

1 **Inter-domain Horizontal Gene Transfer of Nickel-binding Superoxide Dismutase**

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6 7 **Abstract**

8 The ability of aerobic microorganisms to regulate internal and external concentrations of the
9 reactive oxygen species (ROS) superoxide directly influences the health and viability of cells.
10 Superoxide dismutases (SODs) are the primary regulatory enzymes that are used by
11 microorganisms to degrade superoxide. SOD is not one, but three separate, non-homologous
12 enzymes that perform the same function. Thus, the evolutionary history of genes encoding for
13 different SOD enzymes is one of convergent evolution, which reflects environmental selection
14 brought about by an oxygenated atmosphere, changes in metal availability, and opportunistic
15 horizontal gene transfer (HGT). In this study we examine the phylogenetic history of the protein
16 sequence encoding for the nickel-binding metalloform of the SOD enzyme (SodN). A comparison
17 of organismal and SodN protein phylogenetic trees reveals several instances of HGT, including
18 multiple inter-domain transfers of the *sodN* gene from the bacterial domain to the archaeal domain.
19 Nearly half of the archaeal members with *sodN* live in the photic zone of the marine water column.
20 The *sodN* gene is widespread and characterized by apparent vertical gene transfer in some
21 sediment-associated lineages within the Actinobacteriota (Actinobacteria) and Chloroflexota
22 (Chloroflexi) phyla, suggesting the ancestral *sodN* likely originated in one of these clades before
23 expanding its taxonomic and biogeographic distribution to additional microbial groups in the
24 surface ocean in response to decreasing iron availability. In addition to decreasing iron quotas,
25 nickel-binding SOD has the added benefit of withstanding high reactant and product ROS
26 concentrations without damaging the enzyme, making it particularly well suited for the modern
27 surface ocean.

28 **Introduction**

29 Molecular oxygen in the ground state is a diradical. The presence of these two unpaired
30 electrons in parallel spin makes O₂ susceptible to univalent reduction, which will lead to the
31 formation of reactive oxygen species (ROS) (Fridovich, 1978). When O₂ interacts with an electron
32 donor such as a reduced metal or organic carbon and univalent reduction occurs, superoxide (O₂^{•-}
33) is formed. This reaction occurs in association with the core metabolic and physiological functions
34 of microorganisms. Superoxide is produced at photosystem I during photosynthesis (Asada, 2006),
35 at multiple locations along the electron transport chain in aerobic respiration (Larosa & Remacle,
36 2018), and on the outer membrane of cells for a range of purposes including nutrient acquisition
37 (Rose et al., 2008), cell signaling (Buetler et al., 2004), redox homeostasis (Diaz et al., 2019; Yuasa
38 et al., 2020), and cell growth and proliferation (Carlioz & Touati, 1986; Oda et al., 1995; Saran,
39 2003). Superoxide is a highly reactive molecule that typically has a lifetime on the order of a few
40 minutes under physiological conditions (Diaz et al., 2013). It can further act as an oxidant or
41 reductant in a wide range of consequential biogeochemical reactions involving nutrients, metals,
42 organic carbon, and nitrogen species.

43 The evolution of oxygenic photosynthesis and subsequent Great Oxygenation Event (~2.3-
44 2.4 Gya) greatly increased the energy available to fuel microbial metabolisms (Fischer et al., 2016;
45 Ward et al., 2019). However, increasing oxygen levels is in many ways a double-edged sword.
46 The potential to produce superoxide and other ROS would have increased in parallel with
47 atmospheric oxygen levels, which can have a myriad of detrimental effects on cells (Taverne et
48 al., 2018). The reactivity of superoxide with a wide range of organic moieties and metal cofactors
49 can damage various cell components, including lipid membrane peroxidation and iron release from
50 iron-sulfur clusters (Longo et al., 1999; Radi et al., 1991). Superoxide production can precede the
51 formation of hydroxyl radical, which itself can have devastating effects on biomolecules (Buxton
52 et al., 1988; Oda et al., 1992). The ability to manage and mitigate the deleterious consequences of
53 superoxide and its downstream effects and reaction products is an inescapable challenge of life
54 evolving in the presence of molecular oxygen.

