SUPPORTING INFORMATION

Inhibiting Iron Mobilization from Bacterioferritin in *Pseudomonas aeruginosa* Impairs Biofilm Formation Irrespective of Environmental Iron Availability

*Anabel Soldano[1], Huili Yao[1], Josephine R. Chandler[2] and Mario Rivera[1,]**

[1] Department of Chemistry, Louisiana State University, 232 Choppin Hall, Baton Rouge, Louisiana, 70803, United States

[2] Department of Molecular Biosciences, University of Kansas, 1200 Sunnyside Avenue, Lawrence, Kansas, 66045, United States

*Corresponding author. E-mail: mrivera@lsu.edu

ORCID: 0000-0002-5692-5497

Table of Contents:

The supporting information contains four figures comprised in 5 pages.

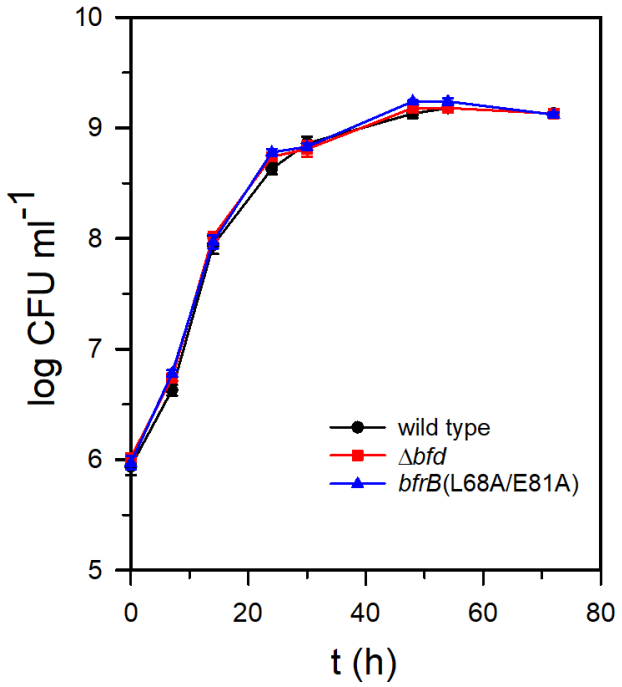

Figure S1. Wild type, Δ*bfd* and *bfrB*(L68A/E81A) cells cultured statically in 24-well plates exhibit similar planktonic growth rates in PI media supplemented with 20 μM Fe. Error bars represent the standard deviation of three independent growth curves.

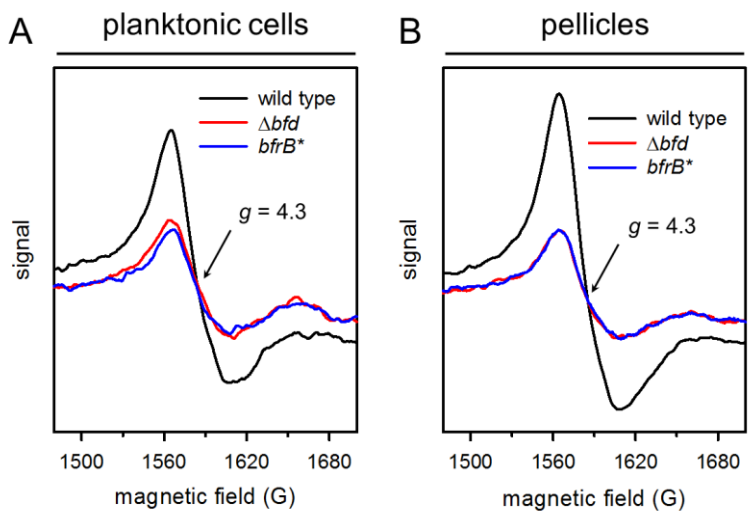

Figure S2. Whole-cell EPR spectra obtained from *P. aeruginosa* (**A**) planktonic and (**B**) pellicle-embedded cells harvested after 48 h of growth under static conditions. Cells were cultured in PI media supplemented with 20 µM Fe and treated as described in Experimental Methods prior to EPR spectroscopic analysis. The arrow indicates the ferric iron characteristic signal with g = 4.3. The *bfrB*(L68A/E81A) variant has been abbreviated as *bfrB** for simplicity.

