Heme protects *Pseudomonas aeruginosa* and *Staphylococcus aureus* from calprotectin-induced iron starvation

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Table S1. Representative ICP-MS analysis of LB and Tris:TSB media.

Element	LB (µM)	Tris:TSB (µM)
Mg	77.8	164.0
Ca	149	79.1
Mn	0.275	0.157
Fe	7.89	4.25
Co	0.164	0.052
Ni	0.001	1.14
Cu	0.214	0.257
Zn	15.6	5.39

Table S2. Strains used in this study.

Strain	Description	Reference
S. aureus USA300	Methicillin-resistant strain	(1)
S. aureus USA300 JE2	Parent strain of NTML Collection; community isolate; methicillin-resistant	(2)
S. aureus Newman	Laboratory strain	(3), (4) Courtesy of Tony Richardson
S. aureus P1	Longitudinal CF isolate, type USA300, #2050	(5)
S. aureus P2	Longitudinal CF isolate, type USA300, #2059	(5)
S. aureus P3	Longitudinal CF isolate, type USA300, #2089	(5)
S. aureus COL	Methicillin-resistant strain	MRSA Clinical Isolate, 1961, Courtesy of Tony Richardson
S. aureus M2	Laboratory strain of MRSA	(6)
P. aeruginosa PAO1	*Pa* laboratory strain	(7)
P. aeruginosa PAO1 Δ*hasRphuR*	*hasR and phuR* knockout strain	(8)
P. aeruginosa PA14	Clinical isolate UCBPP-PA14	(9), Courtesy of Dianne Newman
P. aeruginosa PAO1/P_{antR}-'$lacZ^{-SD}$	PAO1 with the P_{antR}-'$lacZ^{-SD}$ reporter fusion integrated at the chromosomal *att* site	(10)

Table S3. Primers and probes used for RT-PCR.

Target	Sequence	Reference
Primers		
oprf (f)	GCG TTC GCA ACA TGA AGA AC	(11)
oprf (r)	CTT CTT GTT GCC GGT TTC GTA	(11)
phuS (f)	TGC CGA CGA ACA CCA TGA	(12)
phuS (r)	TGG CGA CCT GGC GAA A	(12)
hasA (f)	ATC GAC GCG CTG CTG AA	(13)
hasA (r)	TGG TCG AAG GTG GAG TTG ATC	(13)
hasR (f)	CGT GGC GTC GAG TAC CAG	(13)
hasR (r)	GGT CTT CGA ACA GAA GTC GTT G	(13)
sigA (f)	TGG TGC TGG ATC TCG ACC TA	
sigA (r)	TGC AAT TGC TGA CCA AGC AC	
sirA (f)	ACG CGA CAA TTA AGT CCG GT	
sirA (r)	CCA CTG ACG TCG CTG TAT CT	
isdC (f)	CTG CCA AAG ATG AAC GCA CT	
isdC (r)	GCA CCT GCT ACA TCA GTT GGT	
Probes		
oprf	CGG TGA GTA CCA TGA CGT TCG TGG C	(11)
hasA	TCG ACC CGA GCC TGT	(13)
hasR	CTG GCC TAC GGG CAG CTC TCC TA	(13)
phuS	CTT TCG GCC GCC GCT TCG A	(12)

Table S4. OD_{600} values of *S. aureus* cultures following CP and heme treatment.

Treatment	**JE2 OD_{600}** (range, N = 5)
Untreated	3.7 – 3.9
CP	2.3 – 3.1
Heme	3.2 – 3.8
CP + heme	2.3 – 2.5

