

# Size and strain of zinc sulfide nanoparticles altered by interaction with organic molecules

Maureen Le Bars<sup>a, b, \*1</sup>, Clément Levard<sup>a</sup>, Samuel Legros<sup>b</sup>, Vladimir Vidal<sup>a</sup>, Alejandro Fernandez Martinez<sup>c</sup>, F. Marc Michel<sup>d</sup>, Antoine Thill<sup>e</sup>, Bénédicte Prelot<sup>f</sup>, Gabrielle Dublet-Adli<sup>g</sup>, Daniel Borschneck<sup>a</sup>, Jérôme Rose<sup>a</sup>, Emmanuel Doelsch<sup>b</sup>

<sup>a</sup> Aix Marseille Univ, CNRS, IRD, INRAE, Coll France, CEREGE, Aix-en-Provence, France

<sup>b</sup> UPR Recyclage et Risque, CIRAD, F-34398 Montpellier, France; Recyclage et Risque, Univ. Montpellier, CIRAD, Montpellier, France

<sup>c</sup> Univ. Grenoble Alpes, Univ. Savoie Mont Blanc, CNRS, IRD, IFSTTAR, ISTerre, Grenoble, France

<sup>d</sup> Department of Geosciences, Virginia Polytechnic Institute and State University, VA 24061, United States

<sup>e</sup> NIMBE, UMR 3685 CEA, CNRS, Université Paris-Saclay, CEA Saclay, 91191 Gif-sur-Yvette Cedex, France

<sup>f</sup> ICGM, Univ Montpellier, CNRS, ENSCM, 34290 Montpellier, France

<sup>g</sup> Norwegian Geotechnical Institute, 0806 Oslo, Norway

\* Corresponding author: maureen.lebars@usys.ethz.ch

## Abstract

Nanosized zinc sulfides (nano-ZnS) have size-dependent and tunable physical and chemical properties that make them useful for a variety of technological applications. For example, structural changes, especially caused by strain, are pronounced in nano-ZnS <5 nm in size, the size range typical of incidental nano-ZnS that form in the environment. Previous research has shown how natural organic matter impacts the physical properties of nano-ZnS, but was mostly focused on their aggregation state. However, the specific organic molecules and the type of functional groups that are most important for

---

<sup>1</sup> Present address: Institute of Biogeochemistry & Pollutant Dynamics, ETH Zurich, Universitätstrasse 16, 8092 Zurich, Switzerland

25 controlling nano-ZnS size and strain remain unclear. This study examined the size-dependent strain of  
26 nano-ZnS synthesized in the presence of serine, cysteine, glutathione, histidine, and acetate.  
27 Synchrotron total scattering pair distribution function analysis was used to determine the average  
28 crystallite size and strain. Among the different organic molecules tested, those containing a thiol group  
29 were shown to affect particle size and size-induced strain most strongly when added during synthesis,  
30 but also significantly reduced particle strain when added to as-formed nano-ZnS. The same effects are  
31 useful to understand the properties and behavior of natural nano-ZnS formed as products of microbial  
32 activity, for example, in reducing environments, or of incidental nano-ZnS formed in organic wastes.

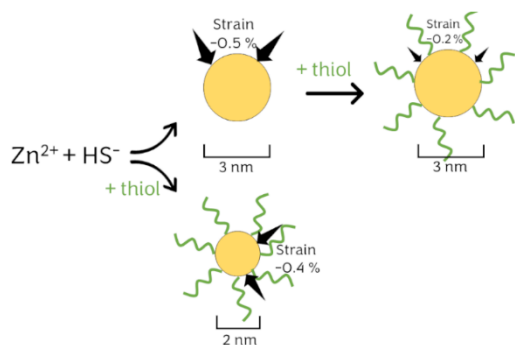
### 33 **Keywords**

34 thiol, pair distribution function, sphalerite, lattice contraction, structure, ZnS

### 35 **Synopsis**

36 Zinc sulfide nanoparticles (nano-ZnS) are incidental nanoparticles that are extensively spread on  
37 cultivated soils during recycling of organic waste. This study investigated how interactions with  
38 organic molecules control the structural properties of nano-ZnS.

### 39 **Table of Content art**



40

41

## 42 **Introduction**

43 ZnS nanoparticles (nano-ZnS) are of great interest due to their size-dependent electric and optical  
44 properties that can be tuned for different technological applications.<sup>1,2</sup> Nano-ZnS can also be found in  
45 nature, for example, in systems undergoing sulfate reduction by bacteria<sup>3,4</sup> or formed incidentally in  
46 liquid and anaerobically digested zinc-rich organic waste (OW) used as agricultural fertilizer.<sup>5-8</sup> Nano-  
47 ZnS formed in OW are 3-5 nm in size and have been shown to undergo transformation in days or  
48 months in OW or amended soils.<sup>5,7,9</sup> Considering the high concentration of Zn in OW combined with  
49 the amount of OW applied on croplands, we previously estimated that 440 000 tons of nano-ZnS are  
50 released into the environment each year.<sup>10</sup> Sulfur in soil is an essential macronutrient for plants<sup>11</sup> and its  
51 reduction to sulfide can lead to the immobilization of chalcophile metals (Fe, Cu, Cd, Hg) through  
52 metal sulfide precipitation.<sup>12</sup> Zinc also plays a crucial role in human functioning<sup>13</sup> and in plant  
53 proteins.<sup>14</sup> However, millions of hectares of cropland suffer from Zn deficiency.<sup>15-17</sup> On the other hand,  
54 Zn can be toxic for plants<sup>18</sup> and Zn toxicity in crops can occur in contaminated soils, for example in  
55 agricultural soils treated with sewage sludge.<sup>19</sup> Considering the high volume of nano-ZnS spread on  
56 soils, understanding their formation and transformation is crucial to be able to predict zinc and sulfur  
57 cycling.