55 Superoxide dismutase is the primary biological tool that microorganisms use to degrade
56 superoxide, both within the cell membrane and in the environment. The presence of at least one
57 copy of superoxide dismutase in the genome of all extant aerobic organisms (and many anaerobes)
58 underscores its necessity in coping with reactive oxygen (Brioukhanov & Netrusov, 2004). The

59 name “superoxide dismutase” is used as a catch-all term to describe four different metalloforms of
60 three distinct enzyme families that have no known homology. These four metalloforms include
61 manganese (Mn) (gene: *sodA*, protein: SodA, enzyme: MnSOD), iron (Fe) (gene: *sodB*, protein:
62 SodB, enzyme: FeSOD), copper and zinc (CuZn) (gene: *sodC*, protein: SodC, enzyme:
63 CuZnSOD), and nickel (Ni) (gene: *sodN*, protein: SodN, enzyme: NiSOD)(Dupont et al., 2008;
64 Wolfe-Simon et al., 2005). Only the Mn- and Fe-binding metalloforms have demonstrable
65 homology (including a cambialistic isoform that can function with either Fe or Mn (Lancaster et
66 al., 2004)), thus, superoxide dismutase is likely an example of convergent evolution in which life
67 reinvented the wheel three separate times (No, 2017).

68 The concentration of oxygen, its influence on metal redox state, and the availability of each
69 metal cofactor in the environment all present important selective pressures on determining the
70 distribution of these different SOD-encoding genes spatially and throughout Earth History. While
71 the true antiquity of these three enzyme families is not known with certainty, the relative ages have
72 been inferred from a combination of phylogenetic arguments and likely environmental conditions
73 on the ancient surface Earth. The FeSOD/MnSOD enzyme family is thought to be the most ancient
74 of these three enzyme families (Hatchikian & Henry, 1977; Yost & Fridovich, 1973). This notion
75 is evidenced by the observation that *sodA/sodB* is phylogenetically wide-spread, and because low
76 atmospheric oxygen in the early Earth would have favored high concentrations of dissolved Fe^{2+}
77 and Mn^{2+} (Wolfe-Simon et al., 2005). Conversely, CuZnSOD, thought to be the youngest of the
78 three enzyme families, is widespread in higher plants and animals, present in some bacteria, and
79 absent from archaea and protists (Case, 2017). This metalloform of SOD can also be localized to
80 the cytoplasm, the nucleus, mitochondrial intermembrane space, chloroplasts, and extracellular
81 space (Case, 2017; Weisiger & Fridovich, 1973). The presumed younger age of the CuZnSOD is
82 supported by its relative scarcity among microorganisms, and the fact that Cu and Zn would have
83 both been highly insoluble in an low-oxygen world (Case, 2017; Saito et al., 2003; Wolfe-Simon
84 et al., 2005).

85 Intermediate in antiquity to the MnSOD/FeSOD and CuZnSOD enzymes, and the primary
86 focus of this study, is Ni-binding superoxide dismutase. NiSOD is the most recently discovered of
87 these three enzyme families (discovered in 1996), and has been the subject of less study than the
88 other two enzyme families (Youn et al., 1996). The evolution and proliferation of NiSOD is
89 thought to coincide with decreasing iron availability resulting from ocean oxygenation (Wolfe-

90 Simon et al., 2005). In fact, NiSOD is the most common metalloform of SOD in modern marine
91 bacteria (Sheng et al., 2014). The abundance of NiSOD among marine Cyanobacteria has been
92 used to suggest that NiSOD may have originated among marine phototrophs (Case, 2017; Wolfe-
93 Simon et al., 2005). However, more recent work suggests that *sodN* originated within the
94 Actinobacteriota clade before appearing in other clades through a combination of vertical and
95 horizontal gene transfer (Schmidt et al., 2009). Currently, *sodN* is thought to be present primarily
96 in bacterial clades, however, some instances of *sodN* in photosynthetic eukaryotes have been
97 identified (Cuvelier et al., 2010). To date, *sodN* has not been identified in archaea (Sheng et al.,
98 2014).

