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On the nature of dissolved copper ligands in the early buoyant plume of hydrothermal vents

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Abstract :

Environmental context Copper released by deep-sea hydrothermal vents has been recognised to be partly stabilised against precipitation by its complexation with strong Cu binding ligands. Yet, the sources and nature of these compounds in such environments are still not fully understood. This study shows that the Cu ligands detected are hydrothermally sourced and could be mainly inorganic sulfur species. Abstract The apparent speciation of Cu in the early buoyant plume of two black smokers (Aisics and Y3) from the hydrothermal vent field Lucky Strike (Mid-Atlantic Ridge) was investigated using competitive ligand exchange-adsorptive cathodic stripping voltammetry (CLE-AdCSV). We have assessed the apparent Cu-binding ligand concentration ([L]) and the corresponding conditional stability constant (log K) for 24 samples. At the smoker Aisics, [L] ranged from 18.2 to 2970nM. Log K-CuL ranged from 12.4 to 13.4. At Y3, the binding capacity of natural ligands was from 32.5 to 1020nM, with Log K-CuL ranging from 12.5 to 13.1. Total dissolved Cu ranged from 7.0 to 770nM and from 12.7 to 409nM at Aisics and Y3, respectively. Our results show that the amount of ligand L increases with dissolved Mn (dMn) concentrations, suggesting a hydrothermal origin of the Cu-binding ligands detected. In addition, such high concentrations of Cu-binding ligands can only be explained by an additional abiotic source differing from organic processes. Based on the massive in situ concentrations of free sulfides (up to 300 μ M) and on the striking similarities between our log K-CuL and the log K-Cu(HS) previously published, we infer that the Cu-binding ligands could be predominantly inorganic sulfur species in the early buoyant plume of the two vent sites studied.

26 **1. Introduction**

27 Speciation studies are crucial for understanding the biogeochemical cycle of trace metals in the
28 open ocean. Commonly used to predict the biological availability and the toxicity of metals on
29 microorganisms ^[1], chemical speciation also brings new insights on which chemical species are
30 controlling the fate of a particular metal in a specific environment.

31 Hydrothermal fluids are the results of the leaching of oceanic crustal basalts by hot and acidic
32 percolated seawater through the seafloor ^[2]; as a consequence, they are enriched in various
33 inorganic ^[3] and in small amount of organic species ^[4,5]. The plumes discharging to the deep
34 ocean are a complex mixture of dissolved and particulate species ^[3,6], mostly reduced, which
35 concentrations depend on the extent of the basalt alteration (i.e. water/rock interactions), as well
36 as the proportion of subsurface mixing with seawater ^[7]. Initial concentrations of species in the
37 plume are also impacted by chemical reactions occurring throughout the mixing of the
38 hydrothermal fluid with seawater.

39 Despite this complex mixture, previous speciation studies have emphasized the ability of
40 inorganic sulfides to reduce the toxicity of divalent iron ^[8] or copper ^[9] by forming metal-sulfide
41 complexes. These previous findings suggest that inorganic sulfides contribute to metal-
42 complexation in hydrothermal vents. More recently, dissolved copper from deep-sea
43 hydrothermal vents ^[10,11] and from shallow hydrothermal sources ^[12] has been recognized to be
44 partly stabilized against precipitation by its complexation with strong organic chelates. These
45 organic ligands, probably thiols ^[10], have been assumed to compete against inorganic sulfide
46 complexation, hence preventing a part of Cu from being precipitated across the mixing with
47 seawater. Model simulations have even shown that such organic compounds likely increase the
48 solubility of copper, impacting thus the global Cu-fluxes in the ocean ^[13]. Specific organic
49 ligands for iron have also been detected in hydrothermal buoyant plumes ^[14-16], suggesting a
50 stabilization in the dissolved phase as well as a higher solubility of iron in the deep ocean.

51 However, the source and nature of these organic compounds in such environments are still
52 poorly known. Organosulfur compounds coming from **biologically-rich diffuse areas**, rather
53 than typical oceanic **ligands** (e.g. humic substances ^[17]) have been suggested to be the main
54 organic species found in such environment and thus to represent these organic ligands ^[10-12].
55 Despite these assumptions, the potential impact of reduced sulfur species on metal speciation
56 is still pointed out ^[11,12]. Because of their massive concentrations in the hydrothermal end-
57 members (from μM to mM levels) ^[11,15], inorganic sulfides should indeed play an important
58 role in metal-complexation in the early buoyant plume.

59 In this study we provide new data on the apparent speciation of Cu in the early buoyant plume
60 of two black smokers located in the Lucky Strike hydrothermal vent field (Mid-Atlantic Ridge).
61 We discuss the apparent ligand concentrations obtained in line with DOC levels and, for the
62 first time, with the *in situ* measurements of total inorganic sulfides in the buoyant plume. We
63 then anticipate on what is the nature of the copper binding ligands in the studied area in line
64 with the corresponding *in situ* parameters measured.

65

66 **2. Experimental**

67 **2.1. Sampling sites**

68 Hydrothermal fluid samples were collected at the Lucky Strike vent field (37°N) during
69 two oceanographic cruises on the RV Pourquoi Pas? and the Atalante (Momarsat 2015: [http://
70 dx.doi.org/10.17600/15000200](http://dx.doi.org/10.17600/15000200) and Momarsat 2016: <http://dx.doi.org/10.17600/16001200>).

71 The Lucky Strike Seamount is located southwest of the Azores Islands on the slow-spreading
72 Mid-Atlantic ridge (MAR) ($\sim 2.2 \text{ cm/yr}$ ^[18,19]) and extends over almost 11 km at depth between
73 1800 and 2000 m ^[20]. The volcano summit is made of three volcanic cones surrounding a flat
74 depression which hosts a fossil lava lake as well as the Lucky Strike vent field (average depth:

75 1690 m). Hydrothermal activity is gathered around the lava lake which prevents hydrothermal
76 fluids from directly discharging through the crust^[20–22]. The Lucky Strike vent field displays a
77 wide variety of emission and structures which are all hosted on a basaltic substratum^[23]. Two
78 black smokers from Lucky Strike were selected to study the nature of the Cu-binding ligands:
79 Aisics (32°17.34'N; 32°16.53'W, 1690 m depth) and Y3 (37°17.51'N; 32°16.67'W, 1726 m
80 depth) (Figure 1).

81

82 **2.2. Sampling method**

83 At each site, *in-situ* filtration was carried out in the early buoyant plume of each smoker using
84 the PEPITO sampler^[24] implemented on the ROV Victor 6000 (Figure 1). Prior to use, all
85 equipment used for sampling and filtration was rigorously washed with diluted hydrochloric
86 acid (pH 2, Suprapur, Merck) and rinsed with ultrapure water (Milli-Q element system). The
87 *in-situ* filtered samples were collected by pumping water into 2L-PVC/DEHP blood bags
88 (Terumo, sterile treated by ethylene oxide, PVC/DEHP, 1BDT200BM) with on-line filtration
89 at 0.45 µm (HATF, Millipore). For each dive, sampling was performed towards increasing
90 temperatures, i.e. from the seawater-dominated to the hydrothermal-dominated part of the
91 mixing zone, thus minimizing potential contamination of the sampler. The acid-cleaned
92 titanium-silicone nozzle of the sampling device was first positioned in the buoyant plume, at
93 1 to 2 m from the vent orifice. A high-temperature probe coupled to the nozzle was used to
94 monitor the temperature at each point of the mixing gradient sampled. When the temperature
95 probe was stabilized at 4°C (coldest part of the mixing), the pumping of the first sample (A1)
96 was started and held for 3 to 5 min. The nozzle was then shifted downwards within the central
97 part of the buoyant plume with temperature increments of ~ 10°C until reaching values ranging
98 from 100 to 150°C. Overall, 15 samples were collected for each of the 2 mixing gradients
99 sampled. Pumping was systematically stopped in case of a steep increase in temperature (e.g.

100 10°C to 100°C in a few seconds) or a chimney structure collapsing to avoid samples
101 contamination by particles.

102 Coupled to the PEPITO sampling, one chemical miniaturized analyzer (CHEMINI [25]) was
103 used for the *in situ* measurement of sulfides ($\Sigma S = HS^- + S^{2-} + H_2S$) [26]. Briefly, ΣS measurement
104 is based on flow injection analysis with colorimetric detection (methylene blue method). The
105 chemical analyzer was calibrated *in situ* at the beginning and at the end of each dive using S(-
106 II) stock solutions. Hydrothermal solutions were directly pumped without any filtration and the
107 signal acquisition (~ 3 min) was initiated at the same time as the PEPITO sampling (the same
108 titanium-Silicon cannula is used for both PEPITO and CHEMINI). The chemical species
109 analyzed corresponded to an operationally defined fraction of sulfides, herein called free
110 sulfides. This fraction included the dissolved and particulate sulfides in unfiltered seawater that
111 are labile enough to react with methylene blue at pH 4.7 (acetate buffer). *In situ* measurement
112 of dissolved oxygen was performed using an optode (Aanderaa).

113 Immediately after recovery of the ROV, samples were processed in the chemical lab on board
114 (clean lab, Class 100 000, ISO 8) and pH was measured on dedicated aliquots using a daily-
115 calibrated pH-meter (NBS buffers: pH 4, 7 and 10 at 25°C). Each bag was then sub-sampled
116 for further spectrometric analysis of the total dissolved concentrations of dissolved manganese
117 (dMn). Aliquots were stored in acid-cleaned 15 mL-polypropylene tubes (Elkay) and
118 immediately frozen at -4°C until on shore analysis. The remaining volume in the PVC/DEHP-
119 bags was dedicated to Cu-speciation measurements.

120 **2.3. Storage strategy and analyses performed for the assessment of Cu speciation**

121 The protocol used for storage and on-shore analyses for the assessment of Cu speciation is
122 summarized in Figure 2. In total, thirty hydrothermal samples were dedicated to the study of
123 Cu-binding ligands. Samples collected in 2015 were poisoned directly inside the PVC/DEHP-
124 bag with sodium azide (NaN₃, extra pure, Merck) and stored at 4°C for one year. For 500 mL

125 of sample, a volume of 150 μL of NaN_3 (0.2 M) was added. It should be mentioned that both
126 frozen and poisoned samples were available in 2015, with the aim at comparing the effect of
127 sampling storage on Cu-speciation measurements (Figure 1). Though no comparative study was
128 done yet, we chose the poisoning method because we suspected freezing to worsen aggregation
129 of colloidal phases (black precipitates were regularly seen inside frozen bags). In addition,
130 freezing at -20°C was reported to modify the original speciation of Cu, suggesting that this
131 storage was not fully adapted for such measurements compared to a storage at 4°C [27].
132 However, storage of our samples (4°C) was longer (1 year), meaning that changes in speciation
133 can occur before Cu titration analyses and that the results obtained for this year should be
134 discussed cautiously. Samples from the second cruise (2016) were immediately transferred in
135 500 mL PFA bottles after recovery on board and also poisoned with azide before storage at 4°C
136 for 2 months. Aliquots from bags and bottles were then analyzed on shore by High Resolution
137 Inductively Coupled Plasma Mass Spectrometry (HR ICP-MS) for total dissolved copper (dCu)
138 concentrations (see paragraph 2.5).