58 Studies of synthetic nano-ZnS have shown that particle size has a significant impact on their  
59 chemical properties and structure. For example, Zhang et al. (2010)<sup>20</sup> showed that total free surface  
60 energy of nano-ZnS was size-dependent at sizes ranging from 2 to 6 nm, and affected their dissolution  
61 rate in an EDTA-containing medium. Gilbert et al. (2004)<sup>21</sup> reported that 3.4 nm ZnS undergoes  
62 significant structural strain, as indicated by an average of 1% lattice contraction compared to in bulk  
63 ZnS. Indeed, Jiang et al. (2001)<sup>22</sup> demonstrated in theory that lattice contraction in face-centered cubic  
64 crystals is linked to their size. A size-strain relationship has already been reported in nano-ZnS (1.5 – 4  
65 nm)<sup>23</sup> and in analogous systems such as nano-CdSe (2.3 – 3.7 nm).<sup>24</sup> Strain-induced lattice contraction

66 is caused by structural stress (the strength applied at the surface of the particle as a result of size  
67 reduction) and can have significant effects on the reactivity of metallic particles.<sup>25-27</sup>

68 Strain has been shown to be affected by surface chemistry and in particular, by the strength of  
69 surface-ligand interactions.<sup>28</sup> It is thus particularly important to take such interactions into  
70 consideration in complex environmental matrices when studying the environmental fate of nano-ZnS.  
71 Interactions between nano-ZnS and organic matter have been observed in situ, e.g. in biofilms.<sup>3, 4, 29</sup> In  
72 addition, laboratory studies highlighted the key properties of organic matter that affect the aggregation  
73 state of nano-ZnS.<sup>29-33</sup> To give an example, when present in solution during the first hours following  
74 nano-ZnS precipitation, organic molecules, including glutamic acid, glycine, glutathione and cysteine,  
75 have been shown to stabilize nano-ZnS by limiting aggregation.<sup>31-33</sup> Finally, surface–ligand interactions  
76 involving organic molecules such as histidine, glutathione, cysteine have been used to control the size  
77 of nano-ZnS photocatalysts.<sup>34-37</sup> Although several authors reported the noticeable effects of surface  
78 interactions on nano-ZnS structural properties (mostly size) and aggregation behavior, to the best of our  
79 knowledge, to date, no systematic study has investigated the effect of surface interactions with a variety  
80 of environmentally relevant organic molecules on both particle size and strain.

81 The aim of this study was thus to examine the size-dependent structural strain of nano-ZnS  
82 synthesized in the presence of organic molecules. For this end, nano-ZnS in the presence of serine,  
83 cysteine, glutathione, histidine, and acetate were used to test how different surface interactions  
84 impacted size and strain. The molecules were chosen specifically to evaluate the effects of the type of  
85 functional group (carboxyl, thiol, hydroxyl, methyl, imidazole), steric hindrance, organic-to-metal(Zn)  
86 ratio, and the effects of adding organic matter during or after nano-ZnS synthesis.

## 87 **Materials and methods**

### 88 *1.1 Nano-ZnS synthesis*

89 A series of nano-ZnS samples were synthesized in the presence of different organic molecules  
90 (OM) including L-serine (99% purity, Acros Organics), L-cysteine (>98% purity, VWR), reduced L-  
91 glutathione (>98% purity, VWR), L-histidine (>99% purity, Sigma Aldrich), and sodium acetate (>99%  
92 purity, Sigma Aldrich). The resulting nano-ZnS are hereafter referred to as ZnS\_ser, ZnS\_cys,  
93 ZnS\_GSH, ZnS\_hist, ZnS\_ace, respectively. To include organic molecules, the synthesis method was  
94 adapted from bare nano-ZnS synthesis methods (see SI Part I, for more details).<sup>5, 7, 9, 28</sup> To be  
95 representative of environmental conditions, we chose contents with excess S and organic molecules  
96 compared to Zn (S/Zn molar ratio of 1.2, and an OM/Zn molar ratio of 2), neutral pH (7) and room  
97 temperature (~25 °C). The final concentration of ZnS was set at 0.01 M. The samples were prepared in  
98 an anoxic glovebox (N<sub>2</sub> atmosphere) using N<sub>2</sub>-purged ultrapure water. To prevent variations in pH  
99 during synthesis due to the potential dissociation of the different protonated groups present in the  
100 organic molecules, the syntheses were performed with 0.07 M HEPES buffer (>99.5% purity, Sigma  
101 Aldrich) to obtain a final pH of 7. Briefly, OM and Na<sub>2</sub>S were dissolved in separated HEPES buffer  
102 solutions, and ZnCl<sub>2</sub> was dissolved in ultrapure water. The OM solution was mixed with Zn solution  
103 and finally with S solution. An additional series of samples was synthesized with increasing  
104 concentrations of cysteine (ZnS\_Cys/Zn\_0.2, ZnS\_Cys/Zn\_0.5 and ZnS\_Cys/Zn\_1 for Cys/Zn molar  
105 ratios of 0.2, 0.5 and 1). Because of the lower concentration of cysteine compared to the previous set of  
106 syntheses, the concentration of HEPES was reduced to 0.035 M. For both series of samples, a nano-  
107 ZnS with no organic molecules was synthesized in the same conditions, referred to hereafter as  
108 “Control A” and “Control B”. The parameters are summarized in Table S2.

109 All the sample nano-ZnS suspensions were aged for 30 hours at room temperature in the dark,  
110 under constant stirring in glass vials that were sealed in the anoxic glovebox. The samples were then

111 dialyzed against ultrapure water (MWCO of 1 kDa) to remove excess salts. The nano-ZnS suspensions  
112 were then freeze-dried and the recovered solids ground to a fine powder using a mortar and pestle  
113 under an anoxic atmosphere for further characterization.

114 Finally, the effect of adding cysteine after ZnS-NP formation was tested on a bare nano-ZnS  
115 (bare\_ZnS\_S/Zn\_0.5, synthesis described in SI part I) with a Cys/Zn molar ratio of 0.2. The resulting  
116 suspension was analyzed after preparation by total X-ray scattering.

## 117 *1.2 Total X-ray Scattering*

118 Synchrotron total scattering measurements were performed on beamline 11-ID-B of the  
119 Advanced Photon Source at Argonne National Laboratory (Argonne, IL USA). All the synthesized  
120 nano-ZnS samples were measured as dry powders loaded in 1 mm O.D. Kapton capillaries. In addition,  
121 the bare nano-ZnS (bare\_ZnS\_S/Zn\_0.5) was characterized immediately after the addition of deionized  
122 water or cysteine solution (cysteine/Zn molar ratio of 0.2) directly in the capillary. Total scattering  
123 intensities were recorded using an amorphous-silicon based area detector (2048x2048 pixel, Perkin  
124 Elmer).<sup>38, 39</sup> Total X-ray scattering signals were obtained with an energy of 86.7 keV ( $\lambda = 0.143 \text{ \AA}$ ,  
125 monochromator Si(422)) and the distance between the sample and the detector was 16 cm. A CeO<sub>2</sub>  
126 crystalline reference was used for calibration (Figure S2).