99 Given the ever-expanding availability of quality genomic data and analysis tools, we revisit
100 the phylogeny of the NiSOD protein to better understand its evolutionary history and its
101 distribution across the tree of life. We examine the role of vertical and horizontal gene transfer in
102 the controlling the phylogeny of NiSOD sequences. With this information, we aim to better
103 understand the environmental pressures that select for NiSOD over other SOD genes and
104 contextualize the history of this gene within the broader scope of global biogeochemical cycles.

105

106 **Methods**

107 A reference database was constructed containing a single high-quality genome from every genus
108 of bacteria and archaea for which one is available based on the GTDB database (Parks et al., 2018,
109 2020). A list of all genomes included in the GTDB database was downloaded from the GTDB
110 servers (<https://data.ace.uq.edu.au/public/gtdb/data/releases/>). Genomes that did not meet current
111 metrics for high quality (e.g. completeness <90% or contamination >5%, (Bowers et al., 2017))
112 were removed. This list was finally dereplicated to select a single genome of the highest available
113 completeness from every genus. Genomes were then downloaded from the NCBI WGS and
114 Genbank databases. This genome database was used to search for homologs of SodN and other
115 proteins using the tblastn function of BLAST+ (Camacho et al., 2009). For organismal
116 phylogenies, concatenated ribosomal protein phylogenies following methods from Hug et al.
117 (2016) (Hug et al., 2016). Protein sequences were aligned with MUSCLE (Edgar, 2004) and
118 phylogenetic trees were produced using RAxML v.8.2.12 (Stamatakis, 2014) on the Cipres
119 Science Gateway (Miller et al., 2010). Node support was calculated using transfer bootstrap
120 support with BOOSTER (Lemoine et al., 2018). Trees were visualized using the Interactive Tree

121 of Life Viewer (Letunic & Bork, 2016). Histories of horizontal gene transfer were assessed via
122 incongruence of organismal and metabolic protein phylogenies as described previously (e.g.
123 (Doolittle, 1986; Ward et al., 2020; Ward & Shih, 2020)). Assessment of genomic neighborhood
124 of sodN genes was performed on the RAST platform (Aziz et al., 2008). In order to verify that
125 NiSOD presence was authentic and not a case of false positives (i.e. gene presence in
126 metagenome-assembled genomes due to contamination, e.g. (Ward et al., 2018)), we repeated
127 analyses with a more stringent quality cutoff of a maximum of 3.5% contamination as determined
128 by CheckM (Parks et al., 2015).

129

130 **Results**

131 We have produced the most complete phylogeny of NiSOD proteins available. The
132 phylogenies of the organisms identified in this study and their respective SodN protein sequences
133 are presented in Figures 1 and 2, respectively. This SodN phylogeny expands substantially on the
134 small collection of characterized NiSOD-producing isolates to include many lineages known only
135 or primarily from metagenome-assembled genomes (e.g. Patescibacteria) as well as cultured
136 organisms in which the presence of NiSOD was previously undescribed (e.g. diverse
137 Proteobacteria and Actinobacteriota). This more comprehensive view of the distribution of NiSOD
138 provides a much more complete context for interpreting the evolutionary history of this enzyme
139 family. At present, no homologous outgroup to the NiSOD protein family is known, and as a result
140 it impossible to robustly root the NiSOD phylogeny. For ease of viewing Figure 2 is shown with
141 an arbitrary midpoint root, but this is not intended to imply directionality of evolution.
142 Nonetheless, our results provide valuable understanding to evolutionary relatedness between
143 NiSOD sequences even if we are unable to pinpoint the origin of this enzyme family.

144 The presence of a small number of deep clades is readily visible in the NiSOD phylogeny.
145 In particular, much of the diversity of NiSOD sequences belongs to a large clade made up primarily
146 of sequences from Actinobacteriota, but also containing large clusters of sequences from
147 Cyanobacteria and smaller lineages from other organisms (bottom half of Figure 2). The remainder
148 of NiSOD diversity belongs to a somewhat smaller but more diverse clade made up largely of
149 Chloroflexota sequences interspersed with sequences from Patescibacteria, Proteobacteria, and
150 other organisms (top half of Figure 2). While each of these clades contains sequences from multiple
151 bacterial phyla (and, surprisingly, multiple archaeal phyla), these clades are made up of a plurality

152 of and bounded by lineages of Actinobacterota and Chloroflexota, respectively. With few
153 exceptions (e.g. Cyanobacteria), NiSOD sequences from a particular phylum are polyphyletic,
154 scattered in multiple lineages separated by many sequences from other phyla. Moreover, the
155 topology of phylogenetic relationships both within and between phyla are largely incongruent with
156 organismal relationships, indicating many instances of interphylum horizontal gene transfer.

