

1 *Assessing the interactions of metal nanoparticles in soil*  
2 *and sediment matrices – A quantitative analytical multi-*  
3 *technique approach*

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11 **Abstract**

12 The impact and behavior of engineered nanomaterials (ENMs) entering the environment is an  
13 important issue due to their growing use in consumer and agricultural products. Their mobility  
14 and fate in the environment are heavily impacted by their interactions with natural particle  
15 components of saturated sediments and soils. In this study, functionalized gold nanoparticles  
16 (AuNPs – used as model ENMs) were spiked into complex solid-containing media (standard  
17 soils and estuarine sediment in moderately hard water). AuNPs were characterized in the  
18 colloidal extract (< 1  $\mu\text{m}$ ) following centrifugal separation of the non-colloidal phase, using  
19 different analytical techniques including asymmetric-flow field-flow fractionation and single  
20 particle inductively coupled plasma mass spectrometry. Attachment of functionalized AuNPs to  
21 the soil particles did not significantly depend on their concentration or surface coating (citrate,  
22 bPEI, PVP, PEG). Similarly, UV degradation of coatings did not substantially alter their  
23 recovery. Conversely, the presence of natural organic matter (NOM) is a key factor in their  
24 adhesion to matrix particles, by decreasing the predicted influence of native surface chemistry  
25 and functional coatings. A kinetic experiment performed over 48 h showed that attachment to  
26 soil colloids is rapid and that hetero-aggregation is dominant. These results suggest that transport  
27 of ENMs away from the point of discharge (or entry) could be limited in soils and sediments, but  
28 additional experiments under more realistic and dynamic field conditions would be necessary to  
29 confirm this more generally. Transport properties may also differ substantially in matrices where  
30 NOM is largely absent or otherwise sequestered or when dissolution of ENMs is an important  
31 factor.

32

33 **Keywords:** metal nanoparticles, engineered nanomaterials, natural colloids, aggregation, field  
34 flow fractionation, single particle mass spectrometry, transport, fate

## 35 **1 Introduction**

36 Because of the wide range of applications for engineered nanomaterials (ENMs) in industry,  
37 medicine, consumer products and agriculture, the nanotechnology field has witnessed rapid  
38 development. Consequently, the number of studies focused on ENMs is increasing rapidly.  
39 However, knowledge of the physico-chemical state and reactivity in environmentally relevant  
40 media and validated tools to measure those properties remain a significant challenge.<sup>1,2</sup>

41 Soils (and sediments) are rich environmental matrices with respect to naturally occurring  
42 colloids (nano- to micro-scale). Soil colloids (operationally defined by a size between 1 nm and 1  
43  $\mu\text{m}^3$ ) typically carry a net electrostatic charge (negative in most cases) and/or have hydrophobic  
44 characteristics that may promote interactions with natural or anthropogenic species (e.g., trace  
45 elements, ENMs, etc.). These colloids are composed of mineral (clay, iron oxides, etc.) and/or  
46 organic (natural organic matter – NOM) components, and play a central role in determining the  
47 behavior, mobility and fate of ENMs that enter the environment. Correspondingly, colloidal  
48 transport through soils has been widely studied for metallic trace element fate<sup>4-8</sup>. The most  
49 important sources of entry for ENMs into soils arise from application of wastewater sludge as a  
50 soil amendment, intentional releases for environmental applications (e.g., zero valence iron  
51 nanoparticles<sup>9,10</sup>) and accidental spillages<sup>11</sup>; ENMs may also enter the environment via aerosols  
52 that subsequently deposit into soil or aquatic systems and from end-of-life disposal in landfills.

53 A number of studies have examined the behavior of ENMs in environmental matrices and  
54 more particularly in soils<sup>12-18</sup>. The aggregation rate including homo-aggregation (ENM-ENM)  
55 and hetero-aggregation (ENM-natural colloid) is a key parameter to assess the reactivity,  
56 transport and bioavailability of ENMs in the soil compartment and their potential impact on the  
57 biota and groundwater<sup>19</sup>. Here we use the term ‘aggregation’ in the generic form (i.e., equivalent  
58 to agglomeration), due to its historical use in this context.

59 The principal methods used to detect and characterize ENMs in soils and sediments include  
60 transmission electronic microscopy (TEM)<sup>15</sup>, hydrodynamic chromatography (HDC)<sup>14</sup>,  
61 asymmetrical flow field-flow fractionation (AF4)<sup>12</sup> and dynamic light scattering (DLS)<sup>13</sup>. Both  
62 HDC and AF4 are typically coupled in tandem to specific detectors, such as DLS, inductively  
63 coupled plasma mass spectrometry (ICP-MS), or fluorescence. The benefits of stable isotope  
64 detection with ICP-MS for quantifying and differentiating ENMs from naturally occurring  
65 species has also been investigated<sup>20</sup>.

66 Given the low environmental concentrations of ENMs, pre-concentration methods are often  
67 needed for analytical techniques with higher limits of quantification (LOQ). Thus, our previous  
68 study focused on cloud point extraction applied to a complex soil matrix (extract) spiked with  
69 gold nanoparticles (AuNPs)<sup>21</sup>. In the present study, however, spiked analyte concentrations are  
70 sufficiently high (above expected environmental levels) to negate the need for pre-concentration,  
71 as the purpose here is to validate the general analytical approach and to demonstrate its potential  
72 to interrogate ENM-matrix interactions. This work builds on our previous studies by assessing  
73 the behavior and fate of model AuNPs in soil and sediment matrices under different conditions  
74 using a standard leaching test procedure. AuNPs were chosen because they are relatively stable  
75 and easy to detect. Moreover, surface coatings tend to play a determinant factor with respect to  
76 the interaction of metal NPs with complex media (unless the cores are significantly unstable)<sup>22-</sup>  
77 <sup>24</sup>. To this end, a method of extraction for complex solid matrices (soils and sediments), derived  
78 from the US Environmental Protection Agency<sup>25</sup> and the International Organization for  
79 Standardization 18772:2008<sup>26</sup>, was applied. EPA 1316 and ISO 18772:2008 are guidance  
80 methods on leaching procedures. They provide information on the liquid/solid partitioning of  
81 contaminants (inorganic, non-volatile organic and natural radionuclides). For comparison  
82 purposes the leaching procedure is generally standardized by setting conventional operating  
83 parameters, allowing a higher degree of robustness, repeatability, reproducibility and a broad  
84 applicability to different types of soils<sup>26</sup>. The leaching method was used as a means to obtain an  
85 extract (eluate) from a solid matrix, which may be used to estimate the release of the most  
86 available fraction of soil colloids from spiked samples under laboratory conditions. Laboratory  
87 conditions do not aim to fully simulate actual field conditions, but rather this approach offers  
88 improved insight into the behavior of ENMs in complex solid matrices and provides proof of  
89 principle for the combined analytical methodology demonstrated here. It is important to note that  
90 batch methods represent a given time frame (which may vary) in which equilibrium is not  
91 necessarily reached<sup>27-29</sup>. The relative merits of batch versus dynamic column tests in predicting  
92 transport and fate is beyond the scope of the present work.