127 Data were optimized using standard methods,<sup>40</sup> Fit2D software<sup>41</sup> was used for 2D scattering  
128 images, and PDFgetX3 software<sup>42</sup> for data correction. The background was removed using the  
129 scattering pattern of an empty polyimide capillary. A q-range from 1.4 to 24.4  $\text{\AA}^{-1}$  was used to obtain  
130 the PDF from the Fourier transform of the reduced structure factor. The structural model for sphalerite  
131 (ZnS) was used to refine the PDF using PDFgui software.<sup>43</sup> The following parameters were refined:  
132 scale factor, lattice parameters ( $a=b=c$ ), anisotropic displacement parameters (constrained to be the  
133 same value for Zn and S), particle diameter, correlated atomic motion factor and Q broad (peak  
134 broadening due to instrumental Q resolution). To avoid correlations between parameters, the Q broad

135 and the anisotropic atomic motion factor were never allowed to vary together. Lattice contraction (%),  
136 i.e. structural strain, is defined as  $(a_{\text{nano}} - a_{\text{bulk}})/a_{\text{nano}}$  (referred to hereafter as  $\Delta a/a$ ), where  $a_{\text{nano}}$  is the  
137 refined lattice parameter for a nano-ZnS sample and  $a_{\text{bulk}}$  is the lattice parameter for bulk ZnS (5.4093  
138 Å).<sup>44</sup>

139 The weighted residual factor  $R_w$  of the refinement is calculated as:

$$R_w = \sqrt{\frac{\sum_{i=1}^N w(r_i)[G_{\text{obs}}(r_i) - G_{\text{calc}}(r_i)]^2}{\sum_{i=1}^N w(r_i)G_{\text{obs}}^2(r_i)}}$$

140

141 where  $w(r_i)$  is a multiplying factor,  $G_{\text{obs}}(r)$  the experimental PDF function, and  $G_{\text{calc}}(r)$  the refined PDF  
142 function. To estimate the uncertainty on each refined structural parameter, additional fits were  
143 performed with different initial values used for the refined parameters (scale factor, lattice parameters,  
144 anisotropic displacement parameters, particle diameter, correlated atomic motion factor and Q broad).  
145 Four fits were selected according to 2 criteria: (i) the values of the refined parameters were physically  
146 consistent and (ii) the quality of the fit was acceptable (i.e. all PDF peaks considered by refinement and  
147  $R_w < 0.3$ ). The values presented in this study (size, lattice parameter) are the average of the 4 values  
148 obtained with the 4 fits and the error bars represent the standard deviation for the same 4 values.

### 149 *1.3 Small Angle X-ray Scattering*

150 Small angle X-ray scattering (SAXS) experiments were performed on nano-ZnS powders placed inside  
151 a 1-mm Kapton capillary using a laboratory SAXS beamline (Xeuss 2.0) equipped with a copper  
152 microfocus X-ray source. The counting time was 1 h for each measurement. The scattering image was  
153 recorded on a direct photon counting 1M pixels Pilatus camera protected from the direct X-ray beam by  
154 a 3mm beamstop. SAXS profiles of intensity ( $I(q)$ ) vs. scattering vectors ( $q$ ) were obtained by radial  
155 averaging of the scattered X-ray 2D images as a function of  $q$ , where  $q = 4\pi\sin\theta/\lambda$  ( $\lambda$  and  $2\theta$  denote the

156 incident wavelength and the scattering angle, respectively). The scattering angles were calibrated using  
157 a silver behenate lamellar-like phase that has a well-known  $d_{001}$  repeat distance of 5.838 nm thus  
158 enabling precise measurement of the distance between the sample and the detector. The radial averaged  
159 SAXS profiles were further corrected for the scattering contribution of the empty Kapton capillary and  
160 the electronic background using pySAXS software (<https://pypi.org/project/pySAXS/>). The scattering  
161 profiles obtained were typical of powders made of aggregated spheres. The increase in intensity at the  
162 lowest angles observed for the cysteine-containing sample originated from inter-particle scattering and  
163 are not discussed in the present work. We estimated the nano-ZnS particle size for each powder using  
164 the fit of the intermediate  $q$  range of the scattering curve obtained by a monodisperse sphere model.

#### 165 *1.4 Statistics*

166 The Scikit-posthocs Python package was used for statistical analysis to compare sizes and lattice  
167 contraction values.<sup>45</sup> A Kruskal-Wallis test was used to compare all the samples ( $n=21$ ) according to  
168 one parameter (size or lattice contraction). The four values obtained from the four different PDF  
169 refinements were used for this comparison. In cases of rejection of the null hypothesis ( $H_0$  = all  
170 samples are from the same population), a Conover post-hoc test was used for multiple pair-wise  
171 comparison with a step-down method using Bonferroni adjustments. Differences were considered  
172 significant for an adjusted  $p$ -value  $< 0.05$ .

173

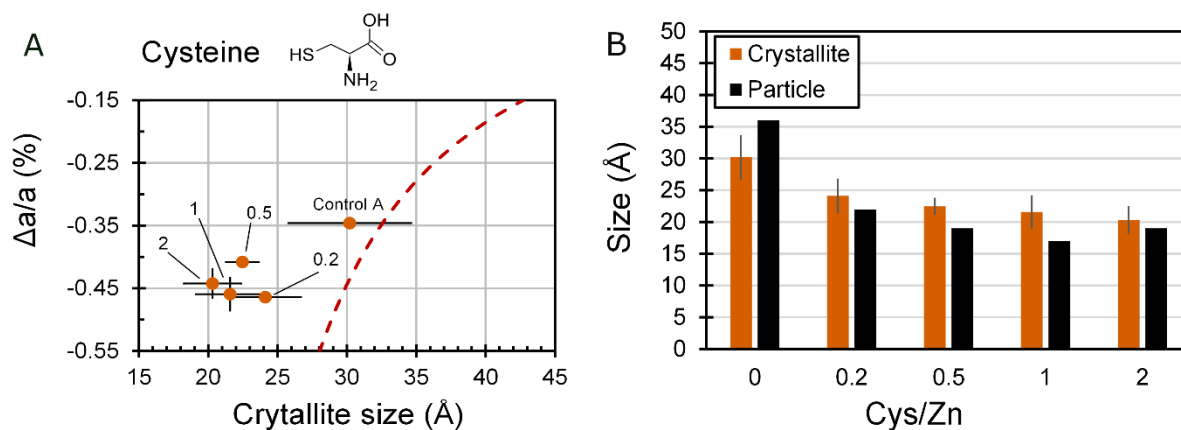


## 174 Results and Discussion

175 Environmentally relevant organic molecules with functional groups of varying affinity for Zn (-OH, -  
176 COOH, -NH<sub>2</sub>, -SH, imidazole) were used to investigate the role of these interactions in the size and  
177 strain of nano-ZnS. In the following, the structural properties of nano-ZnS synthesized in presence of  
178 organic molecules are compared with (i) a control nano-ZnS synthesized in the same conditions with  
179 no organic molecules and with (ii) the set of bare nano-ZnS represented by the quadratic function for  
180 which synthetic conditions were varied to obtain nano-ZnS of different sizes. The size-strain  
181 relationship obtained for bare nano-ZnS is presented in SI, part I. As expected based on previous  
182 literature data, this relationship can be modeled using a quadratic function.<sup>22</sup>

### 183 *Effect of thiol groups on size and strain*

184 Cysteine was used to assess the impact of thiol groups, which are known to have a strong affinity for  
185 ZnS surfaces.<sup>46</sup> In addition, cysteine has the highest stability constant with zinc ( $\log K = 9.11$ )<sup>47</sup>  
186 compared with other amino acids.



