Electronic Supplementary Material (ESI) for Metallomics. This journal is © The Royal Society of Chemistry 2020

## Supplemental Materials for Characterization of the Fe metalloproteome of a ubiquitous marine heterotroph, *Pseudoalteromonas* (BB2-AT2): Multiple bacterioferritin copies enable significant Fe storage

Michael G. Mazzotta,\*a Matthew R. McIlvina and Mak A. Saito\*a

<sup>a</sup> Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA.

**Supplemental Table 1.** ICP-MS instrument conditions. For integration purposes three points per isotope were collected in each run. The acronym ng (no gas) indicates the instrument was not operated in collision or reaction mode.

Parameter/Option	Value
Nebulizer	MicroMist nebulizer
Cones	Nickel (C1) and Platinum (C2-C3)
Plasma gas flow	15 L / min (Ar)
Auxiliary gas flow	0.9 L / min (Ar)
Nebulizer gas flow	0.81-0.87 L / min (Ar)
Blend gas flow	0.15-0.17 L / min (Ar)
RF power	1500 W
Isotopes analyzed (integration time per	<sup>24</sup> Mg (0.1s, He), <sup>51</sup> V (0.2s, He), <sup>52</sup> Cr (0.2s,
point, gas used for collision / reaction)	He), <sup>55</sup> Mn (0.2s, He), <sup>56</sup> Fe (0.1s, He/H2),
	<sup>59</sup> Co (0.2s, He), <sup>60</sup> Ni (0.2s, He), <sup>63</sup> Cu (0.2s,
	He), <sup>66</sup> Zn (0.2s, He), <sup>78</sup> Se (0.5s, He), <sup>95</sup> Mo
	(0.2s, He), <sup>111</sup> Cd (0.5s, ng), <sup>185</sup> Re (0.2s,
	ng), <sup>208</sup> Pb (0.1s, ng), <sup>232</sup> Th (0.1s, ng), <sup>233</sup> U
	(0.1s, ng)
Internal standard	<sup>115</sup> ln

**Supplemental Table 2**. Amino acid sequence of TonB protein putative TonBdependent outer membrane receptor; K02014 Fe complex outermembrane receptor protein identified in metaproteomes of METZYME and ProteOMZ expeditions. This sequence is searchable on the Ocean Protein Portal (oceanproteinportal.org) and the sequence is also available within the FASTA file of identified proteins on BCO-DMO data repository for the METZYME expedition (<u>https://www.bco-</u> <u>dmo.org/dataset/708816</u>).

> NODE\_6889\_length\_5617\_cov\_51.2573\_3249\_5617\_-

ITGSDFVNQGAMDLPDMIRTLVPSFNVNTQPISDAATLIRPANLRGLPPDNMLVLVNGK RRHRGAVISFLGSGISDGAQGPDISAIPSIALKQVEVLRDGASAQYGSDAIAGIMNFVLR DNSEGTQLEIRTGEYKEGDGDAWRIAANVGMPFTSAGYANLSVELQDSKATSRSVQR ADAQGLYDGGNTAIWNYPEPAQIWGSPDVSDDYKFVVNIGLSLDESKDFYLFGNYGE RNVLGGFFFRNPTNRGGVFSTDGGATRLVGDVAEATAGAARTCPVVPVPASGAGSST DTALAAIKADPNCFVFNEMWPGGFTPSFGGDVMDWSIASGVTGTTDAGTFWDVSVSV GENQADYFIMNTVNASLGPDTPKEFNPGSYVQLEKNFNVDFIKPVAVEGFASDLNVAY GLEFREEQFTVISGNKESWEIGPYFKQGFGIGSNGFGGFSPSMAGTWDRSNIALYVDL EADVSEQLLMGLAVRYEDFDTFGSTSNSKLSFLYRATDNLSLRGTTSTGFRAPTPGQA NISNISTVADAGGDLYQKGTLAPTNPVSVYYGGKELTPEESTNTSFGFVWDATDALNV TFDFYSIELEDRITQGDDISITAADIALLQSLGVPGAGDLTNFRFYVNDFDTTTDGFDIVA TYDTEIANGNTSFTFVYSDVETEVDRTGGLIGSGRVDQLEKLLPGKRWNLSAVHNTGD WRILGRMNWVDEWFTEEWGGGGGGYISDMSTIDLEVSRDLGDYTLTFGAQNAFDDYP DKELRGLILGWEYPEASPLGFNGAFYYFKVGMDF



**Supplemental Fig 1.** Comparison of growth curves performed between the SpectraMax Me5 plate reader ( $\mu$  = 0.12 h<sup>-1</sup>; black) and Shimadzu UV-1601 ( $\mu$  = 0.21 h<sup>-1</sup>; red).

	125 259 375 500 625 750 875
Query seq.	N-terminal plug ANAAA ligand-binding site AA
Specific hits	[ ligand_ga_eed_channel
	CirA
	TonB_dep_Rec
Non-specific	PRK13524
1103	TonB-hemlactrns
Superfamilies	OM_channels superfamily
	CirA superfamily
	PRK13524 superfamily
	PRK13524 superfamily TonB-hemlactrns superfamily
<b>{O2014</b> ),	PRK13524 superfamily   TonB-hemlactrns superfamily   TonB-dependent Fe complex outermembrane protein   145 250 375 540 625
(02014), <sup>Query seq.</sup>	PRK13524 superfamily TonB-hemlactrns superfamily
<b>(O2014),</b> Query seq. Specific hits	PRK13524 superfamily TonB-hemlactrns superfamily FonB-dependent Fe complex outermembrane protein
(O2014), Query seq. Specific hits	PRK13524 superfamily TonB-hemlactrns superfamily FonB-dependent Fe complex outermembrane protein CirA CirA TonB_dep_Rec
(O2014), Query seq. Specific hits Non-specific hits	PRK13524 superfamily TonB-hemlactrns superfamily FonB-dependent Fe complex outermembrane protein CirA CirA TonB_dep_Rec Ligand_gated_channel
<b>(O2014),</b> Query seq. Specific hits Non-specific hits	PRK13524 superfamily TonB-hemlactrns superfamily FonB-dependent Fe complex outermembrane protein Lifs 284 075 510 645 CirA CirA TonB_dep_Rec Ligand_gated_channel TonB_dep_Rec
(O2014), Query seq. Specific hits Non-specific hits	PRK13524 superfamily TonB-hemlactrns superfamily FonB-dependent Fe complex outermembrane protein Life 25° 25° 25° 6°° 6°° 6°° CirA CirA TonB_dep_Rec Ligand_gated_channel PRK1342 TonB-hemlactrns Off channels, gumpt family

**Supplemental Fig 2.** BLAST comparison of the sequences of BB2AT2\_1746, ferric enterobactin receptor and KO2014, TonB-dependent Fe complex outermembrane protein.