93 Analytically, we employ hyphenation of AF4 with tandem detection methods, including  
94 ultra-violet visible (UV-Vis) absorption, multi-angle light scattering (MALS) and ICP-MS,  
95 which have proven particularly valuable and flexible in our previous work<sup>20, 21</sup>. This coupling  
96 provides information about the size, but also about the nature and composition of NPs under

97 relevant conditions, and with minimal perturbation of the analyte. Additionally, other  
98 complementary techniques were used, such as single particle (sp) ICP-MS, in order to determine  
99 the AuNP size distribution and to assess the homo-aggregation rate. In this study, AuNPs with  
100 different surface coatings and concentrations were spiked into the matrix before extraction to  
101 assess the Au distribution between the more massive soil particles and the colloidal fraction  
102 (nominally  $< 1 \mu\text{m}$  for the present work). A kinetic study was also performed on soil samples  
103 spiked with citrate-capped AuNPs, up to 48 h. Finally, the potential role of surface coating  
104 degradation on the AuNP-soil interaction was evaluated using UV exposure as a model approach  
105 to induce coating degradation of polyethylene glycol (PEG) capped AuNPs.

## 106 **2 Materials and methods**

### 107 **2.1 Chemicals**

108 Deionized (DI) water ( $>18 \text{ M}\Omega \text{ cm}$ ) was supplied by a Type II biological grade water  
109 purification system (Aqua Solutions, Jasper, GA, USA)<sup>†</sup> and was utilized for all sample  
110 preparation and dilution. AuNPs coated with PVP (10 kDa), PEG (5 kDa) and bPEI (25 kDa)  
111 with a nominal size of 30 nm were purchased from Nanocomposix (San Diego, CA, USA). 30  
112 nm and 60 nm citrate (Cit) AuNPs were obtained from Ted Pella (Redding, CA, USA). To  
113 prepare AuNPs (from Cit-AuNPs) coated with NOM, commercial humic acid (Suwannee River  
114 Humic Acid Standard II, HA) from the International Humic Substances Society (IHSS, St. Paul,  
115 MN, USA) and HA extracted directly from the soil sample (see protocol in Electronic  
116 Supporting Information - ESI) were used. Ammonium nitrate ( $\text{NH}_4\text{NO}_3$ , 99 %) was used for the  
117 AF4 mobile phase after dilution with DI water (to reach a concentration of  $0.5 \text{ mmol L}^{-1}$ ) and  
118 filtration through a  $0.2 \mu\text{m}$  regenerated cellulose membrane. Concentrated nitric,  $\text{HNO}_3$ , ( $\geq 69$   
119 %) and hydrochloric,  $\text{HCl}$ , ( $\geq 37 \%$ ) acids (Fluka TraceSelect, Sigma-Aldrich, St. Louis, MO,  
120 USA) were used to digest AuNPs prior to ICP-MS experiments for trace analysis. Elemental  
121 calibration standards were prepared from NIST SRM 3121 Au and SRM 3140 Pt standard  
122 solutions.

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<sup>†</sup> The identification of any commercial product or trade name does not imply endorsement or recommendation by the National Institute of Standards and Technology.

## 123 2.2 Sample preparation

### 124 2.2.1 Soil extraction

125 In this study, three different environmental matrices were utilized:

- 126 - Nebraska SONE-1 (internal reference agricultural soil) obtained from the US Geological
- 127 Survey (USGS); this sample was used for most experiments reported in the present study,
- 128 - NIST SRM 2709a (San Joaquin Soil), an agricultural soil collected by the USGS from a
- 129 fallow field in central California's San Joaquin Valley<sup>30</sup>,
- 130 - NIST SRM 1646a (Estuarine Sediment) dredged from the Chesapeake Bay<sup>31</sup>.

131 Information provided by the producer on the corresponding certificates indicated that the matrix  
132 materials were sieved at 2 mm for the two soil samples and at 1 mm for the sediment, to remove  
133 the coarsest fraction; materials were then ground to a particle size below 100  $\mu\text{m}$  ( $< 90 \mu\text{m}$  for  
134 SONE-1 and 75  $\mu\text{m}$  for the two SRMs). In general, particulates with a higher specific surface  
135 area are likely to present a more reactive material and to represent the greater portion of available  
136 surface area (on a mass basis) within the matrix.

137 Fig. 1 schematically shows the extraction procedure based on EPA 1316 and ISO  
138 18772:2008 leaching methods and previous work performed in our laboratory<sup>20, 21, 25, 26</sup>.  
139 Moderately Hard Water (MHW) was used as the leachant (i.e., the liquid used in a leaching test)  
140 and was prepared according to the EPA recommendation i.e., 96  $\text{mg L}^{-1}$   $\text{NaHCO}_3$ , 60  $\text{mg L}^{-1}$   
141  $\text{CaSO}_4 \cdot \text{H}_2\text{O}$ , 60  $\text{mg L}^{-1}$   $\text{MgSO}_4$  and 4  $\text{mg L}^{-1}$   $\text{KCl}$ <sup>32</sup>. It is generally preferable to use leachant  
142 that provides an ionic strength closer to typical environmental media rather than DI water alone,  
143 and MHW has been used extensively for environmental studies. The calculated ionic strength is  
144 3.3  $\text{mmol L}^{-1}$ . A liquid/solid (L/S) ratio of 10:1 was used, containing 1 g of solid matrix and 10 g  
145 of leachant. This ratio is somewhat arbitrary in nature, but has been commonly used in previous  
146 studies and proved both workable and reproducible. AuNPs were spiked into soil samples by  
147 addition of a small volume (100  $\mu\text{L}$ ) into the solid-leachant system, to obtain concentrations in  
148 the range from (10 to 5000)  $\mu\text{g kg}^{-1}$ . The resulting preparations were agitated for 24 h using an  
149 end-over-end rotator.

