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1 **Stabilization of fragmental polystyrene nanoplastic by natural organic matter:**

2 **Insight into mechanisms**

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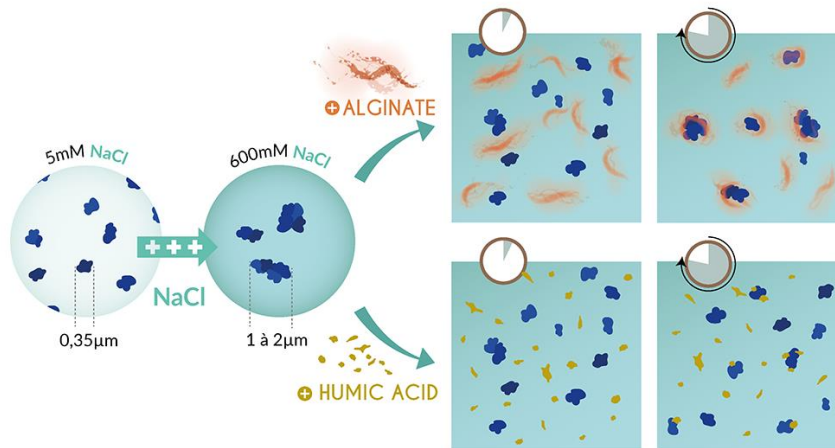
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12 **ABSTRACT**

13 The increasing amount of plastic debris in the environment and its disintegration into
14 submicrometric particles is a cause for concern. Due to the colloidal nature of nanoplastics, their
15 environmental fate should be investigated separately from that of microplastics. Abiotic factors greatly
16 influence nanoplastics' stability. This will affect its residence time in the hydrosphere. So, we
17 investigated the behavior of two different nanoplastic models (with different sizes and shapes) regarding
18 ionic strength, pH, and varying concentrations of two natural organic matters: humic acid and sodium
19 alginate. The results demonstrate that both natural organic matters enhanced the aqueous stability of
20 nanoplastics over time at high ionic strengths. Depending on the organic matter's nature, different
21 stabilizing mechanisms were revealed using dynamic light scattering and asymmetrical flow field flow
22 fractionation coupled to static light scattering. Humic acid provides electrostatic repulsion between
23 particles, and some larger humic acid molecules provide a steric hindrance. Sodium alginate sorbs onto
24 and bridges separate particles and small aggregates of nanoplastics. The covered particles are stabilized
25 by steric hindrance. The results highlight the importance of considering natural organic matters'
26 properties when assessing nanoplastics behavior in the environment.



27

28 **KEYWORDS**

29 Plastic debris, Environmental fate, Aggregation, Ionic strength, Morphology

30

31 **SYNOPSIS**

32 Mechanisms of nanoplastic stabilization will depend on the composition of the natural organic
 33 matter.

34

35 INTRODUCTION

36 As the use of plastic-based materials increases, plastic waste in the environment increases
37 proportionally^{1,2}. These last five years, it was demonstrated that plastic debris could persist as
38 nanoplastic (< 1 μ m) before eventual mineralization of the polymer³⁻⁵. Environmental sampling of
39 plastic debris at the ocean surface, coupled to numerical modeling, suggests that a substantial part of all
40 the plastic debris is composed of nanoplastics⁶⁻⁸. Since nanoplastics are an emerging contaminant, their
41 environmental fate should be better described. While airborne transport of microplastics is increasingly
42 coming under scrutiny^{9,10}, water remains the environmental compartment where most plastic debris is
43 found^{11,12} and where oxidative and hydrolytic conditions are favorable to plastic degradation¹³⁻¹⁶. As
44 such, it is crucial to understand the behavior of nanoplastics in aqueous systems.

45 To describe a colloidal material's environmental fate in aqueous systems, successive and
46 complementary approaches consist of modeling simple environmental systems in the lab, using these
47 results to establish numerical simulations, and, finally, confronting these simulations with the analysis
48 of environmental samples¹⁷. Based on this approach, experimental systems describing nanoplastics' fate
49 have emerged¹⁸, especially concerning porous media¹⁹⁻²¹ and water^{22,23}. Nanoplastics' stability in water
50 is generally determined by measuring changes in their size and sedimentation rates. Using this approach,
51 the stability of nanoplastics has been assessed in natural waters²³⁻³³, in deionized water with various
52 ionic compositions, ionic strengths, and pHs^{21,22,25,31,34-40}, in the presence of NOM and suspended
53 sediments^{22,23,27,31,33,34,36,38-41} and in the presence of extracellular polymeric substances (EPS)^{37,42}. Most
54 studies have used polystyrene (PS) latex spheres, which are perfectly smooth, spherical, and
55 monodisperse in size. Recently, the stability of more environmentally relevant models, such as aged
56 polystyrene (PS) latex spheres, laser-ablated PS, or fragmental PET, has been studied^{37,39,43,44}.

57 While these studies allow the emergence of global trends concerning nanoplastic stability, they also
58 have inconsistent conclusions. For example, nanoplastic models have been observed to be both stable
59 and unstable in artificial seawater^{33,45}, and in the presence of iron^{23,34}. Such discrepancies can be
60 explained first by the physical and chemical properties of the nanoplastics models used (size, shape,
61 surface functionalization, composition, purity, etc.), which are known to strongly affect the behavior of

62 colloidal materials. Additionally, nanoplastics are strongly sensitive to the media's properties (type,
63 concentration, and speciation of electrolytes, nature of the organic matter, pH). Indeed, according to the
64 relative concentration of spherical PS nanoplastic models, NOM, and cations, opposite behaviors have
65 been observed^{23,39}.

66 In light of these observations, the stability of two nanoplastic models was studied. The first model
67 is a monodisperse polystyrene latex (PSL) sphere. The second model is produced from the mechanically
68 degraded primary microplastic (PS pellets) and, as such, is more environmentally relevant due to its
69 irregular, asymmetrical shape and polydisperse size. NPs' aqueous stability was assessed at different
70 ionic strengths (5 to 770 mmol L⁻¹ NaCl) and in the presence of varying concentrations (0.005 to
71 140 mg L⁻¹) of two NOMs which have different properties: humic acid (HA) and sodium alginate (SA).
72 HA has a relatively compact structure and amphiphilic properties, whereas SA has a more linear
73 structure with hydrophilic properties. HA represents terrestrial organic matter, whereas SA represents
74 marine organic matter and is a significant component of EPS produced by microbial communities^{46,47}.
75 It was demonstrated that both NOMs stabilize the environmentally relevant NP model at high ionic
76 strength. However, due to their different physico-chemical properties, the NOMs have different
77 stabilizing mechanisms. These were characterized by asymmetrical flow field flow fractionation
78 coupled to static light scattering (A4F-SLS) and confirmed by dynamic dynamic light scattering (DLS).
79 The present work discusses these mechanisms and their possible implication for the fate of nanoplastics
80 in both terrestrial and marine environments.

