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1 **High spatial resolution imaging of subcellular macro and trace element distribution**
2 **during phagocytosis**

3

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22

23 **Short running head:** Subcellular element distribution during phagocytosis

24

25 **Keywords:** NanoSIMS, ICP-MS, Trace elements, mass spectrometry, macrophage,

26 *Salmonella*

27

28 **Significance to metallomics**

29 Analyses of the composition of the cellular metallome and the spatial resolution of elemental
30 distribution within the cell are key information for a better understanding of cellular processes.
31 Besides a large number of biochemical reactions catalyzed by trace elements as part of the
32 metalloproteome of a cell, these metals play a central role during phagocytosis, the main player
33 of the cellular immune response. Using a combination of quantitative (ICP-MS) and spatially
34 resolved mass spectrometry (NanoSIMS) in combination with optical spectroscopy and
35 Transmission Electron Microscopy (TEM), this work describes the distribution of macro- and
36 trace elements during phagocytosis, using murine macrophages and *Salmonella* as model
37 system.

38

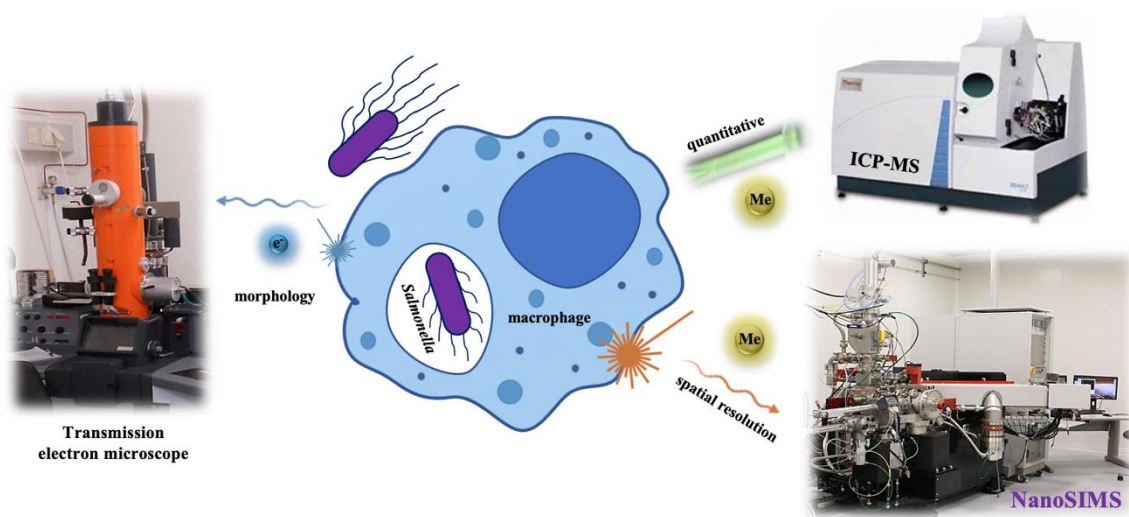
39 **Abstract**

40 The bioavailability of trace elements in the course of evolution had an essential influence on
41 the emergence of life itself. This is reflected in the co-evolution between eukaryotes and
42 prokaryotes. In this study, the influence and cellular distribution of bioelements during
43 phagocytosis at the host-pathogen interface was investigated using high-resolution nanoscale
44 secondary ion mass spectrometry (NanoSIMS) and quantitative inductively coupled plasma
45 mass spectrometry (ICP-MS). In the eukaryotic murine macrophages (RAW 264.7 cell line),
46 the cellular Fe / Zn ratio was found to be balanced, whereas the dominance of iron in the
47 prokaryotic cells of the pathogen *Salmonella enterica* Serovar Enteritidis was about 90%
48 compared to zinc. This confirms the evolutionary increased zinc requirement of the eukaryotic
49 animal cell. Using NanoSIMS, the Cs⁺ primary ion source allowed high spatial resolution
50 mapping of cell morphology down to subcellular level. At a comparable resolution, several low
51 abundant trace elements could be mapped during phagocytosis with a RF plasma O⁻ primary
52 ion source. An enrichment of copper and nickel could be detected in the prokaryotic cells.

53 Surprisingly, an accumulation of cobalt in the area of nuclear envelope was observed indicating
54 an interesting but still unknown distribution of this trace element in murine macrophages.

55

56 **Graphical Abstract**



57

58 **Introduction**

59 Macrophages are key players in innate immunity and the cellular functions of phagocytosis play
60 a central role in the defense against pathogens by cells of the eukaryotic innate immune system
61 at the host-pathogen interface. The occurrence of phagocytotic active cells is not limited to the
62 immune system, but arose early in evolution before the advent of multicellularity [1-5]. The
63 phagocytosis processes of predatory protozoan and their bacteria prey have evolved for the last
64 2 billion years [6]. There are remarkable similarities to mammalian macrophages, one being the
65 major challenge for bacteria to obtain sufficient metal ions for growth in the host environment
66 [7]. The role of iron and manganese sequestration is assumed and the intoxication by zinc and
67 copper as an antimicrobial strategy is a rising hypothesis within the last decade [8]. Thus,
68 antimicrobial host actions, and the corresponding bacterial defenses have a long evolutionary
69 history [9]. Bioavailability of trace metals regulates host-pathogen as well as predator-prey
70 interactions during phagocytosis [10]. In case of host-pathogen relation, the cytoplasm of the
71 host provides the source of metals and changes the metal composition and trafficking after
72 pathogen detection. It has been shown that iron and manganese from the pathogenic vacuole
73 (phagosome) is retained, but the changes in copper and zinc pools are still under discussion.
74 Several mammalian infection studies have focused on the effects of metal deficiency and
75 overloading or poisoning at the cellular and subcellular level [7, 11-13].
76 Element-specific imaging enables the localization of chemical elements including trace metals
77 in biological tissue and cells and, hence, has the potential to investigate biochemical functions,
78 biosorption and bioaccumulation processes of trace elements at the cellular and even at the
79 subcellular level [14]. Nanoscale secondary ion mass spectrometry (NanoSIMS) is a technique
80 where a primary ion beam sputters the surface of a sample and the resulting secondary ions are
81 separated and detected in a mass spectrometer [15]. However, the limitation is the generation
82 of ions from the sample, that depend on the atomic density of the analyte in the surface, the

83 sputtering rate (the rate of ejection of the analyte from the surface by the primary ions) and the
84 ionization yield (the fraction of sputtered atoms that are ionized) [16]. The ionization yield
85 depends, in turn, on the ionization energy for positive secondary ions and the electron affinity
86 for negative ions [17]. Either a cesium (Cs^+) or oxygen (O^-) primary ion beam scans the sample
87 surface and thus produce elemental maps with a resolution down to 50 nm. A cesium ion source
88 is well established to generate and map negative secondary ions (e.g., C^- , CN^- , O^- , and P^-) in
89 biological samples. In the meantime, the recent implementation of a bright RF plasma O^-
90 primary ion source in NanoSIMS for the generation of positive secondary ions [18] (e.g., Na^+ ,
91 Ca^+ , Fe^+ , Co^+ , Ni^+ , and Cu^+) allows the mapping of trace metals in cells with similar spatial
92 resolution than the cesium source, down to 40 nm, and paves the way for metal detection inside
93 bacteria.

94 Up to now, all NanoSIMS studies on bacteria employed the cesium ion source mainly for the
95 investigation of metabolic activities using stable isotopes such as ^{13}C and ^{15}N [19, 20]. Even in
96 those few studies where bacteria metabolisms of metal ions were regarded, the metals
97 themselves were rarely analyzed by NanoSIMS. The exceptional cases in which NanoSIMS
98 was used are limited to those metals whose oxide ions can be generated by the Cs^+ source. For
99 example, to investigate radionuclide uptake by bacteria $^{238}\text{U}^{16}\text{O}^-$ was detected simultaneously
100 with $^{13}\text{C}^{14}\text{N}^-$ to show uraninite (UO_2) nanocrystal precipitation on the surface of
101 *G. sulfurreducens* cells [21]. The same bacteria were used to study microbial iron reduction
102 where iron was detected as $^{56}\text{Fe}^{16}\text{O}^-$ on the substrate surface, but not in the bacteria itself [22].
103 A similar approach was used to investigate the Fe-oxidizing bacterium *Leptothrix ochracea*
104 [23, 24]. In addition, NanoSIMS was employed to observe aluminum and copper (via $^{27}\text{Al}^{16}\text{O}^-$
105 and $^{63}\text{Cu}^{16}\text{O}^-$ secondary ions) in bacteria induced biominerals [25]. A recent work studied
106 arsenic reduction in presence of iron by the *Shewanella* bacterium via subcellular detection of
107 $^{56}\text{Fe}^{12}\text{C}^-$, $^{56}\text{Fe}^{16}\text{O}^-$, and $^{75}\text{As}^-$ [26]. Furthermore, the bioimaging of macrophages with

108 NanoSIMS is limited to few studies too. Again, mainly the stable isotopes ^{13}C and ^{15}N together
109 with the cesium ion source were employed to explore cholesterol metabolism [27, 28] or the
110 intracellular localization of drugs [29-31] in macrophages.

111 Few publications describe metal imaging in macrophages by synchrotron radiation X-ray
112 fluorescence (SXRF), which is a complementary technique to NanoSIMS for elemental
113 mapping in biological samples. Although recent developments have allowed the optimization
114 of some beamlines to achieve spatial resolutions similar to NanoSIMS, macrophage analyses
115 were reported with spatial resolution at the low micrometer level, which provided rather diffuse
116 images and did not allow metal localization at the organelle level. An early work of Kitamura
117 and coworkers showed the local distribution of chromium, iron, zinc, and calcium in single
118 macrophage cell at 5 μm resolution [32, 33]. With the same resolution, mercury, selenium, zinc
119 and copper accumulation was observed in macrophage aggregates of yelloweye rockfish liver
120 [34]. The phagocytosis of asbestos fibers was visualized by the SXRF mapping of calcium,
121 magnesium, iron, and silicon in macrophages with a beam size of 0.2 $\mu\text{m} \times 1 \mu\text{m}$ [35]. In a
122 similar study, iron release from carbon nanotubes was observed in murine macrophages with
123 a beam size of 0.4 $\mu\text{m} \times 0.9 \mu\text{m}$ [36]. To our knowledge, no study investigated so far
124 intracellular metal distribution in macrophages and bacteria during phagocytosis due to the lack
125 of both high-sensitive and high-resolution imaging techniques for trace elements.

126 We report here for the first time high-sensitive and high-resolution imaging of macro and trace
127 elements at cellular and subcellular level in macrophages and bacteria using NanoSIMS
128 equipped with a bright RF plasma O^- primary ion source as well as a conventional cesium ion
129 source. The aim was to visualize the elemental distribution and accumulation of metals during
130 phagocytosis of bacteria. The model system was the murine RAW 264.7 macrophage cell line
131 in combination with the pathogen *Salmonella enterica* Serovar Enteritidis (*Sa*). This model
132 system is well established and has been used for decades to study phagocytosis [37-40].

133 Employing both ion sources in NanoSIMS, non-metals (C, N, P, S) as well as metal (Na and
134 Ca) macroelements and trace metals (Mn, Fe, Co, Ni, Cu,) can be analyzed and associated to
135 subcellular structures allowing the discussion of the biological role and cellular distribution of
136 these elements during phagocytosis.