150 A centrifugation technique was used to isolate soil colloids  $\leq 1 \mu\text{m}$  (total range of the  
151 colloidal fraction) and  $< 0.45 \mu\text{m}$  (commonly used threshold). The settling time to obtain the two

152 different size fractions was determined using the equation:  $t = \frac{18\eta \ln(\frac{R}{S})}{\omega^2 d^2 \Delta\rho}$  where  $\Delta\rho$  is the density

153 difference between the particles and the medium,  $R$  (115 mm) and  $S$  (minimum 64 mm) represent  
154 the distances from the axis of rotation at the bottom and at the top of the solution, respectively,  $\omega$   
155 is the angular velocity of the centrifuge,  $d$  the particle (equivalent spherical) diameter and  $\eta$  is the  
156 viscosity of the suspension medium (i.e., water). The densities considered are  $2.65 \text{ g cm}^{-3}$  for soil  
157 (an estimated mean value) and  $19.3 \text{ g cm}^{-3}$  for Au<sup>33,34</sup>. This method assumes that the particles in  
158 suspension are spheres and behave according to Stokes law; therefore, an unavoidable  
159 uncertainty on the size fraction exists and the obtained fractions should be viewed as nominal  
160 approximations. A Beckman J2-HC centrifuge (Beckmann Coulter Life Sciences, Indianapolis  
161 IN, USA) using a J20.1 fixed angle rotor (15 mL tube volume) was used. The centrifugation  
162 speed was set at 2000 rpm (515 g at  $R$ ). In a first step, a centrifugation time of 2 min was applied  
163 to remove material  $> 1 \mu\text{m}$ . The supernatant was collected and a small part of it was put aside for  
164 analysis. In a second step, the fraction  $\leq 1 \mu\text{m}$  was centrifuged for 10 min to remove material  $>$   
165  $0.45 \mu\text{m}$ . Under these conditions Au particles smaller than 300 nm and 140 nm remain in  
166 suspension for the soil colloidal thresholds of  $1 \mu\text{m}$  and  $0.45 \mu\text{m}$ , respectively.

167 To digest AuNPs in soil extracts, either in solution or attached to soil colloids, undiluted  
168 aqua regia (1:3;  $\text{HNO}_3$ : $\text{HCl}$ ) was added to the aliquots of the supernatants. These solutions were  
169 then left at room temperature overnight in a fume hood. This was followed by dilution with DI  
170 water (containing 0.1 % thiourea to avoid memory effects in the instrument and to stabilize Au)  
171 to reach 2 % aqua regia. Total gold concentration was then determined by ICP-MS. The  
172 digestion procedure was verified by spiking a known mass quantity of AuNPs in a soil extract ( $<$   
173  $0.45 \mu\text{m}$ ) followed by ICP-MS analysis; this yielded total Au content within experimental  
174 uncertainty. The potential for  $\text{AuCl}_3$  precipitation was mitigated due to the large excess of Cl  
175 (from aqua regia) over Au in all samples.

176 To verify that the AuNPs did not adhere substantially to the centrifuge tube walls  
177 (polypropylene), AuNPs were agitated in the absence of the soil matrix under the same  
178 conditions. Results (not shown) indicate the percentage of Au remaining in solution after  
179 agitation is  $> 95 \%$  for Cit-, PVP- and PEG- coatings. bPEI-AuNPs (positively charged)  
180 presented a significant loss (70 %). The affinity of bPEI-AuNPs for the soil particles is relatively  
181 high (compared to other coatings) due to the soil's net negative charge, whereas the container  
182 surface is neutral and has a total area that is relatively small compared with the colloidal fraction.  
183 Based on these results, we conclude that analyte loss to the container surface represents a

184 relatively small effect, of order 5 % or less; this effect may be measurably higher for bPEI coated  
185 particles, but the soil colloids should outcompete the container surface for adsorption in an  
186 agitated system.

### 187 **2.2.2 UV exposure**

188 AuNP suspensions were irradiated at a nominal concentration of 10 mg L<sup>-1</sup> in DI water.  
189 Samples were held in quartz vials in a photoreactor (Rayonet RMR-600, Southern New England  
190 Ultraviolet Co. - SNEUCo, Branford, CT) equipped with a carousel and eight lamps (RMR-  
191 3500A, SNEUCo) emitting UV light centered at 350 nm and ranging from (300 to 400) nm. The  
192 irradiance was estimated to be  $\approx 30 \text{ W/m}^2$  by ferrioxalate actinometry, following previously  
193 described protocols<sup>35</sup> and measurements<sup>36</sup>. After irradiation, the AuNPs were then spiked into  
194 SONE-1 slurry as described before for the hetero-aggregation experiments.

### 195 **2.3 Instrumentation**

196 A Zetasizer Nano ZS (Malvern, Worcestershire, UK) was used offline to determine the  
197 hydrodynamic diameter (z-average) and the zeta-potential (Smoluchowski limit) of the NP  
198 suspensions and soil extracts. The ionic strength and pH for these measurements were  
199 established by the combination of MHW and the soil samples; no additional adjustments were  
200 made. Conductivity and polydispersity index were also determined using this instrument.

201 Total organic carbon (TOC) was determined by UV/persulfate oxidation using a Phoenix  
202 8000 (Teledyne Tekmar, Mason, OH). The default instrument method for TOC concentrations  
203 from (0.1 to 20) mg L<sup>-1</sup> as C was used: 4.0 mL of sample was mixed with 0.5 mL of 21 %  
204 phosphoric acid and sparged with N<sub>2</sub> for 120 s to remove inorganic carbon; then, the acidified  
205 sample and 1.0 mL of 10 % persulfate/5 % phosphoric acid reagent were injected into the UV  
206 reactor and sparged with N<sub>2</sub>. CO<sub>2</sub> from oxidation of the organic carbon was monitored by a  
207 nondispersive infrared detector. Concentrations as mass fraction C were determined against  
208 potassium hydrogen phthalate calibration solutions.

