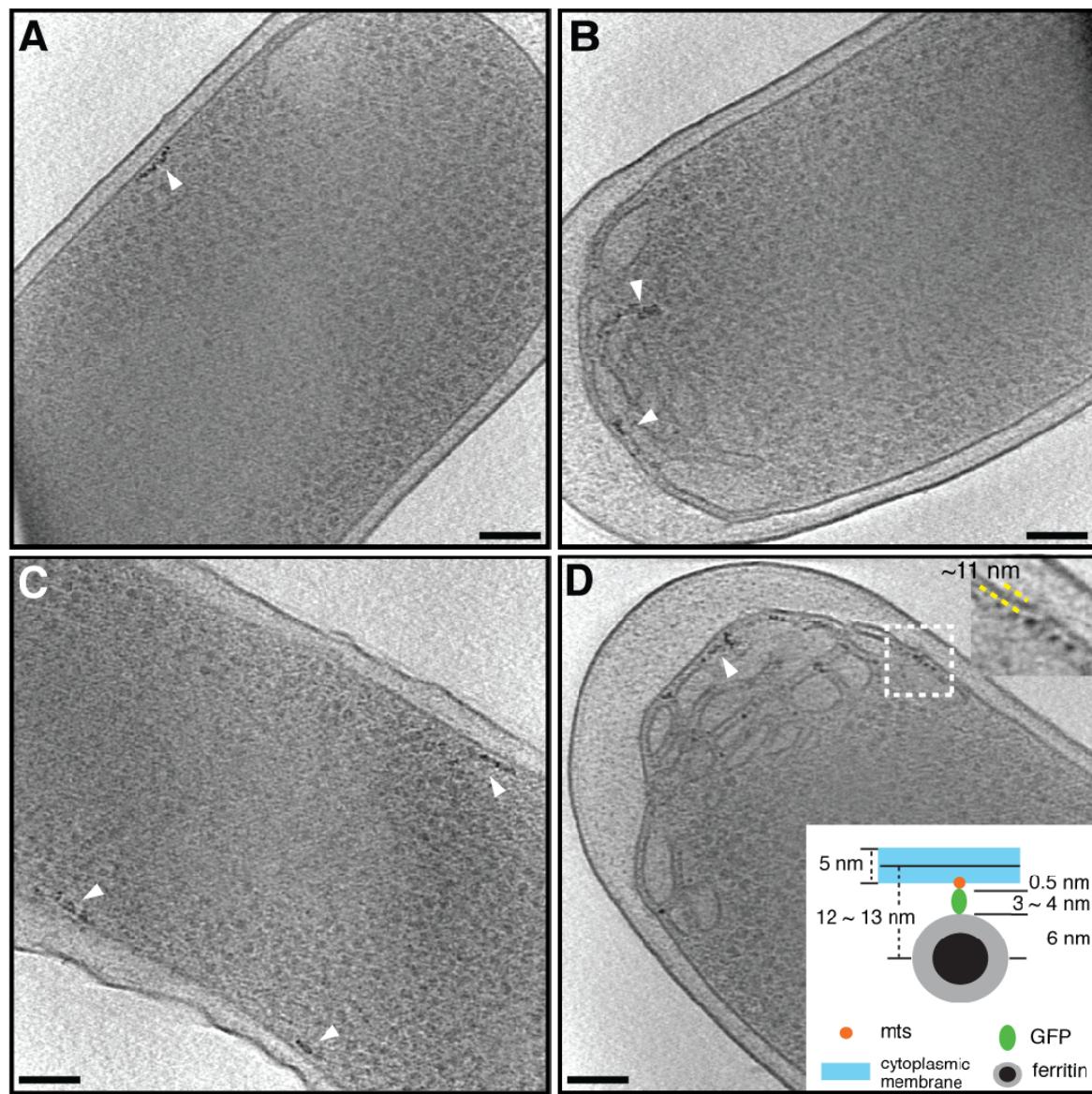
D Characteristics of cells grown with and without iron (n=200)

Growth condition	% of cells with visible invagination	Average cell length in μm (SD)
LB	27.5%	3.8 (0.7)
LB + 0.2 mM FeSO ₄	26.2%	3.8 (0.9)
LB + 1 mM FeSO ₄	25.8%	3.8 (0.8)

(A-C) DIC images of *E. coli* cells grown in LB medium supplemented with 0, 0.2, or 1 mM of Fe(II).