137

138 **Material and Methods**

139 ***Bacterial strain and growth conditions***

140 *Salmonella enterica* Serovar Enteritidis (*Sa*) was cultivated in complex medium LB (Difco™
141 LB Broth, Lennox). Cultures were shaken aerobically at 37 °C. A first pre-culture was
142 incubated for 16 h, and diluted 1 to 400 and incubated for 2 h (second pre-culture), which was
143 then used to inoculate the main culture, also at a dilution of 1 to 400. This allowed highly
144 reproducible steady-state growth to the mid-exponential phase. Samples from culture were used
145 to determine the colony-forming units (cfu) as a function of cell number.

146 ***Cell culture of murine macrophage cell line RAW 264.7***

147 RAW 264.7 (ATCC® TIB-71™) represents a murine (BALB/c; H2^d) adherent growing
148 monocyte/macrophage cell line which is transformed by Abelson murine leukemia virus.
149 RAW 264.7 cells are capable of pinocytosis and phagocytosis, antibody-dependent lysis of
150 tumor cells as well as nitric oxide (NO) and cytokine production and they are responsive to
151 LPS. Cells were cultured in 175 cm² cell culture flasks (Greiner Bio-One, Frickenhausen,
152 Germany) in phenol-red free RPMI 1640 medium supplemented with 10 % FBS, 2 mM L-
153 glutamine, 10 mM HEPES buffer, 100 µg/ml penicillin/streptomycin (Biochrom, Berlin,
154 Germany) and 50 µM β-mercaptoethanol (Sigma Aldrich, Steinheim, Germany) at 37 °C, 5%
155 CO₂ and 95% air humidity. Cell numbers were determined by cell counting chamber.

156 ***Determination of metal content by means of inductively coupled plasma - mass spectrometry***
157 ***analysis (ICP-MS)***

158 To determine the metal content, *Sa* were cultivated until mid-exponential phase of growth. A
159 10 mL cell suspension was centrifuged for 30 min at 4 °C and at 4,500 × g. Cells were washed
160 twice with 50 mM Tris-HCl buffer containing 10 mM EDTA (pH 7). The supernatant was
161 discarded and the residual liquid carefully removed at each step. RAW 264.7 cells were cultured
162 at a density of 1 × 10⁶ cells / ml in 58 cm² cell culture dishes with 10 ml of cell culture medium

163 described above. Afterwards RAW 264.7 cells were incubated and stimulated with 1×10^8 heat
164 inactivated *Sa* (hi*Sa*; inactivation by 60 °C for 1 h) or not as control. After 4 h cells were
165 harvested and washed twice with Phosphate-buffered saline (0.15 M NaCl, pH 7.4; PBS)
166 containing 5 mM EDTA (Sigma Aldrich). The pellet was suspended in concentrated 67% (w/v)
167 HNO₃ (trace metal grade; BDH Prolabo®, VWR, Darmstadt, Germany) and mineralized at
168 70 °C for 2 h. Samples were diluted to a final concentration of 2% (w/v) nitric acid. Indium was
169 added as internal standard at a final concentration of 10 ppb. Elemental analysis was performed
170 via inductively coupled plasma mass spectrometry (ICP-MS) using ESI-sampler SC-2
171 (Elemental Scientific Inc., Omaha, USA) and an X-Series II ICP-MS instrument (Thermo
172 Fisher Scientific, Bremen, Germany) operating with a collision/reaction cell and flow rates of
173 5 ml/min of He/H₂ (93% / 7%), with an Ar carrier flow rate of 0.76 L/min and an Ar make-up
174 flow rate of 15 l/min. An external calibration curve was recorded with ICP-multi element
175 standard solution XVI (Merck, Darmstadt, Germany) in 2% nitric acid. Each sample was
176 introduced via a peristaltic pump and analyzed for its metal content. For blank measurement
177 and quality/quantity thresholds, calculations were performed based on DIN32645 (this standard
178 procedure describes in chemical analysis - decision limit, detection limit and determination
179 limit under repeatability conditions - terms, methods, evaluation; ICS Code – 71.040.01). The
180 results were transformed from concentrations via molar units into atoms per sample and divided
181 by the number of cells per sample.

182 ***Phagocytosis assay with macrophages RAW 264.7 and FITC-labelled Sa***

183 Firstly, 1×10^9 heat inactivated *Sa* were incubated with 0.20 mg Fluorescein isothiocyanate
184 (FITC) in 2.0 mL carbonate buffer (0.10 M) for 1 h protected from light. After incubation the
185 *Sa* were centrifuged and washed for five times with PBS. For phagocytosis assay 4×10^7 FITC-
186 labelled heat inactivated *Sa* were incubated with 1×10^6 RAW 264.7 for 40 min at 37 °C in cell
187 culture medium. Afterwards the cells were plated onto a microscope slide via cytocentrifuge

188 Shandon Cytospin (Thermo, Germany). Pictures were taken with phase contrast and
189 fluorescence microscopy by Primovert (Zeiss, Germany).

190

191 ***Sample preparation of macrophages and Sa for transmission electron microscopy and***
192 ***NanoSIMS***

193 1×10^7 RAW 264.7 cells were co-cultured with 1×10^4 Sa for 15 h in RPMI medium. After
194 cultivation the cells were fixed directly with 3% glutaraldehyde (Sigma, Taufkirchen,
195 Germany) in 0.10 M sodium cacodylate buffer (SCB) for 3 h, centrifuged at 5000 rpm for 5 min
196 and taken up in 4% agar/SCB, followed by one wash-step with SCB-buffer overnight at 4 °C
197 and three wash-steps with the same buffer for 5 min. For TEM analysis the samples were
198 postfixed with 1% osmium (VIII) tetroxide in SCB for 1 h. After washing with H₂O samples
199 were dehydrated in a series of ethanol (2 times for 5 min. with 25%, 50%, 75% and 100%
200 ethanol each). Thereafter the samples were infiltrated with epoxy resin according to Spurr [41]
201 and polymerized at 70 °C. Subsequent ultrathin sections of 80 nm and 300 nm were cut with an
202 Ultracut R ultramicrotome (Leica, Wetzlar, Germany). The 80 nm ultrathin sections were
203 placed on copper grids and observed with an EM900 transmission electron microscope (Carl
204 Zeiss Microscopy, Oberkochen, Germany) operating at 80 kV. The images were recorded using
205 a Variospeed SSCCD camera SM-1k-120 (TRS, Moorenweis, Germany). The 300 nm sections
206 were placed on silicon wafers for NanoSIMS analysis.

207 ***NanoSIMS analysis***

208 Elemental distribution of non-metals, micronutrients and trace metals was analyzed on the
209 ultrathin sections with a NanoSIMS 50L (Cameca, Gennevilliers, France), equipped with seven
210 parallel electron multiplier detectors. A Cs⁺ ion source was used for the mapping of carbon
211 (¹²C₂⁻), nitrogen (¹²C¹⁴N⁻), sulfur (³²S⁻), and phosphorus (³¹P⁻) in selected macrophage cells,
212 with a current of approximately 0.25 pA. Areas between 20 μm × 20 μm and 30 μm × 30 μm
213 were pre-sputtered (10 min at 100 pA, for cesium implantation in order to increase the

214 secondary ion yield) and subsequently analyzed with 512×512 pixels, and a dwell time of
215 10 ms per pixel. Afterwards, regions of interest between $2 \mu\text{m} \times 2 \mu\text{m}$ and $3 \mu\text{m} \times 3 \mu\text{m}$ were
216 analyzed in detail with 128×128 pixels and the same dwell time.

217 A HyperionTM RF plasma O⁻ ion source [18] was used to map sodium ($^{23}\text{Na}^+$), potassium ($^{39}\text{K}^+$),
218 calcium ($^{40}\text{Ca}^+$), manganese ($^{55}\text{Mn}^+$), iron ($^{56}\text{Fe}^+$), nickel ($^{58}\text{Ni}^+$), cobalt ($^{59}\text{Co}^+$) and copper
219 ($^{63}\text{Cu}^+$). For these isotopes, the electron multiplier detectors cannot be placed at mass
220 differences of only 1 amu (atomic mass unit). Therefore, two different mass calibrations were
221 necessary, one for the analysis of C, Na, Ca, Fe and Co and another for C, Na, K, Mn, Ni and
222 Cu. Areas between $20 \mu\text{m} \times 20 \mu\text{m}$ and $65 \mu\text{m} \times 65 \mu\text{m}$ were analyzed with 256×256 pixels,
223 10 ms dwell time and a current of 5 pA. Later, regions of interest of approx. between $5 \mu\text{m}$
224 $\times 5 \mu\text{m}$ and $25 \mu\text{m} \times 25 \mu\text{m}$ were mapped with a beam of 1.5 pA for the detection of the
225 macronutrients Na, Ca, and K, and then the same regions were mapped with 25 pA for trace
226 metal detection.

227 Mass calibration was performed with element standards obtained from standard solutions
228 (PlasmaCAL, SPC Science, Canada) dropped and evaporated on silicon wafers. Mass
229 resolution was optimized to resolve possible interferences, but entrance and aperture slits were
230 open to allow maximum transmission of the ions of the trace elements, which resulted in a mass
231 resolution of approximately 3000-5000. The cluster ion $^{12}\text{C}^{14}\text{N}^-$ was detected to maximize the
232 signal measured for ^{14}N , and mapping of ^{12}C , originating from the resin, was used to control
233 homogeneous sputtering of the area. Image processing was performed using the software
234 WinImage (Cameca). Several maps of the same area were acquired and stacked to obtain the
235 final image. The diameter of the primary ion beam (probe size) determining the spatial
236 resolution was measured and calculated by the knife-edge method (16-84% criterion) [18] in
237 several points of an image and an average was given. An example is presented in Supplementary
238 Figure 1.

239 **Results and Discussion**

240 **Cellular metal content of *S. enterica* Serovar Enteritidis and murine macrophages**

241 The element composition in a cell and the relationship to cellular and subcellular distribution
242 for cell biology functions, are key factors for understanding biochemical processes in cells and
243 multicellular organisms. A quantitative analysis of the cellular metal content, also known as
244 metallome or metal quota, in *Sa* and murine macrophage cell line RAW 264.7 was done by
245 ICP-MS. In both organisms, the macroelement magnesium (Mg) dominates the metal content,
246 surpassing the following most abundant metals calcium (Ca), iron (Fe) and zinc (Zn), by
247 approximately one order of magnitude (Figure 1, Supplementary Table 1). The amounts of these
248 elements found in both organisms are in agreement with their occurrence as metal co-factors of
249 enzymes shown in recent database analyses, where Mg was found to be in 16% of enzymes, Zn
250 in 9%, Fe in 8%, Mn in 6% and Ca in 2% [42, 43]. In comparison, other trace metals such as
251 Cu, Co, Ni and molybdenum (Mo) are present in much lower amounts (Supplementary Table
252 1), as they are also used in less enzymatic processes [42, 43]. All these metals are essential co-
253 factors in enzymatic reactions or have structural functions in metalloproteins. Manganese (Mn),
254 for example, is an essential element for most living organisms and has several biochemical
255 functions as an integral component of enzymes like pyruvate carboxylases, arginases and
256 superoxide dismutases, while several other enzymes, including kinases, transferases,
257 hydrolyses and decarboxylases can be activated by manganese [44, 45]. Copper (Cu) is often
258 employed by metal binding enzymes to catalyze electron transfer for energy production and by
259 superoxide dismutase to perform an antioxidant activity [46-50]. As metal co-factor nickel is
260 found in enzymes like NiFe-hydrogenases and ureases of archaea, bacteria, plants, and
261 primitive eukaryotes [51]. Cobalt (Co) has its primary role in cobalamin as a co-factor of
262 individual enzymes, methylmalonyl-CoA mutase, methionine synthase and methionyl
263 aminopeptidase [48-50]. In order to gain biological activity and function in enzymes, Mo is

264 complexed with pterin, with the exception of the bacterial Mo-nitrogenase, and can be found in
265 small amounts in eukaryotes and prokaryotes [52, 53].