81

82 **EXPERIMENTAL**

83 **Sample preparation**

84 All aqueous solutions and dispersions were prepared with analytical grade deionized (DI) water
85 (Millipore, 18.2 MΩ). A stock solution of NaCl (solid, LabKem ExtraPure) at 1.80 mol L⁻¹ was prepared.
86 The pH of all solutions was fixed at pH of 5, 6.5, or 8 using NaOH (Fisher Scientific, Analytical Grade)
87 and HCl (70%, Sigma Aldrich, ACS Grade). All solutions and dispersions were stored at 4°C in the dark

88 before use. Two nanoplastic (NPs) models were used in this study and are described in Table 1 and
 89 illustrated in Figure S1. Carboxylated polystyrene latex spheres of 200 nm (*PSL COOH*) are purchased
 90 from Polysciences© (Polybead® Carboxylate Orange Dyed Microspheres 0.20 µm, Warrington USA).
 91 A stock dispersion at a concentration of 100 mg L⁻¹ was prepared. A NP model with irregular and
 92 polymorphic shapes (*NPT-P*) was produced by the mechanical abrasion of industrial-grade polystyrene
 93 (PS) pellets (Total, Paris, France) as described by El Hadri et al. (2020)⁴⁸. The pellets are composed of
 94 primary (-P) PS, which contains no additives and has not been aged. Due to the less stable nature of the
 95 *NPT-P* compared to *PSL COOH*, the experiments presented here used different batches of *NPT-P* to
 96 avoid a bias brought about by the aging of the stock dispersion. Each batch was produced using PS from
 97 the same degradation round. Before each experiment, the size of the nanoplastic dispersions was verified
 98 with DLS measurements. Concentration of the stock *NPT-P* solution was measured with a Total Carbon
 99 Analyzer (Shimadzu TOC-V CSH) and varied between 22 and 35 mg L⁻¹. According to the molecular
 100 composition of PS, 1 mg L⁻¹ of organic carbon was converted to 1.08 mg L⁻¹ *NPT-P*.

101

102 *Table 1: Characteristics of the two nanoplastic (NP) models. The polydispersity index (PDI) is defined as the variance of the*
 103 *Gaussian-fitted size distribution. The aspect ratio is defined as the ratio of the length of the major axis and minor axis as*
 104 *determined by TEM images (Figures S1 and S2).*

| Nanoplastic model | z-average diameter (nm) | Polydispersity Index (PDI) | Aspect ratio* | Zeta potential in 5 mmol L ⁻¹ NaCl (mV) | | |
|-------------------|-------------------------|----------------------------|---------------|--|---------------|---------------|
| | | | | pH 5 | pH 6.5 | pH 8 |
| <i>PSL COOH</i> | 197 ± 2 | 0.03 ± 0.01 | 1.02 ± 0.05 | -37.69 ± 1.91 | -38.65 ± 2.23 | -42.80 ± 2.98 |
| <i>NPT-P</i> | 339 ± 7 | 0.18 ± 0.03 | 1.70 ± 0.57 | -31.67 ± 1.01 | -33.54 ± 2.72 | -35.14 ± 2.13 |

105 * Figure S2 illustrates the aspect ratio of *NPT-P* particles

106

107 Sodium alginate (SA) was prepared by introducing 60 mg of SA powder (solid, Acros Organics)
 108 into 0.1 L DI water and mixing at 350 rpm in a square bottle overnight. The humic acid (HA) used in
 109 this work was Leonardite purchased from the International Humic Substance Society (IHSS). The stock
 110 solution of HA was prepared by adding 50 mg of Leonardite powder to 0.1 L of DI water. To solubilize

111 the stock solution, pH was adjusted to 11 (with NaOH at 0.1 mol L⁻¹) under continual agitation with a
112 magnetic stirrer. Then, the solution was mixed at 350 rpm for 24h. pH was then fixed to either 5, 6.5, or
113 8 using 0.1 mol L⁻¹ HCl. The concentrations of the NOM stock solutions were determined with a Total
114 Carbon Analyzer (Shimadzu TOC-V CSH). According to the NOM's molecular composition, 1 mg L⁻¹
115 of organic carbon was converted to 1.6 mg L⁻¹ HA and 2.8 mg L⁻¹ SA.

116

117 **Size characterization**

118 Hydrodynamic diameters (d_H) were determined by dynamic light scattering (DLS) probe
119 (Vasco-Flex, Cordouan Technologies, Pessac, France). The measured d_H of an agglomerating
120 suspension is the average of the d_H of the individual particles and aggregates, weighted by their scattered
121 light intensities⁴⁹. The backscattered light is collected at a geometric angle of 170° with respect to the
122 incident beam direction. For time-resolved DLS, each correlation function was accumulated for 60
123 seconds and were spaced 30 seconds apart. DLS measurements of stock solutions are composed of an
124 average of six measurements of 60 seconds. The z-average hydrodynamic diameter (d_{zH}) was determined
125 by fitting a normal distribution to the raw data using the cumulant algorithm. To analyze the different
126 populations in size present in a dispersion, the Sparse Bayesian learning (SBL) algorithm was used. The
127 distribution of the NPs' gyration radii was measured by static light scattering (DAWN HELEOS 18
128 Angles, Wyatt Technology) with prior size fractionation using an asymmetrical flow field flow
129 fractionation (A4F, Eclipse 3+, Wyatt Technology, Dernbach, Germany) and a UV-vis absorbance
130 detector (1200 series, Agilent Technologies, France) as a concentration detector at 254 nm. The global
131 method of A4F separation was used. It was previously optimized and described by Gigault et al. (2017)⁵⁰
132 (see Supplemental Information, S1).

133

134 **Kinetics of Colloidal Aggregation**

135 The kinetics of nanoplastic aggregation were determined by measuring the z-average
136 hydrodynamic diameters (d_{zH}) of the dispersions over one hour. A total volume of 3 mL was prepared
137 by adding NaCl and DI water to the vial, followed by NOM (when it was studied), and vigorously

138 mixing the solution. Finally, the nanoplastic dispersion was added to the vial, marking the beginning of
 139 the kinetic study. All kinetic studies were performed in triplicate. The aggregation rate (k) was
 140 determined from the slope of the one-hour-long kinetic study, according to equation (1):

$$141 \quad \left(\frac{dd_{zH}(t)}{dt} \right)_{t \rightarrow 0} \propto kN_0 \quad (1)$$

142 where $d_{zH}(t)$ is the hydrodynamic diameter of aggregates as a function of time t and N_0 is the initial
 143 number-based particle concentration. Statistical analyses were operated using one-way ANOVA.
 144 Pairwise comparisons of aggregation rates were made using Tukey's method.