139 Reduced Sulfide Species (RSS) concentrations appeared to be so high in 2016 that a
140 pretreatment of samples was needed to enable any electrochemical measurement of labile Cu.
141 Prior to the complexometric titration experiments, samples were purged with clean air for about
142 1.5 h to remove volatile sulfides. They were left to equilibrate overnight and then filtered at
143 $0.2\ \mu\text{m}$. Samples were not acidified before purging to minimize the chemical changes of the
144 natural inorganic and organic ligands. We also tested the procedure which involves acidification
145 of sample and purging with N_2 , as described by Sander et al. [10], but we did not observe any
146 improvement in the voltammogram shape when compared to the ‘air purging’ method we
147 applied. The clean air purging led to the oxidization of some inorganic RSS as SO_4^{2-} but also
148 as S^0 or polysulfides particles that probably trapped some dissolved Cu. To ensure a correct
149 measurement of the remaining dissolved Cu, pretreated samples were re-analyzed by ASV as

150 described in section 2.4.2.d. The final Cu concentrations obtained for pretreated samples are
151 labelled dCu^* and are used for calculations of L^* concentrations and of $\log K'^*_{CuL}$. L^* and
152 $\log K'^*_{CuL}$ will thus represent the complexing parameters for Cu-binding ligand resistant to
153 oxidation processes. The pre-treatment was not required for samples from 2015 because of the
154 lower apparent RSS concentrations. Dissolved copper concentrations (dCu) obtained by HR
155 ICP-MS were used for calculations of L and $\log K'_{CuL}$. For voltammetric titration experiments,
156 untreated samples refer to samples collected at Y3 and Aisics in 2015.

157 Because samples collected in 2016 were treated differently than those collected in 2015, the
158 two datasets could not be directly compared. Despite this important issue, each dataset enabled
159 to shed different insights on the speciation of Cu in hydrothermal vents and was thus used
160 accordingly. On one hand, samples from 2015, which remained untreated, were the most
161 comparable to the other Cu speciation data that have been previously published. This dataset
162 was also the most reflective of relatively unperturbed Cu speciation, bearing in mind that any
163 hydrothermal sample is perturbed during storage or pH buffering. On the other hand, samples
164 from 2016 enabled to discuss on the relative importance of organic ligands relative to inorganic
165 ligands.

166

167 **2.4. Voltammetric measurements**

168 **2.4.1. Analytical equipment and reagents**

169 Voltammetric measurements were performed using the potentiostat-galvanostats
170 PGSTAT128N or μ Autolab III (Metrohm) controlled by GPES 4.9 software coupled with a
171 three-electrode system 663 VA stand (Metrohm) at the Ruđer Bošković Institute (RBI),
172 Croatia. The working electrode was a Hanging Mercury Drop Electrode (HDME) (drop area:
173 0.25 mm^2). A double junction Ag/AgCl/sat. NaCl/UVSW and a platinum wire were used as the
174 reference and the counter electrodes, respectively. UVSW used for experiments refers to UV-

175 irradiated seawater collected off-shore of Šibenik (Croatia). Solutions were stirred during the
176 deposition step by a quartz or a Teflon stirrer rotating at 3000 rpm. Experiments were conducted
177 in a quartz cell with a working volume of 10 mL. All measurements were carried out at room
178 temperature (~25°C). pH was monitored using a pH-meter (Orion Research, model 920) with a
179 daily calibrated pH electrode (Metrohm). Ultra clean water (ASTM Type I, 18.2 MΩ; Milli-Q)
180 used for final washings and preparation of chemicals was purified by combining Elix 5 and
181 Synthesis 10 purification systems from Millipore. All sample containers were acid-cleaned with
182 10% nitric acid (HNO₃ 65%, analytical reagent grade) and thoroughly rinsed with Milli-Q water
183 before use. Only fluorinated ethylene propylene (FEP) or perfluoroalkoxy (PFA) bottles were
184 used. Prior to voltammetric measurement, an aqueous stock solution containing 0.05 M
185 salicylaldehyde (SA, 98% Sigma) was prepared and stored at 4°C. A 1 mM SA standard was
186 then prepared from the 0.05 M stock solution and used for voltammetric analyses. A pH buffer
187 was made by dissolution of boric acid (Suprapur, Merck) in 0.35 M ammonia (> 25%,
188 TraceSelect, Fluka) to obtain a 1 M solution. The addition of 100 μL of this buffer in 10 mL
189 sample gave a pH of 8.2 in the cell. Slightly acidic Cu standards solutions (10⁻⁶; 10⁻⁵ and 10⁻
190 ⁴ M) were prepared in Milli-Q water (pH ~ 3) from a 1 g.L⁻¹ Cu-stock solution (Fluka).

191

192 **2.4.2. Determination of Cu complexing parameters by CLE-AdCSV**

193 **2.4.2.a Titration procedure**

194 The Cu binding ligands concentration ([L] or [L*]) and the corresponding conditional stability
195 constant (log K' or log K'*) were assessed using Competitive Ligand Exchange – Adsorptive
196 Cathodic Stripping Voltammetry (CLE – AdCSV) with SA as the competing ligand [28].
197 Theoretical background as well as details for the calculation of complexation parameters are
198 provided in supplementary information. In order to perform CLE-AdCSV experiments within

199 the linear range of the method (up to ~ 400 nM of Cu_T), untreated samples with high Cu content
200 (1st set) were generally diluted 10-fold in clean UVSW ($d\text{Cu} < 1$ nM). Pretreated samples (2nd
201 set) were not diluted as the signal measured without addition of Cu was generally low.
202 Preparations for titration experiments were carried out under a laminar flow hood in a 500 mL-
203 acid-cleaned FEP bottle and in fifteen 30-mL acid-cleaned FEP flasks with ETFE screw caps.
204 A 16th 30-mL flask was used for the blank. The analysis was carried out using an automated
205 system equipped with an in-house made sample changer which allowed the addition of the
206 sample in the cell plus a rinse between each aliquot. A volume of 20 mL was needed in each of
207 the 15 flasks (i.e. 10 mL for the rinse and 10 mL for the analysis). When 10-fold diluted, 30 mL
208 of sample was first transferred in the 500-mL FEP bottle. Then, borate buffer and SA were
209 added in order to reach a pH of 8.2 ± 0.2 and a SA concentration of $4 \mu\text{M}$ in final solution.
210 UVSW was finally added to achieve the total volume of 300 mL. 20 mL aliquots of the solution
211 were then pipetted into the 15 FEP flasks. Cu was added to each flask in logarithmic steps [29]
212 progressively increasing the additions from 0 to 300 nM by using automatic burette system
213 (XE1000, TECAN). The blank consisted of UVSW prepared directly in the 30-mL flask with
214 0.01 M borate buffer and $4 \mu\text{M}$ of SA. The 16 flasks were left to equilibrate overnight at room
215 temperature (8 h minimum). Concentration of labile Cu was then determined by CSV using
216 30 to 180 s deposition time. The deposition potential was -0.1 V followed by a 5 s equilibration
217 time and a cathodic scan from 0.05 V to -1.6 V using the square wave (SW) mode (step potential
218 2 mV, frequency 50 Hz). Five repetitions for each aliquot were made for quality control.
219 Overall, precision was better than 2%. The titration experiment performed in UVSW showed
220 fully linear response, confirming that the chosen titration range was adequate.
221 Once the voltammogram treatment was performed (see 2.4.2.c), final titration curves were
222 plotted in the ProMCC software. Conditional stability constant as well as ligand concentrations
223 were modelled using the complete complexation-fitting model [30]. For all samples, the use of

224 1 ligand model gave proper estimations of both conditional stability constant and Cu-binding
225 ligands concentrations.

226 **2.4.2.b Side reaction coefficient**

227 Most of samples were diluted in UVSW of salinity 38 resulting in a final salinity of around 35-
228 36 in the measured samples. As salinity and the SRC of CuSA were close among samples,
229 calculations of stability constants for diluted samples were all conducted with a salinity value
230 of 36. Values of 9.54 and 14.96 for $\log K'_{CuSA}$ and $\log \beta'_{Cu(SA)_2}$ were obtained and used for all
231 further calculations. A value of 13 was chosen for **inorganic** side reaction coefficient α_x and
232 used in calculations ^[31].

233 **2.4.2.c Signal treatment and final titration curves**

234 All voltammograms treatment was performed using the software ECDSOFT available at
235 <https://sites.google.com/site/daromasoft/home/ecdssoft> ^[32]. Raw signals were first corrected
236 from noise using automatic smoothing (Savistky-Golay method ^[33]). Smoothed
237 voltammograms were then automatically subtracted by the blank (which was flat in the potential
238 range of Cu(SA)_x peak). Additionally, the baseline at the position of Cu(SA)_x peak was
239 manually subtracted for each voltammogram using the 'spline' procedure ^[34]. **The reduction**
240 **peak corresponding to the Cu(SA)_x complexes was found at ~ 0.35 V (Figure 3). A 'shoulder'**
241 **was also observed on the positive side of the main Cu-SA peak and could be linked to other**
242 **Cu-complexes existing in the sample, such as Cu-sulfides nanoparticles ^[35-37].**

243

244

245 **2.4.2.d Total dissolved Cu in pretreated samples**

246 Total dissolved Cu concentration in pretreated samples (dCu*) was determined using
247 differential pulse anodic stripping voltammetry (DPASV). Prior to analysis, 10 to 20 mL of
248 hydrothermal fluid samples were acidified to pH < 2 (20 to 40 μ L of HNO₃ 69 %, Suprapur)
249 and then UV-irradiated overnight (250 W high pressure mercury lamp). Electrochemical
250 paramaters were adapted from Omanović et al. [38]. Samples were first deaerated by purging the
251 solution for at least 5 min. The accumulation was performed at a deposition potential of -1 V
252 for 120 or 180 s, followed by an anodic stripping scan from -0.45 to 0 V using the differential
253 pulse (DP) mode (modulation time 40 ms, modulation amplitude 40 mV, step potential 2 mV
254 and interval time 0.1 s). A 2-s potential-jump to -1.3 V was applied at the end of the
255 accumulation time. This step is needed for non-UV irradiated samples at natural pH in order to
256 desorb any layer of natural organic matter from the mercury drop [39], but was also found to
257 slightly improve the baseline shape in acidic and UV-irradiated samples. The equilibration time
258 was set to 5 s. Cu concentration was determined using the standard addition method. Every
259 measurement was repeated 3 times for quality control.