266 Beside Mg, the trace metals zinc and iron are the two most abundant cofactors in proteomes
267 (metalloproteomes) with a variety of biological and biochemical functions [54]. The high
268 cellular iron content determined here in *Sa* and murine macrophages (Figure 1) is explained by
269 its use in the active centers of hundreds of enzymes and in oxygen carrier proteins [55, 56].
270 Furthermore, iron can be bound by glutathione and stored cellularly by ferritin or
271 bacterioferritin in prokaryotes and eukaryotes [56-59]. Iron plays a critical role in energy
272 conservation during electron transfer in the respiratory chain as a component of cytochromes
273 and cytochrome oxidase, coordinated in heme or iron sulfur (2Fe-2S / 4Fe-4S) complexes [60].
274 Furthermore, redox-active iron gains importance as a cofactor of various enzymes in a multitude
275 of metabolic pathways, such as in ribonucleotide reductases for DNA synthesis or in the TCA
276 cycle in succinate dehydrogenases and aconitases.

277 In the case of zinc (Figure 1), this element is redox-inactive, but it is also a structural and
278 catalytic key component of hundreds of proteins and is involved in all levels of cellular signal
279 transduction and regulation. Besides, it has significant functions in cell growth, development,
280 transcription, cell division, oxidative stress, apoptosis and aging [61, 62]. Bioinformatic
281 analyses suggest that iron and zinc proteins represent about 10 % of the prokaryotic and
282 eukaryotic proteomes [63, 64].

283 The determination of the cellular ratio of these two elements shows that the iron quota in *Sa*
284 dominates compared to zinc with 91 % to 6 % in the metal pool. In eukaryotic cells, here the
285 murine macrophages, this ratio shifts towards zinc with a percentage of 53 % iron to 43 % zinc
286 (Figure 1 C & D). Previously studies have shown that the Zn/Fe ratio shifts in the metalloproteomes
287 between prokaryotic and eukaryotic cells, primarily with the recruitment of metal co-factors
288 used in the metalloproteome [63-67]. Further bioinformatic comparisons of coding protein-

289 metal binding motifs between eukaryotes and prokaryotes predict that eukaryotic
290 metalloproteomes require more zinc and less iron due to a higher content of zinc binding
291 proteins, especially by the high number of zinc finger proteins in regulatory processes. [68-70].
292 In addition, eukaryotic organisms are able to store and accumulate zinc in subcellular
293 compartments, whereas prokaryotes cannot. However, both eukaryotes and prokaryotes have
294 storage and buffering capabilities in the form of metallothioneins and metal binding stretches
295 in P-loop GTPases as well as ribosomes as zinc sink [71-78].
296 Exceptions to the dominating abundance of magnesium, iron and zinc can be seen by the rising
297 abundance of single elements, such as calcium, through strong metal fluxes in form of
298 regulation as a second messenger. The functions of calcium ions (Ca^{2+}) in cell signaling
299 processes as universal messenger in eukaryotes and in regulation of gene expression as well as
300 regulation of intracellular processes have been studied for a long time [79-81]. However, the
301 physiological role in prokaryotic cells remains unclear and is still unexplored [82]. Only indirect
302 evidence shows that Ca^{2+} affects several bacterial physiological processes [83-85]. Similar to
303 eukaryotes, bacteria maintain the cytosol free of Ca^{2+} within the nanomolar range even in the
304 presence of mM extracellular concentration [82, 86-90]. This different range of functions in
305 eukaryotes and prokaryotes is reflected in the quantitative determination of cellular calcium
306 content between the organisms shown here, with an order of magnitude higher percentage of
307 calcium in macrophages than in *Salmonella*, compared to the ratio of the other determined
308 cellular metal contents (Figure 1, Supplementary Table 1).

309

310 **Morphology & cell biology imaging**

311 Macrophages play a major role in the immune response to most infections along with other
312 professional phagocytes like neutrophils and monocytes or non-professional dendritic cells
313 [91]. The process of phagocytosis is a type of endocytosis, first noticed by William Osler [92]

314 and studied and named by Élie Metchnikoff [93, 94]. It is described as “the ingestion of solid
315 particulate matter by cells” [95]. Enveloping of the plasma membrane results in the engulfing
316 of large particles ($\geq 0.5 \mu\text{m}$) like bacteria or other pathogens, giving rise to an internal
317 compartment called the phagosome (Figure 2). Macrophages discriminate between infectious
318 agents and self or other body's own cells by conserved pathogen-associated molecular patterns
319 (PAMPs) presented on the pathogen cell surface, such as lipopolysaccharides or lipoteichoic
320 acids of *Sa*, which can be recognized by phagocytotic receptors, like membrane-bound Dectin-
321 1, mannose receptors, CD14 and scavenger receptor A (SR-A) [96, 97]. These molecular and
322 cell biological processes are shown in Figure 2 (A) as a live cell image during the process of
323 phagocytosis. The heat inactivated FITC-labelled *Sa* are represented by green fluorescence. The
324 bacteria (*Sa*) are located outside and inside the macrophages. Figure 2 (B) shows a light
325 microscope image of an $1 \mu\text{m}$ semi-thin section stained with toluidine blue after fixation and
326 epoxy resin embedding of after 15 h of incubation of macrophages and *Sa*. Cell shape and
327 nucleus as well as possibly enclosed bacteria in vesicular structures become visible in the light
328 microscopic resolution as an overview image. Using transmission electron microscopy, in the
329 control with heat inactivated *Sa* incubation (Figure 2 (C)) subcellular structures such as vesicles
330 of different sizes, mitochondria, nucleus, nucleolus and nuclear envelope, also known as nuclear
331 membrane, can be identified. In Figure 2 (D), after incubation with intact *Sa*, these bacterial
332 structures can be shown in vesicles corresponding to the phagosomes or phagolysosomes.

333

334 *NanoSIMS analysis*

335 Further investigation of the elemental distribution and partitioning of the metallome between
336 eukaryote and prokaryote cells was performed qualitatively by NanoSIMS. From the elements
337 that were quantified by ICP-MS, a selection was made for NanoSIMS analyses, based on their
338 relevance and their relative sensitivity. Please note that NanoSIMS shows different sensitivity

339 to different elements, thus it is only semi-quantitative allowing the measurement of the relative
340 distribution of an element in an image but not a quantitative comparison between elements as
341 it is possible by ICP-MS. Furthermore, quantitative elemental imaging by NanoSIMS is limited
342 due to the sample preparation by chemical fixation and resin embedding since a leach of
343 elements and the occurrence of artifacts can never be completely excluded.

344 Concerning non-metals, phosphorus (P) is contained in every living cell and is found at an order
345 of magnitude higher in quantity than magnesium, while the basic elements C, N and O are the
346 main constituents [98, 99]. As a structural component of cell membranes and nucleic acids,
347 phosphorus is involved in many biological processes [100, 101]. Sulfur occurs as the fifth major
348 non-metal element in all the major classes of biomolecules [99]. Sulfur can be found in a lower
349 but close stoichiometry to P compared with C and N (C:N:P:S – 42:6:1:0.4) in cells [102-104].
350 For NanoSIMS detection, N has a low negative ion yield, but is able to form a molecular species
351 with C, as $^{12}\text{C}^{14}\text{N}^-$, which produces a high yield [105]. Thus, the use of the Cs^+ source, with the
352 production of high-count rates by non-metal macroelements, as $^{12}\text{C}^{14}\text{N}^-$, $^{31}\text{P}^-$ and $^{32}\text{S}^-$ ions, leads
353 to a high-resolution cell structure and shape imaging of eukaryotic cells and subcellular
354 structures as well as bacterial cells (Figure 3 and Supplementary Figure 2). The small current
355 beam of 0.25 pA, enables to attain spatial resolution between 100 nm and 130 nm. Vesicles
356 such as endosomes and phagosomes appear as empty spaces in the macrophage cell due to the
357 leaching processes during sample preparation (Figure 3 A_1). Increased subcellular nitrogen
358 and phosphorus concentrations show the nuclear envelope and the nucleolus in the imaging
359 (Figure 3 A_1, A_2 & C_1, C_2). This elemental concentration can be caused by chromosome
360 condensation of the heterochromatin at the nuclear envelope and lamina, as can also be seen in
361 the EM images (Figure 2). This is due to the chemical composition of the DNA, containing
362 carbon and nitrogen as well as phosphorus from the phosphate backbone. Furthermore, regions
363 of different phosphorus and sulfur concentrations can be distinguished in the nucleolus, which

364 points to the different components, the pars granulosa or granular component (GC) and the pars
365 fibrosa, which is composed of the fibrillar center (FC) and the dense fibrillar component (DFC)
366 [106, 107]. In the FC, transcription of rDNA occurs, leading to an accumulation of phosphorus-
367 rich DNA and RNA [108]. In contrast, the DFC and GC have a high protein content due to the
368 fibrillarin, nucleophosmin and pre-ribosomal protein subunits [109], which can lead to an
369 enrichment of the sulfur content due to thiol side chains of the amino acids, cysteine and
370 methionine. With comparable $^{12}\text{C}^{14}\text{N}^-$ count rates and strongly detached from the surrounding
371 cytoplasm as well as from the empty area of the enveloping vesicular structure, the *Salmonella*
372 cells stand out in the phagosome (Figure 3 A_1 & B_1). Due the compact cell shape of a
373 bacterium, the phosphorus content is even higher here compared to the nucleolus and the
374 nuclear envelope of macrophages (Figure 3 A_2 & B_2). Thus, phagocytosed bacteria, with a
375 size of 1-2 μm , can be clearly distinguished from the eukaryotic cell structures in shape and
376 size as well as element composition. In addition, mitochondrial structures with a size of around
377 0.5 μm are characterized by an increased sulfur content, a very low phosphorus content and
378 high $^{12}\text{C}^{14}\text{N}^-$ count rates [110, 111]. This indicates an increased protein and low nucleic acid
379 content (Figure 3 A_3 & C_1, C_2, C_3).

380 The O^- ion source was used to analyze the metal distribution where sodium (Na) and calcium
381 (Ca) were preferred for detailed imaging of the whole cell morphology [112]. A preliminary
382 investigation of the samples showed that Mg had a similar cell distribution than Ca (data not
383 shown). Concerning the trace metals, zinc (Zn) was also excluded based on its low ion yield,
384 which in turn resulted in a lack of sensitivity, and thus no Zn signal was detected in the cells
385 during preliminary sample investigation (data not shown). Thus, the remaining metals were
386 grouped in two sets to be analyzed with the O^- ion source, one including, Na, Ca, Fe and Co
387 and another, Na, K, Mn, Ni and Cu, with Na maintained in each analysis to facilitate the cell
388 localization. Additionally, two different beam currents were used to map the same areas: 1.5 pA

389 for small probe sizes in order to detect the macronutrients Na, Ca, and K, with a higher spatial
390 resolution between 70 nm (small areas) and 100 nm (big areas), and 25 pA for trace metal
391 detection, resulting in larger probe sizes and thus a lower spatial resolution between 120 nm
392 (small areas) and 250 nm (big areas) (Figures 4, 5 & Supplementary Figures 1, 3-5).