145 At low ionic strengths, electrostatic repulsion between particles is high due to a thick electrical double
 146 layer (EDL): the colloidal dispersion is said to be in the reaction-limited aggregation (RLA) regime. As
 147 ionic strength increases, electrostatic repulsion decreases, and the aggregation rate increases. At an ionic
 148 strength corresponding to the critical coagulation concentration (CCC), the interparticle energy barrier
 149 is eliminated, aggregation rate is maximal (k_{fast}), and the diffusion-limited aggregation (DLA) regime is
 150 reached. The attachment efficiency α describes aggregation kinetics by normalizing aggregation rates
 151 under RLA regime (k) by the DLA regime (k_{fast}):

$$152 \quad \alpha = \frac{k}{k_{fast}} = \frac{\left(\frac{dD_{zH}(t)}{dt} \right)_{t \rightarrow 0}}{\left(\frac{dD_{zH}(t)}{dt} \right)_{t \rightarrow 0, fast}} \quad (2)$$

153

154 **Derjaguin Landau Verwey Overbeek (XDLVO) theory of colloidal stability**

155 The total interaction energy as a function of the distance separating the particles, $G^{tot}(h)$, is
 156 calculated as the sum of the Lifshitz-van der Waals attraction, $G^{LW}(h)$, the electrical double layer (EDL)
 157 repulsion, $G^{EDL}(h)$, and the Lewis acid-base energy of interaction $G^{AB}(h)$. The surface interaction energy
 158 was calculated at an ionic strength of 5 mmol L⁻¹. Particle diameters and zeta-potential are presented in
 159 Table 1.

160 The Lifshitz-van der Waals component, $G^{LW}(h)$, was calculated using the expression of the
 161 retarded van der Waals interactions between two identical approaching spheres proposed by Gregory
 162 (1981)⁵¹:

$$163 \quad G^{LW} = - \frac{Hr_{p1}r_{p2}}{6(r_{p1}+r_{p2})h} \left[1 - \frac{bh}{\lambda} \ln \left(1 + \frac{\lambda}{bh} \right) \right] \quad (3)$$

164 where H is the Hamaker constant of polystyrene particles interacting through water, equal to $1.23 \cdot 10^{-20}$
 165 J; r_{p1} and r_{p2} are the radii of particles 1 and 2, respectively; b is an empirically defined constant, $b = 5.32$;
 166 and λ is the characteristic wavelength of the interaction with a value of ~ 100 nm⁵².

167 The electrical double layer repulsion $G^{EDL}(h)$ was calculated using the expression proposed by
 168 Hogg et al. (1966)⁵³:

$$169 \quad G^{EDL} = \pi\epsilon \frac{r_{p1}r_{p2}}{(r_{p1}+r_{p2})} \left[2\xi_{p1}\xi_{p2} \ln \left(\frac{1+e^{-\kappa h}}{1-e^{-\kappa h}} \right) + (\xi_{p1}^2 + \xi_{p2}^2) \ln (1 - e^{-2\kappa h}) \right] \quad (4)$$

170 where ϵ is the permittivity of the medium, equal to $6.95 \cdot 10^{-10} \text{ C}^2 \cdot \text{J}^{-1} \cdot \text{m}^{-1}$, ζ_{p1} , and ζ_{p2} are the surface charges
 171 of particles 1 and 2, respectively, approximated by the zeta potential; and κ is the inverse of the EDL
 172 thickness (Debye Huckel length reciprocal length), determined by the following equation:

$$173 \quad \kappa = \left[\frac{e^2}{\epsilon k_B T} \sum i z_i n_i \right]^2 \quad (5)$$

174 where e is the charge of the electron; k_B the Boltzmann constant, T the temperature, z_i the valency of the
 175 ions i, and n_i the number of ions i per unit volume.

176 The Lewis acid-base energy of interaction $G^{AB}(h)$ of our system is the expression proposed by
 177 van Oss (1993)⁵⁴:

$$178 \quad G^{AB}(h) = 2\pi \frac{r_{p1}r_{p2}}{(r_{p1}+r_{p2})} \lambda_{AB} \Delta G_{(h=h_0)}^{AB} e^{\left(\frac{h_0-h}{\lambda_{AB}} \right)} \quad (6)$$

179 where λ_{AB} is the correlation length, chosen as 1.65 nm, according to Valsesia et al. (2018), and h_0 is the
 180 minimum distance of separation between the particle and the surface, taken as 0.158 nm⁵⁵. The acid-
 181 base potential $\Delta G_{h=h_0}^{AB}$ is expressed as:

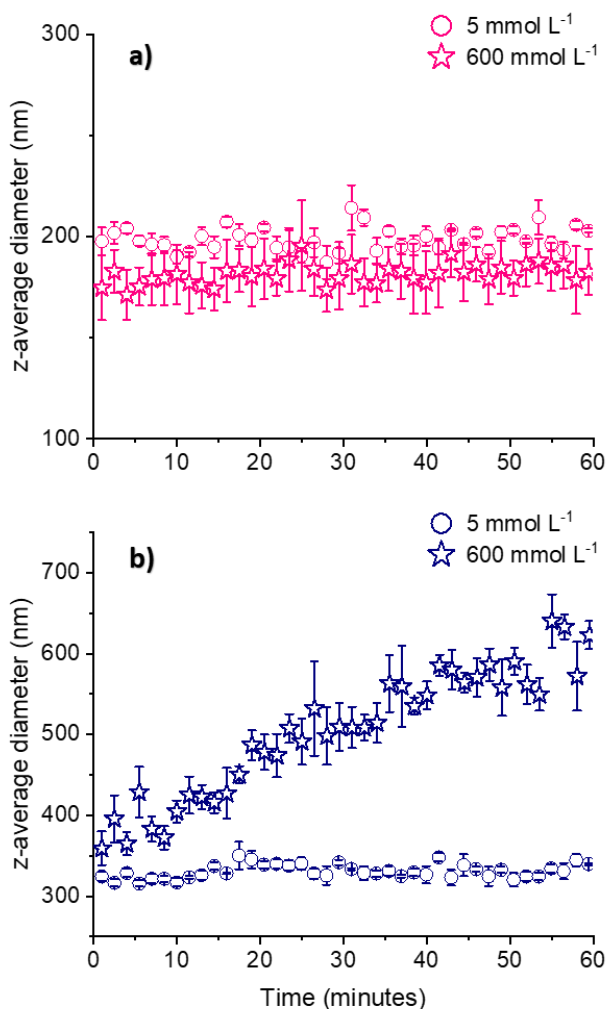
182
$$\Delta G_{(h=h_0)}^{AB} = -2 \left(\gamma_{p1}^{AB} + \gamma_{p2}^{AB} - 2 \sqrt{\gamma_{p1}^{AB} \gamma_{p2}^{AB}} \right) \quad (7)$$

183 With γ_{p1}^{AB} and γ_{p2}^{AB} the polar component of the surface free energy for particles 1 and 2, respectively.

184 γ_p^{AB} was directly quantified using the method by Valsesia et al. (2018) and found to be equal to 33.91

185 and 31.82 mJ.m⁻² for *PSL COOH* and *NPT-P*, respectively⁵⁵.