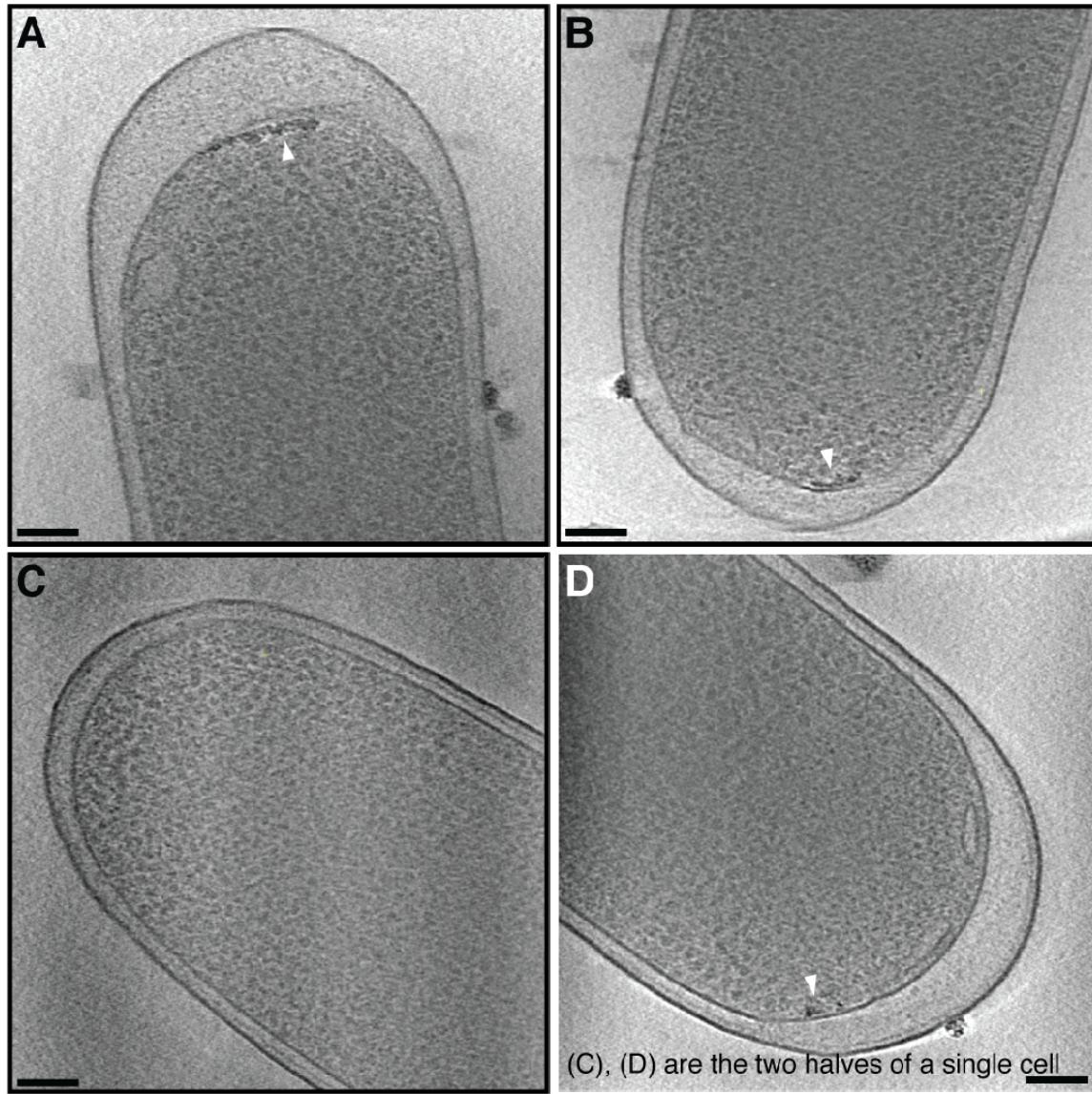
Scale bars: 2 μm . (D) Characteristics of cells grown in the three conditions as in (A-C).