260

261 **2.5. Total dissolved Cu and Mn concentrations in untreated samples**

262 Untreated samples (both sets) were analyzed by HR ICP-MS (Element 2, Thermo Finnigan; at
263 IRB, Zagreb) and by ICP-AES (Ultima 2, Horiba Jobin Yvon; at PSO, Brest) for dCu and dMn
264 concentrations, respectively. The measurement of dCu concentrations was performed after
265 dilution of the samples from bags and PFA-bottles in a 2% HNO₃ solution (dilution factor: 10)
266 spiked with a stock solution of indium (1 g.L⁻¹) as internal standard (final concentration:
267 10 μ g.L⁻¹). External standards of Cu were then prepared in the matrix matching media (UWSV,
268 2% HNO₃) for the assessment of dCu. The analysis of dMn was conducted on aliquots stored

269 in 15 mL pre-cleaned polypropylene tubes (Elkay) dedicated for ICP measurements.
270 Preparation of solutions and standards is more detailed in Cotte et al. ^[40]. Blanks included
271 several replicates of the 2% HNO₃ solution as well as ultrapure water processed in the PEPITO
272 sampler. Repeatability (n = 10) was 3% for dMn and 4% for dCu. Dissolved concentrations
273 presented here were generally well above the detection limits. Blanks were below detection
274 limits and mostly negligible when compared to dissolved concentrations. It should be
275 mentioned that acidification alone in sulfidic waters has been previously reported to result in a
276 significant underestimation of total dissolved Cu because of the formation of stable Cu sulfide
277 phases which can adsorb onto bottle walls ^[41]. However, overlooking total dCu concentrations
278 is much more critical for the correct interpretation of the dCu spatial distribution in relation to
279 the hydrothermal vent distance, than for the complexation study by which we tried to identify
280 possible ligands (organic/sulfur derived) which control Cu speciation. Due to the complexity
281 of such studies, including sample collection, preservation, storage and, finally, measurement, it
282 is certain that some absolute values (e.g. concentrations of dCu) do not correspond to the
283 ambient conditions. However, the observed experimental/voltammetric phenomena are
284 supposed to be mostly unaffected regardless of the total concentrations of some compounds.

285

286 **2.6. Dissolved organic carbon**

287 Samples were pipetted in glass tubes cleaned with 10% HNO₃ (analytical reagent grade), rinsed
288 with Milli-Q water and calcinated for 4 hours at 450°C. Dissolved organic carbon (DOC)
289 measurement was conducted by direct injection of the sample into the furnace for combustion
290 at a temperature of 680°C through a highly sensitive catalyst (Pt) ^[42]. Formed CO₂ was then
291 measured by a non-dispersive infrared analyzer (NDIR). Dissolved inorganic carbon (DIC) was
292 removed from the solution by acidification of each sample with a 2% HCl solution to pH 2-3
293 and bubbling with clean air stream for 10 min. Calibration was performed using potassium

294 hydrogenophthalate as a standard. Sample from each cuvette was measured in triplicate and
295 standard deviation was calculated ($0.01 \text{ mg}\cdot\text{L}^{-1}$). Note that DOC was only measured on the 2016
296 samples.

297

298 3. Results and discussion

299 3.1. Master variables

300 All Cu speciation parameters and the associated master variables measured in the present study
301 are summarized in Table 1, Table 2 and Table 3. Dissolved manganese (dMn) was used as
302 conservative tracer because of its well-known very slow scavenging rate in the plume^[43,44]. As
303 expected, samples from the early buoyant plume were highly enriched in dMn at both sampling
304 sites compared to typical deep-seawater concentrations (dMn $\sim 0.5 \text{ nM}$ at 1700 m depth^[45]).
305 The 3 sets of samples collected at Aisics (2015 and 2016) and Y3 (2015) displayed dMn
306 concentrations ranging from 1 to $\sim 100 \text{ }\mu\text{M}$ indicating that the examined area corresponds to a
307 dilution of the hydrothermal solution with local seawater of ~ 3 to ~ 300 (Table S1). In addition,
308 similar T–dMn linear relationships were observed at both vent sites ($T \sim 1.5\text{dMn}$).

309 Dissolved Cu concentrations (dCu) in untreated samples were generally found in the range 10-
310 800 nM at both vent sites with values from 12 to 771 nM and from 13 to 409 nM at Aisics and
311 Y3, respectively (Table 1). This is much higher (one or two orders of magnitude) than the
312 ambient concentrations reported in deep-seawater of the North Atlantic ($2\text{--}3 \text{ nM}$ ^[46,47]) but
313 typical of hydrothermal input. As opposed to dMn, concentrations for dCu differed between the
314 two vent sites, Y3 displaying globally higher dCu concentrations than Aisics (dCu = 5.6dMn
315 vs. dCu = 3.0dMn , respectively). This discrepancy suggests different physico-chemical
316 properties in the corresponding end-members fluids (e.g. higher dCu at Y3). Note that at Aisics,

317 a low year-to-year variability of dCu in the mixing gradient was observed for the 2 sampling
318 years (Table 1 and Table 2).

319 In pretreated samples, dissolved Cu concentrations (dCu^{*}) were much lower than the original
320 dCu. Indeed, dCu^{*} at Aisics varied between 2.5 and 14 nM whereas corresponding dCu ranged
321 from 7 to 410 nM (Table 2). Such difference arises from the removal of the dCu by adsorption
322 onto the PFA bottle walls over the storage period and by additional scavenging onto the newly-
323 formed particles resulting from the air-purging treatment.

324

325 **3.2. *In situ* concentrations of sulfides and oxygen**

326 Concentrations of *in situ* free sulfides ($\Sigma S = HS^- + S^{2-} + H_2S$) and dissolved oxygen are
327 summarized in Table 3. *In situ* concentrations of ΣS ranged from 1 to 320 μM and from 5 to
328 350 μM at Aisics in 2015 (dive 02598) and in 2016 (dive 09632), respectively. The ΣS
329 concentrations were slightly higher at Y3 as they were ranging from 52 to 415 μM . Like for
330 dCu, this difference could be due to a different chemical signature of each end-member fluid.
331 Note that the ΣS data were not measured in 2015 at Y3. We thus chose to use the ΣS data
332 measured in 2014 at the same site. Such earlier data can be considered representative of free
333 sulfide conditions occurring at Y3 considering the very low variability observed for other
334 chemical parameters at that site between 2014 and 2015^[48]. As expected, *in situ* ΣS levels were
335 increasing with dMn and T, towards the vent orifice (Figure 5, Figure 6 and Figure 7). Note
336 however the positive anomaly in ΣS observed at Aisics where ΣS concentrations reach more
337 than 300 μM at around 50 μM of dMn (Figure 5). Changes in the *in situ* dissolved oxygen (O₂)
338 levels along the mixing gradient were not as steep as it was noticed for ΣS . Concentrations were
339 indeed slowly decreasing with increasing dMn concentrations from 276 to 146 μM
340 (avg = 200 μM) and from 264 to 183 μM (avg = 230 μM) at Aisics and Y3, respectively (Table
341 3). The two vent sites generally displayed similar oxygen levels throughout the mixing gradient,

342 though slightly lower levels were sometimes found at Aisics (e.g. at dMn in the range
343 10–50 μM).

344

345 **3.3. Description of the voltammograms**

346 In our hydrothermal samples, voltammograms differed from those classically obtained in
347 seawater because of the occurrence of interferences on the $\text{Cu}(\text{SA})_x$ signal as well as several
348 additional peaks. As their description can provide an ‘imprint’ of the hydrothermal
349 environment, a complete scan from 0.05 to -1.6 V was performed during stripping. None of
350 these interferences or additional peaks were detectable when analyzing UVSW, indicating a
351 natural origin of the compounds involved and not an artifact from the method. In this section,
352 we investigate on the nature of these additional peaks thanks to laboratory tests and literature.
353 We then discuss on the interferences affecting the $\text{Cu}(\text{SA})_x$ signal.

354 **3.3.1. On the nature of additional peaks**

355 Voltammograms for untreated and pretreated samples are presented in Figure 3. The peak at -
356 1.45 V was related to the reduction of dMn (it is not accumulated) as the peak height was
357 increasing in response to Mn(II) additions (Figure S1). As expected, the closer from the vent
358 orifice, the higher was the dMn signal (Figure S2).

359 The massive signal observed at -0.6 V vs. Ag/AgCl in pretreated samples (Figure 3B) could be
360 related to the high ambient reduced sulfur species (RSS) ^[49,50] in our samples, in line with the
361 high *in situ* concentration of ΣS measured (up to 300 μM). The signal is much lower but still
362 visible in untreated samples (Figure 3A). The peak likely corresponds to the dissolution-
363 reduction of an HgS layer deposited on the mercury drop surface as reported in many studies ^{[50–}
364 ^{55]}. Many sulfur species could contribute to the HgS layer deposition especially sulfides (HS^-
365 and S^{2-}), but also dissolved elemental sulfur S^0 (< 0.2 μm), polysulfides (S_n^{2-}) ^[52,56] or metal

366 sulfide nanoparticles^[35–37,57,58]. According to the excess of the *in situ* free sulfide concentrations
367 measured, free sulfides should be the dominant RSS in our samples. In the experimental
368 conditions used (pH ~ 8), HS⁻ might be the dominant free sulfide species and the formation of
369 the insoluble HgS layer at deposition potential more positive than -0.5 V could therefore be
370 expressed according to the following reversible two-electron process^[49] (1):

371



372

373 As dissolved Fe concentrations are relatively high in untreated samples (~2–200 μM^[48]), we
374 cannot exclude that some dissolved Fe would still persist as Fe(II) species, even after the pre-
375 treatment. If so, the diffusion of labile Fe(II) to the Hg electrode may change the HgS layer
376 formed with sulfides to a FeS layer^[58] (2):

377



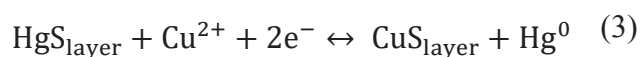
378

379 FeS layers could also be generated by the direct deposition of FeS nanoparticles at the electrode
380 surface^[58]. Since the FeS layers deposited are re-dissolved at around -0.6 V^[58], they could
381 partly contribute to the massive signal observed in pre-treated samples. In addition to sulfide
382 species, thiols could also contribute to the large peak observed, this compounds providing a
383 peak at the same potential^[59,60]. It is also important to underline that the height of the peak at -
384 0.6 V was decreasing with additions of Cu (Figure 3B). This suggests that the specie(s)
385 involved, i.e. sulfides or thiols, is(are) reacting with the added Cu throughout the titration
386 experiment^[50,60].