187

Figure 1: (A) Size-strain relationship of nano-ZnS formed in presence of cysteine (ZnS\_Cys/Zn\_0.2, ZnS\_Cys/Zn\_0.5, ZnS\_Cys/Zn\_1 and ZnS\_cys synthesized at a Cys/Zn molar ratio of 0.2, 0.5, 1 and 2, respectively) and a control nano-ZnS synthesized in the same conditions but with no OM (Control A). The Cys/Zn molar ratio are given for each dot in panel A. Crystallite sizes and strain were obtained

*from PDF refinement (see material and methods, section 1.2); the red dashed line corresponds to the fit of the bare nano-ZnS set of particles shown for the sake of comparison (described in SI, part I); (B) Crystallite and particle sizes obtained by PDF and SAXS refinements, respectively, depending on Cys/Zn. The values and error bars are averages and standard deviation of the 4 values obtained from 4 different fits of the experimental PDF function of one particle.*

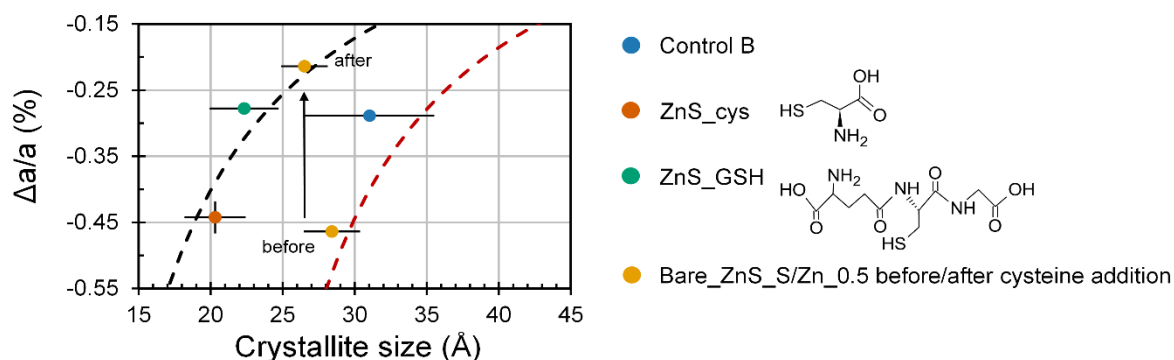
188 Nano-ZnS was synthesized using an increasing Cys/Zn ratio of from 0.2 to 2 and compared to a control  
189 synthesized with no cysteine (Control A). The size-strain relationship is shown in Figure 1 A.

190 The nano-ZnS synthesized in the presence of cysteine exhibited both smaller average crystallite  
191 domains and higher strain than the control nano-ZnS. The decrease in the size of the crystallite domain  
192 was accompanied by a decrease in particle size, as revealed by SAXS (Figure 1 B). In addition, for  
193 each particle, the sizes of the crystallite domain and of the particle were found to be identical within the  
194 range of the refinement error (Figure 1 B), strongly suggesting the particles formed were made of a  
195 single crystal (i.e. monocrystalline) and did not have a highly disordered surface layer and/or internal  
196 disorder.

197 Interestingly, when cysteine was added in the system at the beginning of the synthesis, the size-strain  
198 properties of the synthesized nano-ZnS did not vary as a function of the Cys/Zn ratio in the range 0.2 to  
199 2. At the lowest Cys/Zn ratio (ZnS\_Cys/Zn\_0.2), the estimated coverage of the surface of the nano-ZnS  
200 by cysteine molecules was 40% (Table S4) which seems sufficient to inhibit particle growth in a similar  
201 way as when cysteine was added in excess (ZnS\_cys with a Cys/Zn ratio of 2).

202 The surface density of thiol-surface interactions not only depends on the thiol-Zn ratio but also on the  
203 size of the thiol molecule (steric hindrance), which may also affect the size-strain properties of the  
204 nano-ZnS formed. In particular, if we consider environmental conditions, thiol molecules are often be  
205 bigger than cysteine molecules. The biomolecule glutathione (GSH) was chosen to investigate the

206 effect of the size of the organic molecules because it exhibits a thiol and other functional groups that  
207 are similar to cysteine but is significantly larger than cysteine (307.3 versus 121.16 g.mol<sup>-1</sup>).



208

Figure 2: Sizes and strains of nano-ZnS formed in the presence of cysteine, glutathione (ZnS\_cys and ZnS\_GSH), a control nano-ZnS synthesized in the same conditions but with no OM (Control B) and the bare nano-ZnS before and after exposure to cysteine (bare\_ZnS\_S/Zn\_0.5 and bare\_ZnS\_S/Zn\_0.5\_cys) obtained from PDF refinement (see material and methods, section 1.2). The red and black dashed lines correspond to the quadratic function that fits the size-strain relationship of the bare nano-ZnS set of particles (SI, part I) and the nano-ZnS particles formed in the presence of the thiol functional group (i.e. ZnS\_cys, ZnS\_GSH, bare\_ZnS\_S/Zn\_0.5\_cys), respectively. The values and error bars are averages and standard deviations of the 4 values obtained from 4 different fits of the experimental PDF function of one particle.

209 Nano-ZnS formed in the presence of GSH at a GSH/Zn ratio of 2 was found to be slightly bigger than  
210 nano-ZnS formed in the presence of cysteine (22 and 20 Å, for ZnS\_GSH and ZnS\_cys respectively,  
211 for an OM/Zn ratio of 2, Figure 2) but based on our reported refinement errors, the difference was not  
212 significant. However, ZnS\_GSH exhibited a lattice contraction of -0.28%, significantly lower than  
213 ZnS\_cys (-0.44%). Taken together, these two observations suggest that the presence of GSH during  
214 synthesis influenced nano-ZnS growth, but that the effects were less marked than with cysteine. Indeed,  
215 the higher steric hindrance for GSH could limit the number of thiol groups on the surface of nano-ZnS,

216 compared with cysteine. The lesser strain observed with GSH than with cysteine was in line with the  
217 size-strain relationship observed for bare nano-ZnS, which showed a decrease in strain with bigger  
218 crystallite sizes (see SI, part I).

