

Crystal Structure of the Siderophore Binding Protein BauB Bound to an Unusual 2:1 Complex Between Acinetobactin and Ferric Iron

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Table S1. Summary of BauB crystallization and data collection.....	S2
Table S2. Structural homologs of BauB.....	S3
Figure S1. BauB purification and oligomerization state	S4
Figure S2. Overlay of BauB chains A and B.....	S5
Figure S3. Sequence alignment of structurally characterized SBPs	S6
Figure S4. Electron density of ferric acinetobactin.....	S7
Figure S5. Overlay of BauB and CeuE	S8
Figure S6. Structural representation of <i>cis</i> -[Acb ₂ Fe]	S9
Figure S7. Siderophore dependent fluorescence quenching of BauB	S10
Figure S8. Overview of the acinetobactin pathway in <i>A. baumannii</i>	S11

Table S1. Summary of BauB crystallization and data collection.

Data Set Entry	1	2
Crystal Identifier	BB_41	BB_5
Protein	CHis_BauB-SS (50 mg/mL) + ferric Acb (3 mM)	
Protein Buffer	10 mM Tris, 30 mM NaCl, 0.04 mM TCEP, pH 8.0	50 mM Tris, 150 mM NaCl, 0.2 mM TCEP, pH 8.0
Crystallization Cocktail	25% PEG MME 5k, 50 mM MES pH 6.0 (14 °C)	34% PEG 4k, 100 mM EPPS pH 8.0 (14 °C)
Cryoprotection	Serial transfer through 8,16,20% ethylene glycol in cocktail	
Beamline	SSRL 12-2	APS GM/CA 23ID-D
Detector Distance (mm)	375	325
Oscillation Range (°)	360	180
Oscillation Angle (°)	0.25	0.20
No. of Images	1,440	900
Exposure Time (s)	0.5	0.2
Beam size (w × h, μm)	100 × 100	200 × 100
Wavelength (Å)	0.9795	1.0332
Data Collection		
Resolution range (Å)	68.6 – 1.9 (2.0 – 1.9)	45.6 – 2.0 (2.04 – 2.0)
Space group	P 2 ₁	P 2 ₁
Unit cell a, b, c (Å)	37.6, 137.3, 56.3	37.9, 136.8, 56.3
α, β, γ (°)	90.0, 90.0, 90.0	90.0, 90.1, 90.0
Total Observations	260338	123410
Unique reflections	39102	38149
Multiplicity	6.7	3.2
Completeness (%)	87.3 (90.5)	97.7 (91.1)
Mean I/sigma(I)	11.8 (6.7)	3.2 (3.0)
R_{MERGE}	0.09 (0.17)	0.19 (0.58)
R_{MEAS}	0.11 (0.20)	0.26 (0.80)
CC1/2	0.99 (0.97)	0.94 (0.42)

Table S2. Structural homologs of BauB

PDB	% ID	RMS Disp.	Residues Aligned	Name
3GFV	36	2.0	276/286	<i>B. subtilis</i> YclQ
3TEF	32	2.1	274/279	<i>V. cholerae</i> VctP
5AD1	29	2.2	272/290	<i>C. jejuni</i> CeuE
4JCC	29	1.9	270/284	<i>S. pneumoniae</i> PiuA
4MX8	26	2.3	272/302	<i>X. cellulolytica</i> Periplasmic BP
3MWF	26	3.1	258/292	<i>S. aureus</i> SirA
3TNY	26	3.2	263/280	<i>B. cereus</i> YfiY
2WHY	21	3.2	266/283	<i>B. subtilis</i> FeuA
4FNA	21	3.2	252/278	<i>S. aureus</i> FhuD2

The top hits the search for structural homologs were filtered to remove PDB coordinates for identical proteins with multiple ligands. FeuA and FhuD2 were not among the most highly homologous but are included for comparison with the discussion in the main text.