157 In contrast to previous claims (Sheng et al., 2014), we found that NiSOD are not absent
158 from the archaeal domain. In fact, several clades of archaeal NiSOD exist, scattered throughout
159 the enzyme phylogeny (Figure 2). The lack of reciprocal monophyly between bacterial and
160 archaeal NiSOD sequences, and the incongruence between organismal and protein phylogenies
161 (Figure 1, Figure 2), strongly suggests that the distribution of NiSOD genes throughout the tree of
162 life is the result of multiple instances of interdomain horizontal gene transfer.

163

164 **Discussion**

165 Horizontal gene transfer (HGT) is commonplace in the microbial world. It is invoked to
166 explain the distribution of a wide variety of genes that play a role in all aspects of metabolism and
167 physiology. Evidence of inter-domain horizontal gene transfer, however, is much less
168 commonplace. Generally speaking, the frequency of horizontal gene transfer between prokaryotes
169 is thought to increase with increasing genetic similarity, meaning inter-domain horizontal gene
170 transfer should be the rarest genetic transfer (Andam & Gogarten, 2011). In fact, clear instances
171 of horizontal gene transfer between archaea and bacteria are fleeting and thought to be quite rare
172 (Avni & Snir, 2020). It is clear from this study that NiSOD has indeed undergone multiple
173 instances of horizontal gene transfer, including several instances of transfer from the bacterial
174 domain to the archaeal domain.

175 NiSOD sequences are distributed throughout the bacterial domain, with most protein
176 diversity distributed between two large clades made up primarily of Actinobacteriota and
177 Chloroflexota. Substantial diversity of sequences derived from other bacterial and archaeal taxa
178 appears interspersed within these two clades, apparently reflecting a long history of horizontal
179 gene transfer within and between deep taxonomic groups including at the phylum and domain
180 level. While support values for some shallow nodes are relatively poor (e.g. within closely related
181 clades of Actinobacteriota sequences), support values for deep nodes including those between
182 archaeal and bacterial lineages are robust, providing strong confidence in interpretations of

183 horizontal gene transfer. Because of our stringent quality cutoff for metagenome-assembled
184 genomes (MAGs), we can confidently assert that the organismal source of NiSOD proteins is
185 accurate, and does not reflect contamination or incorrect recruitment of sequences into MAGs,
186 further strengthening interpretations of HGT.

187 Many apparent instances of HGT of NiSOD—including the bulk of interdomain transfer
188 events—appear to have occurred between taxa that tend to inhabit similar niches within the same
189 environment. For example, most Chloroflexota NiSOD sequences are from members of the
190 Dehalocoiccoidia class. Members of Dehalocoiccoidia are abundant and diverse in marine
191 sediment, terrestrial soil, and groundwater primarily as anaerobic heterotrophs (Taş et al., 2010;
192 Wasmund et al., 2016). Nested within the Chloroflexota NiSOD sequences are at least three
193 clusters of sequences from the Patescibacteria. Nested even deeper within Patescibacteria NiSOD
194 clades are a few sequences derived from archaea including the Woesearchaea. Both Patescibacteria
195 and Woesearchaea are thought to exploit similar strategies in the terrestrial subsurface as
196 symbionts with highly reduced genomes and cell sizes (Brown et al., 2015; Castelle et al., 2015).
197 This suggests the possibility that communities of predominantly anaerobic heterotrophs in
198 terrestrial sediment may have exchanged NiSOD genes repeatedly over geologic time, likely as an
199 adaptation to tolerate trace amounts of oxidants. This appears to have resulted in HGT of NiSOD
200 from Chloroflexota to Patescibacteria in at least three events, followed by two transfers from
201 Patescibacteria to Woesearchaea. In this case, it appears that colocalization and overlapping
202 metabolic strategies has enabled repeated HGT between lineages that have diverged on the
203 superphylum and even the domain level.