186



189

190 *Figure 1: Aggregation kinetics of 4 mg L⁻¹ a) PSL COOH and b) NPT-P in either 5 mmol L⁻¹ or 600 mmol L⁻¹ NaCl at pH 6.5*

191

(Error bar = standard deviation)

192 Figure 1 shows that both particles are stable and low ionic strengths (5 mmol L⁻¹). However, as ionic
 193 strength increases, the particles show differences in stability: *PSL COOH* is stable at high ionic strength
 194 (600 mmol L⁻¹), while *NPT-P* aggregates, with d_{zH} increasing from 359 to 623 nm in one hour. While
 195 the aggregation kinetics presented in Figure 1 took place at a pH of 6.5, the trends in stability were the
 196 same at pH 5 and 8, representing the pH range of natural waters (Figure S3). Based on these kinetics of
 197 aggregation, the *NPT-P*'s critical coagulation concentration (CCC) in NaCl was determined to be 59 and
 198 67 mmol L⁻¹ NaCl at pH 6.5 and 8, respectively (Fig. S4). These values are lower than the CCC of 260

199 mmol L⁻¹ (NaCl, unadjusted pH) previously determined by El Hadri et al. (2020) and show no significant
200 increase in stability with pH⁴⁸. This suggests that the *NPT-P* studied here has lower surface oxidation
201 than those studied by El Hadri et al. (2020), as confirmed by a lower zeta (-33 vs. -44 mV). For *NPT-P*,
202 the concentration of -COOH on the surface is lower, inhomogeneous, and uncontrolled. The mechanical
203 degradation method used to produce *NPT-P* cannot control the -COOH functionalization of their surface,
204 which induces possible variability on the CCC. The CCC value of *PSL COOH* was not assessed since
205 these particles were stable up to 1 M NaCl, which is above environmentally relevant concentrations.
206 The CCC of *NPT-P* was lower than that of *PSL* models, as illustrated in Table 2. The reasons behind
207 differences in stability between our *PSL* and *NPT-P* models are discussed below. Differences in stability
208 between *PSL* particles and environmentally relevant nanoplastic models are commonly observed.
209 Indeed, Yu et al. (2019) determined that non-functionalized and carboxylated *PSL* spheres have a CCC
210 of 310 and 308 mmol L⁻¹ NaCl, respectively³⁹. Mao et al. (2020) found an even greater CCC of 591
211 mmol L⁻¹ NaCl for non-functionalized *PSL* spheres³⁷. The aging of these particles by UV-irradiation
212 strongly oxidized their surface. This caused stronger electrostatic repulsion, and consequently, the CCC
213 increased up to 1108 mmol L⁻¹. Singh et al. (2019) found a lower CCC of 140 mmol L⁻¹ NaCl for non-
214 functionalized *PSL* and attributed this difference to the removal of surfactants³¹. The CCC calculated
215 for *NPT-P* was coherent with observations made on other non-spherical, non-emulsified and surfactant-
216 free, nanoplastic models. For example, NPs produced by laser ablation of PS show strong aggregation
217 in 300 mmol L⁻¹ NaCl³⁹. NPs produced from mechanical fragmentation polyethylene glycol
218 terephthalate (PET-G) had a CCC of 54 mmol L⁻¹ NaCl at pH 6, and 110 mmol L⁻¹ NaCl at pH 10⁴³.
219 These recent observations confirm that particles' surface functionalization and morphology, as well as
220 the presence of surfactants play key roles in the kinetics of aggregation.

221

222

Table 2: Summary of different critical coagulation concentrations (CCC) of NaCl for various NP models

| NPs models studied Nomenclature : Composition Type of particle <i>Surface functionalization</i> * Nominal size (Charge) | CCC (mmol L⁻¹) | Reference |
|--|--|-------------------------------------|
| PS Latex sphere <i>NF</i> 100nm (-) | 310 in NaCl at pH 7.4 | Yu et al., 2019 (39) |
| PS Latex sphere <i>COOH</i> 100nm (-) | 308 in NaCl at pH 7.4 | |
| PS Laser ablation 60 nm (-) | Not determined. Strong aggregation in 300 mmol L ⁻¹ NaCl pH 7.4 | |
| PS Latex sphere <i>NF</i> 100 nm (-) | 591 in NaCl pH 7.5 | Mao et al., 2020 (37) |
| PS Latex sphere aged by UV-irradiation during 60 hours <i>NF</i> 100 nm (-) ** | 957 in NaCl pH 7.5 | |
| PS Latex sphere aged by UV-irradiation during 120 hours <i>NF</i> 100 nm (-) ** | 1108 in NaCl pH 7.5 | |
| PS Latex sphere <i>NF</i> 240(-) | 140 in NaCl pH 6 | Singh et al., 2019 (31) |
| PET-G Mechanical degradation 500 nm (-) | 54 in NaCl pH 6 110 in NaCl pH 10 | Dong et al., 2020 (43) |
| PS Mechanical degradation 350 nm (-) | 260 in NaCl pH unadjusted | El Hadri et al., 2020 (48) |
| PS Mechanical degradation 350 nm (-) | 59 in NaCl pH 6.5 | <i>NPT-P</i> particles studied here |
| PS Mechanical degradation 350 nm (-) | 67 in NaCl pH 8 | |

223 **NF* = Non-functionalized, *COOH* = carboxylated224 ** Initial particles were non-functionalized. Aging produced an increasing amount of carbonyl functional groups on the
225 surface.