209 Total Au concentrations were determined with an ICP-MS model 7900 from Agilent  
210 Technologies (Santa Clara, CA, USA). The instrument was equipped with a concentric nebulizer  
211 and a refrigerated Scott chamber (2 °C). To obtain maximum sensitivity, the tune solution  
212 provided by Agilent containing multi-element standards (1 µg L<sup>-1</sup> each of <sup>7</sup>Li, <sup>89</sup>Y, <sup>140</sup>Ce and  
213 <sup>205</sup>Tl in 2 % v/v HNO<sub>3</sub>) was measured before analysis. The instrument was optimized for  
214 minimum oxide (<sup>156</sup>CeO/<sup>140</sup>Ce) and doubly charged (<sup>70</sup>Ce<sup>++</sup>/<sup>140</sup>Ce<sup>+</sup>) level (< 2 %). Platinum (Pt)

215 was added as an internal standard to both the Au solution calibration standards and to the  
216 samples. Data were collected at  $m/z$  197 for Au and  $m/z$  195 for Pt. Dissolved Au calibration  
217 standards were prepared over a mass fraction range from (0.05 to 5)  $\mu\text{g kg}^{-1}$  in aqua regia (2.0 %  
218  $v/v$ ). Single particle (sp) ICP-MS measurements were also performed on the Agilent 7900 using  
219 the time resolved analysis (TRA) mode, with a dwell time of 10 ms and measurement time of  
220 300 s. The transport efficiency was determined each day using dissolved Au calibration  
221 standards and NIST Reference Materials 8012 and 8013, with nominal AuNP size of 30 nm and  
222 60 nm, respectively <sup>37, 38</sup>. Samples were diluted to appropriate levels (less than 15,000  
223 particles/mL). Serial dilutions were performed to exclude coincident artefacts. Additionally,  
224 standard suspensions containing only monomers were evaluated at different concentrations to  
225 test for onset of significant coincidence.

226 The AF4 model used in this study was an Eclipse 3+ system from Wyatt Technology (Santa  
227 Barbara, CA). It was equipped with an 1100 series isocratic pump (Agilent Technologies) to  
228 generate mobile phase flow and a degasser (Gastorr TG-14, Flom Co., Ltd., Tokyo, Japan). All  
229 injections were performed with an Agilent Technologies 1260 ALS series autosampler. The  
230 detection system was formed by a 1200 series UV-vis absorbance diode array detector (Agilent  
231 Technologies) and a multi-angle laser light scattering (MALS) detector (DAWN HELEOS,  
232 Wyatt Technology). Due to the large size range present in these samples, a 250  $\mu\text{m}$  spacer (with  
233 dimensions of 26.5 cm length and narrowing width from 2.1 to 0.6 cm) was used to set the AF4  
234 channel height. The main flow rate and the cross flow rate were set at 0.5  $\text{mL min}^{-1}$  and 0.3  $\text{mL}$   
235  $\text{min}^{-1}$ , respectively <sup>21</sup>. The retention time ( $t_R$ ) starts after the focus step (flow of 2  $\text{mL min}^{-1}$ ).  
236 Polyethersulfone (PES) 10 kDa membranes were purchased from Wyatt Technology and used  
237 for the accumulation wall. Data from the AF4 detectors was processed using Astra ver. 6.1.4.25  
238 (Wyatt Technology) and OpenLab CDS Rev. C.01.06 (Agilent Technologies) software. The  
239 Berry formalism (first order) was used to determine the radius of gyration ( $R_g$ ) from MALS data  
240 <sup>39</sup>. The AF4 selectivity was determined using polystyrene latex beads (Thermo Fisher Scientific,  
241 Waltham, MA, USA) of known size and the relationship  $S_d = \left| \frac{d \log D_H}{d \log R} \right|$ . For the AF4 conditions  
242 used in this study, a selectivity of  $0.9 \pm 0.1$  was calculated, which indicates high quality  
243 separation (i.e., value close to unity) <sup>40</sup>. During hyphenation with the ICP-MS (Agilent 7900), in  
244 addition to <sup>197</sup>Au, characteristic isotopes were monitored to track the natural colloids: <sup>27</sup>Al, <sup>55</sup>Mn

245 and  $^{57}\text{Fe}$ . The collision cell was used in these measurements, with He at  $3.5 \text{ mL min}^{-1}$ , to mitigate  
246 interferences from polyatomic species (See ESI, Table S1).

## 247 **2.4 Uncertainty analysis**

248 Uncertainty intervals and error bars reported in this study, unless otherwise noted, represent one  
249 standard deviation about the mean determined under repeatability conditions with measurements  
250 on at least two replicate samples.

# 251 **3 Results and discussion**

## 252 **3.1 Sample characterization**

253 Table 1 summarizes the sample characteristics of the solid (soils and sediment) extracts  
254 (below  $0.45 \mu\text{m}$ ) and the AuNP samples. The hydrodynamic diameter and zeta-potential (ZP) of  
255 the natural solid colloid samples were measured for the  $< 0.45 \mu\text{m}$  fraction after extraction with  
256 MHW without modification. Prior to measurements, AuNP solutions were diluted in MHW  
257 (leachant), with a pH of 7.8. pH was also measured in the solid extracts and was 6.8, 8.0 and 8.1  
258 for SONE-1, San-Joaquin and Estuarine sediment, respectively. Given that the same leachant  
259 was used (MHW), the difference in pH, is attributed to the soil characteristics (e.g., SONE-1 is  
260 the most acidic solid). ZP is pH dependent, therefore ZP of SONE-1 is not directly comparable  
261 to the other natural samples. However, it appears that all colloids are negatively charged,  
262 especially in the estuarine sediment with  $-24.2 \pm 0.8 \text{ mV}$ . For the AuNPs, the ZP is negative for  
263 all samples except for bPEI, which is positive as expected. Cit-AuNPs and HA-AuNPs are the  
264 most negatively charged with a ZP near  $-20 \text{ mV}$ . PEG-AuNPs exhibit a small residual negative  
265 ZP of  $-2.2 \text{ mV}$ , which has been commonly observed for this coating. The  $d_H$  (z-average)  
266 determined by DLS shows that the PEG coating increases the size of the nominally  $30 \text{ nm}$   
267 AuNPs to  $51 \text{ nm}$ , compared to  $42 \text{ nm}$  and  $33 \text{ nm}$ , for PVP and HA coatings, respectively. The  
268 soils/sediment extracts contain polydisperse colloids, therefore the measured sizes are at best  
269 rough estimates of the mean size. The average  $d_H$  is below  $450 \text{ nm}$  for all extracted matrices,  
270 though the estuarine sediment, at  $400 \text{ nm}$ , is significantly larger than the soils. We note that after  
271 centrifugation the sediment derived particles were more easily resuspended relative to the soil  
272 particles.