393 The high sensitivity to Ca and Na, based on the high NanoSIMS ion yield using the O⁻ ion
394 source, allows to image elemental distribution and resolve cell shape and structure of large
395 eukaryotic cells, such as the macrophage cell line used here, as well as subcellular
396 compartments and the *Sa* in the phagosomes. It is possible to visualize large cells in a larger
397 image (up to 65 μm × 65 μm) as well as very small cell components or bacteria, with a high
398 spatial resolution of 70 nm, and to track simultaneously the enrichments of these two macro
399 elements (Figures 4 & 5). Increased calcium accumulation occurs in the areas of the nuclear
400 envelope, the heterochromatin condensation areas and partially in the nucleolus in comparison
401 to the cytosol (Figure 4 B_1 & B_2; Figure 5 & Supplementary Figures 3-5). In the function of
402 calcium in signal transduction, the nuclear envelope serves as a source for nuclear Ca²⁺ waves
403 that turn on gene expression required for adaptive responses [113]. The construction of the core
404 envelope from the inner nuclear membrane (INM) and the outer nuclear membrane (ONM)
405 creates the perinuclear space as intracellular Ca²⁺ storage. The nuclear pore complex (NPC)
406 connects INM with OPNM and enables the separation to the nucleoplasm [114]. Eukaryotic
407 cells use different mechanisms to keep the cytosolic calcium concentration very low. For
408 example, sarcoendoplasmic reticulum calcium ATPase (SERCA), localized in the endoplasmic
409 reticulum (ER) membrane, is used to eliminate calcium from the cytoplasm, as well as the
410 plasma membrane calcium ATPases and sodium–calcium exchangers (NCXs) [115]. This
411 creates a calcium gradient at the membranes and leads here to the differentiation of calcium-
412 enriched subcellular structures (Figure 4 B_1 & B_2). Likewise, the bacterial cells (*Salmonella*)
413 stand out due to an increased calcium but also sodium concentration (Figure 4 A_3 & B_3). It

414 should be mentioned, that NanoSIMS is extremely sensitive to calcium, even if it is present
415 only in trace amounts, resulting in bright Ca images (Figure 4). This is based on the high
416 secondary ion yield of calcium in NanoSIMS using the oxygen primary ion source and high
417 local Ca concentrations such as in the nuclear envelope. However, these measured intensities
418 do not reflect the Ca quantity compared to other elements.

419 The greatest challenge for all analytical methods available today, however, lies in the detection
420 of trace elements under non-artificial enrichment. Techniques based on synchrotron X-ray
421 fluorescence (SXRF), scanning x-ray fluorescence microscope (SXFEM), Energy-dispersive X-
422 ray spectroscopy (EDS) or mass spectrometry (NanoSIMS and LA-ICP-MS) often reach the
423 limits of detection and spatial resolution in order to visualize these element distributions in
424 detail on a subcellular level [116, 117]. By use of the NanoSIMS 50L in combination with the
425 new Hyperion RF plasma O⁻ ion source, it is possible to detect normal cell physiological
426 enrichment of trace elements such as Mn, Ni, Cu and Co in individual compartments of the
427 eukaryotic cell as well as bacterial cells with a stand-alone resolution down to 70 nm in this
428 work.

429 Figure 4 C_3, D_3 & E_3 shows that there is an increased concentration of Mn, Ni and Cu in
430 the *Sa* cells compared to the surrounding cytoplasm of the phagosome of the macrophage cell
431 (Figure 5 & Supplementary Figure 4). Thus, a smaller number of atoms per cell (Figure 1,
432 Supplementary Table 1) due to a much smaller cell volume of the bacteria leads to a
433 concentration increase in this area compared to the distribution and compartmentalization in
434 the eukaryotic cell. Iron and manganese, on the other hand, occurs in macrophage cells with a
435 relatively even distribution (Figure 5).

436 Unlike all other detectable trace elements, cobalt appears to accumulate in the areas of the
437 nucleus envelope. It is striking that calcium and cobalt show a coincidence in their spatial
438 localization (Figure 5 & Supplementary Figures 4 & 5). The reasons for this are unclear.

439 However, cobalt accumulation in the area of the cell nucleus and in perinuclear structures was
440 also shown in human HaCaT cells (keratinocytes) during intoxication with high micromolar Co
441 concentrations [118]. The mapping also points to an accumulation of cobalt in the ER, Golgi
442 apparatus or in the perinuclear space. Furthermore, cobalt accumulation can be shown in the
443 bacterial cells. This agrees with a higher percentage ratio of cobalt amounts in the small-volume
444 bacterial cells compared to the eukaryotic cell (Figure 1 & Supplementary Table 1). The
445 biological relevance of cobalt is given in the bacterial cobalamin biosynthesis as well as the
446 eukaryotic use of the resulting vitamin B12 precursors [119, 120]. However, this only gives an
447 indication of the biological relevance but not of the cellular distribution in the prokaryotic cells.

448 **Conclusion**

449 Using the NanoSIMS 50L in combination with the novel Hyperion RF plasma O⁻ primary ion
450 source, several trace elements can be mapped in cells such as eukaryotic macrophages and small
451 prokaryotic *Salmonella* cells during phagocytosis at the cellular and subcellular level. In
452 particular, the accumulation of cobalt in the nuclear envelope respectively at the nuclear lamina
453 indicates an interesting but still unknown distribution of this trace element in murine
454 macrophages. In addition, an enrichment of copper and nickel could be detected at region of
455 prokaryotic cells. Furthermore, the Cs⁺ ion source allows high spatial resolution mapping of
456 cell morphology at subcellular level. In this way, the relevant metal macro- and trace elements
457 detected by the O⁻ ion source can be assigned to their biological use through allocation to
458 cellular structures and compounds.

459 **Declaration of competing interest**

460 The authors declare that they have no known competing financial interests or personal
461 relationships that could have appeared to influence the work reported in this paper.

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470

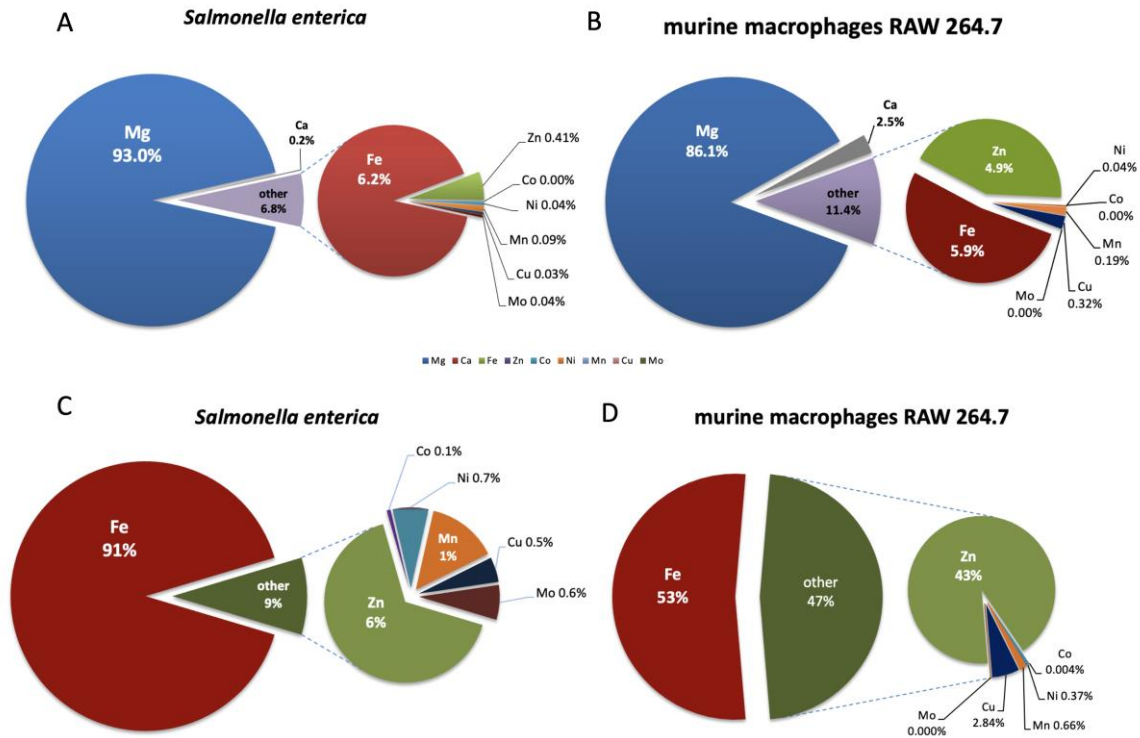
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473 **Data Availability Statements**

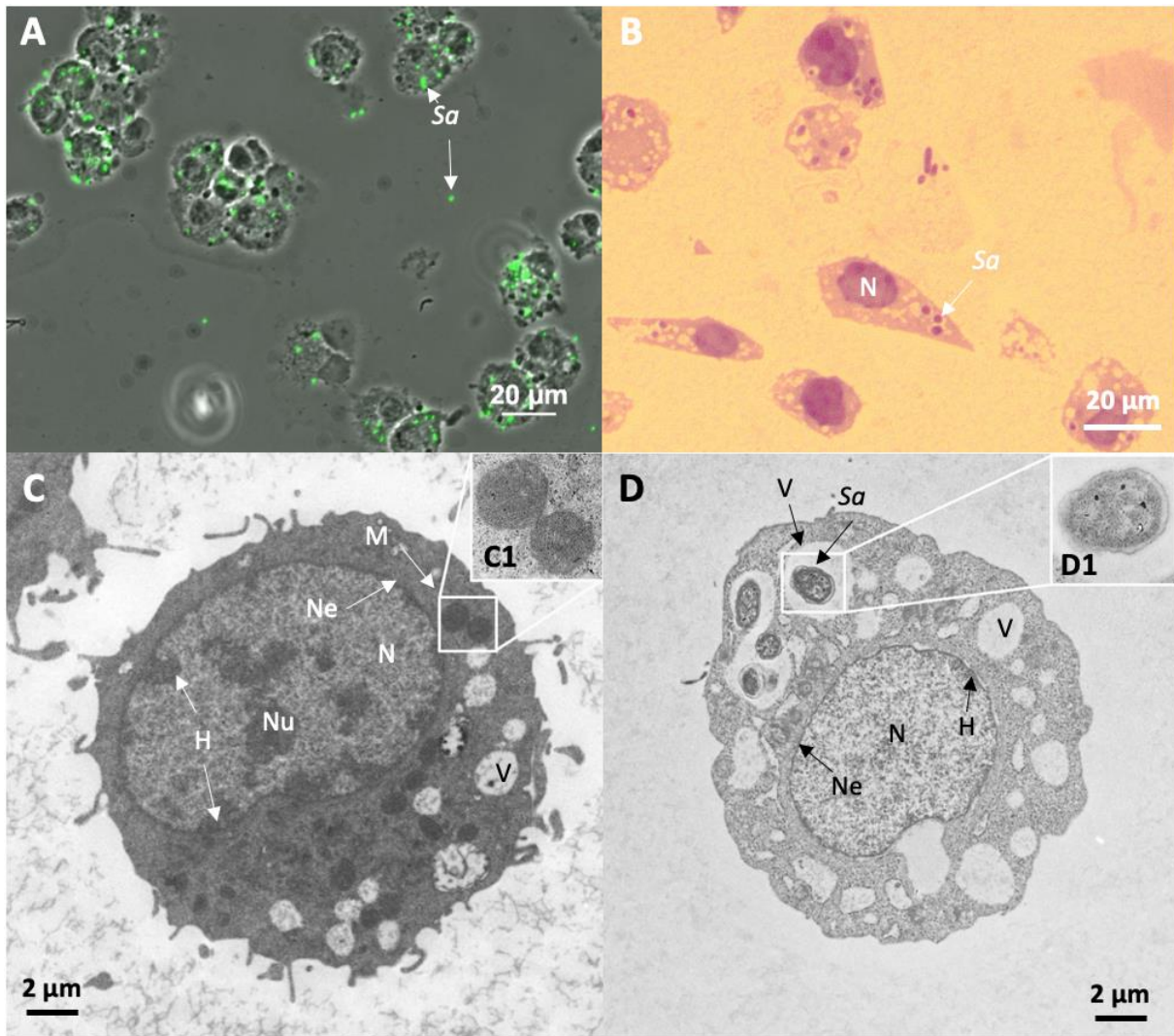
474 The data underlying this article are available in the article and in its online supplementary
475 material.