226

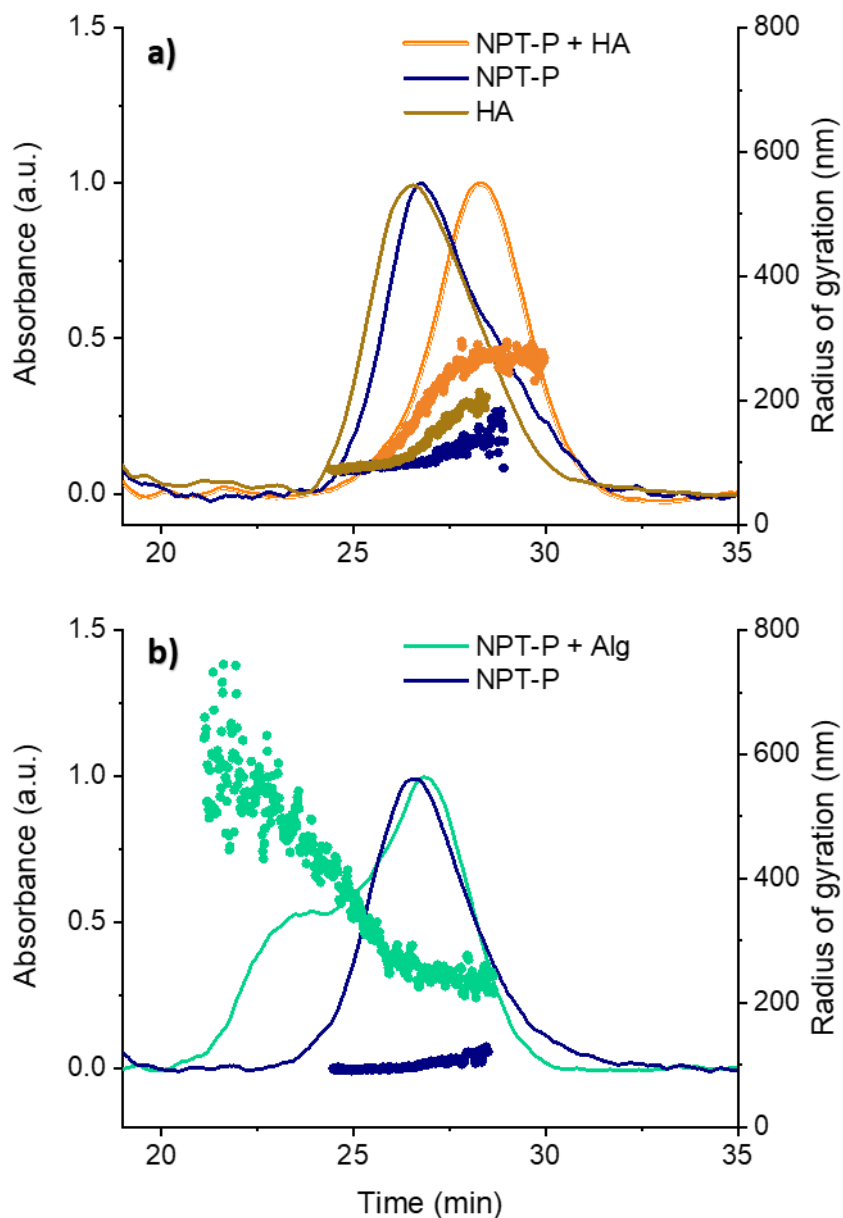
227 To characterize the effect of the particles' properties (size, surface potential, etc.) on their
228 stability, the level of repulsion (energy barrier) between particles can be modeled by the extended
229 Derjaguin Landau Verwey Overbeek (XDLVO) theory. According to the XDLVO theory, the
230 interaction energies between *NPT-P* and *PSL COOH* are not significantly different (Table S1 and Figure
231 S5). This demonstrates that size, surface charge, and hydrophobicity (Table 1), which are used to
232 calculate interaction energy profiles, do not explain differences in stability. Instead, it suggests that *NPT-*
233 *P*'s morphologies and polydispersity (Table 1 and Figure S1), as well as the lack of surfactants in the
234 dispersion, are responsible for the aggregation rates observed. Indeed, particle morphology will affect
235 their attachment efficiency. *NPT-P* has an aspect ratio of 1.70 ± 0.57 and asperities on their surface,
236 whereas *PSL COOH* has an aspect ratio of 1.02 ± 0.05 and a smooth surface. At close approach, particles
237 with elongated shapes (high aspect ratios) have larger van der Waals attraction when their major axii
238 face each other⁵⁶. While this has been difficult to demonstrate experimentally⁵⁷, a few aggregation
239 experiments support this theory^{43,58}. Also, the collision of irregular and rough particles is likely to occur
240 between particle protrusions and edges⁵⁹.

241 For this reason, it is more accurate to model the interaction energy between *NPT-P* and
242 asperities, using the smallest radius of curvature as the asperity radius⁶⁰. This significantly reduces the
243 level of repulsion (Fig. S5) since the final volume of interaction is reduced and repulsive forces
244 (electrostatic and acid-base) decay more quickly with distance than attractive forces (Lifshitz-van der
245 Waals)^{61,62}. Secondly, particle collision rate during perikinetic aggregation (i.e., induced by collisions
246 driven by the Brownian motion) is always more significant for dispersions containing different particle
247 sizes⁶³. Finally, *NPT-P* particles are free of surfactants, which have a stabilizing effect^{63,64}. Since the
248 more environmentally relevant nanoplastic model, *NPT-P*, is not stable at high ionic strengths, the
249 stabilizing effect of natural organic matters (NOMs) was studied.

250 **Stabilization of *NPT-P* by natural organic matters**

251 Two different NOMs were chosen to represent the wide variety of physico-chemical properties
252 of naturally occurring organic matters. The interaction of the *NPT-P* with the natural organic matter was
253 described by characterizing the size of *NPT-P* with NOM at high ionic strength. Asymmetrical flow
254 field-flow fractionation coupled to static light scattering (A4F-SLS) was used to characterize the
255 assembly of *NPT-P* with NOM as this technique can discriminate different size populations and changes
256 in polydispersity. Figure 2 illustrates the fractograms obtained for *NPT-P* in the presence of HA (Fig
257 2a) and SA (Fig. 2b). *NPT-P* and HA alone have similar times of elution and variation of their radii of
258 gyration (R_g) over time. For *NPT-P* with HA, the peak's elution time range is identical, suggesting that
259 *NPT-P* and HA stay dispersed and retain their initial sizes. However, the maximum of the peak increases
260 from 26 to 28 minutes, with the corresponding R_g increasing from 100 nm up to 270 nm. This shift
261 suggests that a specific size fraction of HA is associated with *NPT-P* and formed larger hetero-
262 aggregates. Concerning the mixture of *NPT-P* with SA (Figure 3b), no fractograms were obtained for
263 SA in these fractionation conditions due to its low scattering properties at this concentration
264 (57 mg L⁻¹). In the presence of SA, two peaks are observed: one eluted around 23 minutes and another
265 around 26 minutes. The first peak corresponds to a $R_g > 400$ nm, while the second corresponds to a
266 smaller R_g , around 250 nm. In A4F, the normal elution mode occurs when the relative diffusion between
267 the different populations through the channel's height allows their separation according to the parabolic

268 profile of the main velocity flow. However, an earlier peak with a high R_g is indicative of steric elution
 269 mode⁶⁵. In this mode, the particles become too large to be separated based on diffusion coefficient and
 270 are instead eluted by dragging forces. This first peak, in steric mode, corresponds to SA bridging
 271 separate *NPT-P* particles and sorbing onto small aggregates of *NPT-P*. The second peak overlaps with
 272 that of *NPT-P* alone and can be explained by the association of SA with single *NPT-P* or smaller
 273 aggregates.

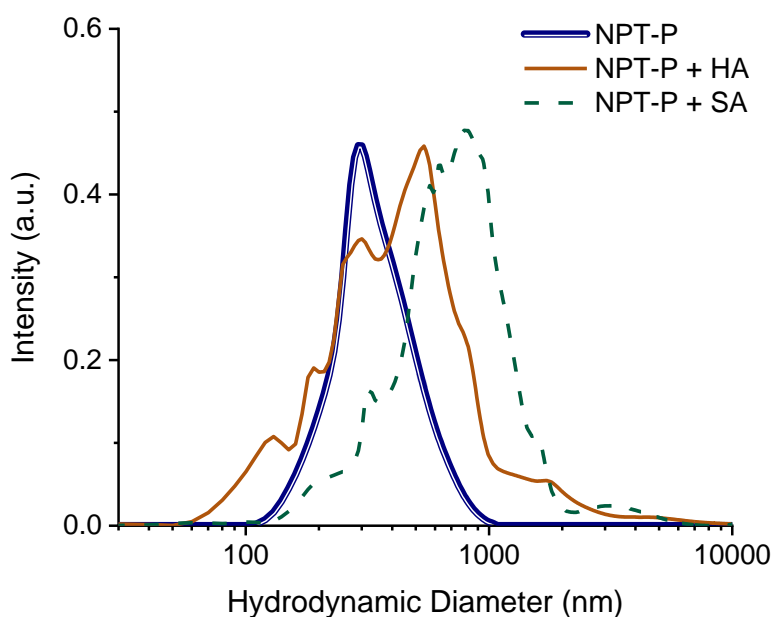


274

275 *Figure 2: Fractograms showing absorbance (line) and radius of gyration (R_g) (points) of a) NPT-P in 5 mmol L⁻¹ NaCl,
 276 NPT-P with 30 mg L⁻¹ HA in 600 mmol L⁻¹ NaCl, and 30 mg L⁻¹ HA in 600 mmol L⁻¹ NaCl at pH 6.5 and b) NPT-P in
 277 5 mmol L⁻¹ NaCl and NPT-P with 57 mg L⁻¹ SA in 600 mmol L⁻¹ NaCl at pH 8, as a function of retention time*