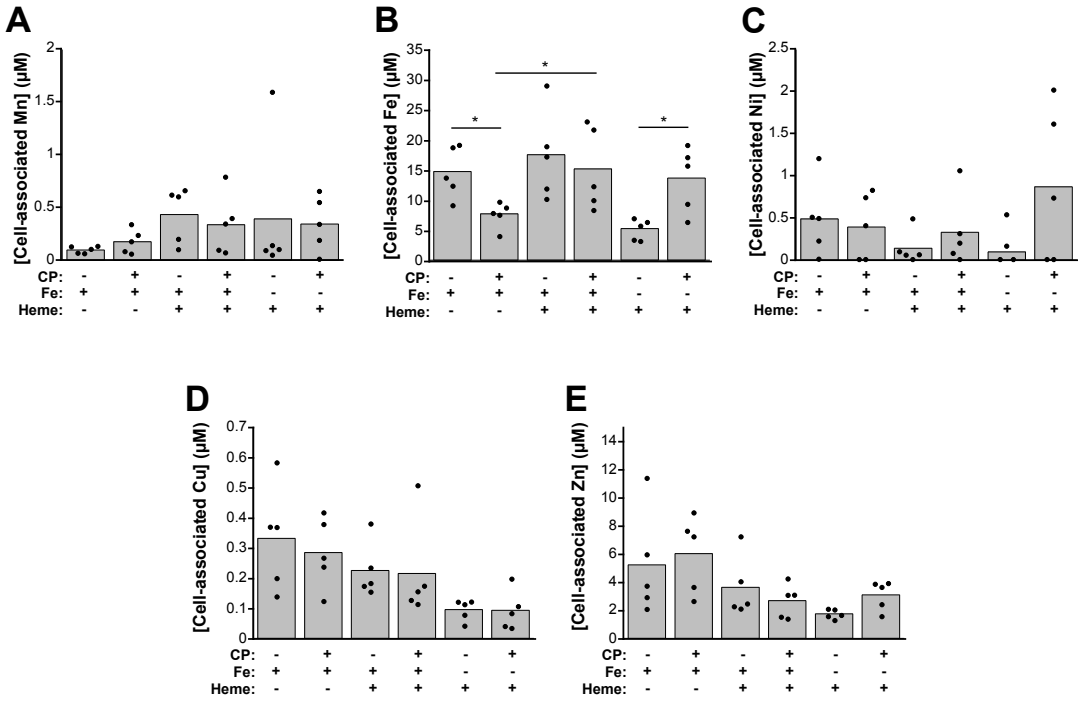

Figure S1. Effect of CP and heme on PAO1 metal uptake. Cell-associated metal in *P. aeruginosa* PAO1 grown in CDM or Fe-depleted CDM in the absence or presence of CP (10 μM) and heme (5 μM) (N = 5, **P* < 0.05). CP, calprotectin; CDM, chemically defined medium.

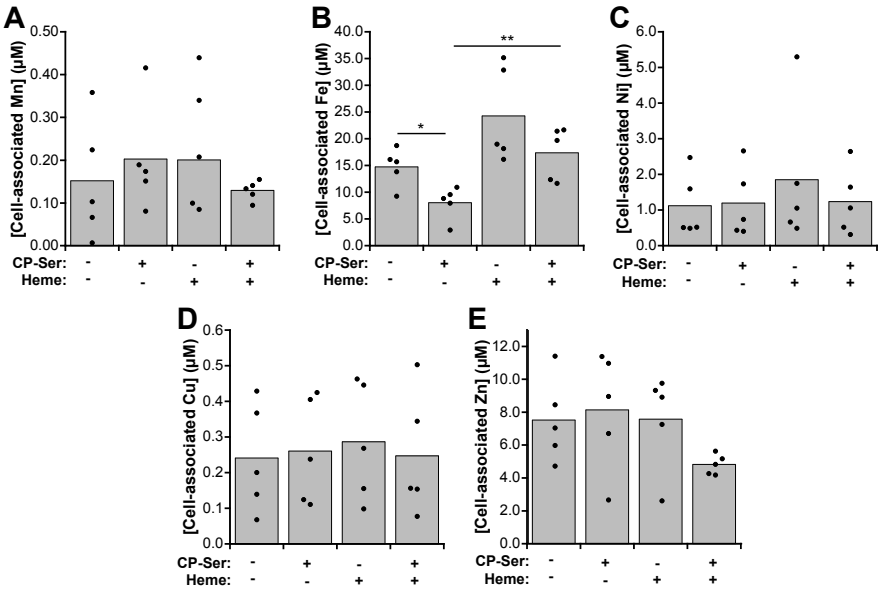

Figure S2. Effect of CP and heme on PA14 metal uptake in CDM. Cell-associated metal in *P. aeruginosa* PA14 grown in CDM in the absence or presence of CP-Ser (10 μM) and heme (5 μM) (N = 5, **P* < 0.05, ** *P* < 0.01). CP, calprotectin; CDM, chemically defined medium.