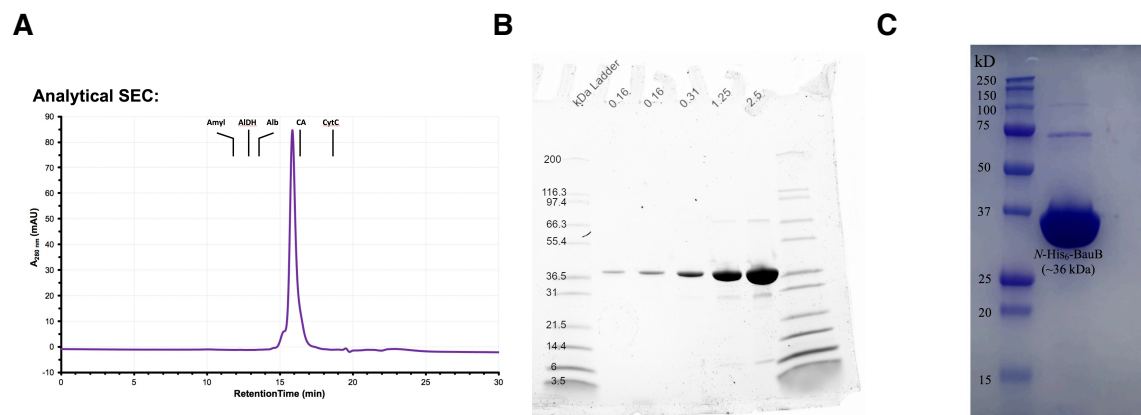


Figure S1. BauB purification. (A) Analytical gel filtration of full length BauB showing monomeric status in solution. Retention times for β -Amylase (200 kD), Alcohol Dehydrogenase (150 kD), Albumin (66 kD), Carbonic Anhydrase (29 kD) and Cytochrome C (12.4 kD) standards are indicated. (B) SDS-PAGE gel recombinant C-His₆-BauB purified from *E. coli* BL21. Molecular weight markers and mass of protein loaded are labeled. (C) SDS-PAGE analysis of Ni-NTA purified N-His₆-BauB (~36.1 kDa).

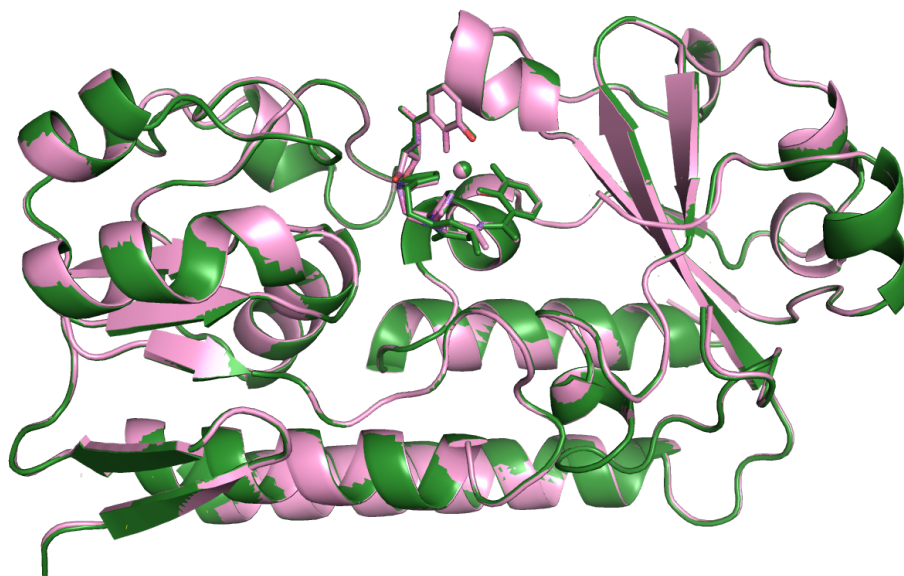


Figure S2. Overlay of two BauB chains (chain A, pink; chain B, green) superimposed with RMS displacement of 0.15 Å over 279 residues.