278

279 To validate the variations in size populations and distributions, Figure 3 shows the size
280 distributions of *NPT-P* with HA and with SA at high ionic strength based on the Sparse Bayesian
281 Learning (SBL) algorithm. This algorithm allows investigating differences in size population within the
282 limits of the DLS resolution. With HA, the *NPT-P* size distribution is large but still covering the size
283 range of the initial *NPT-P*. However, HA induces a shift towards a higher d_H of 530 nm compared to the
284 initial d_H of 320 nm for *NPT-P* alone. This shift may be due to the non-covalent adsorption of larger HA
285 molecules onto the *NPT-P* surface. In the presence of SA, the size distribution is less polydisperse but
286 with a maximum d_H around 820 nm. This larger size population can be explained by the physical
287 association of SA with several ($n > 2$) *NPT-P* particles.



288

289 *Figure 3: Average of intensity-based size distributions according to SBL algorithm of NPT-P with 30 mg L⁻¹ humic acid (HA)*
290 *at pH 6.5 or 57 mg L⁻¹ sodium alginate (SA) at pH 8, in 600 mmol L⁻¹ NaCl measured between 45 and 60 minutes (n ≥ 18).*
291 *The SBL algorithm reveals the most probable continuous size distribution among a set of 25 solutions.*

292 The A4F-SLS fractograms and DLS size distributions (Figures 2 and 3) suggest that HA and SA have
293 different stabilization mechanisms. On the one hand, the HA molecules that are free in solution stabilize
294 *NPT-P* by providing electrostatic repulsion. Some low molecular weight HA molecules are also
295 adsorbing onto the *NPT-P* surface and providing steric repulsion. The co-occurrence of these two
296 mechanisms is supported by the fact that leonardite humic acid is one of the more large and polydisperse

297 humic acids⁶⁶. Indeed, electrostatic repulsion is attributed to the smaller size fraction of humic and fulvic
298 acids^{38,40}, while surface adsorption of the larger size fraction of HAs may occur via π - π interactions
299 (with the aromatic structures of HA) and result in steric hindrance^{22,31,39,67}. Saavedra et al. (2019) noted
300 that both HA and SA⁴¹ stabilized negatively charged particles. Due to HA's compact structure,
301 adsorption of HA onto colloids did not increase their size. However, SA's high molecular weight (282
302 kDa, Fig. S6) and semi-rigid chains can lead to the formation of larger aggregates¹⁷. Indeed, SA chains
303 stabilize *NPT-P* particles by wrapping around single particles and small aggregates and bridging
304 separate particles. These hetero-aggregates are then prevented from further aggregating by steric
305 hindrance. Since SA is highly hydrophilic, its adsorption onto nanoplastics can be attributed to hydrogen
306 bonds and van der Waals interactions^{42,46,68,69}. Indeed, Bhattacharya et al. (2010) demonstrated a
307 significant affinity between negatively charged carboxylated PSL and negatively charged algae⁷⁰. This
308 affinity has been attributed to hydrogen bonds forming between the cellulosic component of algae and
309 the PSL. Finally, using TEM, it appears that *NPT-P* is embedded in SA, while HA does not seem to
310 have such a strong affinity with the *NPT-P* surface (Fig. S7).

311

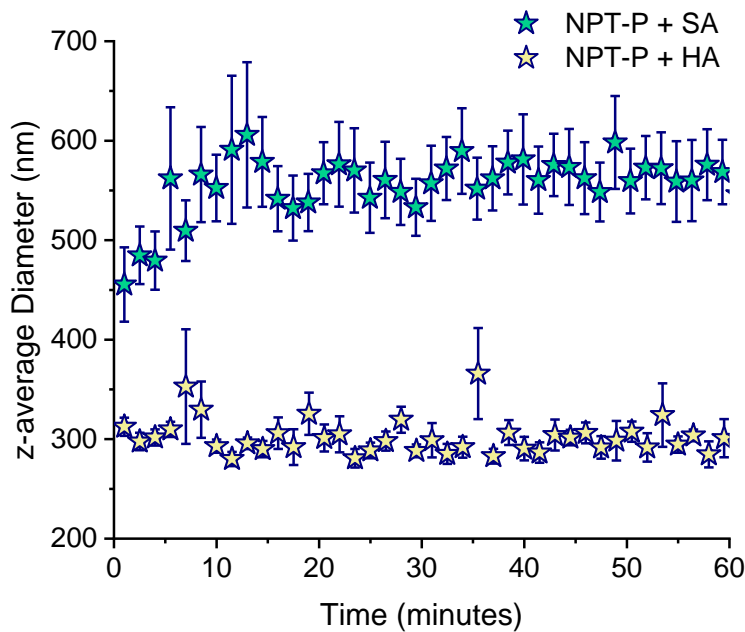
312 **Colloidal stability of NPT-P according to the nature and concentrations of NOM**

313 These two mechanisms of stabilization will have distinct impacts on nanoplastics' colloidal stability
314 in aqueous media. Figure 4 illustrates the size variation of *NPT-P* in 600 mmol L⁻¹ NaCl with either HA
315 or SA. At 600 mmol L⁻¹ NaCl, the size of *NPT-P* increases in the absence of NOM (Fig. 1). Figure 4
316 shows that HA can stabilize *NPT-P* immediately, while SA stabilizes *NPT-P* within 10 minutes. The
317 final sizes obtained (i.e., 300 nm for HA and 560 nm for SA) corroborate that different interactions
318 occur between *NPT-P* and the two NOMs. In the presence of HA, the d_{zH} of *NPT-P* remains constant
319 around 300 nm. HA is highly polydisperse with a colloidal fraction centered around 230 nm. So, for
320 kinetics of *NPT-P* with HA, the d_{zH} presented in Figure 4 is a combination of both the d_{zH} of *NPT-P*
321 (339 nm) and the d_{zH} of HA (230 nm). Despite this significant contribution of HA to the DLS signal,
322 DLS will rapidly detect if aggregation occurs since scattering is highly sensitive to increases in size⁷¹.

323 In the presence of SA, the size of *NPT-P* increases within the first 10 minutes and stabilizes around 560
324 nm. Without NOM, such an increase in size takes more than 30 minutes (Fig. 1).