273 TOC was measured in the natural samples after extraction in the  $< 0.45 \mu\text{m}$  fraction, it was  
274 higher in SONE-1 and the estuarine sample with  $(44 \pm 3 \text{ and } 40 \pm 1) \text{ mg C kg}^{-1}$ , respectively.

275 The San-Joaquin soil contained  $(29.3 \pm 0.4)$  mg C kg<sup>-1</sup>. The estimated carbon content in the  
276 initial solid soil and sediment samples was 1.9 %, 1.2 % and 1.6 % mass fraction for SONE-1,  
277 San Joaquin and estuarine sediment, respectively (Table S2)<sup>41,42</sup>. For AuNPs coated by HA, the  
278 TOC in the HA solution was set at 20 mg C kg<sup>-1</sup>, prepared by dilution from the stock solution.  
279

## 280 **3.2 Influence of AuNP concentration, coating and sample type**

### 281 **3.2.1 Concentration**

282 A range of concentrations of Cit-AuNPs and bPEI-AuNPs from (10 to 5000) µg kg<sup>-1</sup>  
283 were spiked into the SONE-1-leachant system (Fig. 2). Au recovery is determined as the ratio  
284 between the quantity of Au measured by ICP-MS following digestion of the soil extract  
285 (fractions < 1 µm and < 0.45 µm) and the initial spiked amount (measured by ICP-MS after room  
286 temperature acid digestion). The Au recovery after centrifugation is below 20 % mass fraction  
287 (Fig. 2), indicating that more than 80 % of AuNP mass is retained by the non-colloidal (massive)  
288 soil particles or aggregated with a size > 300 nm within the colloid fraction < 1 µm (counter-  
289 indicated by spICP-MS results).

290 For the fraction below 0.45 µm, the extracted Au recovery is around 3 % except for Cit-  
291 AuNPs at 5000 µg kg<sup>-1</sup>, which is  $(7.9 \pm 0.3)$  %. The AuNPs are either free or adsorbed onto the  
292 sub-0.45 µm soil colloids in the extracted suspension. Extracted Au recovery for < 1 µm fraction  
293 varies from  $(7.5 \pm 0.4)$  % to  $(16.2 \pm 0.9)$  %. For Cit-AuNPs, it is comparable at low  
294 concentrations ( $\leq 500$  µg kg<sup>-1</sup>). However, as for the < 0.45 µm fraction, the recovery for 5000  
295 µg kg<sup>-1</sup> is higher with  $(16.2 \pm 0.9)$  %. For bPEI-AuNPs, the recovery is similar from 100 µg kg<sup>-1</sup>  
296 at around  $(10.5 \pm 2.2)$  % and is lower  $(7.5 \pm 0.4)$  % for 10 µg kg<sup>-1</sup>. These observed differences in  
297 recovery are not substantial, and the attachment may be similar, but appears to vary somewhat  
298 between the larger and colloidal (< 1 µm) soil particles.

299 In both fractions < 0.45 µm and < 1 µm, the recoveries of Au as a function of the  
300 concentration are similar for the cit-AuNPs (up to 500 µg kg<sup>-1</sup>), and for the bPEI-AuNPs (at all  
301 concentrations tested), which is consistent with basic hetero-aggregation theory as described by a  
302 modified Smoluchowski model (Equation 1)<sup>29, 43</sup>, assuming that the processes of homo-  
303 aggregation and breakup of hetero-agglomerates are insignificant:

$$304 \quad \frac{dn}{dt} = -\alpha\beta nB \quad (1)$$

305 where  $n$  is the number concentration of AuNPs,  $B$  is the number of soil particles,  $\alpha$  is the  
306 attachment efficiency between the AuNPs and soil particles,  $\beta$  is the second-order collision rate  
307 constant between the AuNPs and soil particles, and  $t$  is time. For  $B$ ,  $\alpha$ , and  $\beta$  all remaining  
308 constant over the duration of the hetero-aggregation experiment:

$$309 \quad \frac{n}{n_0} = e^{-\alpha\beta Bt} \quad (2)$$

310 where  $n_0$  is the initial concentration of AuNPs. Hence, the fraction or percent of AuNPs  
311 remaining unaggregated, as well as the fraction of AuNPs hetero-aggregated to the soil particles,  
312 is not expected to depend on the initial AuNP concentration when comparing the same type (i.e.,  
313 surface chemistry) of AuNP over the same time duration (and thus the measured recoveries are  
314 not strongly dependent on the initial spiked concentration of AuNPs). We note that at sufficiently  
315 high concentrations of AuNPs, the rate of homo-aggregation will become significant (vida infra).