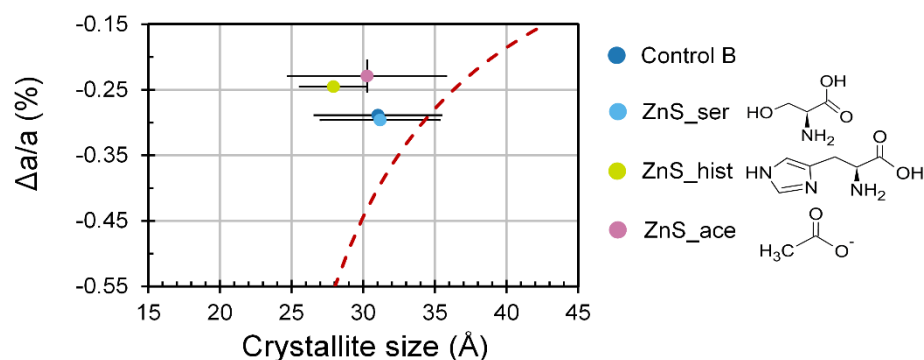
219 The results described above were obtained with thiol-bearing molecules that were added during the  
220 synthesis of nano-ZnS and that affected both the degree of particle growth and structural strain. To  
221 study the effect of thiol functional groups on lattice contraction independently of its effect on size,  
222 samples of as-prepared bare nano-ZnS (bare\_ZnS\_S/Zn\_0.5, with a size of 28 Å and a lattice  
223 contraction of -0.46 %, synthesized using the bare nano-ZnS method, SI Part I) were mixed with  
224 cysteine. This bare nano-ZnS sample was chosen because of its small size and high strain. In Figure 2,  
225 the dot corresponding to the bare nano-ZnS before the addition of cysteine (bare\_ZnS\_S/Zn\_0.5) fits  
226 on the red dashed line representing the bare nano-ZnS set (Figure S6). As shown by the two yellow  
227 dots in Figure 2, adding cysteine after nano-ZnS synthesis did not significantly alter the size of the  
228 nano-ZnS (27 Å), however, lattice contraction dropped to -0.21%. This suggests that binding functional  
229 groups (e.g., thiol) strongly to the surface reduces nano-ZnS internal strain and that this effect is  
230 independent of particle size. This strain release could be due to a similar mechanism to that reported by  
231 Zhang et al. (2003)<sup>48</sup> i.e., a decrease in internal energy and an increase in crystallinity induced by the  
232 interaction between water and the surface of the ZnS. However, additional observations with thiols  
233 added after the synthesis are needed to conclude on a systematic strain release.

234 The size-strain relationship of the cysteine-coated nano-ZnS formed in 2 steps  
235 (bare\_ZnS\_S/Zn\_0.5\_cys) is in line with the nano-ZnS formed directly in presence of cysteine or GSH  
236 (ZnS\_cys and ZnS\_GSH). In contrast, the nano-ZnS synthesized with no organic molecules (Control B  
237 and bare\_ZnS\_S/Zn\_0.5) are in line with the bare nano-ZnS size-strain relationship (red dashed line).  
238 Interestingly, as shown in Figure 2, the presence of thiol groups caused the size-strain relationship to  
239 shift to an approximately 1 nm size smaller than the one of bare nano-ZnS. Indeed, the black dashed

240 line in Figure 2 corresponds to the red dashed line (bare nano-ZnS size-strain relationship, Figure S6)  
241 offset by 1 nm. This suggests thiol-bearing molecules lead to the formation of smaller nano-ZnS when  
242 present before ZnS precipitation.

243 *Organic molecules with no effects or only slight effects on particle size and strain.*

244 To check if the cysteine and GSH effects on nano-ZnS structural properties were due to the affinity of  
245 thiol for the surface of ZnS, a set of nano-ZnS were synthesized in the presence of organic molecules  
246 that are known to have lower affinities for ZnS surface than thiol groups. Serine and histidine were  
247 chosen to study the effect of -hydroxyl and -imidazole functional groups compared with the effect of  
248 the thiol functional group present in cysteine. Indeed, these three molecules have a similar composition  
249 (-COOH and -NH<sub>2</sub> groups) except for the third functional group that varies (-SH, -OH and -imidazole  
250 groups, Figure 3). Acetate was chosen to investigate the effect of the carboxyl functional group (-  
251 COOH) alone.



252

Figure 3: Sizes and strains of nano-ZnS formed in the presence of serine, histidine, acetate (ZnS\_ser, ZnS\_hist and ZnS\_ace, respectively); a control nano-ZnS synthesized in the same conditions but with no OM (Control B), obtained from PDF refinement (see material and methods, section 1.2). The red dashed line corresponds to the fit of the bare nano-ZnS set of particles (SI, part I) shown for the sake of comparison. The values and error bars are averages and standard deviations of the 4 values obtained from 4 different fits for the experimental PDF function of one particle.

253 The size and strain values of ZnS\_ser, ZnS\_hist and ZnS\_ace, the nano-ZnS synthesized in the  
254 presence of serine, histidine, acetate (OM/Zn ratio of 2) and the corresponding control nano-ZnS  
255 synthesized in the same conditions but with no OM are presented in Figure 3 and SI part II.

256 No significant effect on crystallite domain size was observed for nano-ZnS formed in presence of  
257 serine, acetate and histidine (within the range of refinement error). This can be explained by the fact  
258 that the surface interactions of the organic molecules and nano-ZnS were not strong enough to impact  
259 particle growth.

260 However, the presence of histidine and acetate reduced the amount of strain of the as-formed nano-ZnS  
261 (-0.25 and -0.23% for ZnS\_hist and ZnS\_ace compared to -0.29% and -0.30% for the control nano-ZnS  
262 and ZnS\_ser). Molecular dynamics simulations were used to calculate binding energies between the  
263 surface of ZnS and various organic molecules, and showed that most molecules including serine,  
264 acetate and histidine, bind poorly to the surface of ZnS.<sup>46</sup> Histidine binding energy (3.06 kJ/mol) is  
265 slightly higher than for serine (1.95 kJ/mol). This suggests that the imidazole group of histidine has a  
266 slightly higher affinity for the nano-ZnS surface than for the hydroxide functional group of serine,<sup>46</sup>  
267 which could explain the slight decrease in strain. For acetate, the interaction mechanism is not clear  
268 (not considered in Nawrocki and Cieplak (2014))<sup>46</sup> but may originate from the hydrophobicity of the -  
269 CH<sub>3</sub> group in addition to potential surface affinity. Indeed, the ZnS surface is mostly hydrophobic<sup>46</sup> and  
270 the hydrophobic -CH<sub>3</sub> extremity of acetate has been shown to initiate interactions on other hydrophobic  
271 surfaces including gold.<sup>49</sup>

272 Nano-ZnS synthesized with serine (ZnS\_ser) exhibited similar crystallite domain size and strain to  
273 those of the control nano-ZnS. This suggests that serine and its functional groups, -COOH, -OH and -  
274 NH<sub>2</sub>, have no effects on the crystallite domain size and strain of nano-ZnS. As the thiol group was the  
275 only group that differed between cysteine and serine, we conclude that the interaction between the thiol  
276 group of cysteine and the nano-ZnS surface was responsible for affecting nano-ZnS structural

277 properties. This conclusion is supported by molecular dynamics simulations that showed a binding  
278 energy of 98 kJ/mol for cysteine on ZnS (110) surface, which is 1 to 2 orders of magnitude higher than  
279 for histidine and serine.<sup>46</sup> Such a difference can be explained by the formation of a covalent disulfide  
280 bond for cysteine at the ZnS surface.<sup>46</sup>

281 Based on nucleation and growth principles,<sup>50</sup> the thiol binding with Zn or S surface atoms during nano-  
282 ZnS formation could inhibit growth by blocking growth sites and consequently favor the formation of  
283 smaller nano-ZnS compared to the cysteine-free system. Also, if the surface energy decreases as a  
284 consequence of thiol binding, the system is thermodynamically more favorable for smaller crystal sizes  
285 (reduced critical nucleus size) than a cysteine-free system.