204 Similarly, interdomain HGT of NiSOD appears to have occurred between organisms in
205 shared environments with similar life strategies. An example of this is seen within the
206 Actinobacteriota clade of NiSOD sequences. Here, NiSOD sequences derived from the archaeal
207 Poseidoniales clade appear nested within a deeper group of bacterial Marinisomata sequences.
208 Both Marinisomata and Poseidoniales are primarily known to exist as water column heterotrophs,
209 with Poseidoniales primarily restricted to the photic zone while Marinisomata exist throughout the
210 water column (Huang & Wang, 2020; Rinke et al., 2019). This suggests a possible route of
211 transmission of NiSOD genes from marine sediment Actinobacteriota, to water column
212 Marinisomata, and ultimately to surface ocean Poseidoniales. A similar transmission process
213 through different taxa moving up the water column may have led to the acquisition of NiSOD by

214 Cyanobacteria. However, Cyanobacteria and Poseidoniales NiSOD sequences are not closely
215 related, suggesting that NiSOD sequences were introduced into the photic zone multiple times
216 from multiple lineages. This repeated, directional migration of NiSOD genes through the
217 environment, rather than extensive sharing of a single set of NiSOD genes simply within the photic
218 zone, highlights that the successful horizontal transfer of genes can require more than simple
219 colocalization of organisms and that multiple routes of transfer can be occurring into and within
220 an environment at any given time.

221 There are two likely candidates for the clade in which *sodN* originated: Actinobacteriota
222 and Chloroflexota. The *sodN* gene is widely distributed across both clades, and the phylogeny of
223 the SodN protein largely resembles the organismal tree of these two clades. In the absence of a
224 known outgroup, it is not formally possible to root SodN protein phylogenies (Figure 2 is
225 arbitrarily midpoint rooted for clarity). As a result we cannot conclusively determine the origin or
226 earliest representatives of the SodN enzyme. However, the abundance, diversity, and phylogenetic
227 relationships of SodN sequences from Actinobacterota and Chloroflexota makes them prime
228 candidates for the originators or earliest adopters of SodN. As previously highlighted, the
229 Actinobacteriota clade encompasses organisms that live in a variety of environments, many of
230 which are found in soil and sediment. The Chloroflexota phylum also contains incredible diversity
231 in metabolism and environmental requirements. Notably, this phylum includes members that are
232 thermophilic, heterotrophic, autotrophic, aerobic, and anaerobic (Islam et al., 2019).
233 Representatives of the class Dehalococcoidia are well represented among the Chloroflexota
234 members with *sodN*; these organisms are primarily found in terrestrial and marine sedimentary
235 environments (Wasmund et al., 2014). Anaerolineae is the second most well represented
236 Chloroflexota class in *sodN* diversity. This class contains members isolated from marine sediment,
237 aquifers, and wastewater (Yamada & Sekiguchi, 2009). Although this class has been previously
238 thought to include primarily obligate anaerobes that make a living from fermentation, several
239 members contain genes for respiration (Hemp et al., 2015; Pace et al., 2015; Ward et al., 2015).
240 The presence of NiSOD in diverse members of these two deeply rooted classes of Chloroflexota
241 suggests the possibility that NiSOD was present in their last common ancestor. Molecular clock
242 analyses have suggested that Anaerolineae and Dehalococcoidia diverged ~2.2 Gya (Shih et al.,
243 2017), providing an indirect estimate for the antiquity of this enzyme family.