387 The peak at -0.1 V, which does not increase with the accumulation time, observable for both
388 untreated and pretreated samples could be related to three different reduction processes (Figure

389 3). The first one could be iodide which was found to occur at that potential in estuarine samples,
390 though easily removed through the application of a desorption step at more negative potential
391 [17]. Unfortunately, neither the addition of potassium iodide in the buffered samples (pH 8.2, not
392 shown), nor the application of the 1-s jumping potential did produce any increasing or removal
393 of the peak of interest, respectively. Combined with the fact that iodide is not a major compound
394 of hydrothermal fluid compared to chloride, this first hypothesis was thus excluded. Another
395 possible explanation could be the deposition of a copper-sulfide layer on the drop surface as a
396 result of an exchange reaction between Hg^{2+} from the HgS layer and labile Cu in solution,
397 according to the reversible reaction (3) [55]:

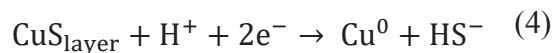
398



399

400 Labile Cu could correspond to weak Cu-complexes or, potentially, Cu-sulfides nanoparticles
401 (clusters). Such process has been shown to happen for many metal as Zn^{2+} or Fe^{2+} but only the
402 formation of the CuS layer provides a signal at -0.1 V vs Ag/AgCl (e.g. $E \sim -0.6$ V for the FeS
403 layer) [53,55]. Once deposited, the CuS layer will be reduced at ~ -1.0 V vs Ag/AgCl [35-37,55] as
404 described in reaction (4) :

405

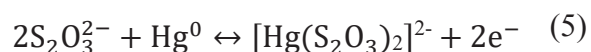


406

407 Among voltammograms of untreated samples, this reduction peak was sometimes noticeable
408 (up to 15 nA, Figure 3A). In pretreated samples, this signal was not observed, probably because
409 hindered by the massive HgS reduction peak. The peak potential of the CuS formation process
410 (3) is determined by the solubility product K_{sp} of CuS [55]. Through the titration experiment, K_{sp}
411 will increase with increasing addition of Cu whereas $\text{p}K_{\text{sp}}$ will decrease. Consequently, the peak

412 potential will be slightly shifted towards more negative potential as shown by Milanović et al.
413 ^[55]. This feature was not observed when performing titrations, except some slight shifting of
414 the presumed CuS peak toward more positive potential in few cases (Figure 3A). In addition
415 with the potential shifting observed, the peak height was not dependent on the accumulation
416 time. However, the peak height was increasing when getting closer to the vent source (Figure
417 S2). This led us to the final assumption that the peak of interest could be related to thiosulfate,
418 which is known to be formed in hydrothermal vents by the slow oxidization of sulfides ^[61].
419 Thiosulfate (S₂O₃²⁻) reduction occurs at around -0.1 V vs Ag/AgCl and is well separated from
420 the global sulfides reduction peak (-0.6 V) at pH 8 ^[51,52]. The interaction between thiosulfate
421 and mercury results in the formation of a soluble complex via the oxidation of Hg according to
422 the two electron process (5):

423



424

425 The soluble complex cannot be pre-concentrated to the drop surface as it immediately goes back
426 to solution after its formation (as mentioned above, this peak does not increase with the
427 accumulation time). The cathodic scan enables then to strip only the quantity of [Hg(S₂O₃)₂]²⁻
428 formed on the drop surface at that precise moment, the current measured corresponding to the
429 reduction of Hg²⁺ to Hg⁰ ^[51]. Unfortunately, addition of sodium thiosulfate was not performed
430 on samples to confirm this assumption. Besides, the aforementioned studies were working on
431 laboratory solutions with known concentrations of all species in solution or on samples not truly
432 representative of hydrothermal samples. Accordingly, the identification of each electroactive
433 species in such unusual samples must be more problematic and more tests should be further
434 carried out to decide which process is truly happening. Hence, the peak at -0.1 V vs Ag/AgCl
435 could be both related to the CuS layer formation and to the thiosulfate reduction.

436

437 3.3.2. Interferences on the Cu(SA)_x signal

438 Interferences on the Cu(SA)_x signal were observed in most of hydrothermal samples (Figure 3).
439 At low addition of Cu, unknown peak/shoulder at ~ -0.27 V was systematically overlapping
440 with the Cu(SA)_x signal in both untreated and pretreated samples. A similar shoulder is
441 observable in voltammograms presented on the 'Figure 1.b' of Sander et al.'s paper^[10]. In both
442 datasets, the corresponding reduction signal was thus fitted and **de-convolved** from the
443 interferences observed at ~ -0.27 V **using the ECDSOFT program**. Depending on the sample, 1
444 or 2 peaks were considered to overlap with the main Cu(SA)_x reduction peak. Signal **de-**
445 **convolution** was performed by starting from the signal corresponding to the highest Cu addition,
446 where interferences no longer overlook the Cu(SA)_x reduction peak, towards the signal
447 corresponding to the lowest Cu addition, where extraction was more liable to errors. The height
448 of the **de-convolved** peak of Cu(SA)_x was then measured and used to construct the final titration
449 curves which consist in the relationship between the measured current (*i_p*) and the total
450 concentration of Cu (Figure 4).

451 Note that the Cu(SA)_x signal at ~ -0.3 V obtained for pretreated samples was not well defined,
452 especially at low additions of Cu where it appears flattened and narrower compared to the one
453 observed in untreated samples (see boxed window (*), Figure 3B). The spike observed did not
454 correspond to a reversible process which suggest that it was not related to the reduction of the
455 Cu(SA)_x complex. With increasing additions of Cu, the spike was shifting towards more
456 positive potential (-0.27 V) until the appearance of a second peak corresponding to the
457 reversible reduction of the Cu(SA)_x complexes at -0.3 V. In addition, the maximum reduction
458 current for the Cu(SA)_x signal, corresponding to the last addition of Cu (same final
459 concentration), was ~ 200 nA in pretreated samples whereas it reached almost 400 nA for

460 untreated samples. These issues are likely related to the HgS layer formed at the mercury drop.
461 By occupying most of the space available on the drop, the HgS layer probably prevents the
462 Cu(SA)_x complexes from adsorbing on the drop surface. This feature has already been observed
463 by Powell ^[11] who mentioned both an over-scale reduction peak at -0.5 V (vs Ag/AgCl) related
464 to RSS and a poorly defined Cu(SA)_x peak, but before any pre-treatment of his hydrothermal
465 samples. Consequently, the HgS layer formation as well as its reduction are interfering on the
466 Cu(SA)_x signal, although the two reduction peaks are not overlapping.

467 In an attempt to remove the HgS interference, a 1-s potential-jump to -0.65 V to desorb the HgS
468 layer from the drop was performed to the air-purged samples at the end of the accumulation
469 step. This desorption step effectively removed > 95% of the HgS interference. However,
470 comparative experiments in model and real samples showed that this procedure is providing
471 good results on model samples whereas its application on real hydrothermal samples still needs
472 to be more explored, because of problems with the linearity. Though unsuccessful, the
473 procedure employed is described in supplementary information. Note that the HgS interference
474 should also be high in untreated samples since the *in situ* concentrations of ΣS are similar than
475 for pretreated samples. It was not the case because untreated samples were 10-fold diluted and
476 were analyzed a year after collection, meaning that most of the free RSS could have been slowly
477 oxidized with time by O₂ excess in samples (e.g. ~ 200 μM, Figure 5) or potentially by slight
478 air passing through sampling bags. By contrary, pretreated aliquots were analyzed only 2
479 months after sampling, meaning that a short time is required between sampling and
480 electrochemical analysis for speciation studies. In conclusion, fairly good titration curves were
481 obtained from the classical analysis used for speciation studies ^[28] but titration of the Cu-
482 binding ligands was more problematic than expected in 2016 because of the high ambient sulfur
483 species. Despite not fully identified, sulfur species obviously play a major role in the early
484 buoyant plume and likely interfere on Cu speciation measurement in this area.

485

486 3.4. Cu-complexing ligand parameters

487 Concentrations for Cu-binding ligands ([L]) in the untreated samples ranged from 0.032 to
488 3 μM (avg. 700 nM, $n = 19$; Table 1). Aisics and Y3 both exhibited similar concentrations for
489 [L] which varied between 0.23 and 3 μM and from 0.032 to 1 μM , respectively. Interestingly,
490 an increasing trend towards the plume core clearly appears when plotting both [L] and ΣS
491 against the dMn concentrations at Y3 (Figure 6). This suggests a common source for all these
492 parameters including the Cu-binding ligands. This feature has already been observed close to
493 deep-sea vents for Fe-binding ligands which were highly correlated with the hydrothermally
494 sourced Fe concentrations ($r^2 = 0.996$)^[16]. The trend was less clear at Aisics where [L] were
495 globally flattened by two samples displaying the highest concentrations measured (02598 B1
496 and 02598 D3) (Figure 5). Note that the corresponding dCu concentrations in these two samples
497 were also the highest measured (770 and 460 nM, resp.), indicating that these two exceptions
498 for [L] were not an artefact from the titration experiments. However, it should be mentioned
499 that, when these two ‘outliers’ are not considered, a very good match between [L] and the
500 anomaly of ΣS is observed (not shown). Stability constants ($\log K'_{\text{CuL}}$) remained globally
501 similar at both sites: calculations provided $\log K'_{\text{CuL}}$ values ranging from 12.37 to 13.38
502 (avg. 12.89).