325 Contrary to HA, 57 mg L⁻¹ SA does not contribute to the DLS signal. Therefore this increase in size
326 followed by a stabilization is explained by a rapid hetero-association between *NPT-P* (particles and/or
327 aggregates) and SA. Instead of keeping all particles separate as HA does, the SA biopolymer wraps
328 separate or slightly aggregated plastic nanoparticles. Alginate molecules have been observed to form a
329 layer with an approximate thickness of 20 nm around positively charged PSLs²³. Also, when studying
330 the aggregation of NPs with the dissolved (< 0.22 μm) fraction of organic matter that is naturally present
331 in seawater, Chen et al. (2018) obtained similar kinetics of hetero-aggregation, with a rapid increase in
332 size followed by a plateau around micrometric sizes⁶⁸.

333 The relative quantity of particles and aggregates present in the dispersions over time was estimated
334 based on the hydrodynamic diameter (d_{zH}), the intensity of scattered light (I_{θ}), and a spherical form
335 factor (P_{θ}), as described in Supplemental Information (S7). At high ionic strength, results show that the
336 relative concentration of *NPT-P* plummets without NOM (Fig. S8). The relative *NPT-P* particle
337 concentration does not decrease in the presence of HA, indicating colloidal stabilization induced by
338 electrostatic repulsion. On the contrary, with SA, the relative *NPT-P* particle concentration decreases
339 significantly and then stabilizes. Such behavior can be explained by the hetero-association of SA with
340 *NPT-P* leading to a final state where all the *NPT-P* are associated with SA. Consequently, the rate of
341 collision is reduced because of (i) the low rate of diffusion of large aggregates, (ii) the reduced number
342 of separate particles and aggregates, and (iii) the effective repulsion between SA-coated surfaces.



343

344 Figure 4: Aggregation kinetics of 4.0 mg L^{-1} NPT-P, in 600 mmol L^{-1} NaCl, with 57 mg L^{-1} sodium alginate (SA) at pH 8
 345 and 30 mg L^{-1} humic acid (HA) at pH 6.5 (Error bar = standard deviation)

346

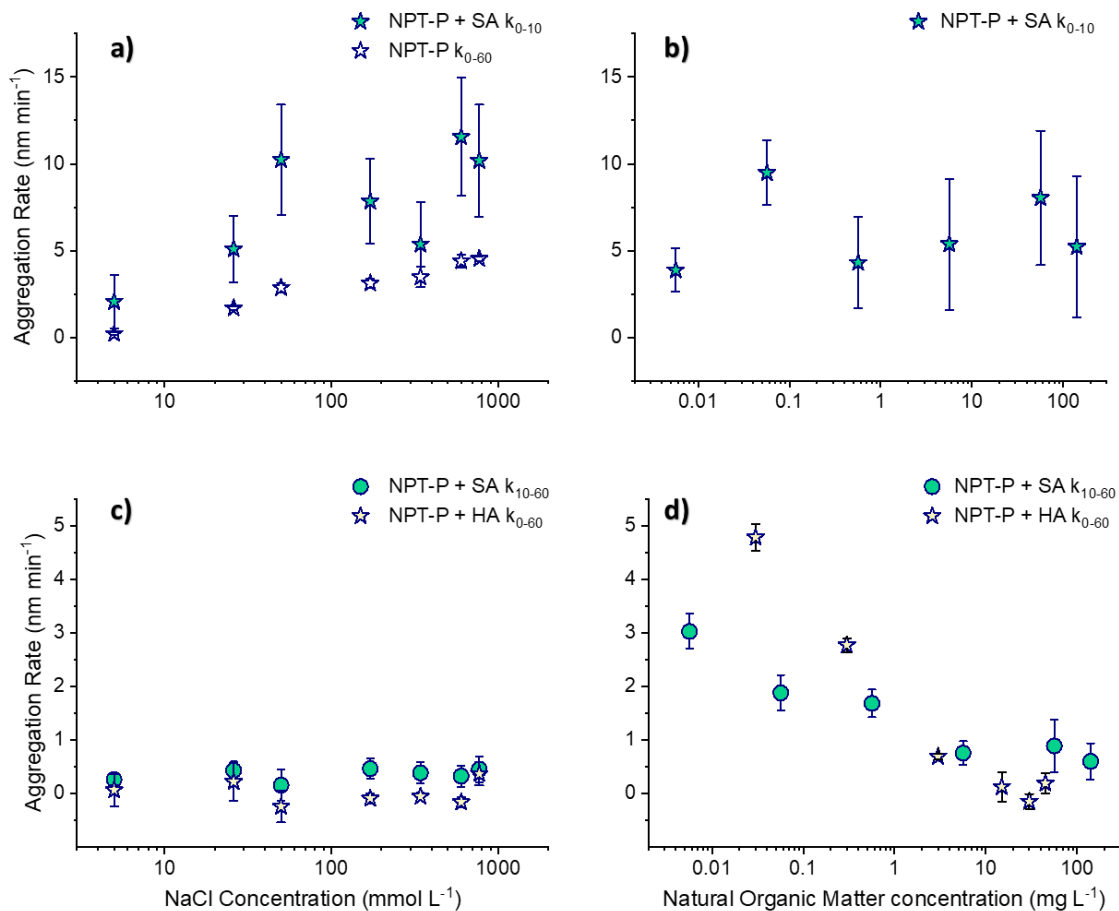
347 To validate this hypothesis, Figure 5 illustrates the different kinetics of association according to
 348 NaCl and NOM concentration. Due to the nature of the kinetics of aggregation of *NPT-P/SA* in the
 349 presence of SA, two different slopes were compared: k_{0-10} , which represents the fast rate of aggregation
 350 from 0 to 10 minutes, and k_{10-60} representing the plateau, observed from 10 to 60 minutes. Figure 5a
 351 shows that NPT-P and SA's association is always faster than the homo-aggregation of *NPT-P* for the
 352 whole range of the ionic strength investigated (5 to 770 mmol L^{-1} , pH 8). So, the aggregation kinetics
 353 are initially accelerated by SA, which sorbs onto and bridges *NPT-P* particles. Figure 5b shows no
 354 significant increase in the rate of the initial, fast aggregation rate (k_{10-60}) as a function of SA
 355 concentration. This suggests that small SA concentrations are sufficient to cover and stabilize *NPT-P*.
 356 The SA that remains free in the solution (non-adsorbed) may have a stabilizing effect by increasing the
 357 solution's electrostatic repulsion. This agrees with Summers et al. (2018), who observed that a low
 358 concentration ($\leq 1 \text{ mg L}^{-1}$) of EPS in a nanoplastic dispersion could play a dispersant effect⁴².