316 Fig. 3 presents the size distribution of the AuNPs at different concentrations for both Cit  
317 and bPEI coating using spICP-MS in the  $< 0.45 \mu\text{m}$  fraction after 24 h of agitation. To quantify  
318 the aggregation rate, an aggregation number (AN) can be determined as described in a previous  
319 study <sup>21</sup> as the ratio of the mass sum of the aggregates (dimer, trimer, and larger oligimers),  
320  $m_{aggr}$ , over the mass sum of the monomer ( $m_{mono}$ ):

$$321 \quad AN = \frac{\sum m_{aggr}}{\sum m_{mono}} \quad (3)$$

322  
323 For citrate coating, the increase of concentration clearly involved the formation of dimers  
324 (at around 38 nm) and then trimers (and higher order oligomers). It is important to note that the  
325 sample concentrations were adjusted (multiple dilutions) to avoid particle event coincidence  
326 during the dwell time. The number of particles is determined in such a way that the particle  
327 number is below  $300 \text{ min}^{-1}$ , at which level coincidence was not observed for the control sample  
328 (AuNPs in DI water). For (100 and 500)  $\mu\text{g kg}^{-1}$ , AN increased to 0.5, and it was 10 times higher  
329 for 5000  $\mu\text{g kg}^{-1}$  (Table 2). bPEI-AuNPs, to a lesser extent, exhibited aggregates starting at 100  
330  $\mu\text{g kg}^{-1}$ . However, the aggregation rate remained relatively constant up to 5000  $\mu\text{g kg}^{-1}$ , in  
331 contrast to Cit-AuNPs. The increase of the AuNP number concentration increased the collision  
332 rate for homo-aggregation of AuNPs, and, therefore, the presence of dimers or higher order  
333 species in the spICP-MS data. It is important to note that, in the soil extract samples, AuNPs may  
334 be homo-aggregated or hetero-aggregated with soil colloids (i.e., single and multiple AuNPs

335 either in a free oligomer or attached to the same colloid can produce similar frequency patterns  
336 under certain conditions).

337 To verify hetero-aggregation, AF4-UV-MALS-ICP-MS was performed on samples with  
338 a spiked concentration of  $5000 \mu\text{g kg}^{-1}$ . Fig. 4a presents the fractograms (detector signal as a  
339 function of  $t_R$ ) for the soil extract samples spiked with Cit-AuNPs. Based on  $R_g$ , fractionation is  
340 effective from about (50 to 250) nm in the main peak ( $t_R$  from 15 min to 60 min). The Au ICP-  
341 MS trace is present throughout the separation (from  $t_R = 5$  min). For comparison, the 30 nm Cit-  
342 AuNPs injected alone (no extract) under the same conditions are eluted at  $t_R = 6$  min. However,  
343 Au is mainly coeluted with the soil colloids, represented in the graph by Al, Fe and Mn for the  
344 clay and the (oxy)hydroxides of Fe or Mn present<sup>44</sup>. Therefore, these results appear to confirm  
345 that hetero-aggregation is dominant. Collections of fractions were performed at three  $t_R$  values:  
346 5.5 min (F1), 16.5 min (F2) and 45 min (F3) for a 1 min collection time. F1 corresponds to the  
347 retention of AuNP monomers injected alone. F2 was collected because of the high Au intensity  
348 at this  $t_R$  (Fig. 4a); moreover, it may correspond to principal aggregates of AuNPs. According to  
349 the number of spherical primary particles and the configuration of aggregates (linear,  
350 spherical...), the hydrodynamic diameter can vary<sup>45</sup>. Finally, F3 was collected at the maximum  
351 of the main colloid population. spICP-MS analysis was performed on each fraction and results  
352 are presented in Fig. 4b. Aggregation numbers for each fraction were calculated from the spICP-  
353 MS size distribution as described in Eq. (3) and were as follows:  $0.21 \pm 0.06$ ,  $7 \pm 1$  and  $4.3 \pm 0.4$ ,  
354 for F1, F2 and F3, respectively. It shows that the higher aggregation rate is associated with F2,  
355 where a more important frequency signal (Fig. 4b) between (37 and 80) nm (spherical equivalent  
356 diameter) is observed.

357 The monomer population (single AuNPs), though also present, may simply reflect  
358 coelution with soil colloids (i.e., AuNPs attached to colloids). F3 indicates a lower homo-  
359 aggregation rate and further confirms that hetero-aggregation is dominant. Additionally, more  
360 than one AuNP can be attached to a single colloid. When aggregates are observed in spICP-MS  
361 and the  $t_R$  in AF4 matches the colloid trace (typically for F3), it represents two NPs (if dimers)  
362 attached to the same soil particle; these AuNPs might be in contact or not (homo/hetero-  
363 aggregation). To help verify which mode is occurring or dominant, one option is to use electron  
364 microscopy imaging. Another possible method would be to use fast scanning with microsecond  
365 dwell times to assess characteristic changes in peak shape associated with hetero- versus

366 homoaggregation, but this was beyond the scope of the present work and would involve  
367 extended studies to validate the approach.

368 For bPEI-AuNPs, the fractionation profile was similar to Cit-AuNPs. However, for the  
369 fractions analyzed by spICP-MS, the rate of homo/hetero-aggregation was lower compared to  
370 citrate coating, with AN values of  $0.23 \pm 0.05$ ,  $0.82 \pm 0.08$  and  $0.77 \pm 0.07$ , for F1, F2 and F3,  
371 respectively (see ESI, Fig. S1). As previously shown, F1 corresponds to the monomer and yields  
372 a low AN whereas F2 and F3 have the same AN associated with a small amount of homo/hetero-  
373 aggregation. It is worth noting that the recovery in the  $< 0.45 \mu\text{m}$  fraction, for a spiked  
374 concentration of  $5000 \mu\text{g kg}^{-1}$ , is higher for citrate ( $8.3 \% \pm 0.9 \%$ ) compared to bPEI coating  
375 ( $3.5 \% \pm 0.2 \%$ ) involving a higher attachment rate for bPEI on larger soil particles. Moreover, in  
376 a simple solution (DI water only or MHW) with only AuNPs, Cit-AuNPs tend to homo-  
377 aggregate more readily compared to bPEI-AuNPs due to the additional polymer-induced steric  
378 stabilization present for bPEI. The difference in recovery can be due to the competition between  
379 homo- and hetero-aggregation. Therefore, oligomers of Cit-AuNPs are formed more easily in  
380 contrast with bPEI-AuNPs.

381 For the remainder of this study, a concentration of  $500 \mu\text{g kg}^{-1}$  was chosen to produce  
382 sufficient signal to be detected by the analytical method and to limit the homo-aggregation  
383 observed at  $5000 \mu\text{g kg}^{-1}$ . Moreover, according to equation 1 the influence of concentration up to  
384  $500 \mu\text{g kg}^{-1}$  is negligible. For real environmental samples a prerequisite step of preconcentration,  
385 such as cloud point extraction<sup>21</sup>, is often needed to detect ENMs by the standard analytical  
386 methods, as environmental concentrations are generally well below the  $\mu\text{g kg}^{-1}$  level<sup>46</sup>.