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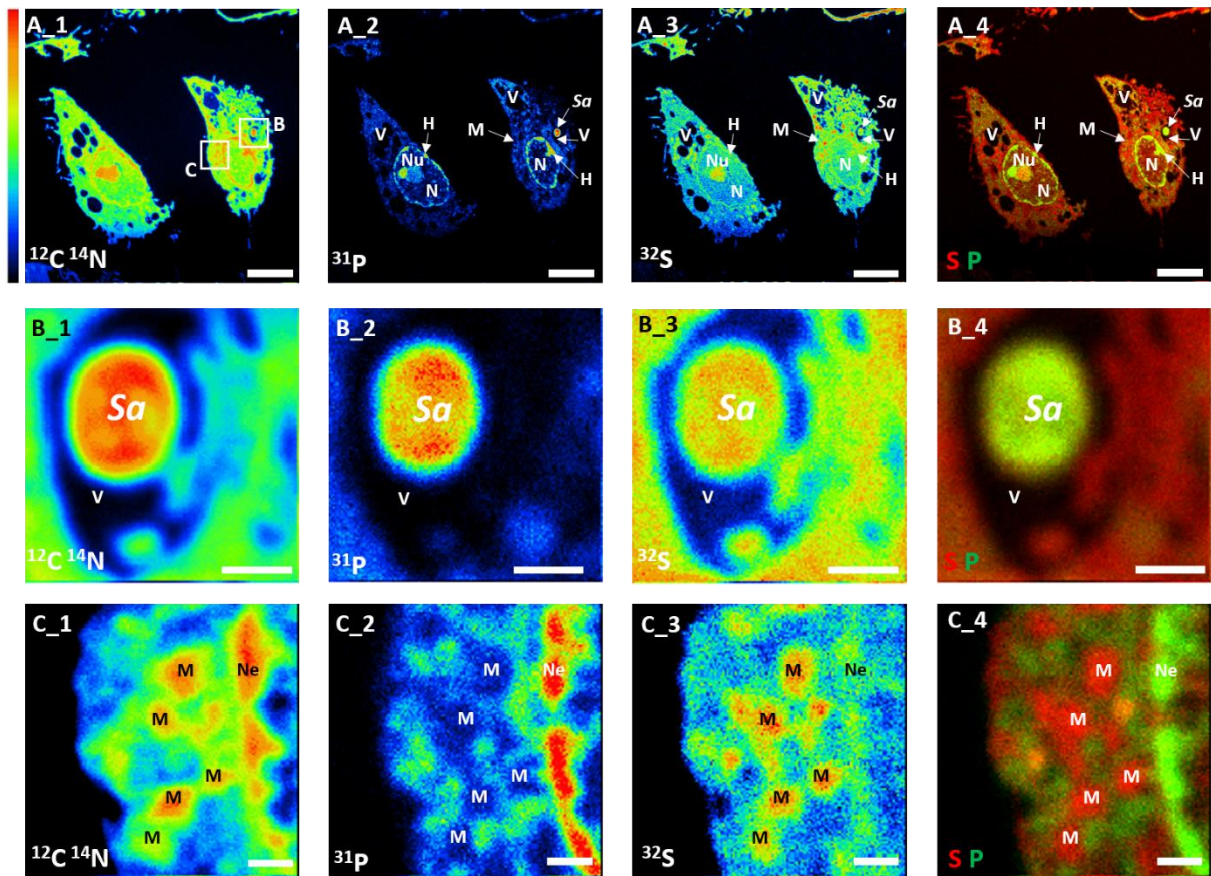
479 **Figure 1 - Cellular metal content ratios of *Salmonella enterica* Serovar Enteritidis and**
 480 **murine macrophage cell line RAW 264.7.** Cellular metal content ratios of *Sa* (A & C) and
 481 RAW 264.7 (B & D) was measured by ICP-MS and given as percentage ratios (metal quota).
 482 The sum of all elements (Mg – magnesium, Fe – iron, Zn – zinc, Co – cobalt, Ni – nickel, Mn
 483 – manganese, Cu – copper and Mo – molybdenum) displayed was set to 100% for normalization
 484 and the individual percentage was calculated in A & B and the magnesium and calcium content
 485 were excluded in C & D.



487

488 **Figure 2 - Morphology of *Salmonella enterica* Serovar Enteritidis (*Sa*) and murine**
 489 **macrophage cell line RAW 264.7 during phagocytosis.** A) Merged bright field and
 490 fluorescence light microscope image of FITC-marked heat inactivated *Sa* with macrophages,
 491 B) Toluidine blue stained semithin section of macrophages co-cultured with vital *Sa*, C) TEM
 492 images of a macrophage co-cultured with inactivated bacteria, mitochondria (C1) and D) with
 493 vital bacteria like B, *Sa* (D1). *Salmonella* (*Sa*), mitochondria (M), nucleus (N), nucleolus (Nu),
 494 nuclear envelope (Ne), vesicle / phagosome (V) and heterochromatin (H).

495

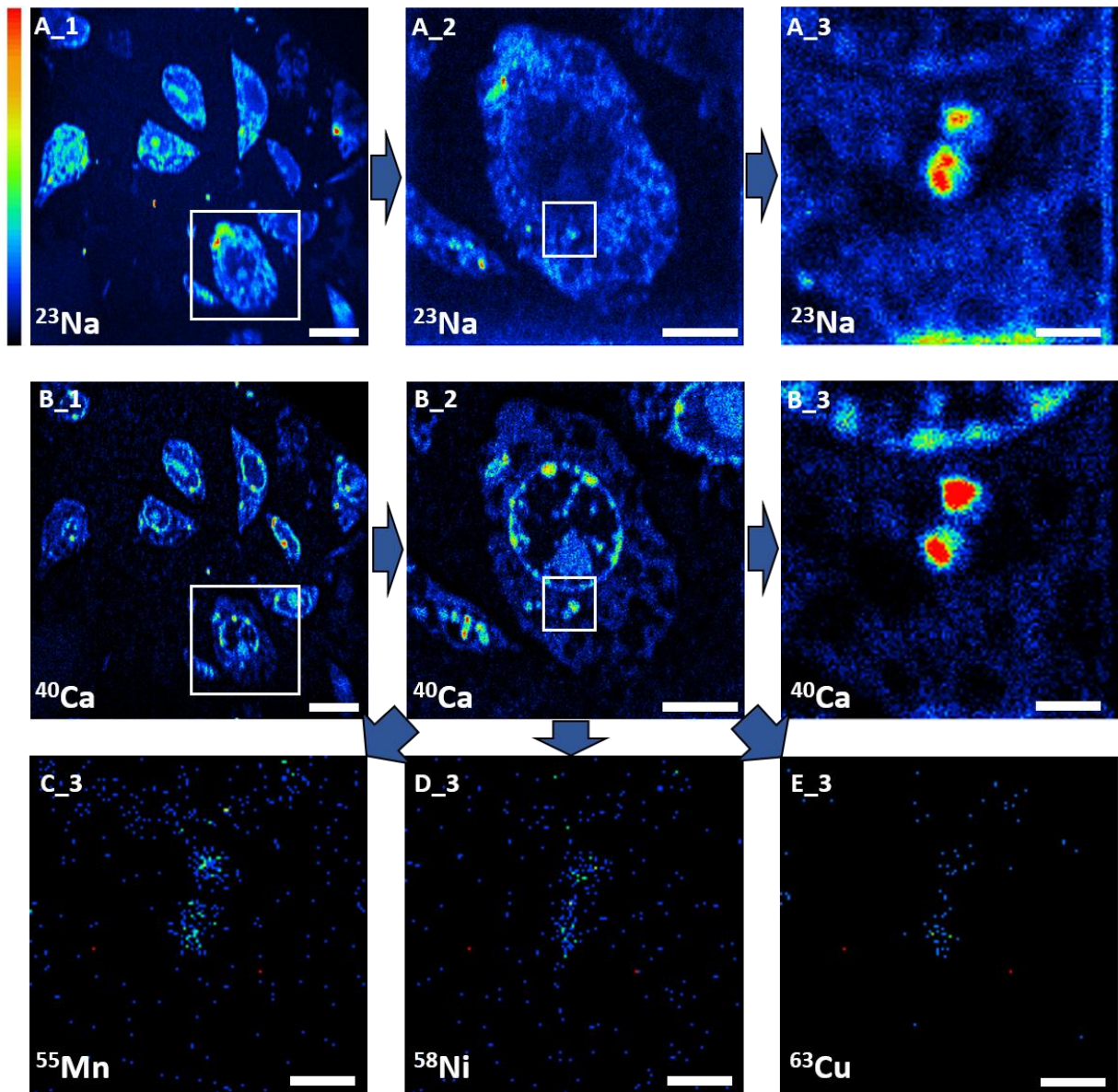


496

497 **Figure 3 - Mapping** of 1) $^{12}\text{C}^{14}\text{N}$ -, 2) ^{31}P - and 3) ^{32}S - with Cs^+ ions and 4) color overlay of ^{31}P
 498 and ^{32}S , of A) two macrophages, and detailed mapping in two regions of interest on the cells,
 499 marked with a white square on the first image, showing B) a *Sa* cell inside a phagosome and C)
 500 ribosomes and nuclear envelope inside the macrophage cell. Color scale on the top left gives
 501 relative intensities of the isotopes count rates. Scale bar: A) $5\ \mu\text{m}$, B) $0.5\ \mu\text{m}$, C) $0.5\ \mu\text{m}$.
 502 *Salmonella* (*Sa*), mitochondria (M), nucleus (N), nucleolus (Nu), nuclear envelope (Ne),
 503 vesicle/ phagosome (V).