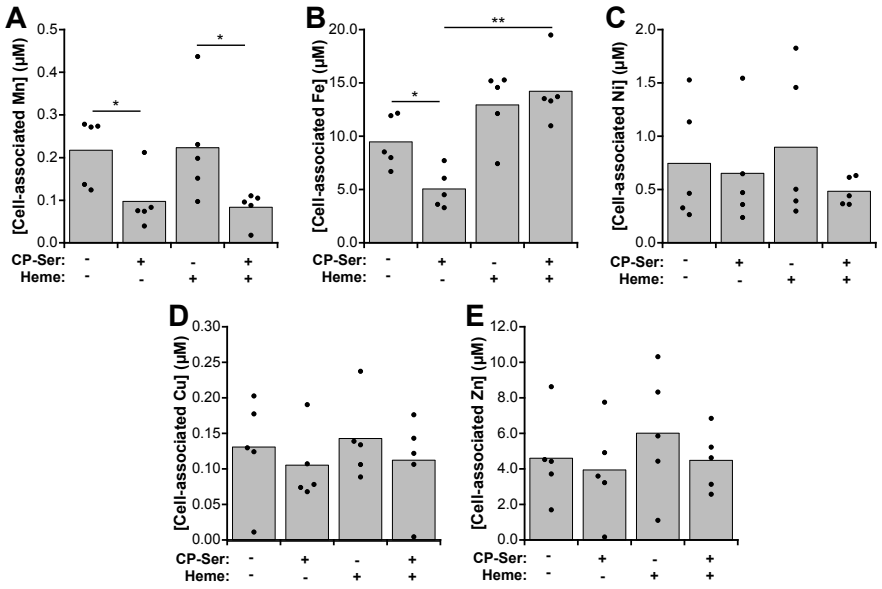

Figure S3. Effect of CP and heme on PA14 metal uptake in Tris:TSB. Cell-associated metal in *P. aeruginosa* PA14 grown in Tris:TSB in the absence or presence of CP-Ser (10 μM) and heme (5 μM) (N = 5, **P* < 0.05, ** *P* < 0.01). CP, calprotectin.