503 To our knowledge, Sander et al.^[10] and Klevenz et al.^[62] are the only studies for which titration
504 experiments have been conducted on untreated hydrothermal samples (i.e. non-acidified, non-
505 purged) and thus to which our results for L can be directly compared. Our untreated samples
506 displayed [L] in similar range than concentrations previously measured at the Kermadec Arc in
507 Pacific^[10]. The authors have indeed reported concentrations for Cu-binding ligands ranging
508 from 125 nM to 4.5 μM (avg. 1.7 μM , $n = 4$). However, Klevenz et al.^[62] have reported
509 narrower concentration ranges (50 - 200 nM) from diffuse hydrothermal fluids of the MAR,

510 with an average value of 125 nM ($n = 2$). Interestingly, our obtained stability constants
511 (12.37–13.38, avg. 12.89 ± 0.11) are also very close to those observed by Sander et al. [10],
512 i.e. $\log K'_{CuL}$ ranging from 12.53 to 13.46 (avg. 12.88 ± 0.61). A similar ligand class has
513 therefore been measured in both cases, suggesting that the Cu-binding ligands found are likely
514 to be of same nature. Although Sander and co-workers have attributed their findings to the
515 occurrence of strong organic ligands (i.e. thiols), it is also worth mentioning that a $\log K'_{CuL}$
516 value of 12.9 has been found for Cu-bisulfide complexes using ligand competition with oxine
517 at pH 8 [50]. Regarding their similar stability constants, both strong organic and inorganic
518 bisulfide ligands **could** therefore be considered as part of the Cu-binding ligands mixture. **It**
519 **should be mentioned that both Cu(I) or Cu(II) species could be part of the Cu-complexes**
520 **detected. The existence of Cu(I) is probable in hydrothermal samples because of the complex**
521 **redox reactions in which Cu is involved [63]. The existing Cu(I) species could be further**
522 **stabilized by complexation with thiols, as previously suggested [64,65]. In our case, the oxidation**
523 **of Cu(I) during sample storage is likely to occur [66] and it would be too speculative to claim**
524 **that the observed Cu speciation is mainly related to Cu(I)-thiol compounds. However, the**
525 **formation of Cu(I)-thiol complexes should also be considered with specific experiment**
526 **designed in order to prove such complexation scenario.**

527

528 **3.5. On the nature of dissolved copper ligands in the buoyant plume**

529 Below, we discuss the potential sources of Cu-binding ligands (organic and/or inorganic) which
530 could explain at best the high concentrations for L measured in our samples, in line with our
531 available data set for DOC and for *in situ* free sulfides. We then consider copper speciation
532 under laboratory conditions and discuss the implications deduced from $[L^*]$ on the persistence
533 of hydrothermal Cu-binding ligands in the oxic water column.

534 **3.5.1. Organic Cu-ligands**

535 DOC in deep ocean waters is mainly composed of refractory organic matter originally coming
536 from sea surface ^[67] and resistant enough to removal processes such as microbial activity. In
537 deep waters, background concentrations of refractory DOC are averaging 45 μM ^[68,69] whereas
538 concentrations detected in focused and diffuse vents range on average from 15 to $\sim 100 \mu\text{M}$
539 ^[70,71] and from 50 to $\sim 100 \mu\text{M}$ ^[70-72], respectively. The oldest (or most refractory) forms of the
540 deep DOC pool are almost all removed ($> 90\%$) through thermal degradation in subseafloor
541 circulation ^[71,73] but, still bulk DOC concentrations remain important because of carbon-rich
542 sediment remobilization as well as local input of abiotically-produced organics compounds
543 ^[4,71].

544

545 Our samples display DOC concentrations ranging from 25 to 90 μM (avg. 53 μM) which is
546 slightly above background DOC in deep seawater (45 μM) but in the range of previous finding
547 in hydrothermal vents. The highest value corresponded to one of the closest sample from the
548 vent source (09632 C3) (Table 2) though no particular trend has been noticed along the mixing
549 gradient. In surrounding deep Atlantic waters, concentrations of organic ligands have been
550 reported to be in the range of 2-4 nM ^[47]. So, basically, organic ligands from deep seawater
551 account for 0.01% of the deep recalcitrant DOC pool. More importantly, those organic ligands
552 can only provide a very limited part of the Cu-binding ligands observed here (up to 3 μM). A
553 deep local source must therefore explain the enhanced concentrations measured for L.

554 First, organic compounds including organosulfur Cu-ligands could be directly produced in the
555 hydrothermal fluid, before venting ^[74]. Synthesis of thiols through abiotic reduction of the
556 hydrothermally-sourced inorganic carbon (CO_2 or CO) has indeed been suggested for H_2 -rich
557 fluids ^[75]. However, this process has been reported to be limited. In addition, the hydrothermal
558 fluids from Lucky Strike show relatively low levels in H_2 when compared to other sites from

559 the MAR such as Rainbow ^[76]. Instead, thermal degradation of microbial communities with the
560 associated dissolved organic matter in subsurface has been suggested to produce methanethiols.
561 The abundance of such specific compounds has been reported to be around 10 nM in high
562 temperature fluids (> 200°C) ^[4]. Indeed, the authors have performed measurements of
563 methanethiols in the end-members fluids of 4 smokers located in the Lucky Strike vent field.
564 The resulting concentrations were around 6–7 nM with a maximum of 23 nM (avg. 10 nM).
565 Since the Aisics and Y3 end-members both display temperatures greater than 300°C (Table S1),
566 the abundance of methanethiols are likely to be in a similar range, i.e. lower than 20 nM.
567 Second, organosulfur ligands with available binding sites could be supplied by **microbial**
568 production in diffuse areas. If located in the close vicinity of focused black smokers
569 (e.g. Aisics), then **microbially-produced** ligands could be laterally entrained in the buoyant
570 plume, as suggested for larvae ^[77]. Cultured microbes from two hydrothermal vents of the MAR
571 have indeed been reported to produce organic Cu-binding ligands as a protection against the
572 enhanced Cu concentrations ^[62]. In response to the addition of 100 nM of Cu, around 200 nM
573 of Cu-binding ligands were produced in one of the cultures, which is not negligible but still not
574 completely explaining the enhanced level for L in the present study.
575 Finally, laterally entrained microbial communities could produce organics directly in the early
576 buoyant plume, where temperature falls down rapidly under 100°C. However, we anticipate
577 that the early mixing of the studied gradients (on the order of ten seconds) should be much
578 faster than **microbial** production of organic compounds. Although not known for hydrothermal
579 microbes, the kinetic of production for organic ligands by phytoplankton in response to metal
580 enrichments has been found to be around 1 nM per day for specific zinc-binding compounds
581 ^[78], which is much longer than the mixing time.
582 **Overall**, deep local sources of potential organic Cu-ligands are existing in deep-sea
583 hydrothermal vents but the input appears to be relatively limited and can only explain a small

584 part of the Cu-binding ligands measured in the buoyant plume. As none carbon-rich sediment
585 remobilization has been noticed during sampling – the lava lake is moderately covered by
586 sediment ^[20] – other sources as well as other type of ligands should be considered such as the
587 input of inorganic Cu-binding ligands by vent sources. It is worth noting however, that the
588 fraction of Cu-ligands resistant to the air purging treatment (L^*) could correspond to organic
589 species. Their concentrations, up to 20 fold lower than those of the ligands L (range 18–44 nM;
590 Table 2) appear to be on the same order of magnitude than the aforementioned potential organic
591 sources. Their stability constants ($\log K^*_{CuL}$) range from 12.57 to 13.04 (avg. 12.79) and are
592 thus comparable to those found for thiols ^[10,60]. When plotting $[L^*]$ against dMn at Aisics
593 (Figure 7), the highest concentrations for L^* were observed closer to the hydrothermal end-
594 member, at dMn concentrations about 75 nM (corresponding to a temperature of ~ 120°C). This
595 feature suggests that the ligands L^* are hydrothermally sourced. Nevertheless, more samples
596 would have been needed in the hotter part of the mixing to confirm this assumption.

597

598 3.5.2. Inorganic Cu-ligands

599 Chloride and sulfides are recognized to be the dominant inorganic Cu-binding ligands in
600 hydrothermal fluids ^[9,79]. While Cu(I)-chloride complexes dominate in hot (~ 300°C) and acidic
601 (pH < 3) fluids ^[80], Cu-sulfides complexes become predominant at temperature lower than
602 150°C with increasing pH ^[79]. Upon mixing with seawater, the temperature of hydrothermal
603 fluids rapidly falls down under 150°C leading to weaker Cu-chloride complexes. Cooling
604 promptly establishes a competition between chloride and sulfides for Cu and Cu-sulfides
605 complexes progressively overlook the Cu-chloride complexes ^[80].

606 Because of their high stability constant, comparable to that of strong organic ligands and their
607 massive concentrations measured *in situ* (> 100 μ M) (Figure 5, Figure 6 and Figure 7), we infer
608 that inorganic sulfides could be the main contributors of the enhanced Cu-binding ligands

609 concentrations observed in the early mixing. This assumption is supported by the fact that the
610 $\log K'_{\text{CuL}}$ value we found at deep-sea vents were very similar to those reported by Al-Farawati
611 and van den Berg ^[50] for Cu(HS) complexes (i.e. 12.9). Moreover, the *in situ* concentrations of
612 inorganic sulfides are greater than 100 μM in most of our samples (Table 3). At such level of
613 inorganic sulfides, Al-Farawati and van den Berg have shown that the competition between
614 organic ligands and bisulfide for Cu does not exist anymore since the effect of organic
615 compounds on Cu-speciation becomes negligible when bisulfide concentrations are greater than
616 1 nM. The slow kinetics reported for the abiotic oxidation of sulfides by oxygen in diffuse
617 hydrothermal vents strengthen this hypothesis ^[61]. The authors have indeed calculated sulfides
618 oxidation rate lower than 1 nM.min⁻¹ which is negligible when compared to the mixing velocity
619 of the early buoyant plume (22-119 cm.s⁻¹ ^[81]) and to the total sulfides concentrations.
620 Furthermore, using *in situ* voltammetry, they did not find enhanced concentrations of sulfur
621 oxidation intermediate (i.e. thiosulfate or polysulfides), attesting to the slow oxidization process
622 of H₂S and HS⁻ in the mixing ^[61].

623 As pH is around 5 for all samples, the inorganic sulfide species should be mainly found as H₂S
624 and HS⁻. With the progressive mixing with seawater, pH will gradually increase until 7–8, and
625 sulfides will be mostly found as bisulfides HS⁻. Consequently, the inorganic Cu-sulfide
626 complexes should be mainly found as Cu(HS)⁺ or as Cu(HS)₂ ^[50] and, in any case, should
627 largely overtake the organic ligands in the early mixing. Elemental sulfur S⁰ could also interact
628 with the ambient Cu ^[11], though it is hard to know to which extent in the present study. However,
629 with the aging of the plume, local mixing of the plume with biologically rich diffuse vents
630 should lead to the input of organic ligands (e.g. thiols) which would mainly come from biotic
631 interactions. Such input would then increase the impact of organic ligands on the speciation of
632 Cu. We hypothesize that organosulfur compounds and stabilized sulfides (i.e. bound to Cu or

633 another metal) might coexist in the neutrally buoyant plume, until the complete oxidation of the
634 inorganic copper-sulfides complexes.