### 387 **3.2.2 Coating**

388 Surfactants, ligands and macromolecules are commonly used as surface coatings to  
389 stabilize (by steric and/or electrostatic repulsion) and functionalize NPs<sup>47</sup>. NOM encountered in  
390 the environment can also change the aggregation behavior of NPs<sup>19</sup>. To assess the influence of  
391 AuNP coatings on their attachment behavior, in addition to Cit and bPEI, soil samples were also  
392 spiked with PVP or PEG functionalized AuNPs, which are intrinsically neutral polymers, or  
393 NOM (SRHA and SONEHA) coated AuNPs (protocol in ESI). Fig. 5 presents the recovery  
394 (calculated as described in section 3.2.1) for the SONE-1 fractions. Less than 15 % of Au is  
395 extracted from the colloidal material for all coating types, with less than 5 % for the  $< 0.45 \mu\text{m}$   
396 fraction, regardless of the coating type. No substantial difference is observed between the

397 coatings. Several explanations may be advanced to explain these results. For instance, the end-  
398 over-end agitation method at 40 rpm for 24 h may increase the collision rate and enhance  
399 attachment efficiency between the AuNPs and the soil particles, overriding resistance due to the  
400 AuNP coating. Additionally, the presence of NOM in the soil sample may mitigate the influence  
401 of the native surface coating. Indeed, Stankus *et al.*<sup>48</sup> showed that regardless of surface  
402 functionalization (anionic, neutral or positive), functionalized AuNPs adsorb SRHA. Their work  
403 indicates that the initial coating may not be a major factor for NP transport and mobility in  
404 environment matrices containing substantial NOM.

405 spICP-MS measurements were performed on each soil extract spiked with AuNPs with  
406 different coatings (PVP, PEG, SRHA and SONE-HA) (see ESI, Fig. S2). The size distributions  
407 indicate that homoaggregation is not present; the frequency distributions are similar to the initial  
408 AuNP samples (diluted stock solution). It should be noted that HA stabilizes AuNPs initially  
409 coated with citrate. AF4 fractograms show that AuNPs are coeluted with the soil colloids; similar  
410 results were found for the Cit and bPEI samples (data not shown).

### 411 3.2.3 Matrix type

412 In addition to SONE-1 agricultural soil, another agricultural soil (San Joaquin, SJ) and an  
413 estuarine sediment (Sed) were also tested. An initial concentration of 500  $\mu\text{g kg}^{-1}$  of Cit-AuNPs  
414 was spiked into each matrix-leachant and the time of contact (agitation) was set at 24 h. After  
415 digestion of the extracted samples, recoveries for SJ and Sed represented  $(1.72 \pm 0.03) \%$  and  
416  $(1.2 \pm 0.1) \%$  of the initial spiked Au for the  $< 0.45 \mu\text{m}$  fraction, whereas below  $1 \mu\text{m}$ , recovery  
417 was  $(8.6 \pm 0.1) \%$  and  $(4.7 \pm 0.1) \%$ , respectively (Fig. 6). For the fraction  $< 0.45 \mu\text{m}$ , these  
418 recoveries are low relative to SONE-1, with a recovery of  $(3.4 \pm 0.1) \%$ . For the fraction  $< 1 \mu\text{m}$ ,  
419 the Au recoveries for the two agricultural soils (SJ and SONE-1) are statistically identical (8.6  
420 versus 8.9), but two-fold higher than Sed.

421 AF4-UV-MALS fractograms for all samples ( $< 0.45 \mu\text{m}$  fraction) present a broad peak  
422 between (20 and 75) min (See ESI, Fig. S3). The maximum of the UV peak corresponds to a size  
423  $R_g \approx 200 \text{ nm}$  for the soil samples (SONE-1 and SJ) and  $\approx 180 \text{ nm}$  for Sed. To the naked eye, we  
424 observed that SJ and Sed were less turbid after centrifugation, compared to SONE-1. Moreover,  
425 for the same injected quantity in AF4, the integrated intensity signals (for SJ and Sed) are  
426 reduced. The UV signal (at 254 nm) was integrated for each matrix and normalized to the largest  
427 area (i.e., that measured for SONE-1). The integrated and normalized UV for SONE-1, SJ and

428 Sed extracts are 1, 0.1 and 0.1, respectively. Similarly, Al, Fe and Mn intensities (determined by  
429 ICP-MS) in the extracts ( $< 0.45 \mu\text{m}$ ) are also lower for SJ and Sed (data not shown). The  
430 normalization for Al, Fe and Mn contents (combined together) in the different extracts represents  
431 1, 0.45 and 0.06 for SONE-1, SJ and Sed, respectively. These results suggest that Sed and SJ  
432 contain a lower fraction of colloids relative to SONE-1. NOM content (as described previously),  
433 is higher in SONE-1 and Sed with  $\geq 40 \text{ mg C kg}^{-1}$  compared with about  $29 \text{ mg C kg}^{-1}$  for SJ.  
434 Thus, the non-negligible Au recovery in Sed extract relative to the number of colloids may be  
435 explained by the presence of NOM. NOM can adsorb<sup>48</sup> or replace the initial coating and adsorb  
436 on other mineral soil particles. Frequency size distributions for AuNPs obtained by spICP-MS  
437 show that aggregation (homo or homo/hetero) is most prevalent in the Sed extracts (See ESI, Fig.  
438 S4), where dimer, trimer and higher order oligomer peaks are clearly present. The Sed particles  
439 are more negatively charged (Table 1) and therefore could be less of a sink for the negatively  
440 charged Cit-AuNPs; this in turn could promote homo-aggregation, but this is speculative without  
441 further confirmation.