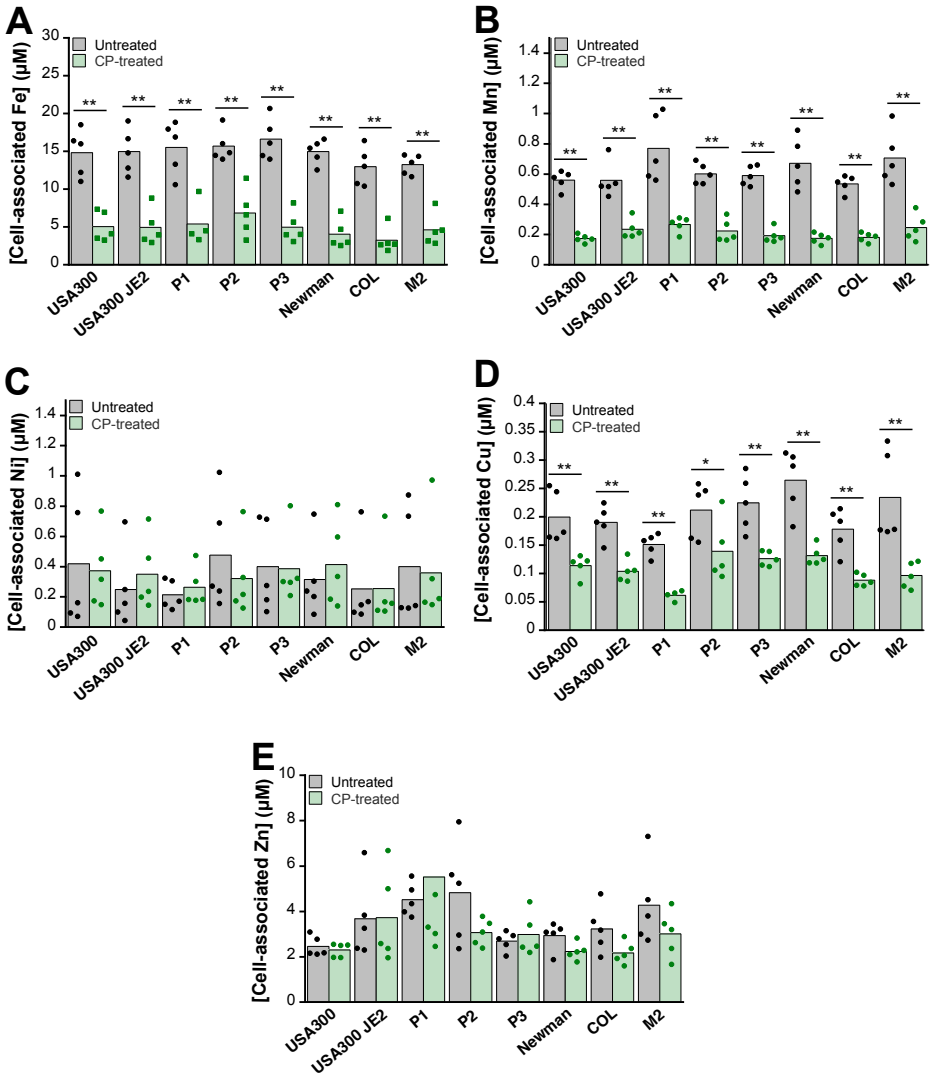

Figure S4. CP inhibits metal uptake by multiple *S. aureus* strains. Cell-associated metal levels for *S. aureus* strains (Table S2) grown in LB in the absence or presence of CP (20 μM) (N = 5, **P* < 0.05, ***P* < 0.01). CP, calprotectin.

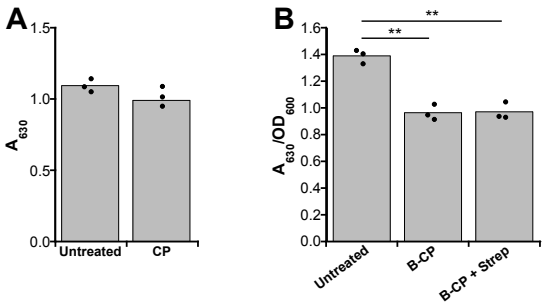

Figure S5. CP does not affect the CAS assay. (A) CAS dye (500 µL) was incubated with 500 µL Tris buffer (untreated) or 500 µL 20 µM CP prepared in Tris buffer (CP) for one hour before measuring the absorbance at 630 nm. (B) *S. aureus* USA300 JE2 was grown in LB the absence or presence of B-CP (20 µM). Supernatants harvested from B-CP treated cultures were split into two separate aliquots and B-CP was pulled down via treatment with streptavidin agarose resin (+ Strep) in one aliquot while the other was left untreated. A CAS assay was performed on each supernatant aliquot. A_{630} measurements were normalized to culture OD_{600} (N = 3, $^{**}P < 0.01$). CP, calprotectin.