244 Comparison of organismal and SodN protein phylogenies can provide some limited insight
245 into the timing of NiSOD evolution. If, as discussed above, the root of the NiSOD tree is near the
246 branch separating the Chloroflexota and Actinobacterota clades (i.e. the midpoint root shown in
247 Figure 2), this suggests that NiSOD was acquired by early members of these clades and has
248 subsequently been vertically inherited into crown groups. Both Actinobacterota and Chloroflexota
249 are phyla with obligately anaerobic basal lineages that are thought to have originated in Archean
250 time before the Great Oxygenation Event, subsequently acquired aerobic respiration, and
251 underwent radiation of class-level lineages during early Proterozoic time (Lewin et al., 2016; Shih
252 et al., 2017). It therefore follows that NiSOD originated at about this time, most likely under low
253 oxygen conditions in marine sediment or the water column. Though it may seem logical to
254 constrain the timing of the evolution of NiSOD with the GOE and associated introduction of
255 significant oxygen to marine environments, small amounts of oxygen can be produced by abiotic
256 processes including photolysis or radiolysis of water—providing an evolutionary impetus for SOD
257 evolution decoupled from biological oxygen production. The origin of NiSOD in late Archean or
258 early Proterozoic time is further consistent with changing marine Ni availability through Earth
259 history. Dissolved Ni concentrations have previously been hypothesized to be on the order of 400
260 nM in the Archaean ocean, decreasing to ~200nM by 2.5 Gya (Konhauser et al., 2009, 2015).
261 These concentration estimates are more than 2 orders of magnitude greater than modern seawater
262 concentrations (Sclater et al., 1976). Whether the ancestral NiSOD evolved in the marine water
263 column or in underlying sediment, it almost certainly evolved in an environment where Ni exceeds
264 modern marine concentrations.

265 There are several reasons why the presence *sodN* in a microbial genome may provide an
266 advantage for organisms over its Fe-binding counterpart. One curious aspect of NiSOD is that it
267 is the only metalloform whose metal cofactor does not react with superoxide as a hydrated ion.
268 While the “ping-pong” mechanism of catalysis is shared by all SODs (i.e. higher valent metal
269 center oxidize superoxide, lower valent metal center reduces superoxide in an alternating fashion),
270 the redox potential of Ni(II/III) redox pair is tuned to the appropriate redox potential by its
271 coordination within the enzyme (Herbst et al., 2009). Therefore, the presence of Ni and its
272 availability is not influenced by O₂ and ROS in the same way that Fe would be. The concentration
273 of soluble Fe in seawater has decreased as a result of atmospheric oxygenation. Although estimates
274 vary, Fe concentrations likely dropped from ~100 μM in Archaean seawater, to low μM or high

275 nM concentrations in the Proterozoic, to the near 1 nM dissolved Fe seen in the open ocean today
276 (Holland, 1984). Today, Fe can be a limiting nutrient for primary productivity in many surface
277 ocean regions, stemming from its use as cofactor in many different essential enzymes (Behrenfeld
278 et al., 1996; Behrenfeld & Kolber, 1999). The utilization of NiSOD over FeSOD under such iron
279 limitation provides a competitive advance in its ability to reduce iron quotas.

280 NiSOD may offer an additional competitive advantage beyond reducing cellular Fe quotas.
281 The superoxide-dismutating activity of both FeSOD and NiSOD are inhibited by excess hydrogen
282 peroxide, one of the two primary products of the dismutation reaction (Sheng et al., 2014).
283 However, the H₂O₂ inhibition of FeSOD and NiSOD work in fundamentally different ways.
284 FeSOD inhibition by H₂O₂ occurs through irreversible peroxidative damage of the enzyme that
285 proceeds through a Fenton-type reaction. Inhibition of NiSOD, conversely, operates by reducing
286 Ni(III) to Ni(II) without damaging the enzyme itself (Herbst et al., 2009). This inhibition is
287 reversible, and SOD catalytic activity is restored once hydrogen peroxide levels drop below the
288 inhibition threshold. Similar to FeSOD, CuZnSOD may also be irreversibly inhibited by excess
289 H₂O₂ (Gabbianelli et al., 2004). While MnSOD is not known to be inhibited by H₂O₂, its SOD
290 activity is lessened slightly at higher concentrations of superoxide (Sheng et al., 2014), which, to
291 our knowledge, has not been demonstrated for the other metalloforms.

292 In the surface ocean, superoxide and hydrogen peroxide production is wide-spread and
293 results from a combination of light-dependent and light-independent reactions (Diaz et al., 2019;
294 Sutherland et al., 2019; Vermilyea et al., 2010). Surface superoxide concentrations range from low
295 pM to several nM in the open ocean, with higher concentrations typically found in productive
296 surface waters (Sutherland, Grabb, et al., 2020; Sutherland, Wankel, et al., 2020). In some cases,
297 superoxide may approach 100-200 nM in productive coral reef ecosystems (Diaz et al., 2016;
298 Grabb et al., 2019). Hydrogen peroxide has a lifetime in seawater that is ~3 orders of magnitude
299 longer than superoxide (i.e. typical H₂O₂ lifetime is hours to days), and correspondingly has
300 concentrations that range from low nM in the deep ocean to as high as several hundred nM in
301 productive sunlit surface water (Hopwood et al., 2017; Yuan & Shiller, 2005). The ability of
302 NiSOD to catalyze superoxide dismutation at near diffusion limited rates and withstand elevated
303 concentrations of hydrogen peroxide may make it uniquely suited for microbial fitness in the
304 surface ocean with modern oxygen levels.