### 286 *Environmental implications*

287 Our results support the results obtained by Zhang et al. (2003)<sup>48</sup> showing that the composition of the  
288 medium determines the structure of nanoparticles through surface interactions. Here, we specifically  
289 investigated the role of different organic functional groups in nano-ZnS size and strain. These results  
290 also represent a step forward in understanding the formation of other analogous metallic nanoparticles  
291 and their fate in the environment. Indeed, nano-ZnS can be a model nanoparticle for several other  
292 sulfide metallic particles with a cubic structure that are formed in anoxic environments or manufactured  
293 (e.g. HgS, CdS).

294 There is also a critical need to understand the specific formation of nano-ZnS in the environment. One  
295 of the biggest pools of nano-ZnS formation is Zn-rich organic waste (OW)<sup>10</sup>, as nano-ZnS are  
296 systematically formed either during storage of liquid OW or during anaerobic digestion of solid and  
297 liquid OW.<sup>7</sup> Our results revealed the key role of thiol containing molecules in nano-ZnS formation. The  
298 organic composition of OW is continuously changing as organic matter is broken down into  
299 progressively smaller molecules during the process of digestion.<sup>51</sup> Thiols are one of the main species of  
300 sulfur involved in these systems.<sup>52</sup> Therefore, thiols are expected to interact with nano-ZnS either

301 during or after their formation. For example, if they are present during nano-ZnS formation, the  
302 thiolated molecules in OW could be a factor explaining the small size of nano-ZnS in OW<sup>5, 6</sup> or in other  
303 organic-rich matrices such as biofilms.<sup>4</sup>

304 The mechanisms controlling the fate of these small nano-ZnS also need further investigation. Indeed,  
305 small nano-ZnS (3-5 nm) were seen to undergo transformation in soil much faster (within months)<sup>5, 9</sup>  
306 than their 25-40 nm homologs (within years).<sup>53</sup> The size threshold below which new properties of  
307 nanomaterial are observed is typically 20-30 nm.<sup>54</sup> These specific nanoscale properties could be related  
308 to size-dependent properties such as lattice contraction, thermal properties, or interfacial reactivity.<sup>54</sup>  
309 More specifically, Huang et al. (2007)<sup>55</sup> showed that the effect of size on lattice contraction was  
310 significant only in the case of metallic nanoparticles less than 5 nm in size. Consequently, we  
311 hypothesize that such a size-strain relationship in nano-ZnS smaller than 5 nm could explain the  
312 difference in reactivity between them and their 25-40 nm homologs. Beyond intrinsic nano-ZnS  
313 properties (size, strain, organic coating), extrinsic properties (medium composition, e.g. soil properties)  
314 also influence the fate of nano-ZnS. For example, we recently showed that the dissolution kinetics of  
315 nano-ZnS applied on soil and the availability of released ions ( $Zn^{2+}$ ) were controlled by the soil  
316 properties (e.g. mineralogy, texture).<sup>9</sup> However, we still need to see if, in such complex environments,  
317 i.e. amended soils, the effect of intrinsic properties plays a more important role in the transformation of  
318 these incidental nano-ZnS than extrinsic properties.

## 319 **Supporting information**

320 Size-strain relationship for the bare nano-ZnS set, experimental data for the nano-ZnS synthesized with  
321 organic molecules (Pair Distribution Function, Small Angle X-ray Scattering), surface coverage  
322 estimation.



## 323 **Conflicts of interest**

324 The authors have no conflicts to declare

## 325 **Acknowledgments**

326 We are grateful to the French Environment and Energy Management Agency (ADEME) and the French  
327 Agricultural Research Centre for International Development (CIRAD) for funding the PhD scholarship  
328 of Maureen Le Bars. This study was part of the DIGESTATE project, funded by the French National  
329 Research Agency (ANR) under Grant ANR-15-CE34-0003-01. We acknowledge Advanced Photon  
330 Source (APS) for access to synchrotron radiation facilities and we would like to thank Olaf J.  
331 Borkiewicz for assistance in using the 11-ID-B beamline. F.M.M. acknowledges support from the  
332 National Science Foundation (NSF) through CAREER-1652237 and the Virginia Tech Center for Earth  
333 and Environmental Nanotechnology “NanoEarth” (NNCI-1542100).

## 334 **References**

- 335 1. X. S. Fang, T. Y. Zhai, U. K. Gautam, L. Li, L. M. Wu, B. Yoshio and D. Golberg, ZnS  
336 nanostructures: From synthesis to applications, *Prog Mater Sci*, **2011**, 56, 175-287.
- 337 2. G. J. Lee and J. J. Wu, Recent developments in ZnS photocatalysts from synthesis to  
338 photocatalytic applications - A review, *Powder Technol*, **2017**, 318, 8-22.
- 339 3. M. Labrenz, G. K. Druschel, T. Thomsen-Ebert, B. Gilbert, S. A. Welch, K. M. Kemner, G. A.  
340 Logan, R. E. Summons, G. De Stasio, P. L. Bond, B. Lai, S. D. Kelly and J. F. Banfield,  
341 Formation of sphalerite (ZnS) deposits in natural biofilms of sulfate-reducing bacteria, *Science*,  
342 **2000**, 290, 1744-1747.
- 343 4. J. W. Moreau, R. I. Webb and J. F. Banfield, Ultrastructure, aggregation-state, and crystal  
344 growth of biogenic nanocrystalline sphalerite and wurtzite, *Am Mineral*, **2004**, 89, 950-960.
- 345 5. T. A. Formentini, S. Legros, C. V. S. Fernandes, A. Pinheiro, M. Le Bars, C. Levard, F. J. K.  
346 Mallmann, M. da Veiga and E. Doelsch, Radical change of Zn speciation in pig slurry amended  
347 soil: Key role of nano-sized sulfide particles, *Environmental Pollution*, **2017**, 222, 495-503.
- 348 6. B. Kim, C. Levard, M. Murayama, G. E. Brown and M. F. Hochella, Integrated Approaches of  
349 X-Ray Absorption Spectroscopic and Electron Microscopic Techniques on Zinc Speciation and  
350 Characterization in a Final Sewage Sludge Product, *J Environ Qual*, **2014**, 43, 908-916.