635

636 **3.6. Chemical changes of *in situ* speciation in laboratory conditions**

637 As soon as samples are collected, free sulfides start going through progressive oxidation
638 because of the excess of O₂ in samples as well as some potential slight air-passing through the
639 bags/bottles. Despite this slow removal, our results show that free sulfides still control Cu
640 speciation at least 2 months after sampling. Facing such dominance, all previous studies aiming
641 at defining organic copper complexation in hydrothermal vents have performed an acidification
642 pre-treatment (pH < 2) followed by N₂-purging to remove any ambient RSS [82] in order to
643 measure only labile Cu released from the remaining organic ligands [10–12]. As mentioned
644 previously, we tested this protocol, but did not observe any difference in the voltammogram
645 shapes and final results.

646 In an attempt to distinguish the organic part from the ligand pool and to fasten the process of
647 sulfur species oxidation which already started inside of the PFA bottle, we simply air-purged
648 and re-filtered at 0.2 μm samples (2nd set) for titrations of Cu-binding ligands. Lower Cu-
649 binding ligands concentrations were systematically found in these pretreated samples, attesting
650 that sulfides species removed are part of the Cu-ligands measured. On the contrary, air purging
651 would not have impacted organic ligands that much. Assuming that L* mostly stand for organic
652 ligands, i.e. strong organosulfur ligands as thiols [10], the organic pool still constitute a limited
653 part of the global ligand pool found in untreated samples. Furthermore, L* could also be used
654 as the utmost example to show how much Cu-complexes are resistant to strong and fast
655 oxidization. Note that the extent to which RSS were removed is unknown for both sets, but the
656 shape of voltammograms clearly show that these compounds still have an influence on Cu-

657 speciation, in accordance with the known stability of Cu-sulfides complexes in oxic conditions
658 [82,83]. In deep waters, the mixture of Cu-binding ligands including organosulfur compounds and
659 stabilized sulfides which results from the progressive mixing with seawater, is therefore able to
660 cope with the rapid mixing between hydrothermal fluid and seawater which could enable
661 dissolved Cu to be transported far from the venting source.

662

663 **4. Conclusion**

664 We assessed the apparent speciation of Cu in the early mixing with seawater of two different
665 black smokers at the Lucky Strike hydrothermal vent field (Mid-Atlantic Ridge). The Cu
666 binding ligand concentrations were increasing with the dMn concentrations suggesting a
667 hydrothermal origin of the Cu-ligands detected. For the first time, the apparent L concentrations
668 were discussed in line with the *in situ* concentrations of free inorganic sulfides. In the early
669 mixing, it appeared that L concentrations were following the trend of ΣS when plotting both
670 parameters against dMn. The corresponding stability constants of Cu-ligands ($\log K'_{CuL}$; avg.
671 12.9) were similar to those already reported for strong organic ligands (e.g. thiols, $\log K'_{CuL}$
672 ~ 13) in deep sea hydrothermal vents [10] but also to that of inorganic sulfide complexes (\log
673 K'_{CuHS}) [50]. Taking into account the experimental conditions used in the present work, the
674 apparent copper binding capacity measured in all samples was likely a combination of strong
675 organosulfur complexes and stable inorganic Cu-sulfide complexes, able to resist to the
676 progressive oxidization of samples. However, the inorganic sulfides, present at massive
677 concentrations *in situ*, are probably the major ligand involved in the stabilization of
678 hydrothermally sourced copper in this area, as suggested in earlier work [8,9]. Based on the work
679 of Al-Farawati and van den Berg [50] and on sulfides concentrations greater than 100 μM in
680 most of our samples, we inferred that Cu bindings ligands could be predominantly inorganic

681 sulfur species in the early mixing. This does not invalidate the previous finding on organic
682 copper complexation in hydrothermal vents. The assumption made here is rather that the impact
683 of the organosulfur ligands on Cu-stabilization should be much lower than the one of inorganic
684 sulfides in the buoyant plume because of their low concentration in comparison to sulfides.
685 However, with the aging of the plume, the progressive oxidization of free sulfides and some
686 local mixing with biologically rich diffuse vents, the part of organic ligand should increase, as
687 well as its role on Cu-stabilization. Organosulfur compounds and stabilized sulfides might
688 therefore coexist in the buoyant and neutrally buoyant plume, until the complete oxidation of
689 inorganic sulfides complexes. Finally, our results indicate that this mixture partly resists to
690 strong oxidizing conditions (air-purging) suggesting that copper could be transported far from
691 the vent sources.

692

693

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983 **Table 1.** Calculated complexing parameters for Cu (L and K'_{CuL}) obtained in untreated samples (2015)
 984 from Aisics and Y3 hydrothermal vents (Lucky Strike, MAR). Mean temperature (recorded during
 985 sampling) as well as natural pH, total dissolved manganese (dMn) and total dissolved copper (dCu)
 986 concentrations measured are also provided.

Site	Sample	T_{PEPITO} °C	pH	dMn μM	dCu nM	L nM	$\log K'_{CuL}$
Aisics	02598 A1	5.27	7.5	1.08	12	-	-
	02598 A3	17.2	6.1	11.1	129	-	-
	02598 B1	31.1	5.6	19.9	771	2104 ± 126	12.86 ± 0.15
	02598 B3	47.0	5.1	23.6	32	230 ± 12	13.03 ± 0.07
	02598 C1	57.0	5.3	37.2	39	377 ± 15	12.68 ± 0.05
	02598 C2	72.6	5.2	48.8	212	746 ± 30	13.38 ± 0.07
	02598 C3	67.6	5.2	44.9	119	1059 ± 74	12.95 ± 0.10
	02598 D1	55.0	5.3	39.4	26	351 ± 23	12.95 ± 0.08
	02598 D2	96.7	5.0	64.3	63	768 ± 88	12.91 ± 0.17
	02598 D3	108	4.9	72.9	456	2972 ± 156	12.97 ± 0.08
	02598 E1	121	4.9	83.4	50	336 ± 25	12.37 ± 0.07
02598 E3	154	4.7	102	312	742 ± 89	12.99 ± 0.20	
Y3	10606 A1	4.97	7.4	0.65	13	32.5 ± 2.6	12.55 ± 0.09
	10606 A2	10.0	6.7	4.26	32	61.5 ± 5.5	12.96 ± 0.14
	10606 A3	20.3	6.2	11.8	76	175 ± 12	12.89 ± 0.08
	10606 B2	35.4	5.8	26.9	365	519 ± 20	13.15 ± 0.09
	10606 B3	50.3	5.6	36.2	234	294 ± 46	12.69 ± 0.32
	10606 C1	60.1	5.5	44.4	102	499 ± 25	12.57 ± 0.05
	10606 C2	68.8	5.5	47.9	409	740 ± 37	12.95 ± 0.10
	10606 C3	80.4	5.4	70.8	325	585 ± 35	12.98 ± 0.10
10606 D1	88.8	5.3	65.2	335	1024 ± 33	13.13 ± 0.05	

987

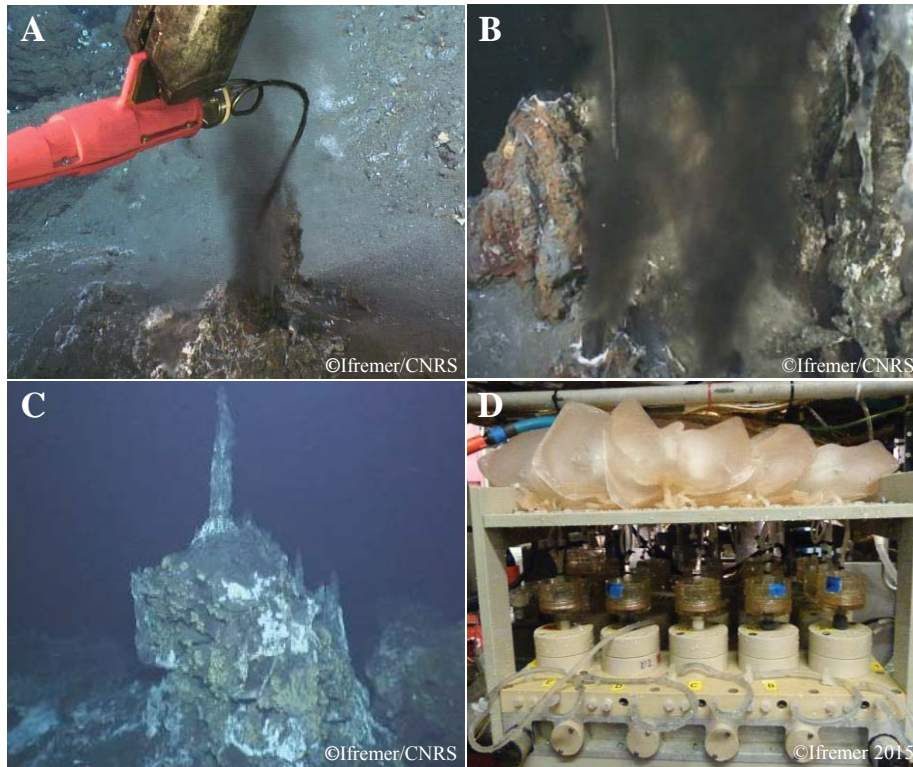
988 **Table 2.** Calculated complexing parameters for Cu (L^* and K^{**}_{CuL}) and dissolved Cu concentrations
 989 (dCu^*) obtained in pretreated (air purged-filtered) samples from Aisics hydrothermal vent (2016). Mean
 990 temperature (recorded during sampling) as well as natural pH, total dissolved manganese (dMn), total
 991 dissolved copper (dCu) and DOC concentrations obtained before any pretreatment of these samples are
 992 also provided.

Site	Sample	T_{PEPITO} °C	pH	dMn μM	dCu nM	dCu^* nM	DOC μM	L^* nM	$\log K^{**}_{CuL}$
Aisics	09632 A1	5.40	7.5	1.90	-	2.5	55.5	-	-
	09632 A2	12.7	5.8	5.03	7	3.6	55.6	-	-
	09632 A3	19.1	5.4	9.85	121	3.5	49.3	-	-
	09632 B1	32.2	5.1	14.0	277	10	59.1	22.8 ± 1.2	13.04 ± 0.05
	09632 B2	56.4	5.4	42.5	55	14	51.2	26.9 ± 7.5	12.75 ± 0.32
	09632 B3	73.6	5.2	38.4	410	-	-	-	-
	09632 C1	87.5	4.6	68.8	-	6.2	24.8	18.2 ± 1.0	12.61 ± 0.05
	09632 C2	114	4.7	77.5	-	6.3	40.8	41.8 ± 2.9	12.57 ± 0.06
	09632 C3	126	4.6	70.9	42	3.1	89.8	43.9 ± 5.1	12.96 ± 0.10

993

994 **Table 3.** Concentrations of ΣS (H_2S , HS^- , S^{2-}) and O_2 obtained with *in situ* instruments at Aisics and
 995 Y3 hydrothermal vents. In order to plot all *in situ* parameters as a function of dMn, the corresponding
 996 manganese concentrations (dMn $_{\Sigma S}$) were estimated from $T_{CHEMINI}$ thanks to the linear dMn- T_{PEPITO}
 997 relationship obtained at Aisics and Y3 (see SI).