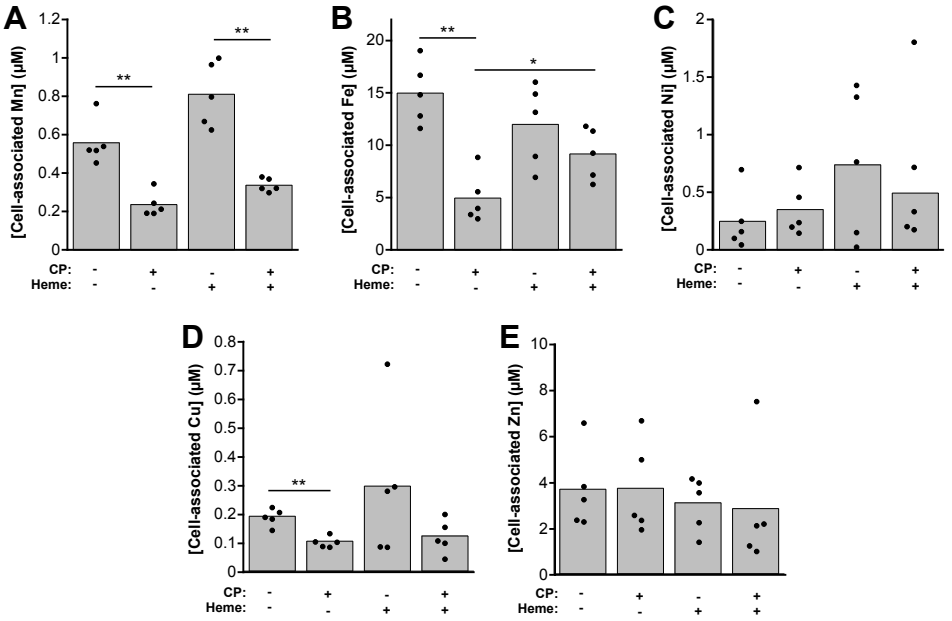

Figure S6. Effect of CP and heme on *S. aureus* metal uptake. Cell-associated metal for *S. aureus* USA300 JE2 grown in LB in the absence or presence of CP (20 μM) and heme (5 μM). (N = 5, **P* < 0.05, ***P* < 0.01). CP, calprotectin.