Abaum_BauB	MNWKKKYGGVALIIAAAVTLQACDQKVADTTQASQKLA	EPITVKHAL	-----G	48
3MWF SaSirA	MNKVIKMLVVTLAFLLVLGCGSNGSNKQSSDRKDKK	TTSIKHAM	-----G	45
3TEF VcVctF	MVLIIVRTLMLRISIKMIPLAYLNHLWKEHMKSRIHCAALGLLAAFAAQA	ETVTIEHRL	-----G	60
3GFV BsYc1Q	MRSMKKFALLFIALVTVAVVISACGNQSTSSKGSDTKKEQ	ITVKHQ	-----D	47
4MX8 XcPBP	MSRTRPIARASMLAVLALTLAACAPSSAGTADDSAETTP	PATASYTWRNRTATEEGADPVYE	-----D	61
4JCC SpPiuA	MKTSLKLYFTALVASFLLLLGACSTNSSTSQTETSS	SAPTEVTIKSSL	-----D	49
5AD1 CjCeue	MKKSILVFAFFAFFFLSLILTACNSNSNENNASSSTTKTNTATVKV	LPISMSDEGDSFLVKDSL	-----G	62
2WHY BsFeuA	MKKISLTLILLALLTAAACGSKNESTASKASGTASEK	KKKIEYLD	-----K	46
4FNA SaFhud2	MKKLLPLIIMLLVLAACGNQGGK	NNKAETKSYKMDD	-----G	38
Abaum_BauB	--TTVIDHLPQR-VAVLDM-NE-ADFLDQLNVP--IMGM-PKDY	---VP-HFLEKYKKDAQI-QDLGA-IVQ-	106	
3MWF SaSirA	--TTEIKGKPKRVVVTLQ-GA-TDVAVSLGVKK-PVGA-VESWTQKPKFEEYIKNDL--KDT-KIVGQOQEP	---	105	
3TEF VcVctF	--KTTLEQKQQR-VVVIQV-GA-LDAIDSFGE--PVAV-SKFDG---TP-DYLAKYK-SDKY-PSAGS-LFE-	---	118	
3GFV BsYc1Q	KNGTKVPKNPKK-VVVFDF-GS-LDTLDKLGDDIVAGL-PKQV---LP-KYLSKFK-DDKY-ADVGS-LKE-	---	108	
4MX8 XcPBP	ETTVEVPVDPQR-IVVFDMAA-LDTIGALGGE--IAGA-PLDS---VP-DYLEEYL-ADDA-FNAGT-LFE-	---	120	
4JCC SpPiuA	--EVVSKLVPEEKIVTFDL-GA-ADTIRALGFAGNIVGMMPTKTT---VP-TYLDLV-GTVKKNVGSMMKEP	---	108	
5AD1 CjCeue	--ENKKIPKNPSK-VVILDLGI-LDTFDALKLNDKVVGV-PAKN---LPKYIQQQF--KNK-PSVGG-VQQ-	---	119	
2WHY BsFeuA	--TYEVTVPTDK-IAITGSVESMMEADAKLLDVH--PQGA-ISFSGK--FP-DMFKDIT--DKA-EPTGE-KME-	---	105	
4FNA SaFhud2	-KTVDIPKDKPR-IAVVAP-TY-AGGLKLLGAN--IVAV-NQQV---DQS-KVLKDKFKGV---TKIGD----	---	93	
Abaum_BauB	PNMERIYALKPD-LILMTP-LHVNYQE-LSKIAPTIIHYDINFN	NSESNHIGLVKDHM-MTLG-KIFN--KED-	172	
3MWF SaSirA	PNLEEISKLKPD-LIVASKVRNEKVDQQLSKIAPTVSSTDTV----	FKFKDIT-KLMG-KALG--KEK-	163	
3TEF VcVctF	PDFETIYTQKPD-LIVIGP-RASKSYDE-LSKIAPTVFAAEA---	DQGYWESTQQQW-RNLG-KVFA--IEP-	181	
3GFV BsYc1Q	PDPDKVAELDPP-LIIISA-RQSESYPE-FSKIAPTYLGVDTA---	KYMEFSKSDA-ETIG-KIFD--KED-	170	
4MX8 XcPBP	ADLIAIEAQQPD-LIVVGG-RSSGLWAD-LNEIAPTIDLRLG----	SYLDTLEQNT-TFLG-KVLG--AEA-	183	
4JCC SpPiuA	PDLAIAALEPDDLIASPR-TQKVFDFKKEIAPTVLFQASKD----	DYWTSTKANIEESLA-SAFGETGTQK	171	
5AD1 CjCeue	VDFEAINALKPD-LIIISG-RQSKFYDK-LKEIAPTLFVGLDNA---	NFLSSFENNV-LSVAKKLYG--LEK-	186	
2WHY BsFeuA	PNIEKILEMKPD-VILASTKFPKTLQK-ISTAGTIPVSHISS-----	NWKENMMLLA-QLTG--KEK-	163	