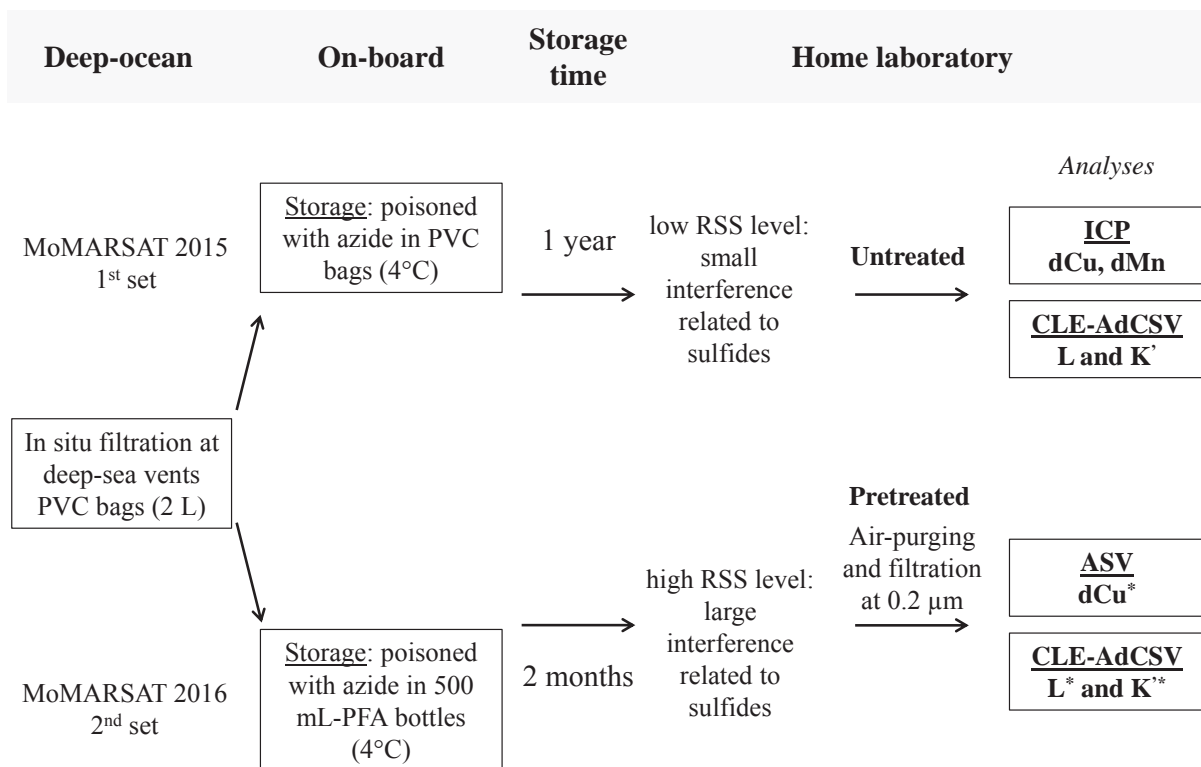
Site and date	Sample	$T_{CHEMINI(\Sigma S)}$	dMn $_{\Sigma S}$	ΣS	O_2
		°C	μM	μM	μM
Aisics (02598-2015)	02598 A1	5.18	0.83	0.98	276
	02598 A3	17.2	11.1	27.7	230
	02598 B1	31.1	19.9	62.6	243
	02598 B3	47.0	23.6	138	210
	02598 C1	57.0	37.2	321	190
	02598 C2	72.6	48.8	265	215
	02598 C3	67.6	44.9	237	189
	02598 D1	55.0	39.4	250	219
	02598 D2	96.7	64.3	231	186
	02598 D3	108	72.9	191	143
	02598 E1	121	83.4	169	162
02598 E3	154	102	187	146	
Aisics (09632-2016)	09632 A1	7.98	2.71	5.25	-
	09632 A2	11.4	5.03	89.0	-
	09632 A3	19.5	10.6	160	-
	09632 B1	30.3	17.9	224	-
	09632 B2	54.1	34.1	263	-
	09632 B3	60.0	38.1	286	-
	09632 C1	108	70.7	320	-
	09632 C2	117	76.6	335	-
	09632 C3	120	78.9	352	-
Y3 (07582-2014)	07582 A1	5.98	1.47	77.7	-
	07582 A2	9.90	4.37	52.2	-
	07582 A3	23.4	14.4	132	-
	07582 B1	45.1	30.4	275	-
	07582 B2	54.7	37.5	286	-
	07582 B3	78.2	55.0	363	-
	07582 C1	97.5	69.3	415	-
	07582 C2	70.8	49.5	266	-
Y3 (10606-2015)	10606 A1	-	-	-	247
	10606 A2	-	-	-	-
	10606 A3	-	-	-	264
	10606 B2	-	-	-	249
	10606 B3	-	-	-	240
	10606 C1	-	-	-	209
	10606 C2	-	-	-	242
	10606 C3	-	-	-	183
	10606 D1	-	-	-	183



999

1000 **Figure 1.** (A) Main black smoker Aisics (Endmember temperature: 307°C) at the Lucky strike vent field
 1001 (MAR) sampled during the Momarsat 2015 and 2016 cruises using the PEPITO sampler. The titanium-
 1002 Silicon inlet coupled to the ROV temperature probe is also shown. (B) Main black smoker Y3
 1003 (Endmember temperature: 326°C) during sampling. (C) Overall view of the site Y3, Lucky Strike
 1004 (MAR). (D) PEPITO sampler implemented on the ROV with freshly collected samples filtered *in-situ*.
 1005 The reader might see that 2 PVC-bags were attached to each filter holder: 2 bags for each sample were
 1006 thus collected in 2015. After recovery, one bag was frozen at -20°C whereas the other one was poisoned
 1007 with NaN₃ and stored at 4°C with the aim to compare the impact of sample storage on Cu speciation.
 1008 As freezing was suspected to worsen the aggregation of colloidal phases, only poisoned bags were
 1009 analyzed at that moment. Samples collected in 2016 were treated the same way, as indicated in the text.

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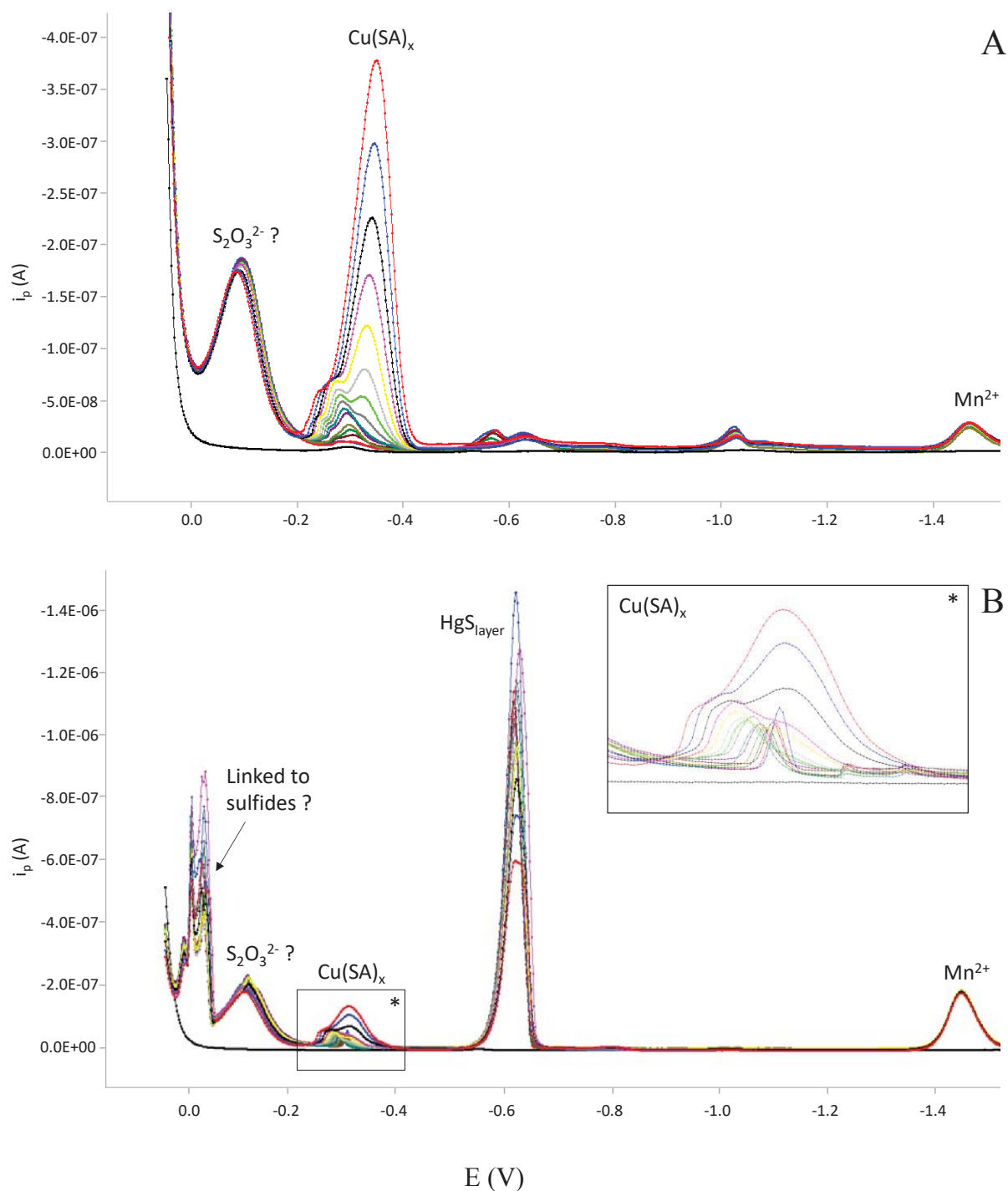
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1012 **Figure 2.** Summary diagram of the strategy used from the storage to the analyses for the assessment of
 1013 the Cu complexing parameters. Samples stored in PFA-bottles were also analyzed by ICP for total
 1014 dissolved copper (dCu) before being pretreated (see paragraph 2.5). RSS denote Reduced sulfide
 1015 species.