305 There is observational evidence to support the notion that not all SOD enzymes are perfect
306 replacements for one another, even when cofactor concentrations are replete. For example, one
307 study examined the growth of two strains of *Synechococcus* under low and high Ni concentrations
308 to compare Ni requirements for NiSOD and urease (Dupont et al., 2008). In that study, one strain's
309 genome encoded for only NiSOD, while the other's genome encoded for both NiSOD and
310 CuZnSOD. The authors showed that under low Ni concentrations and adequate fixed nitrogen
311 (resulting in reduced Ni quotas for urease), Cu/ZnSOD could not replace NiSOD activity.
312 However, not all cyanobacteria have NiSOD; FeSOD is represented across cyanobacterial
313 diversity. Even still, this observation demonstrates that there are enzyme characteristics beyond
314 the metal cofactor identity that select for certain SOD metalloforms; these are not well known and
315 should be the target of future study.

316 Interestingly, 9 of the 26 archaea that we identified with NiSOD genes also contain genes
317 for proteorhodopsins. All nine of these organisms belong to the Poseidoniales clade, which is one
318 of the most widespread archaeal groups in surface ocean waters (Rinke et al., 2019). The selective
319 pressures experienced by these archaea, including nutrient availability, light levels, and ROS
320 levels, mirrors those experienced by cyanobacteria such as *Synechococcus* and *Prochlorococcus*,
321 suggesting that the acquisition and expression of *sodN* plays a key role in microbial success in the
322 modern ocean.

323

324 **Summary and Conclusion**

325 Using the Genome Taxonomy Database, we examined the phylogenetic relation of the
326 nickel-binding metalloform of superoxide dismutase to better understand its evolutionary history
327 and biogeography in the context of environmental constraints. We compared organismal
328 phylogenies and NiSOD gene phylogenies to demonstrate that NiSOD has undergone multiple
329 instances of inter-domain horizontal gene from bacteria to archaea. Actinobacteriota and
330 Chloroflexota as two clades in which NiSOD is pervasive and in which the organismal phylogeny
331 resembles that of the *sodN* gene. As such, these two clades are the most likely candidates for the
332 evolutionary root of *sodN*. The distribution of NiSOD sequence diversity is consistent with one or
333 more vertical migrations in biogeography, starting in soil or marine sediment and migrating to the
334 photic zone. The acquisition of *sodN* as a means to mitigate iron-limitation is consistent with the
335 biogeochemical history of the marine environment, however, other factors such as hardiness

336 against damage by the product hydrogen peroxide may also contribute to its proliferation in the
337 surface ocean.