504

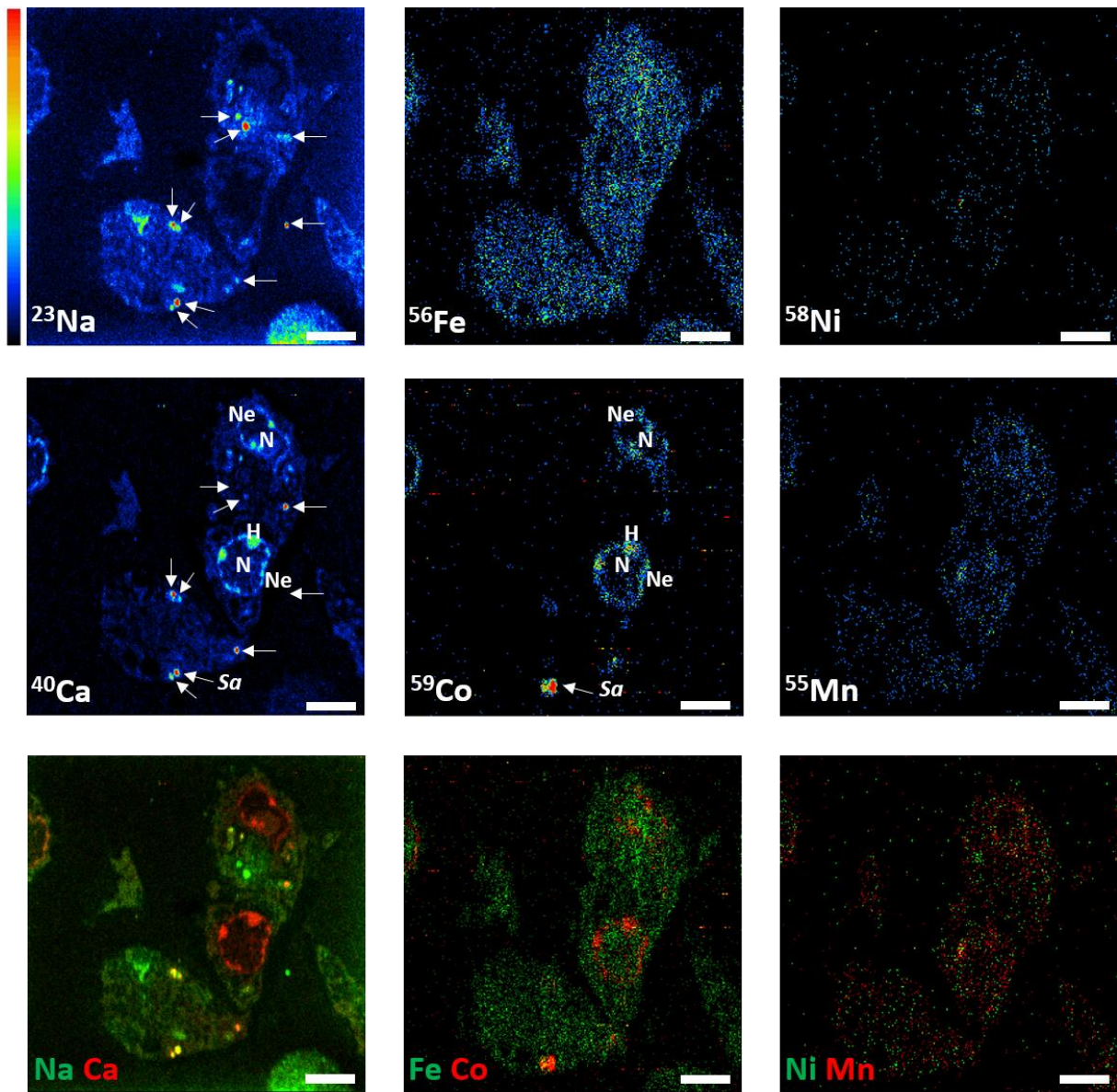
505



507

508 **Figure 4 - Mapping** of A) $^{23}\text{Na}^+$, B) $^{40}\text{Ca}^+$, C) $^{55}\text{Mn}^+$, D) $^{58}\text{Ni}^+$ and E) $^{63}\text{Cu}^+$ with O^- ions of 1)
 509 several macrophages, 2) mapping of a selected macrophage (marked in 1 with a white square)
 510 and 3) mapping of two *Sa* cells inside a phagosome (marked in 2 with a white square) with high
 511 resolution for $^{23}\text{Na}^+$ and $^{40}\text{Ca}^+$ (A_3 and B_3) and low resolution for $^{55}\text{Mn}^+$, $^{58}\text{Ni}^+$ and $^{63}\text{Cu}^+$
 512 (C_3, D_3 and E_3). Color scale on the top left gives relative intensities of the isotopes count
 513 rates. Scale bar: 1) 10 μm , 2) 5 μm , 3) 1 μm .

514



516

517 **Figure 5 - Mapping** of $^{23}\text{Na}^+$, $^{40}\text{Ca}^+$, $^{55}\text{Mn}^+$, $^{56}\text{Fe}^+$, $^{58}\text{Ni}^+$ and $^{59}\text{Co}^+$ with O^- ions of three
 518 macrophages, and colour overlay of two elements, with *Salmonella* (*Sa*) marked with white
 519 arrows on Na^+ and Ca^+ , nucleus (N), heterochromatin (H) and nuclear envelope (Ne). Color
 520 scale on the top left gives relative intensities of the isotopes count rates. Scale bar: 5 μm .

521

522 **References**

- 523 1. Anbar AD, Knoll AH. Proterozoic ocean chemistry and evolution: a bioinorganic bridge? *Science*
524 2002;**297**(5584):1137-42. doi: 10.1126/science.1069651
- 525 2. Knoll AH, Javaux EJ, Hewitt D *et al.* Eukaryotic organisms in Proterozoic oceans. *Philosophical*
526 *Transactions of the Royal Society B: Biological Sciences* 2006;**361**(1470):1023-38. doi:
527 10.1098/rstb.2006.1843
- 528 3. Ruiz-Trillo I, Burger G, Holland PW *et al.* The origins of multicellularity: a multi-taxon genome
529 initiative. *Trends Genet* 2007;**23**(3):113-8. doi: 10.1016/j.tig.2007.01.005
- 530 4. Donoghue PC, Antcliffe JB. Early life: Origins of multicellularity. *Nature* 2010;**466**(7302):41-2.
531 doi: 10.1038/466041a
- 532 5. Niklas KJ. The evolutionary-developmental origins of multicellularity. *Am J Bot* 2014;**101**(1):6-
533 25. doi: 10.3732/ajb.1300314
- 534 6. Hao X, Luthje F, Ronn R *et al.* A role for copper in protozoan grazing - two billion years selecting
535 for bacterial copper resistance. *Mol Microbiol* 2016;**102**(4):628-41. doi: 10.1111/mmi.13483
- 536 7. Hood MI, Skaar EP. Nutritional immunity: transition metals at the pathogen-host interface.
537 *Nature Reviews Microbiology* 2012;**10**(8):525-37. doi: 10.1038/nrmicro2836
- 538 8. Chandrangsu P, Rensing C, Helmann JD. Metal homeostasis and resistance in bacteria. *Nat Rev*
539 *Microbiol* 2017;**15**(6):338-50. doi: 10.1038/nrmicro.2017.15
- 540 9. Hao XL, Luthje FL, Qin Y *et al.* Survival in amoeba-a major selection pressure on the presence
541 of bacterial copper and zinc resistance determinants? Identification of a "copper pathogenicity island".
542 *Applied Microbiology and Biotechnology* 2015;**99**(14):5817-24. doi: 10.1007/s00253-015-6749-0
- 543 10. Capdevila DA, Wang J, Giedroc DP. Bacterial Strategies to Maintain Zinc Metallostasis at the
544 Host-Pathogen Interface. *J Biol Chem* 2016;**291**(40):20858-68. doi: 10.1074/jbc.R116.742023
- 545 11. Buracco S, Peracino B, Andreini C *et al.* Differential Effects of Iron, Zinc, and Copper on
546 *Dictyostelium discoideum* Cell Growth and Resistance to *Legionella pneumophila*. *Front Cell Infect*
547 *Microbiol* 2017;**7**:536. doi: 10.3389/fcimb.2017.00536
- 548 12. Soldati T, Neyrolles O. Mycobacteria and the intraphagosomal environment: take it with a
549 pinch of salt(s)! *Traffic* 2012;**13**(8):1042-52. doi: 10.1111/j.1600-0854.2012.01358.x
- 550 13. Botella H, Stadthagen G, Lugo-Villarino G *et al.* Metallobiology of host-pathogen interactions:
551 an intoxicating new insight. *Trends Microbiol* 2012;**20**(3):106-12. doi: 10.1016/j.tim.2012.01.005
- 552 14. Schaumlöffel D, Hutchinson R, Malherbe J *et al.* Novel Methods for Bioimaging Including LA-
553 ICP-MS, NanoSIMS, TEM/X-EDS, and SXRF. *Metallomics*, 2016, 83-116
- 554 15. Nunez J, Renslow R, Cliff JB *et al.* NanoSIMS for biological applications: Current practices and
555 analyses. *Biointerphases* 2018;**13**(3). doi: Artn 03b301
556 10.1116/1.4993628
- 557 16. Lechene C, Hillion F, McMahon G *et al.* High-resolution quantitative imaging of mammalian
558 and bacterial cells using stable isotope mass spectrometry. *J Biol* 2006;**5**(6):20. doi: 10.1186/jbiol42
- 559 17. MacDonald RJ. Surface Analysis Methods in Materials Science. In: O'Connor J, Sexton, Brett,
560 Smart, Roger S.C.s (ed), 2003
- 561 18. Malherbe J, Penen F, Isaure MP *et al.* A New Radio Frequency Plasma Oxygen Primary Ion
562 Source on Nano Secondary Ion Mass Spectrometry for Improved Lateral Resolution and Detection of
563 Electropositive Elements at Single Cell Level. *Anal Chem* 2016;**88**(14):7130-6. doi:
564 10.1021/acs.analchem.6b01153
- 565 19. Gao D, Huang X, Tao Y. A critical review of NanoSIMS in analysis of microbial metabolic
566 activities at single-cell level. *Crit Rev Biotechnol* 2016;**36**(5):884-90. doi:
567 10.3109/07388551.2015.1057550
- 568 20. Chadwick GL, Otero FJ, Gralnick JA *et al.* NanoSIMS imaging reveals metabolic stratification
569 within current-producing biofilms. *Proc Natl Acad Sci U S A* 2019;**116**(41):20716-24. doi:
570 10.1073/pnas.1912498116