### 442 3.3 Kinetic study

443 For this set of experiments, the time of contact (i.e., agitation time) between SONE-1 soil  
444 and Cit-AuNPs was set between (0 and 48) h with a fixed Au concentration of  $500 \mu\text{g kg}^{-1}$ . Fig. 7  
445 shows that the leachable AuNP content is decreasing rapidly. At  $t = 0$ , less than 60 % and 40 %  
446 of total Au is extracted from the fraction  $< 1$  and  $< 0.45 \mu\text{m}$ , respectively. Therefore, attachment  
447 on soil particles is rapid. At  $t = 2$  h, less than 20 % and 10 % of Au is found in the fraction  $< 1$   
448  $\mu\text{m}$  and  $< 0.45 \mu\text{m}$ , respectively. Extracted Au continued to decrease and reached, after a contact  
449 time of 48 h,  $(8.8 \pm 0.7) \%$  and  $(2.0 \pm 0.2) \%$ , for  $< 1 \mu\text{m}$  and  $< 0.45 \mu\text{m}$ , respectively. Thus, in  
450 this system, equilibrium is not reached<sup>28</sup>.

451 For each reported contact (agitation) time, AF4-UV-MALS-ICP-MS analysis was  
452 performed on the  $< 0.45 \mu\text{m}$  fraction. Fig. 8 presents the ICP-MS trace ( $^{197}\text{Au}$ ) for the  
453 fractograms at five contact times (In Fig. S5 MALS, UV and major elements fractograms are  
454 presented). It shows that at  $t_0$ , Au is eluted principally at 6 min, which corresponds to  $t_R$   
455 associated with free singlet AuNPs. The Au fraction eluted with the natural colloids between (13  
456 and 65) min is lower. When the contact time increases, it is observed that AuNPs are associated  
457 more with the colloidal component. The ratio between the areas of peak 2 (13 min to 75 min) and  
458 peak 1 (0 min to 13 min) increase from 0.9 to 7.6, with a linear correlation ( $y = 0.15x + 0.97$ ,  $R^2$

459 = 0.92). Therefore, larger soil particles (Fig. 7) and colloids (Fig. 8) tend to capture AuNPs over  
460 time, removing them from the solution phase.

### 461 **3.4 UV irradiation**

462 UV exposure was previously observed to transform PEG and PVP coatings on AuNPs<sup>36</sup>,  
463<sup>49</sup>. More specifically, PEG was found to degrade and detach from the AuNP surface, while PVP  
464 oxidized but remained attached to the AuNP as a compressed surface coating. Both  
465 transformations resulted in diminished stability of the AuNPs against homo-aggregation.<sup>36</sup>  
466 While UV exposure would likely have a muted impact on soil associated NPs, NPs used in foliar  
467 applications or those deposited onto the top layer of soil or associated with disturbed surface  
468 layers can be affected. Here, we also employ UV exposure as a well-defined, model approach to  
469 induce coating loss<sup>36, 49</sup>. Other likely routes of coating degradation (e.g., biodegradation) have  
470 not yet been so thoroughly characterized. In this study, we exposed the PEG-AuNPs to UV  
471 irradiation for 4 days in DI water to induce nearly complete removal of the PEG coating (Fig.  
472 S6) and then evaluated the effect on hetero-aggregation after 2 h of mixing with the SONE-1 soil  
473 slurry. Details of the results are reported in the ESI.

474 Similar percent recovery of spiked Au was obtained in the  $< 0.45 \mu\text{m}$  fraction regardless of  
475 UV exposure:  $(1.25 \pm 0.03) \%$  and  $(1.39 \pm 0.1) \%$ , for the unexposed and UV-exposed PEG-  
476 AuNPs, respectively. Likewise, similar percent recovery was also obtained in the  $< 1 \mu\text{m}$   
477 fraction:  $(10.6 \pm 0.6) \%$  and  $(11.8 \pm 1.1) \%$ , for the unexposed and UV-exposed PEG-AuNPs,  
478 respectively. These results are consistent with those from the coating comparison experiments,  
479 where similar recoveries of Au in the different size fractions from the soil slurry were obtained  
480 regardless of coating type. These results differ notably from our previous study on UV-  
481 irradiated PEG-AuNPs, where loss of the coating significantly changed the homo-aggregation  
482 rate of the AuNPs<sup>36</sup>. The results in the soil slurry imply that, for low concentrations of AuNPs  
483 undergoing primarily hetero-aggregation with naturally occurring particles, the presence or  
484 absence of the polymeric coating may not be significant with respect to the hetero-aggregation  
485 process, despite the steric forces imparted by the dense PEG coating. A likely explanation is that  
486 nearly all of the AuNPs have attached to a soil colloid or particle within the mixing time probed  
487 here (2 h), because of the high concentration of soil colloids and hence high collision rate.  
488 Alternatively, the presence of NOM in the soil slurry may play a role in mitigating differences  
489 between coatings. Differences in the attachment efficiency,  $\alpha$ , attributable to the presence of the

490 PEG coating may only be measurable at either shorter mixing times (minutes) or lower soil  
491 colloid concentrations.

## 492 **4 Conclusions**

493 In this work, an extraction procedure, based on standard leaching methods and previous  
494 studies, was applied to soil and sediment matrices spiked with various AuNP concentrations and  
495 surface coatings (as a model ENM). The low percentage of AuNPs extracted (recovered) from  
496 soil suspensions after 24 h of contact, suggests that their environmental mobility is greatly  
497 reduced. Additionally, the kinetic study has shown that the attachment of AuNPs onto soil  
498 particles is rapid (Au loss > 80 % in first 2 h of contact) and begins as soon as the AuNPs are  
499 exposed to soil particles and leachant. Thus, if AuNPs or other ENMs are released into the  
500 environment and enter soil or sedimentary compartments, interactions with naturally occurring  
501 particles are expected to play a substantial role in mitigating transport and determining fate. The  
502 influence of surface coating, including UV degradation of surface coatings, on the attachment of  
503 AuNPs to soil particles was not evident. Furthermore, we conclude that NOM plays a substantial  
504 role in the fate and transport of ENMs by decreasing the predicted influence of native surface  
505 chemistry and functional coatings, much as serum proteins adhere to and mask the intrinsic  
506 surface properties of ENMs in biological systems. We have also demonstrated how an approach  
507 based on the combination of in situ complementary and hyphenated analytical techniques can  
508 effectively be used to interrogate and quantify these complex interactions involving natural  
509 matrices. The approach utilized here can be easily extended to other metal containing ENMs.

510

### 511 **Electronic Supporting Information Available**

512 Additional methods, results and parameters for humic acid extraction, ICP-MS, AF4-UV-  
513 MALS-ICP-MS, and spICP-MS, are presented in the electronic supporting information.

514

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