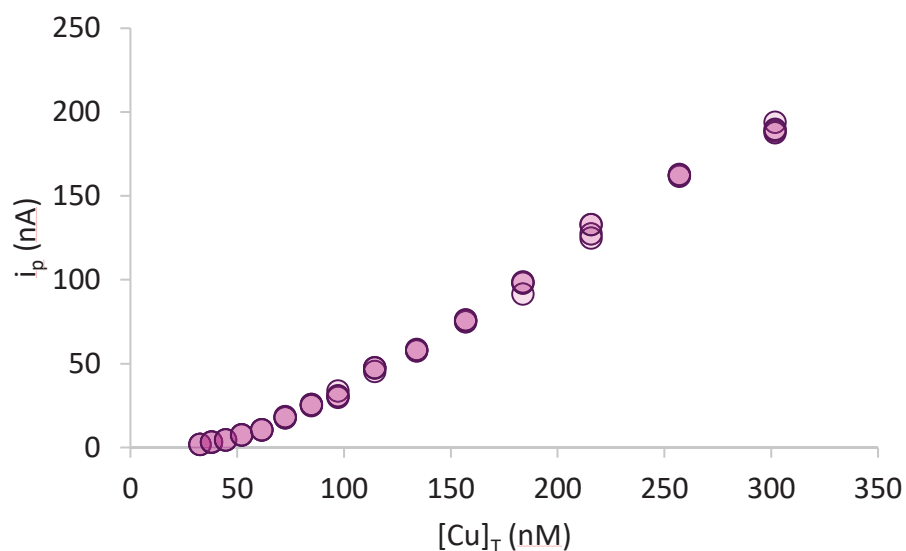
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1019 **Figure 3.** A) Typical voltammogram obtained for complexometric titrations in the untreated samples
 1020 (1st set; e.g. 02598 C1). Untreated samples were 10-fold diluted in UVSW. B) Typical voltammogram
 1021 obtained for the pretreated samples (e.g. 09632 C3). The right panel shows all $Cu(SA)_x$ signals for the
 1022 whole titration experiment. The detailed scale of potential in the inset is provided in Figure S3. All
 1023 titration experiments were performed after overnight equilibration at $pH \sim 8.2$ and $4 \mu M$ SA. Black
 1024 signals correspond to the blank (UVSW). The $Cu(SA)_x$ signal was extracted from the interfering signals
 1025 (i.e. ‘shoulders’ at -0.27 V vs Ag/AgCl) observed at low additions of Cu for both sets of samples.
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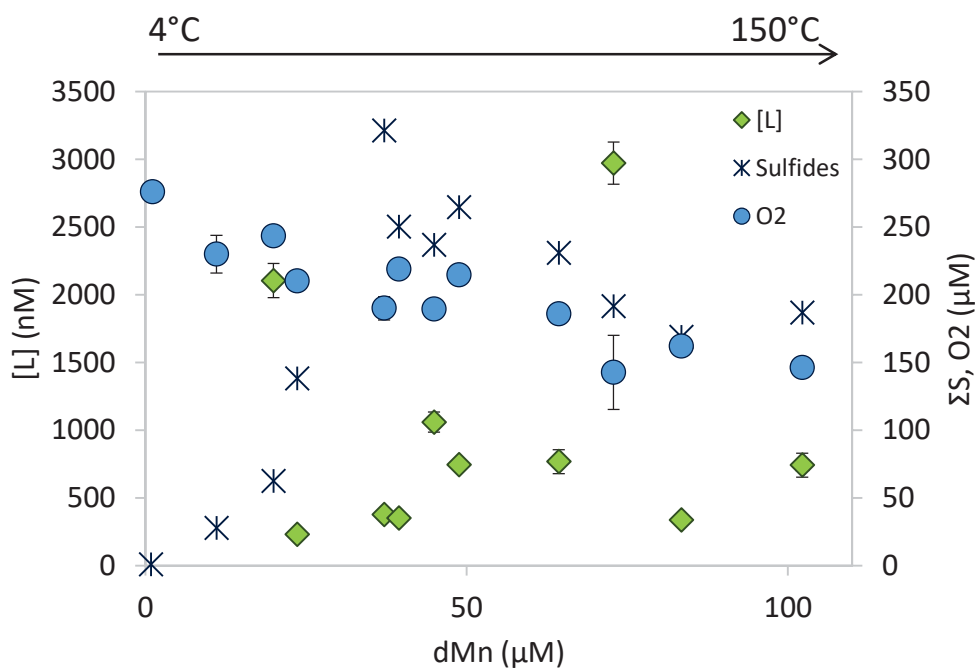


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1029 **Figure 4.** Typical titration curve obtained after voltammogram treatment and signal extraction (e.g.
 1030 10606 C3, 2015) with 14 Cu-standard additions (5 or 4 replicates), 4 μM of SA, 0.01 M borate buffer
 1031 (pH \sim 8.2).

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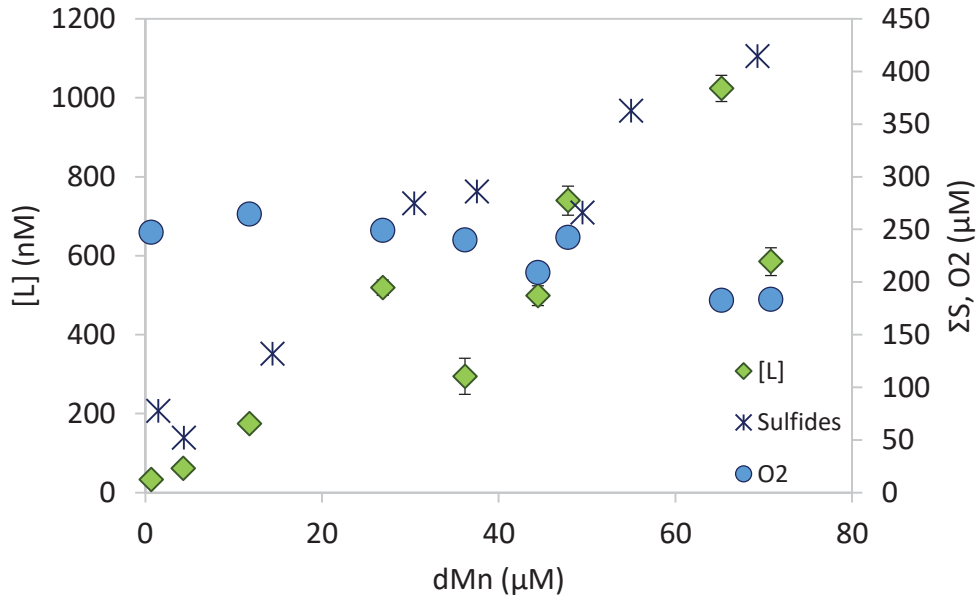
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1035 **Figure 5.** Concentrations of Cu-binding ligands and *in-situ* concentrations of free sulfides ($\Sigma\text{S} = \text{H}_2\text{S} +$
 1036 $\text{HS}^- + \text{S}^{2-}$) measured with the CHEMINI analyzer at the main smoker of Aisics in 2015 (dive 02598).
 1037 Blue dots refer to the *in-situ* concentrations of dissolved oxygen (O_2) measured by the Optode
 1038 (Aanderaa). The linear relationship between temperature and dMn is $T (^{\circ}\text{C}) = 1.43(\text{dMn}) + 4$; $r^2 = 0.99$
 1039 (Figure S4), where the intercept refers to the deep seawater temperature. Errors bars for [L] are
 1040 summarized in Table 1.

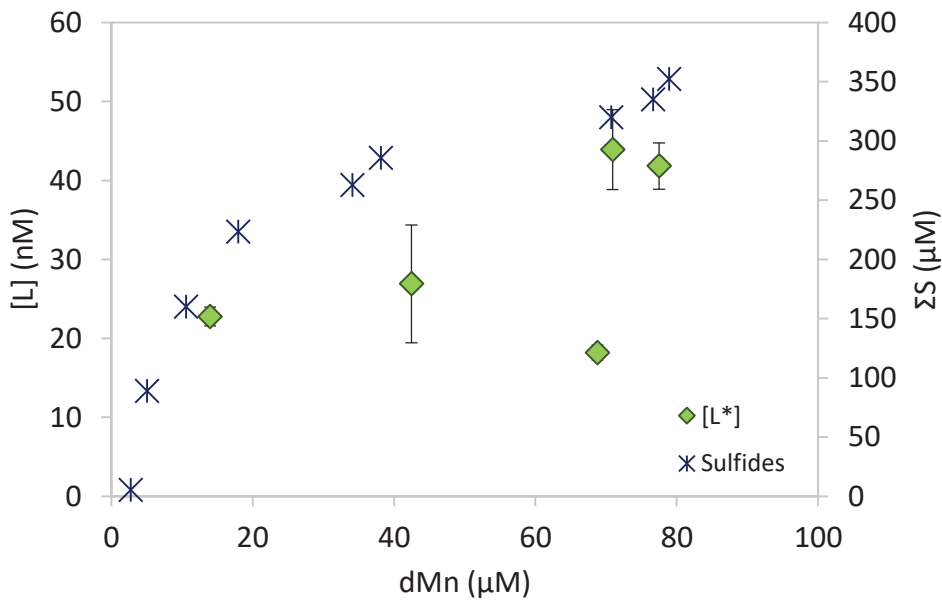
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1043 **Figure 6.** Concentrations of Cu-binding ligands ([L], dive 10606) and *in-situ* concentrations of free
 1044 sulfides ($\Sigma S = H_2S + HS^- + S^{2-}$) measured with the CHEMINI analyzer at the main smoker of Y3 in
 1045 2014. The linear relationship between temperature and dMn is $T (^{\circ}C) = 1.35(dMn) + 4$; $r^2 = 0.99$ (Figure
 1046 S5).

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1049 **Figure 7.** Concentrations of Cu-ligands and *in-situ* concentrations of free sulfides ($\Sigma S = H_2S + HS^- + S^{2-}$)
 1050 measured with the CHEMINI analyzer at the main smoker of Aisics in 2016. ΣS can be plotted as a
 1051 function of dMn thanks to the linear relationship $T (^{\circ}C) = 1.47(dMn) + 4$; $r^2 = 0.94$ (Figure S6).

1052