- 351 7. M. Le Bars, S. Legros, C. Levard, P. Chaurand, M. Tella, M. Rovezzi, P. Browne, J. Rose and E.  
352 Doelsch, Drastic Change in Zinc Speciation during Anaerobic Digestion and Composting:  
353 Instability of Nanosized Zinc Sulfide, *Environmental Science & Technology*, **2018**, 52, 12987-  
354 12996.
- 355 8. S. Legros, C. Levard, C. E. Marcato-Romain, M. Guiresse and E. Doelsch, Anaerobic Digestion  
356 Alters Copper and Zinc Speciation, *Environmental Science & Technology*, **2017**, 51, 10326-  
357 10334.
- 358 9. M. Le Bars, S. Legros, C. Levard, C. Chevassus-Rosset, M. Montes, M. Tella, D. Borschneck,  
359 A. Guihou, B. Angeletti and E. Doelsch, Contrasted fate of zinc sulfide nanoparticles in soil  
360 revealed by a combination of X-ray absorption spectroscopy, diffusive gradient in thin films and  
361 isotope tracing, *Environ Pollut*, **2022**, 292, 118414.
- 362 10. C. Levard, M. Le Bars, T. Formentini, S. Legros and E. Doelsch, Organic waste-borne ZnS  
363 nanoparticles: The forgotten ones, *Environmental Pollution*, **2022**, 308.
- 364 11. M. Hawkesford, W. Horst, T. Kichey, H. Lambers, J. Schjoerring, I. S. Moller and P. White;  
365 Functions of Macronutrients. In *Marschner's Mineral Nutrition of Higher Plants (Third  
366 Edition)*, Marschner, P., Eds., 3rd edn., 2012, ch. 6, pp. 135-189.
- 367 12. A. E. Lewis, Review of metal sulphide precipitation, *Hydrometallurgy*, **2010**, 104, 222-234.
- 368 13. C. Andreini, L. Banci, I. Bertini and A. Rosato, Counting the zinc-proteins encoded in the  
369 human genome, *J Proteome Res*, **2006**, 5, 196-201.
- 370 14. M. Broadley, P. Brown, I. Cakmak, Z. Rengel and F. J. Zhao; Function of Nutrients:  
371 Micronutrients. In *Marschner's Mineral Nutrition of Higher Plants (Third Edition)*, Marschner,  
372 P., Eds., 3rd edn., 2012, ch. 7, pp. 191-248.
- 373 15. B. J. Alloway, Soil factors associated with zinc deficiency in crops and humans, *Environ  
374 Geochem Hlth*, **2009**, 31, 537-548.
- 375 16. N. M. Lowe, The global challenge of hidden hunger: perspectives from the field, *P Nutr Soc*,  
376 **2021**, 80, 283-289.
- 377 17. E. J. M. Joy, E. L. Ander, S. D. Young, C. R. Black, M. J. Watts, A. D. C. Chilimba, B. Chilima,  
378 E. W. P. Siyame, A. A. Kalimpira, R. Hurst, S. J. Fairweather-Tait, A. J. Stein, R. S. Gibson, P. J.  
379 White and M. R. Broadley, Dietary mineral supplies in Africa, *Physiol Plantarum*, **2014**, 151,  
380 208-229.
- 381 18. A. M. Chaudri, C. M. G. Allain, V. L. Barbosa-Jefferson, F. A. Nicholson, B. J. Chambers and  
382 S. P. McGrath, A study of the impacts of Zn and Cu on two rhizobial species in soils of a long-  
383 term field experiment, *Plant Soil*, **2000**, 221, 167-179.
- 384 19. M. R. Broadley, P. J. White, J. P. Hammond, I. Zelko and A. Lux, Zinc in plants, *New Phytol*,  
385 **2007**, 173, 677-702.
- 386 20. H. Z. Zhang, B. Chen and J. F. Banfield, Particle Size and pH Effects on Nanoparticle  
387 Dissolution, *J Phys Chem C*, **2010**, 114, 14876-14884.
- 388 21. B. Gilbert, F. Huang, H. Z. Zhang, G. A. Waychunas and J. F. Banfield, Nanoparticles: Strained  
389 and stiff, *Science*, **2004**, 305, 651-654.
- 390 22. Q. Jiang, L. H. Liang and D. S. Zhao, Lattice contraction and surface stress of fcc nanocrystals,  
391 *J Phys Chem B*, **2001**, 105, 6275-6277.

- 392 23. S. H. Lu, T. F. Chen, A. J. Wang, Z. L. Wu and Y. S. Wang, Lattice and optical property  
393 evolution of ultra-small ZnS quantum dots grown from a single-source precursor, *Appl Surf Sci*,  
394 **2014**, 299, 116-122.
- 395 24. A. S. Masadeh, E. S. Bozin, C. L. Farrow, G. Paglia, P. Juhas, S. J. L. Billinge, A. Karkamkar  
396 and M. G. Kanatzidis, Quantitative size-dependent structure and strain determination of CdSe  
397 nanoparticles using atomic pair distribution function analysis, *Phys Rev B*, **2007**, 76, 115413.
- 398 25. M. Mavrikakis, B. Hammer and J. K. Nørskov, Effect of Strain on the Reactivity of Metal  
399 Surfaces, *Phys Rev Lett*, **1998**, 81, 2819-2822.
- 400 26. P. Strasser, S. Koh, T. Anniyev, J. Greeley, K. More, C. F. Yu, Z. C. Liu, S. Kaya, D. Nordlund,  
401 H. Ogasawara, M. F. Toney and A. Nilsson, Lattice-strain control of the activity in dealloyed  
402 core-shell fuel cell catalysts, *Nat Chem*, **2010**, 2, 454-460.
- 403 27. A. Pratt, L. Lari, O. Hovorka, A. Shah, C. Woffinden, S. P. Tear, C. Binns and R. Kroger,  
404 Enhanced oxidation of nanoparticles through strain-mediated ionic transport, *Nat Mater*, **2014**,  
405 13, 26-30.
- 406 28. B. Gilbert, F. Huang, Z. Lin, C. Goodell, H. Zhang and J. F. Banfield, Surface Chemistry  
407 Controls Crystallinity of ZnS Nanoparticles, *Nano Letters*, **2006**, 6, 605-610.
- 408 29. J. W. Moreau, P. K. Weber, M. C. Martin, B. Gilbert, I. D. Hutcheon and J. F. Banfield,  
409 Extracellular proteins limit the dispersal of biogenic nanoparticles, *Science*, **2007**, 316, 1600-  
410 1603.
- 411 30. A. Deonaraine, B. L. T. Lau, G. R. Aiken, J. N. Ryan and H. Hsu-Kim, Effects of Humic  
412 Substances on Precipitation and Aggregation of Zinc Sulfide Nanoparticles, *Environmental*  
413 *Science & Technology*, **2011**, 45, 3217-3223.
- 414 31. A. P. Gondikas, E. K. Jang and H. Hsu-Kim, Influence of amino acids cysteine and serine on  
415 aggregation kinetics of zinc and mercury sulfide colloids, *J Colloid Interf Sci*, **2010**, 347, 167-  
416 171.
- 417 32. A. P. Gondikas, A. Masion, M. Auffan, B. L. T. Lau and H. Hsu-Kim, Early-stage precipitation  
418 kinetics of zinc sulfide nanoclusters forming in the presence of cysteine, *Chem Geol*, **2012**, 329,  
419 10-17.
- 420 33. B. L. T. Lau and H. Hsu-Kim, Precipitation and growth of zinc sulfide nanoparticles in the  
421 presence of thiol-containing natural organic ligands, *Environmental Science & Technology*,  
422 **2008**, 42, 7236-7241.
- 423 34. W. Bae and R. K. Mehra, Cysteine-capped ZnS nanocrystallites: Preparation and  
424 characterization, *J Inorg Biochem*, **1998**, 70, 125-135.
- 425 35. W. O. Bae, R. Abdullah, D. Henderson and R. K. Mehra, Characteristics of glutathione-capped  
426 ZnS nanocrystallites, *Biochem Bioph Res Co*, **1997**, 237, 16-23.
- 427 36. L. M. Devi and D. P. S. Negi, Effect of starting pH and stabilizer/metal ion ratio on the  
428 photocatalytic activity of ZnS nanoparticles, *Mater Chem Phys*, **2013**, 141, 797-803.
- 429 37. R. Kho, L. Nguyen, C. L. Torres-Martinez and R. K. Mehra, Zinc-histidine as nucleation centers  
430 for growth of ZnS nanocrystals, *Biochem Bioph Res Co*, **2000**, 272, 29-35.
- 431 38. P. J. Chupas, K. W. Chapman and P. L. Lee, Applications of an amorphous silicon-based area  
432 detector for high-resolution, high-sensitivity and fast time-resolved pair distribution function  
433 measurements, *J Appl Crystallogr*, **2007**, 40, 463-470.