Figure S2. *E. coli* cells with mts-ferritin labelling membrane



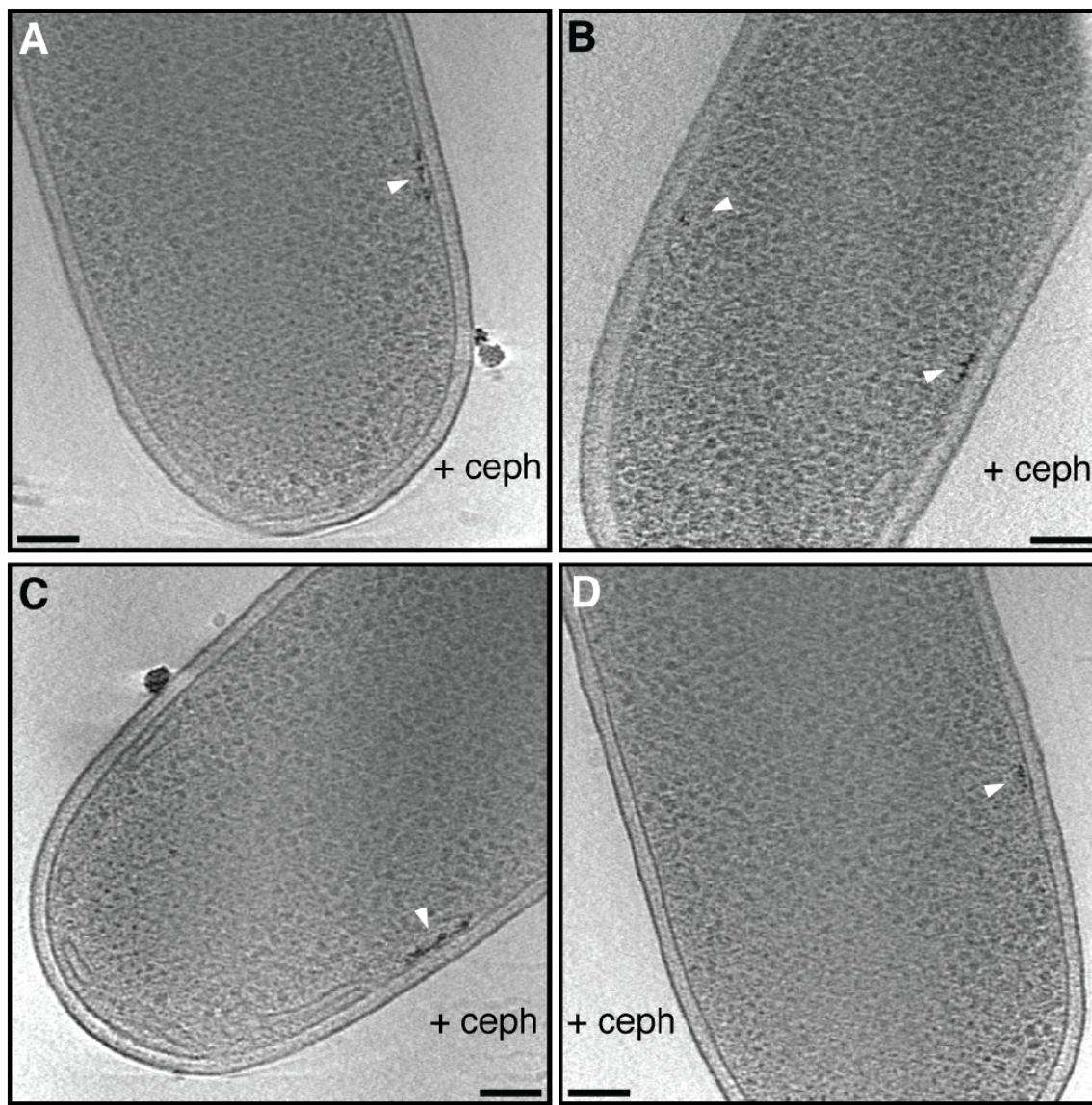
(A-D) 10 nm tomogram slices of *E. coli* cells with ferritin-labelling membrane. Mts-ferritin particles (some of which are marked with white arrowheads) localise to membrane vesicles formed near cell poles or localise along the cytoplasmic membrane. (D) also has the distance from centre of ferritin dots to the cytoplasmic membrane and the expected size of mts-ferritin annotated. Scale bars: 100 nm.