4FNA SaFhud2	GDVEKVAKEKPD-LIIVYS--TDKDIK-YQKVAPTVVDYNKH-----	KYLEQQ-EMLG-KIVG--KED-	150	
Abaum_BauB	LARQVSELDE-QVK-QVQAVTA-----NRPERALVVL--HNN-GAFSNFGIQ-----	S-RYGF-IFNNAFVGV	227	
3MWF SaSirA	EAEDLLKKYDD-KVA-AFQKDAKAKYKDAWPLKASVNN--FRA-DHTRIYAG-----	G-YAGE-IL-NDLGF	222	
3TEF VcVctF	AVEAKIEQVDA-QFK-SIMQYNQ-----QHKSDAMLMV--SSG-GNLTTFGAN-----	S-RFSS-VY-KDFGF	236	
3GFV BsYc1Q	KVKDELANIDHHSIADVVKKTAE-----KLNKGLVIM--AND-GKISAFGPK-----	S-RYGL-IH-DVFGV	225	
4MX8 XcPBP	EAEVLAELEA-GIA-EAKAAVT-----EASGTGLGIM--VSG-GQLSALSPNTGNDPRGARGGL-IY-DVFGV	243		
4JCC SpPiuA	KAKEELAKLDE-SIQ-EVATKNE-----SSDKKALAIL--LNE-GKMAAFGAK-----	SRFFSF-LY-QTLKF	226	
5AD1 CjCeue	EALEKISDIKN-EIE-KAKSIV-----DEDKKRALIILLTNSNKNKISAFGPQ-----	S-RFGI-IH-DVLGI	235	
2WHY BsFeuA	KAKKIADYEQ-DLK-ETKTKIN--DRAKSKALVIR--IRQ-GNIYIYPEQ-----	V-YFNSTLY-GDLGL	222	
4FNA SaFhud2	KVKAWKKDWE-TTA-KDGKEIKKAI--GQDATVSLFD--EFD-KKLYTYGDN-----	WGRGGEVLY-QAFGL	210	
Abaum_BauB	KPASG-----VVDTSLHGQPISSE-FI IKKADPDILYIVDR	TAVMEH-RPNINAASVE-NPL--LRQTKAWKNG	290	
3MWF SaSirA	KRNKDLQKQVDNGKD-IQLTSKE-SIPLMN-ADHIFVVKSDPNAKD-AALVKKTES	EWTSKSKWKNLDAVKNN	292	
3TEF VcVctF	SETVPV----SKESS-HGDLISFE-YIREHN-PKTLVVDRDKVVTK-GETNIRQTFE	-NDL--VKATAYKNG	299	
3GFV BsYc1Q	APADQN----IKASTHGGQSVSYE-YISKTN-PDYLFVIDRGTAGE--TSSTKQVVE	-NDY--VKNVAVKNG	287	
4MX8 XcPBP	QPVED-----IKAA-THGEPISFE-FLEHD-PQWLWVVDRAATGAEGAQAQVLD-NEI--	VNRRTAATED	307	
4JCC SpPiuA	KPTDTK----FEDSRGGQEVSFESSVKEIN-PDILFVINRTLAIGGDNSSNDGVLE	-NAL--IAETPAKNG	289	
5AD1 CjCeue	NAVDEN----IKVGT-HGKSINSE-FILEKNNPDYIFVVDNRNVLGN-KERAQQGILD	-NAL--VAKTKAAQNK	297	
2WHY BsFeuA	KAPNEVKA---AKAQ-ELLISLLEKLLSEMNP-DHIFVQFSD-DENADKPDALKDLEK	-NPI--WKSLEKAVKED	284	
4FNA SaFhud2	KMQPEQQK--LTAKA-GNAEVKQE-EIEKYA-GDYIVTSTSEK-----	PTPGYES-TNM--WKNLKAATKEG	268	
Abaum_BauB	RVI-FVDADAWTT-AASPTSL--KIVMED-VKKGQY	322		
3MWF SaSirA	QVSDDLDEITWNLA-GGYKSSS--LKLIDDLYEKLNNIEKQSK	330		
3TEF VcVctF	HIA-YLDVNAWIA-ISGVKAT--EQMVAD-MRASVGMQ	333		
3GFV BsYc1Q	HVI-YLDSATWLS-GGGLESMT-QQMIKE-VKDGLEK	320		
4MX8 XcPBP	HVL-YLNPTAWIVFFGVEETT-RIMIDD-VLQ-VAAR	340		
4JCC SpPiuA	KKI IQLTPDLWLS-GGGLESTKLMMEID-IQKALK	321		
5AD1 CjCeue	KII-YLDPPEYWLASNGLES--KTMILE-IRNAVK	330		
2WHY BsFeuA	HVY-VNSVDPLAQ--GGTAWSKV-VRFKA-AAEKLTQNKLTQN	317		
4FNA SaFhud2	HIV-KVDAGTYW---YNDPYTL--DFMRKD-LREKLIKAAK	302		