- 571 21. Fayek M, Utsunomiya S, Pfiffner SM *et al.* The application of HRTEM techniques and nanosims
572 to chemically and isotopically characterize *Geobacter sulfurreducens* surfaces. *Can Mineral*
573 2005;**43**:1631-41. doi: DOI 10.2113/gscanmin.43.5.1631
- 574 22. Newsome L, Adams RL, Downie HF *et al.* NanoSIMS imaging of extracellular electron transport
575 processes during microbial iron(III) reduction. *FEMS Microbiol Ecol* 2018;**94**(8). doi: ARTN fiy104
576 10.1093/femsec/fiy104
- 577 23. Iarovaia OV, Minina EP, Sheval EV *et al.* Nucleolus: A Central Hub for Nuclear Functions. *Trends*
578 *Cell Biol* 2019;**29**(8):647-59. doi: 10.1016/j.tcb.2019.04.003
- 579 24. Fleming EJ, Woyke T, Donatello RA *et al.* Insights into the Fundamental Physiology of the
580 Uncultured Fe-Oxidizing Bacterium *Leptothrix ochracea*. *Applied and Environmental Microbiology*
581 2018;**84**(9). doi: ARTN e02239-17
582 10.1128/AEM.02239-17
- 583 25. Du HH, Huang QY, Zhou M *et al.* Sorption of Cu(II) by Al hydroxide organo-mineral
584 coprecipitates: Microcalorimetry and NanoSIMS observations. *Chem Geol* 2018;**499**:165-71. doi:
585 10.1016/j.chemgeo.2018.09.026
- 586 26. Lopez-Adams R, Newsome L, Moore KL *et al.* Dissimilatory Fe(III) Reduction Controls on Arsenic
587 Mobilization: A Combined Biogeochemical and NanoSIMS Imaging Approach. *Front Microbiol* 2021;**12**.
588 doi: ARTN 640734
589 10.3389/fmicb.2021.640734
- 590 27. Ferrari A, He C, Kennelly JP *et al.* Aster Proteins Regulate the Accessible Cholesterol Pool in the
591 Plasma Membrane. *Mol Cell Biol* 2020;**40**(19). doi: 10.1128/MCB.00255-20
- 592 28. Hu X, Weston TA, He C *et al.* Release of cholesterol-rich particles from the macrophage plasma
593 membrane during movement of filopodia and lamellipodia. *Elife* 2019;**8**. doi: 10.7554/eLife.50231
- 594 29. He CW, Migawa MT, Chen K *et al.* High-resolution visualization and quantification of nucleic
595 acid-based therapeutics in cells and tissues using Nanoscale secondary ion mass spectrometry
596 (NanoSIMS). *Nucleic Acids Res* 2021;**49**(1):1-14. doi: 10.1093/nar/gkaa1112
- 597 30. Fearn A, Greenwood DJ, Rodgers A *et al.* Correlative light electron ion microscopy reveals in
598 vivo localisation of bedaquiline in *Mycobacterium tuberculosis*-infected lungs. *PLoS Biol* 2020;**18**(12).
599 doi: ARTN e3000879
600 10.1371/journal.pbio.3000879
- 601 31. Jiang HB, Passarelli MK, Munro PMG *et al.* High-resolution sub-cellular imaging by correlative
602 NanoSIMS and electron microscopy of amiodarone internalisation by lung macrophages as evidence
603 for drug-induced phospholipidosis. *Chem Commun* 2017;**53**(9):1506-9. doi: 10.1039/c6cc08549k
- 604 32. Kitamura N, Ektessabi AM. XAFS in a single macrophage cell. *J Synchrotron Radiat* 2001;**8**(Pt
605 2):981-3. doi: 10.1107/s0909049500014436
- 606 33. Ishihara R, Ide-Ektessabi A, Kitamura N *et al.* Investigation of interactions of nano-particles
607 within cells using micro-beam imaging techniques. *X-Ray Spectrom* 2003;**32**(6):418-22. doi:
608 10.1002/xrs.663
- 609 34. Barst BD, Bridges K, Korbas M *et al.* The role of melano-macrophage aggregates in the storage
610 of mercury and other metals: an example from yelloweye rockfish (*Sebastes ruberrimus*). *Environ*
611 *Toxicol Chem* 2015;**34**(8):1918-25. doi: 10.1002/etc.3009
- 612 35. Pascolo L, Gianoncelli A, Schneider G *et al.* The interaction of asbestos and iron in lung tissue
613 revealed by synchrotron-based scanning X-ray microscopy. *Sci Rep* 2013;**3**:1123. doi:
614 10.1038/srep01123
- 615 36. Bussy C, Paineau E, Cambedouzou J *et al.* Intracellular fate of carbon nanotubes inside murine
616 macrophages: pH-dependent detachment of iron catalyst nanoparticles. *Part Fibre Toxicol* 2013;**10**.
617 doi: Artn 24
618 10.1186/1743-8977-10-24
- 619 37. Liang-Takasaki CJ, Makela PH, Leive L. Phagocytosis of bacteria by macrophages: changing the
620 carbohydrate of lipopolysaccharide alters interaction with complement and macrophages. *J Immunol*
621 1982;**128**(3):1229-35

- 622 38. Muniz-Junqueira MI, Prata A, Tosta CE. Factors influencing phagocytosis of *Salmonella*
623 *typhimurium* by macrophages in murine schistosomiasis. *Rev Soc Bras Med Trop* 1997;**30**(2):101-6. doi:
624 10.1590/s0037-86821997000200003
- 625 39. Shi L, Chowdhury SM, Smallwood HS *et al.* Proteomic investigation of the time course
626 responses of RAW 264.7 macrophages to infection with *Salmonella enterica*. *Infect Immun*
627 2009;**77**(8):3227-33. doi: 10.1128/IAI.00063-09
- 628 40. Hurley D, Hoffmann M, Muruvanda T *et al.* Atypical *Salmonella enterica* Serovars in Murine
629 and Human Macrophage Infection Models. *Infect Immun* 2020;**88**(4). doi: 10.1128/IAI.00353-19
- 630 41. Spurr AR. A low-viscosity epoxy resin embedding medium for electron microscopy. *Journal of*
631 *ultrastructure research* 1969;**26**(1):31-43
- 632 42. Waldron KJ, Rutherford JC, Ford D *et al.* Metalloproteins and metal sensing. *Nature*
633 2009;**460**(7257):823-30. doi: 10.1038/nature08300
- 634 43. Foster AW, Osman D, Robinson NJ. Metal preferences and metallation. *J Biol Chem*
635 2014;**289**(41):28095-103. doi: 10.1074/jbc.R114.588145
- 636 44. Juttukonda LJ, Skaar EP. Chapter 31 - Manganese and Nutritional Immunity. In: Collins JFs (ed).
637 *Molecular, Genetic, and Nutritional Aspects of Major and Trace Minerals*. Boston: Academic Press,
638 2017, 377-87
- 639 45. Spears JW, Engle TE. Feed Ingredients: Feed Supplements: Microminerals. *Reference Module*
640 *in Food Science*: Elsevier, 2016
- 641 46. Festa RA, Thiele DJ. Copper at the Front Line of the Host-Pathogen Battle. *Plos Pathogens*
642 2012;**8**(9):e1002887. doi: ARTN e1002887
643 10.1371/journal.ppat.1002887
- 644 47. Kim BE, Nevitt T, Thiele DJ. Mechanisms for copper acquisition, distribution and regulation.
645 *Nat Chem Biol* 2008;**4**(3):176-85. doi: 10.1038/nchembio.72
- 646 48. Thorgersen MP, Downs DM. Cobalt targets multiple metabolic processes in *Salmonella*
647 *enterica*. *J Bacteriol* 2007;**189**(21):7774-81. doi: 10.1128/JB.00962-07
- 648 49. Rodionov DA, Vitreschak AG, Mironov AA *et al.* Comparative genomics of the vitamin B12
649 metabolism and regulation in prokaryotes. *J Biol Chem* 2003;**278**(42):41148-59. doi:
650 10.1074/jbc.M305837200
- 651 50. Arfin SM, Kendall RL, Hall L *et al.* Eukaryotic methionyl aminopeptidases: two classes of cobalt-
652 dependent enzymes. *Proc Natl Acad Sci U S A* 1995;**92**(17):7714-8. doi: 10.1073/pnas.92.17.7714
- 653 51. Alfano M, Cavazza C. Structure, function, and biosynthesis of nickel-dependent enzymes.
654 *Protein Sci* 2020;**29**(5):1071-89. doi: 10.1002/pro.3836
- 655 52. Mayr SJ, Mendel RR, Schwarz G. Molybdenum cofactor biology, evolution and deficiency.
656 *Biochim Biophys Acta Mol Cell Res* 2021;**1868**(1):118883. doi: 10.1016/j.bbamcr.2020.118883
- 657 53. Mendel RR, Kruse T. Cell biology of molybdenum in plants and humans. *Bba-Mol Cell Res*
658 2012;**1823**(9):1568-79. doi: 10.1016/j.bbamcr.2012.02.007
- 659 54. Watly J, Potocki S, Rowinska-Zyrek M. Zinc Homeostasis at the Bacteria/Host Interface-From
660 Coordination Chemistry to Nutritional Immunity. *Chemistry (Easton)* 2016;**22**(45):15992-6010. doi:
661 10.1002/chem.201602376
- 662 55. Muckenthaler MU, Rivella S, Hentze MW *et al.* A Red Carpet for Iron Metabolism. *Cell*
663 2017;**168**(3):344-61. doi: 10.1016/j.cell.2016.12.034
- 664 56. Gao G, Li J, Zhang Y *et al.* Cellular Iron Metabolism and Regulation. *Adv Exp Med Biol*
665 2019;**1173**:21-32. doi: 10.1007/978-981-13-9589-5_2
- 666 57. Carrondo MA. Ferritins, iron uptake and storage from the bacterioferritin viewpoint. *EMBO J*
667 2003;**22**(9):1959-68. doi: 10.1093/emboj/cdg215
- 668 58. Lane DJ, Merlot AM, Huang ML *et al.* Cellular iron uptake, trafficking and metabolism: Key
669 molecules and mechanisms and their roles in disease. *Biochim Biophys Acta* 2015;**1853**(5):1130-44.
670 doi: 10.1016/j.bbamcr.2015.01.021
- 671 59. Hider RC, Maret W. Iron and zinc sensing in cells and the body. *Metallomics* 2015;**7**(2):200-1.
672 doi: 10.1039/c4mt90051k

673 60. Johnson DC, Dean DR, Smith AD *et al.* Structure, function, and formation of biological iron-
674 sulfur clusters. *Annu Rev Biochem* 2005;**74**:247-81. doi: 10.1146/annurev.biochem.74.082803.133518
675 61. Prasad AS. Zinc: role in immunity, oxidative stress and chronic inflammation. *Curr Opin Clin*
676 *Nutr Metab Care* 2009;**12**(6):646-52. doi: 10.1097/MCO.0b013e3283312956
677 62. Bonaventura P, Benedetti G, Albaredo F *et al.* Zinc and its role in immunity and inflammation.
678 *Autoimmun Rev* 2014. doi: 10.1016/j.autrev.2014.11.008
679 63. Andreini C, Banci L, Bertini I *et al.* Zinc through the three domains of life. *J Proteome Res*
680 2006;**5**(11):3173-8. doi: Doi 10.1021/Pr0603699
681 64. Andreini C, Banci L, Bertini I *et al.* Non-heme iron through the three domains of life. *Proteins*
682 2007;**67**(2):317-24. doi: 10.1002/prot.21324
683 65. Maret W. Metalloproteomics, metalloproteomes, and the annotation of metalloproteins.
684 *Metallomics* 2010;**2**(2):117-25. doi: 10.1039/b915804a
685 66. Andreini C, Banci L, Bertini I *et al.* Counting the zinc-proteins encoded in the human genome. *J*
686 *Proteome Res* 2006;**5**(1):196-201. doi: 10.1021/pr050361j
687 67. Andreini C, Bertini I, Rosato A. Metalloproteomes: a bioinformatic approach. *Acc Chem Res*
688 2009;**42**(10):1471-9. doi: 10.1021/ar900015x
689 68. Dupont CL, Yang S, Palenik B *et al.* Modern proteomes contain putative imprints of ancient
690 shifts in trace metal geochemistry. *Proc Natl Acad Sci U S A* 2006;**103**(47):17822-7. doi:
691 10.1073/pnas.0605798103
692 69. Dupont CL, Butcher A, Valas RE *et al.* History of biological metal utilization inferred through
693 phylogenomic analysis of protein structures. *Proc Natl Acad Sci U S A* 2010;**107**(23):10567-72. doi:
694 10.1073/pnas.0912491107
695 70. Miloch A, Krezel A. Metal binding properties of the zinc finger metallome - insights into
696 variations in stability. *Metallomics* 2014. doi: 10.1039/c4mt00149d
697 71. Krezel A, Maret W. Thionein/metallothionein control Zn(II) availability and the activity of
698 enzymes. *J Biol Inorg Chem* 2008;**13**(3):401-9. doi: 10.1007/s00775-007-0330-y
699 72. Blindauer CA. Bacterial metallothioneins: past, present, and questions for the future. *J Biol*
700 *Inorg Chem* 2011;**16**(7):1011-24. doi: 10.1007/s00775-011-0790-y
701 73. Isani G, Carpena E. Metallothioneins, unconventional proteins from unconventional animals: a
702 long journey from nematodes to mammals. *Biomolecules* 2014;**4**(2):435-57. doi:
703 10.3390/biom4020435
704 74. Ma Z, Chandrangsu P, Helmann TC *et al.* Bacillithiol is a major buffer of the labile zinc pool in
705 *Bacillus subtilis*. *Mol Microbiol* 2014;**94**(4):756-70. doi: 10.1111/mmi.12794
706 75. Hensley MP, Tierney DL, Crowder MW. Zn(II) binding to *Escherichia coli* 70S ribosomes.
707 *Biochemistry* 2011;**50**(46):9937-9. doi: 10.1021/bi200619w
708 76. Chandrangsu P, Huang X, Gaballa A *et al.* *Bacillus subtilis* FoIE is sustained by the ZagA zinc
709 metallochaperone and the alarmone ZTP under conditions of zinc deficiency. *Mol Microbiol*
710 2019;**112**(3):751-65. doi: 10.1111/mmi.14314
711 77. Chandrangsu P, Rensing C, Helmann JD. Metal homeostasis and resistance in bacteria. *Nature*
712 *Reviews Microbiology* 2017;**15**(6):338-50. doi: 10.1038/nrmicro.2017.15
713 78. Haas CE, Rodionov DA, Kropat J *et al.* A subset of the diverse COG0523 family of putative metal
714 chaperones is linked to zinc homeostasis in all kingdoms of life. *BMC Genomics* 2009;**10**. doi: Artn 470
715 Doi 10.1186/1471-2164-10-470
716 79. Campbell AK. *Intracellular Calcium*. Wiley, 2014
717 80. Clapham DE. Calcium signaling. *Cell* 1995;**80**(2):259-68. doi: 10.1016/0092-8674(95)90408-5
718 81. Chang CJ. Searching for harmony in transition-metal signaling. *Nat Chem Biol* 2015;**11**(10):744-
719 7. doi: 10.1038/nchembio.1913
720 82. Dominguez DC, Guragain M, Patrauchan M. Calcium binding proteins and calcium signaling in
721 prokaryotes. *Cell Calcium* 2015;**57**(3):151-65. doi: 10.1016/j.ceca.2014.12.006
722 83. Dominguez DC. Calcium signalling in bacteria. *Mol Microbiol* 2004;**54**(2):291-7. doi:
723 10.1111/j.1365-2958.2004.04276.x