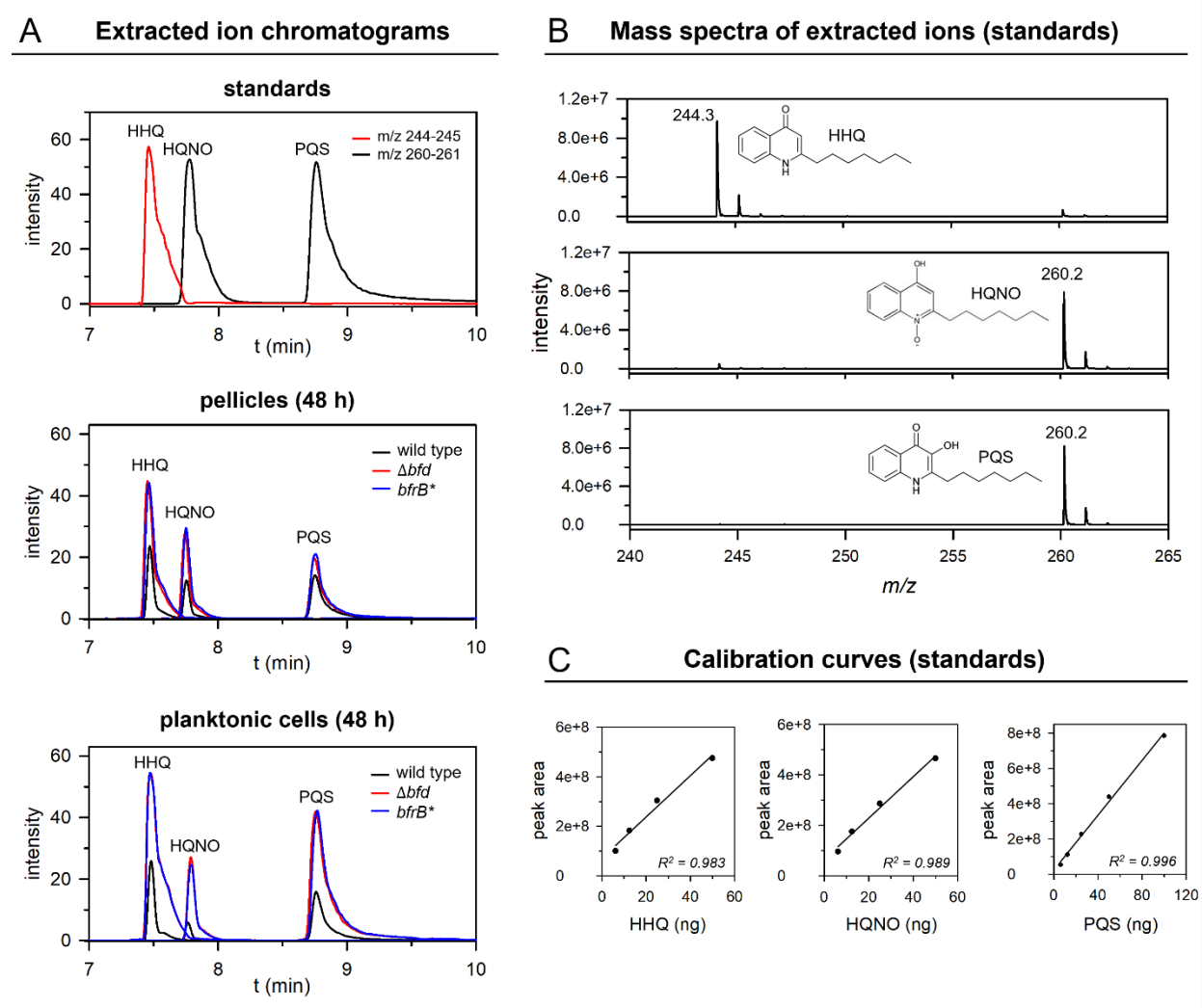

Figure S3. Representative mass spectral data from the LC-MS determination of HHQ, PQS and HQNO extracted from pellicles and planktonic cells. (A) Extracted ion chromatograms showing the elution times of HHQ, HQNO and PQS from the standards, pellicles sampled at 48 h, and planktonic cells sampled at 48 h. (B) Representative mass spectra of extracted ions from the standards, and (C) calibration curves constructed using pure HHQ, HQNO and PQS. The *bfrB*(L68A/E81A) variant has been abbreviated as *bfrB** for simplicity.

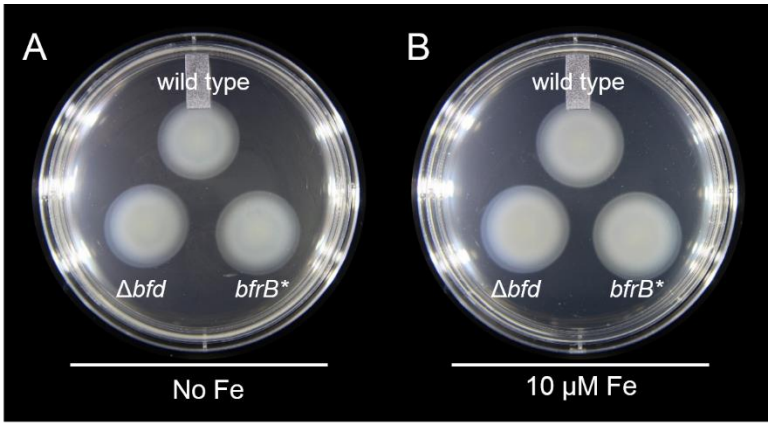

Figure S4. Swimming plates (20 mL of 1% tryptone, 0.5% NaCl, 0.3% agar) (**A**) not supplemented and (**B**) supplemented with 10 μM Fe were stab inoculated at the agar surface with wild type, Δ*bfd* and *bfrB** cells and incubated at 30 °C for 15 h. The *bfrB*(L68A/E81A) variant has been abbreviated as *bfrB** for simplicity.