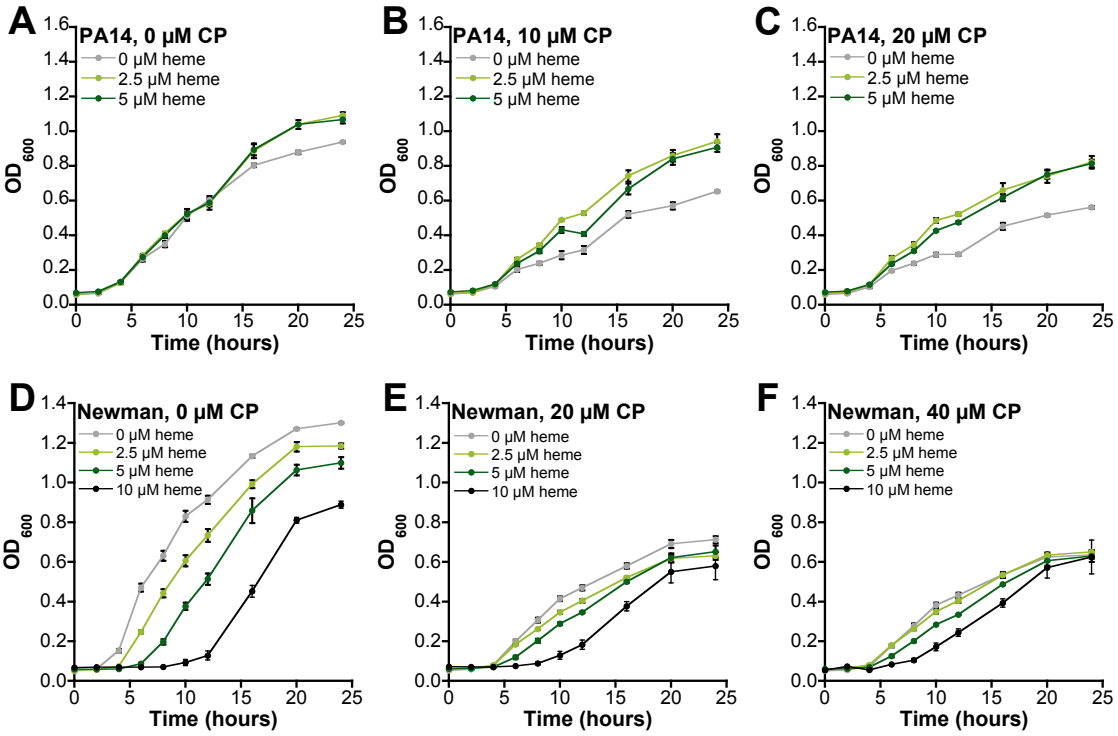

Figure S7. Effect of CP and heme on the growth of *P. aeruginosa* and *S. aureus*. (A-C) *P. aeruginosa* PA14 was grown in Tris:TSB and treated with 0, 10, or 20 µM CP and 0, 2.5, or 5 µM heme. (D-F) *S. aureus* Newman was grown in LB with 0, 20, or 40 µM CP and 0, 2.5, 5, or 10 µM heme (N = 3, SEM, **P* < 0.05). CP, calprotectin.

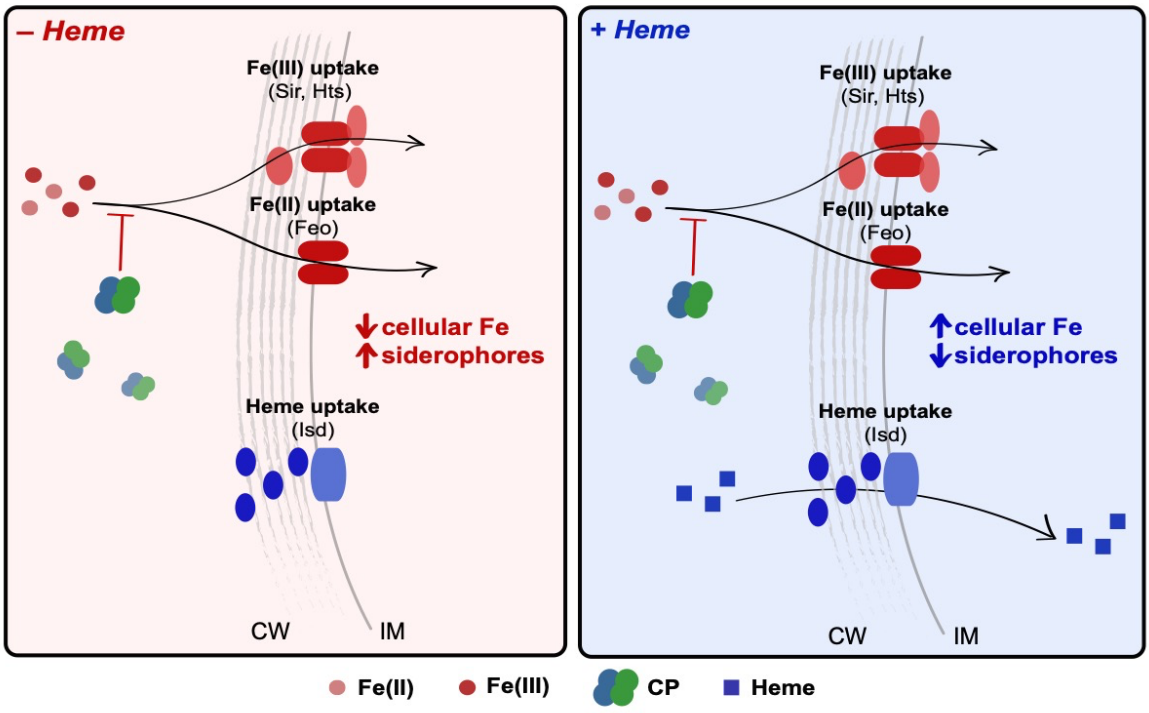

Figure S8. Model for the effect of heme on CP-mediated iron starvation in *S. aureus* based on the current work. (Left) When heme is unavailable, CP inhibits *S. aureus* iron uptake and promotes siderophore production. (Right) When heme is available, *S. aureus* can utilize heme to mitigate the effect of CP on iron uptake, which alleviates its iron starvation responses to CP. CP, calprotectin; CW, cell wall; IM, inner membrane.

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