Figure S3. PROMALS3d structure-based alignment of BauB with homologous siderophore binding proteins. Sequences are identified by PDB code as described in Table S2. Conserved sequences are identified in red, while homologous residues are identified in blue. His239 and Tyr301 of BauB are highlighted in green. BauB residues in Figure 3B are highlighted in yellow. Disordered residues are shown in orange.

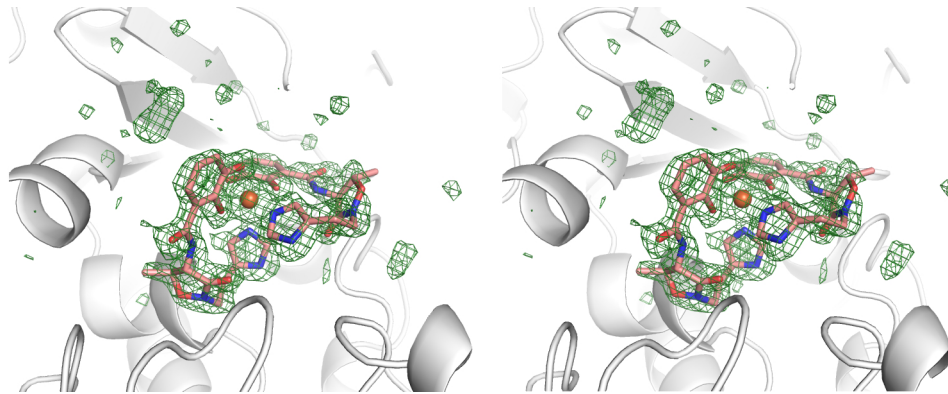


Figure S4. Modeling of acinetobactin₂Fe structure into the electron density in the BauB substrate binding pocket. Stereorepresentation of the final model of ligands bound to BauB. Omit map electron density, contoured at 2.5 σ , was calculated with coefficients of the form Fo-Fc using the final model from which the ligands were removed and subjected to a round of simulated annealing refinement.