- 724 84. Asmat TM, Tenenbaum T, Jonsson AB *et al.* Impact of calcium signaling during infection of
725 *Neisseria meningitidis* to human brain microvascular endothelial cells. *PLoS One* 2014;**9**(12):e114474.
726 doi: 10.1371/journal.pone.0114474
- 727 85. Hay AJ, Yang M, Xia X *et al.* Calcium Enhances Bile Salt-Dependent Virulence Activation in *Vibrio*
728 *cholerae*. *Infect Immun* 2017;**85**(1). doi: 10.1128/IAI.00707-16
- 729 86. Gangola P, Rosen BP. Maintenance of intracellular calcium in *Escherichia coli*. *J Biol Chem*
730 1987;**262**(26):12570-4
- 731 87. Knight MR, Campbell AK, Smith SM *et al.* Recombinant Aequorin as a Probe for Cytosolic Free
732 Ca²⁺ in *Escherichia coli*. *FEBS Lett* 1991;**282**(2):405-8. doi: Doi 10.1016/0014-5793(91)80524-7
- 733 88. Jones HE, Holland IB, Baker HL *et al.* Slow changes in cytosolic free Ca²⁺ in *Escherichia coli*
734 highlight two putative influx mechanisms in response to changes in extracellular calcium. *Cell Calcium*
735 1999;**25**(3):265-74. doi: DOI 10.1054/ceca.1999.0028
- 736 89. Torrecilla I, Leganes F, Bonilla I *et al.* Use of recombinant aequorin to study calcium
737 homeostasis and monitor calcium transients in response to heat and cold shock in cyanobacterial.
738 *Plant Physiol* 2000;**123**(1):161-75. doi: DOI 10.1104/pp.123.1.161
- 739 90. Guragain M, Lenaburg DL, Moore FS *et al.* Calcium homeostasis in *Pseudomonas aeruginosa*
740 requires multiple transporters and modulates swarming motility. *Cell Calcium* 2013;**54**(5):350-61. doi:
741 10.1016/j.ceca.2013.08.004
- 742 91. Rabinovitch M. Professional and non-professional phagocytes: an introduction. *Trends Cell Biol*
743 1995;**5**(3):85-7. doi: 10.1016/s0962-8924(00)88955-2
- 744 92. Ambrose CT. The Osler slide, a demonstration of phagocytosis from 1876 Reports of
745 phagocytosis before Metchnikoff's 1880 paper. *Cell Immunol* 2006;**240**(1):1-4. doi:
746 10.1016/j.cellimm.2006.05.008
- 747 93. Gordon S. Phagocytosis: An Immunobiologic Process. *Immunity* 2016;**44**(3):463-75. doi:
748 10.1016/j.immuni.2016.02.026
- 749 94. Tauber AI. Immunity: How Elie Metchnikoff Changed the Course of Modern Medicine. *Bull Hist*
750 *Med* 2017;**91**(1):140-2. doi: DOI 10.1353/bhm.2017.0015
- 751 95. Walters MN, Papadimitriou JM. Phagocytosis: a review. *CRC Crit Rev Toxicol* 1978;**5**(4):377-
752 421. doi: 10.3109/10408447809081012
- 753 96. Aderem A, Underhill DM. Mechanisms of phagocytosis in macrophages. *Annu Rev Immunol*
754 1999;**17**:593-623. doi: 10.1146/annurev.immunol.17.1.593
- 755 97. Rosales C, Uribe-Querol E. Phagocytosis: A Fundamental Process in Immunity. *Biomed Res Int*
756 2017;**2017**:9042851. doi: 10.1155/2017/9042851
- 757 98. Kirsten A, Herzberg M, Voigt A *et al.* Contributions of five secondary metal uptake systems to
758 metal homeostasis of *Cupriavidus metallidurans* CH34. *J Bacteriol* 2011;**193**(18):4652-63. doi:
759 10.1128/JB.05293-11
- 760 99. Biology and brimstone. *Nat Chem Biol* 2006;**2**(4):169. doi: 10.1038/nchembio0406-169
- 761 100. Fernandez-Garcia C, Coggins AJ, Powner MW. A Chemist's Perspective on the Role of
762 Phosphorus at the Origins of Life. *Life-Basel* 2017;**7**(3). doi: ARTN 31
763 10.3390/life7030031
- 764 101. Gross M. Where is all the phosphorus? *Curr Biol* 2017;**27**(21):R1141-R4
- 765 102. Fagerbakke KM, Heldal M, Norland S. Content of carbon, nitrogen, oxygen, sulfur and
766 phosphorus in native aquatic and cultured bacteria. *Aquat Microb Ecol* 1996;**10**(1):15-27. doi: DOI
767 10.3354/ame010015
- 768 103. Vrede K, Heldal M, Norland S *et al.* Elemental composition (C, N, P) and cell volume of
769 exponentially growing and nutrient-limited bacterioplankton. *Appl Environ Microbiol* 2002;**68**(6):2965-
770 71. doi: 10.1128/aem.68.6.2965-2971.2002
- 771 104. Xu X, Hui D, King AW *et al.* Convergence of microbial assimilations of soil carbon, nitrogen,
772 phosphorus, and sulfur in terrestrial ecosystems. *Sci Rep* 2015;**5**:17445. doi: 10.1038/srep17445
- 773 105. Storms HA, Brown KF, Stein JD. Evaluation of a Cesium Positive-Ion Source for Secondary Ion
774 Mass-Spectrometry. *Anal Chem* 1977;**49**(13):2023-30. doi: DOI 10.1021/ac50021a034

775 106. Hernandez-Verdun D, Roussel P, Thiry M *et al.* The nucleolus: structure/function relationship
776 in RNA metabolism. *Wires Rna* 2010;**1**(3):415-31. doi: 10.1002/wrna.39
777 107. O'Sullivan JM, Pai DA, Cridge AG *et al.* The nucleolus: a raft adrift in the nuclear sea or the
778 keystone in nuclear structure? *Biomol Concepts* 2013;**4**(3):277-86. doi: 10.1515/bmc-2012-0043
779 108. Sirri V, Urcuqui-Inchima S, Roussel P *et al.* Nucleolus: the fascinating nuclear body. *Histochem*
780 *Cell Biol* 2008;**129**(1):13-31. doi: 10.1007/s00418-007-0359-6
781 109. Raska I, Shaw PJ, Cmarko D. Structure and function of the nucleolus in the spotlight. *Curr Opin*
782 *Cell Biol* 2006;**18**(3):325-34. doi: 10.1016/j.ceb.2006.04.008
783 110. Gupta A, Becker T. Mechanisms and pathways of mitochondrial outer membrane protein
784 biogenesis. *Bba-Bioenergetics* 2021;**1862**(1). doi: ARTN 148323
785 10.1016/j.bbabi.2020.148323
786 111. Becker T, Gebert M, Pfanner N *et al.* Biogenesis of mitochondrial membrane proteins. *Curr*
787 *Opin Cell Biol* 2009;**21**(4):484-93. doi: 10.1016/j.ceb.2009.04.002
788 112. Wilson RG, Stevie FA, Magee CW. *Secondary ion mass spectrometry : a practical handbook for*
789 *depth profiling and bulk impurity analysis / R. G. Wilson, F. A. Stevie, C. W. Magee.* New York: Wiley,
790 1989
791 113. Samer S, Raman R, Laube G *et al.* The nuclear lamina is a hub for the nuclear function of Jacob.
792 *Mol Brain* 2021;**14**(1). doi: ARTN 9
793 10.1186/s13041-020-00722-1
794 114. Secondo A, Petrozziello T, Tedeschi V *et al.* Nuclear localization of NCX: Role in Ca(2+) handling
795 and pathophysiological implications. *Cell Calcium* 2020;**86**:102143. doi: 10.1016/j.ceca.2019.102143
796 115. Bading H. Nuclear calcium signalling in the regulation of brain function. *Nature Reviews*
797 *Neuroscience* 2013;**14**(9):593-608. doi: 10.1038/nrn3531
798 116. Kashiv Y, Austin JR, 2nd, Lai B *et al.* Imaging trace element distributions in single organelles and
799 subcellular features. *Sci Rep* 2016;**6**:21437. doi: 10.1038/srep21437
800 117. Matsuyama S, Shimura M, Mirnura H *et al.* Trace element mapping of a single cell using a hard
801 x-ray nanobeam focused by a Kirkpatrick-Baez mirror system. *X-Ray Spectrom* 2009;**38**(2):89-94. doi:
802 10.1002/xrs.1123
803 118. Ortega R, Bresson C, Fraysse A *et al.* Cobalt distribution in keratinocyte cells indicates nuclear
804 and perinuclear accumulation and interaction with magnesium and zinc homeostasis. *Toxicol Lett*
805 2009;**188**(1):26-32. doi: 10.1016/j.toxlet.2009.02.024
806 119. Banerjee R. B12 trafficking in mammals: A for coenzyme escort service. *ACS Chem Biol*
807 2006;**1**(3):149-59. doi: 10.1021/cb6001174
808 120. Yamada K. Cobalt: its role in health and disease. *Met Ions Life Sci* 2013;**13**:295-320. doi:
809 10.1007/978-94-007-7500-8_9
810