Figure S3. *E. coli* with CheY-ferritin labelling chemosensory machinery



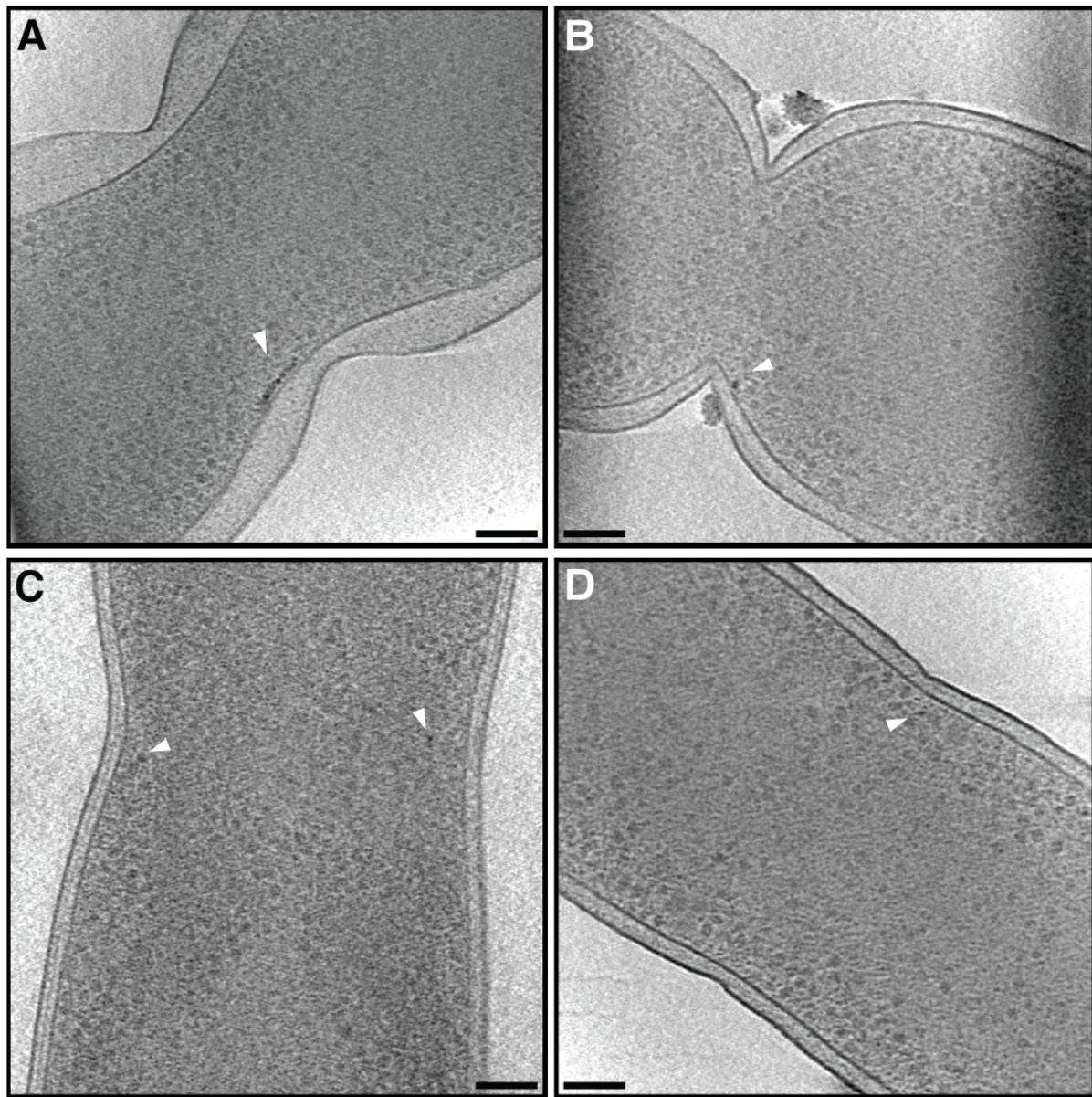
(A-D) 10 nm tomogram slices of *E. coli* cells with ferritin-labelling the chemosensory machinery. CheY-ferritin particles all localised to the chemoreceptors at the cell poles (marked with white arrowheads). (C) and (D) are tomograms of the two halves of a single cell, illustrating CheY-ferritin only ever labels one pole of the cell. Scale bar: 100 nm.

Figure S4. Cephalexin-treated *E. coli* cells with CheY-ferritin labelling chemosensory machinery



(A-D) 10 nm tomogram slices of cephalexin-treated *E. coli* with ferritin-labelling the chemosensory machinery. CheY-ferritin particles localised to the lateral chemoreceptors along the cytoplasmic membrane (marked with white arrowheads). Scale bars: 100 nm.

Figure S5. *E. coli* cells with ZapA-ferritin labelling cell septum



(A-D) 10 nm tomogram slices of *E. coli* cells with ferritin-labelling the cell septum. ZapA-ferritin particles (marked with white arrowheads) all localised close to the invaginating cytoplasmic membrane in the septal region. Scale bars: 100 nm.