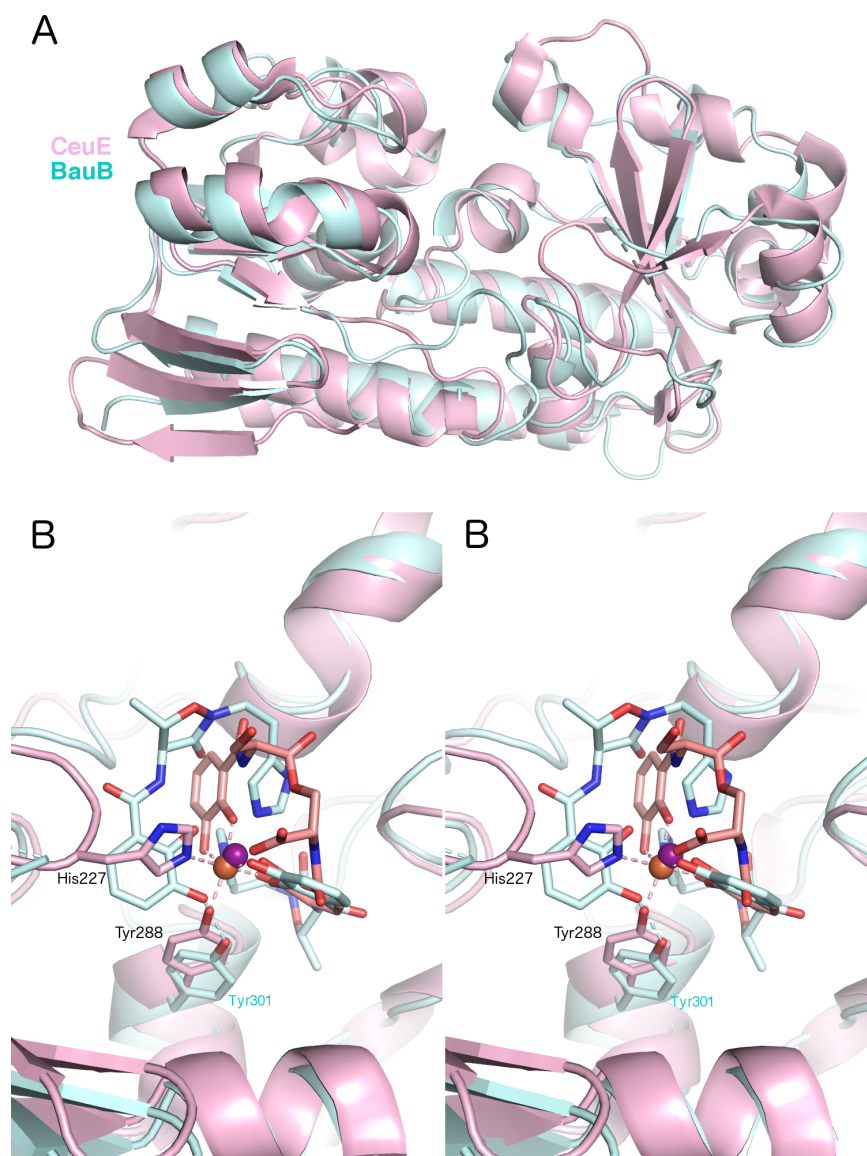


Figure S5. Overlay of BauB and CeuE. A. Ribbon diagram showing BauB (6MFL, cyan) and CeuE (5ADW, pink). B. Stereorepresentation of the siderophore binding pocket of BauB bound to [Acb₂:Fe]⁻¹ anion superimposed with CeuE bound to bis-(2,3-dihydroxybenzoyl-L-Ser). His227 and Tyr288 from CeuE directly coordinate the ferric ion. Tyr301 of BauB interacts with a catechol oxygen of the more exposed acinetobactin molecule. The side chain of His239 in BauB is adjacent to a disordered loop and no density is present for the side chain.