- 434 39. P. J. Chupas, K. W. Chapman, G. Jennings, P. L. Lee and C. P. Grey, Watching nanoparticles  
435 grow: The mechanism and kinetics for the formation of TiO<sub>2</sub>-supported platinum nanoparticles,  
436 *J Am Chem Soc*, **2007**, 129, 13822-13824.
- 437 40. A. Hoehner, S. Mergelsberg, O. J. Borkiewicz, P. M. Dove and F. M. Michel, A new method for  
438 in situ structural investigations of nano-sized amorphous and crystalline materials using mixed-  
439 flow reactors, *Acta Crystallogr A*, **2019**, 75, 758-765.
- 440 41. A. P. Hammersley, S. O. Svensson, M. Hanfland, A. N. Fitch and D. Hausermann, Two-  
441 dimensional detector software: From real detector to idealised image or two-theta scan, *High*  
442 *Pressure Res*, **1996**, 14, 235-248.
- 443 42. P. Juhas, T. Davis, C. L. Farrow and S. J. L. Billinge, PDFgetX3: a rapid and highly  
444 automatable program for processing powder diffraction data into total scattering pair  
445 distribution functions, *J Appl Crystallogr*, **2013**, 46, 560-566.
- 446 43. C. L. Farrow, P. Juhas, J. W. Liu, D. Bryndin, E. S. Bozin, J. Bloch, T. Proffen and S. J. L.  
447 Billinge, PDFfit2 and PDFgui: computer programs for studying nanostructure in crystals, *J*  
448 *Phys-Condens Mat*, **2007**, 19.
- 449 44. B. J. Skinner, Unit-Cell Edges of Natural and Synthetic Sphalerites, *Am Mineral*, **1961**, 46,  
450 1399-1411.
- 451 45. M. Terpilovskii, scikit-posthocs: Pairwise multiple comparison tests in Python, *J. Open Source*  
452 *Softw.*, **2019**, 4, 1169.
- 453 46. G. Nawrocki and M. Cieplak, Interactions of aqueous amino acids and proteins with the (110)  
454 surface of ZnS in molecular dynamics simulations, *J Chem Phys*, **2014**, 140, 095101.
- 455 47. D. R. Burgess, NIST SRD 46. Critically Selected Stability Constants of Metal Complexes:  
456 Version 8.0 for Windows, *National Institute of Standards and Technology*, **2004**, DOI:  
457 <https://doi.org/10.18434/M32154>.
- 458 48. H. Z. Zhang, B. Gilbert, F. Huang and J. F. Banfield, Water-driven structure transformation in  
459 nanoparticles at room temperature, *Nature*, **2003**, 424, 1025-1029.
- 460 49. M. Hoefling, F. Iori, S. Corni and K. E. Gottschalk, Interaction of Amino Acids with the  
461 Au(111) Surface: Adsorption Free Energies from Molecular Dynamics Simulations, *Langmuir*,  
462 **2010**, 26, 8347-8351.
- 463 50. J. J. De Yoreo and P. G. Vekilov, Principles of crystal nucleation and growth, *Rev Mineral*  
464 *Geochem*, **2003**, 54, 57-93.
- 465 51. D. Deublein and A. Steinhauser, *Biogas from waste and renewable resources: an introduction*,  
466 John Wiley & Sons, 2011.
- 467 52. E. D. van Hullebusch, S. S. Yekta, B. Calli and F. G. Fermoso; Biogeochemistry of major  
468 elements in anaerobic digesters: carbon, nitrogen, phosphorus, sulfur and iron. In *Trace*  
469 *Elements in Anaerobic Biotechnologies*, Fermoso, F. G., van Hullebusch, E. D., Collins, G.,  
470 Roussel, J., Mucha, A. P. and Esposito, G., Eds., 2019, ch. 1, pp. 1-22.
- 471 53. A. Voegelin, O. Jacquat, S. Pfister, K. Barmettler, A. C. Scheinost and R. Kretzschmar, Time-  
472 Dependent Changes of Zinc Speciation in Four Soils Contaminated with Zincite or Sphalerite,  
473 *Environmental Science & Technology*, **2011**, 45, 255-261.

- 474 54. M. Auffan, J. Rose, J. Y. Bottero, G. V. Lowry, J. P. Jolivet and M. R. Wiesner, Towards a  
475 definition of inorganic nanoparticles from an environmental, health and safety perspective, *Nat*  
476 *Nanotechnol*, **2009**, 4, 634-641.
- 477 55. Z. X. Huang, P. Thomson and S. L. Di, Lattice contractions of a nanoparticle due to the surface  
478 tension: A model of elasticity, *J Phys Chem Solids*, **2007**, 68, 530-535.
- 479