338

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342

343 **Figures:**

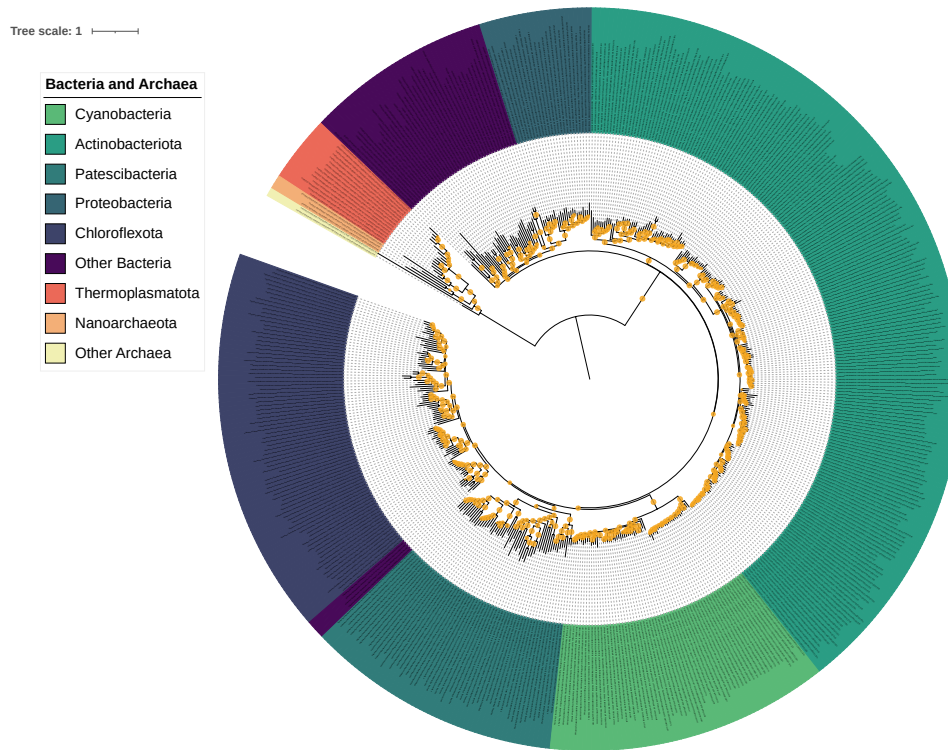
344 Fig 1: Organismal phylogenetic tree determined using concatenated ribosomal protein sequences
345 of organisms from the Genome Taxonomy Database that contain a *sodN* gene. We note that there
346 are slightly fewer organisms in the organismal tree than in the SodN protein tree (Figure 2), this is
347 due to either low completeness or poorly aligned concatenated ribosomal sequences. See methods
348 for a complete description of tree production and quality cutoff metrics.

349

350 Fig 2: SodN protein sequence tree for organisms containing at least one copy of the *sodN* gene in
351 the Genome Taxonomy Database.

352

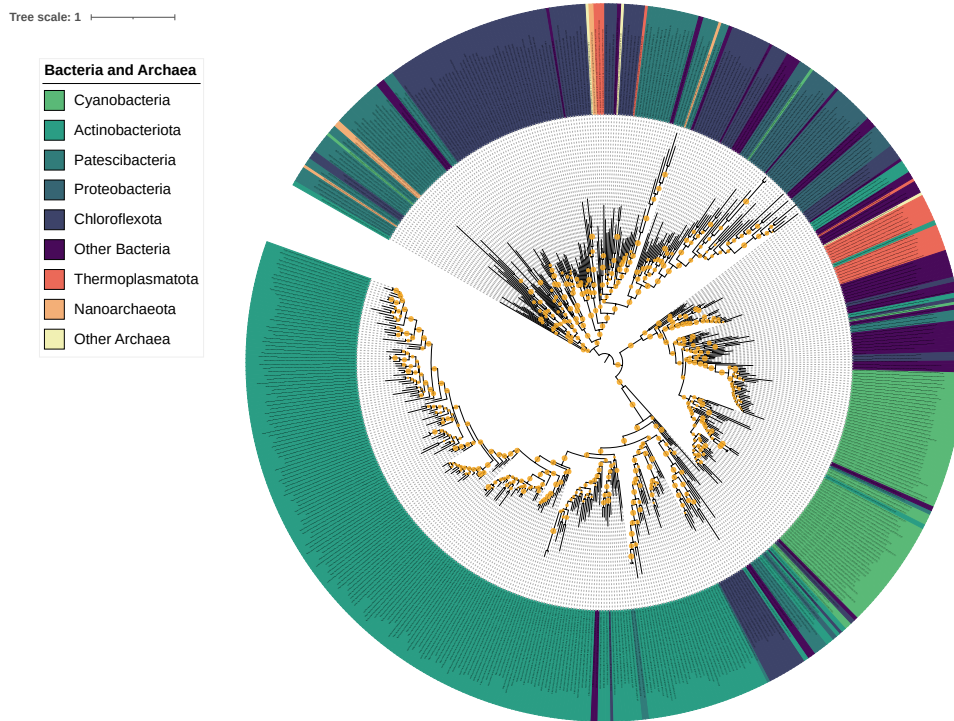
353 Figure 1:



354

355

356 Figure 2:



357

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