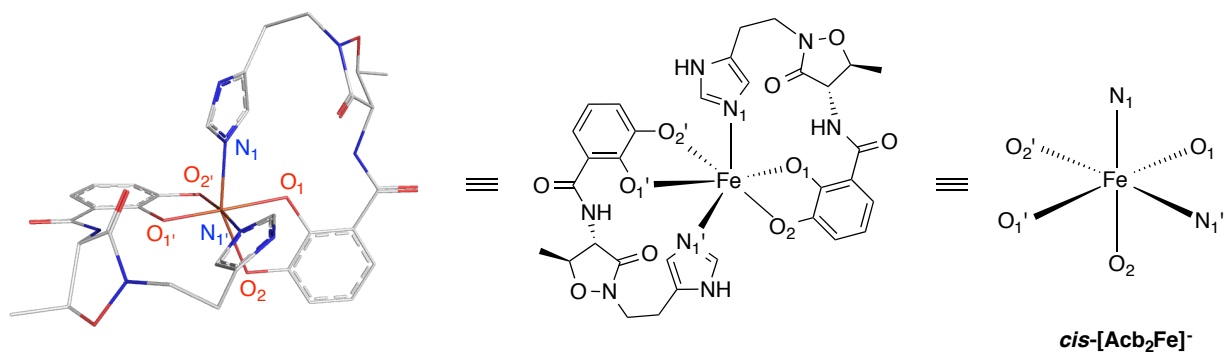


Figure S6. Structural representations of the experimentally observed *cis*-[Acb₂Fe]⁻ geometric and optical isomer. The *cis*-[Acb₂Fe]⁻ structure is isomeric with ML₄^aL₂^b octahedral metal complexes where L^a is oxygen and L^b is nitrogen represented as [FeO₄N₂]⁻.

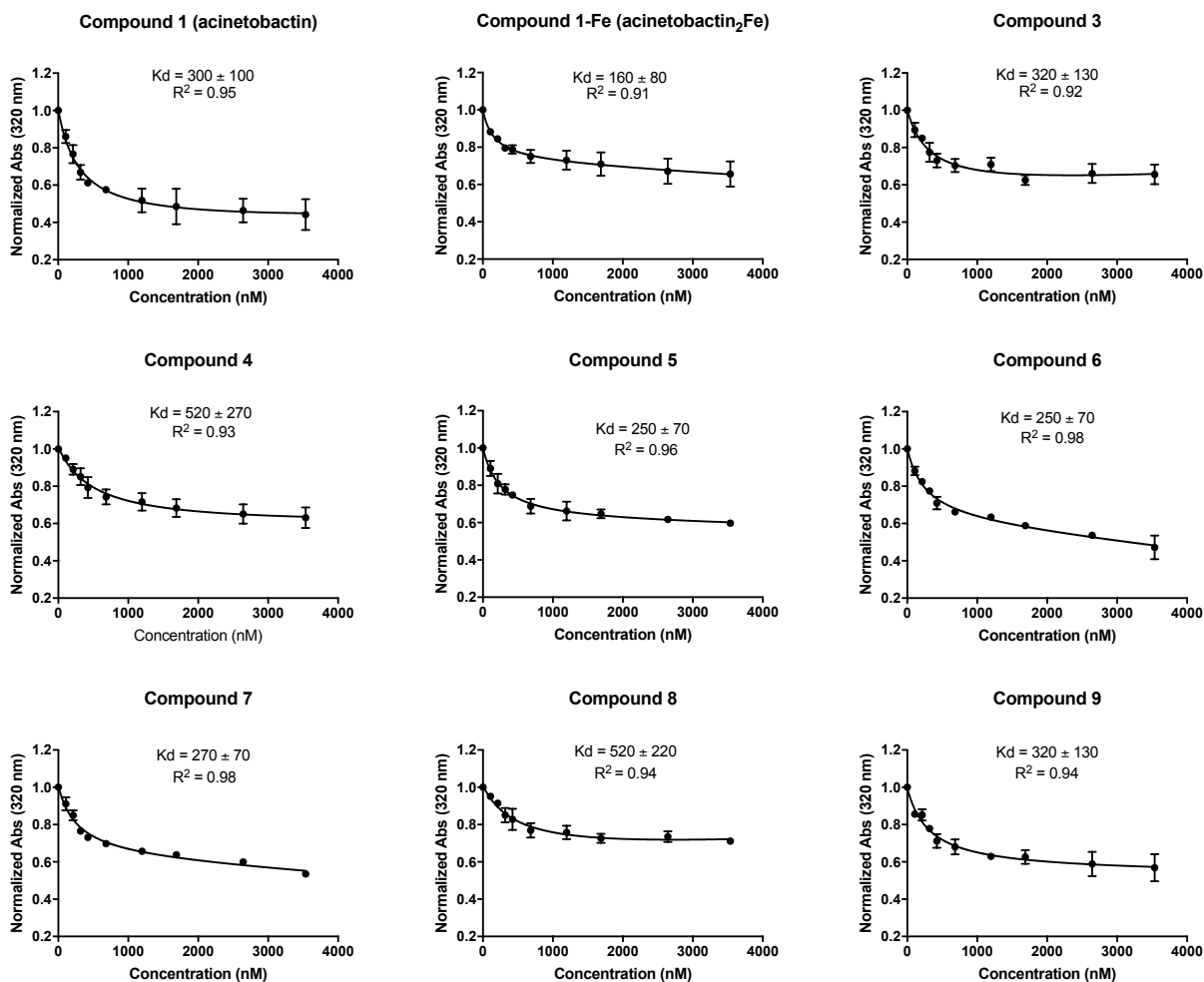


Figure S7. Siderophore-dependent fluorescence quenching of *C*-His₆-BauB. Graphs depict intrinsic tryptophan fluorescence quenching (y-axis; $\lambda_{\text{excitation}} = 280 \text{ nm}$; $\lambda_{\text{emission}} = 340 \text{ nm}$) of *C*-His₆-BauB in the presence of variable siderophore concentrations (x-axis). Apparent K_d values were calculated using a single-binding mode curve fitting model in GraphPad Prism version 7.0b.

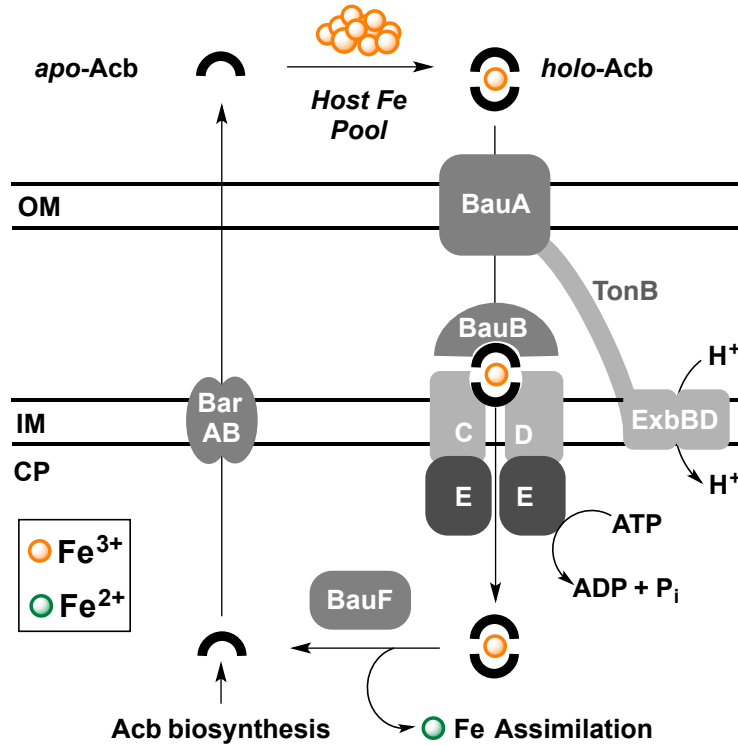


Figure S8. Overview of the acinetobactin pathway in *A. baumannii*. BarAB is a putative efflux pump for cytoplasmic *apo*-Acb. Once formed, the *holo*-Acb ferric complex is imported to the periplasm by the TonB-dependent outer membrane protein BauA. Presumably, BauA is selective for importing various forms of Acb. Periplasmic *holo*-Acb is delivered to the inner membrane permease BauCDE by the SBP BauB (the focus of this work). Once imported to the cytoplasm, the *holo*-Acb ferric complex is likely reduced giving ferrous iron and *apo*-Acb, which is recycled.