359 Figure 5c presents the aggregation rate of *NPT-P* with NOM as a function of ionic strength
 360 during the stable section of aggregation's kinetics. In the presence of NOM, all aggregation rates were

361 lower than without NOM, except at 5 mmol L⁻¹ NaCl, where there was no significant difference (Fig.
362 5c and S3). In the presence of HA, *NPT-P's* aggregation rate is not significantly different from 0 nm
363 min⁻¹ except at 26 and 770 mmol L⁻¹ (p < 0.05). At 770 mmol L⁻¹, the aggregation rate increases,
364 suggesting that HA is losing its stabilizing effect due to a strong electrostatic screening by this high
365 ionic strength. In the presence of SA, the aggregation rate hovers around 0.4 nm min⁻¹, especially at
366 higher ionic strengths. This suggests that HA may have a stronger stabilizing effect than SA. The NOM
367 concentration in Fig. 5c was the minimum concentration required best stabilize the nanoplastic models
368 at 600 mmol L⁻¹ NaCl (cf: Fig. 5d). Figure 5d shows that at high ionic strength HA rapidly reduces
369 *NPT-P's* attachment efficiency. This is supported by the observations made by Singh et al. (2019),
370 indicating that low concentrations of HA increased the CCC of negatively charged PSL spheres almost
371 4-fold³¹. However, even high SA concentrations do not reduce k_{10-60} under 0.6 nm min⁻¹. This agrees
372 with Summers et al. (2018), who show that high alginate concentrations can have a flocculant effect⁴².

373 Furthermore, Lodeiro et al. (2016) noted that SA only slightly increased the stability of silver
374 nanoparticles⁷². Saleh et al. (2010) also noted that HA was more effective than SA at stabilizing carbon
375 nanotubes in NaCl⁷³. The slightly more effective stabilizing capacity of HA compared to SA can be
376 attributed to the fact that SA sorbs onto particles while HA causes repulsion between them. Indeed, the
377 first mechanism is more likely to form flocs that are large enough to be affected by gravity.

378



379

380 *Figure 5: a) Aggregation rate of NPT-P and fast aggregation rate (k_{0-10}) of NPT-P with 57 mg L⁻¹ SA, at pH 8 as a function of*
 381 *NaCl concentration b) fast aggregation rate (k_{0-10}) of NPT-P with varying concentrations of SA at 600 mmol L⁻¹ NaCl and pH*
 382 *8. Aggregation rates of NPT-P with c) 57 mg L⁻¹ SA (k_{10-60}) at pH 8 and with 30 mg L⁻¹ HA (k_{0-60}) at pH 6.5 as a function of*
 383 *NaCl and d) in 600 mmol L⁻¹ NaCl, with varying concentrations of HA at pH 6.5 and different concentrations of SA at pH 8.*

384

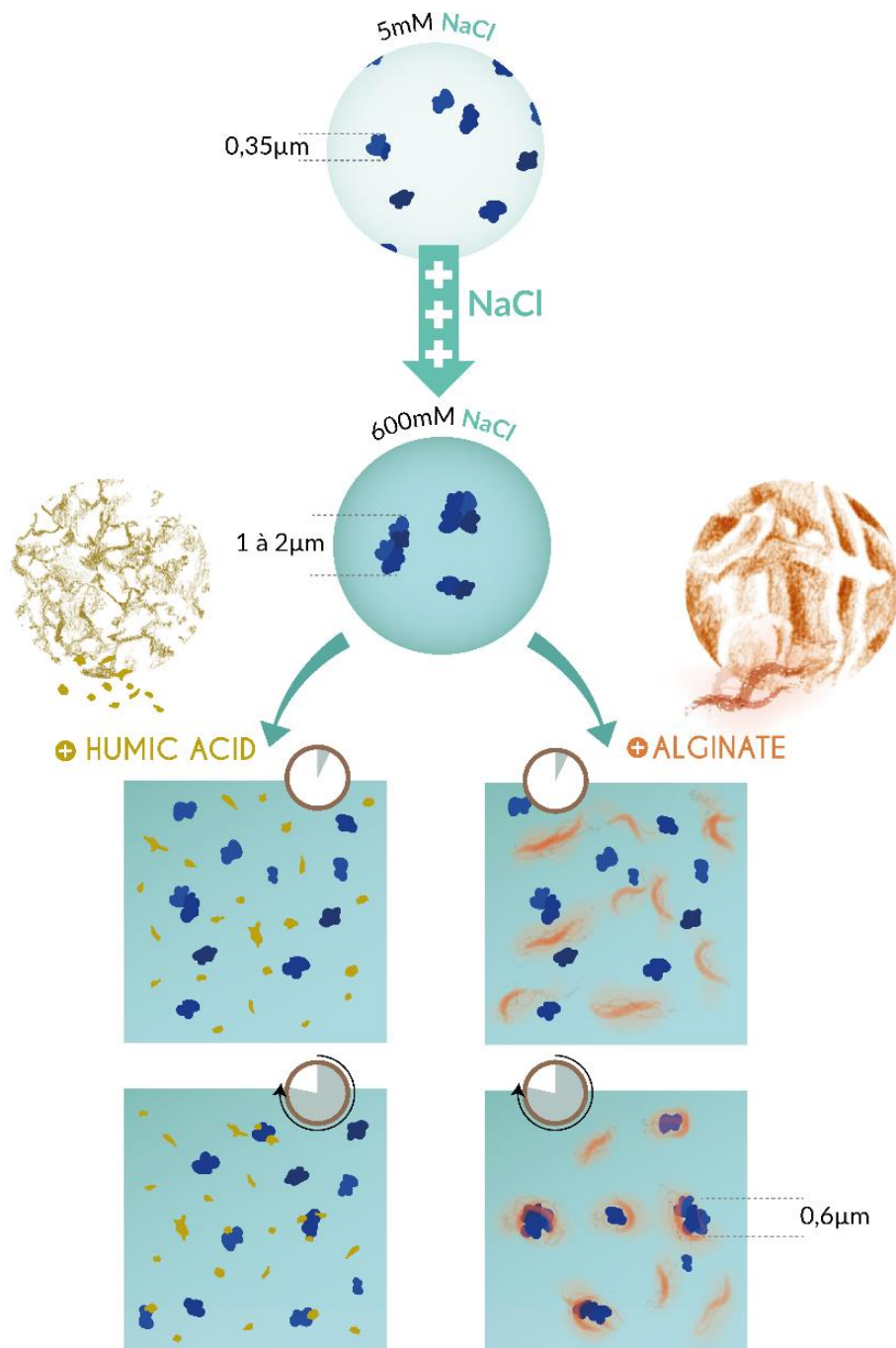
385 Environmental Implications of NOM-NP interactions

386 The aggregation rates presented in Figure 5 were determined at pH 6.5 for HA and pH 8 for SA,
 387 as these pHs are representative of terrestrial and marine aquatic systems, respectively. Kinetic studies
 388 performed at pH 5, 6.5, and 8 for both NOMs show that the same stabilizing mechanisms operate in the
 389 pH range of natural waters (Fig. S9). At a given ionic strength, aggregation rates did not significantly
 390 differ with pH ($p < 0.05$). This minimal pH-dependency is to be expected since (i) *NPT-P's* stability is
 391 not pH-dependent, (ii) HA stabilizes these particles by electrostatic and steric repulsion, which only
 392 requires the NOM to be significantly negatively charged and unfolded, (iii) SA stabilizes *NPT-P* by
 393 sorption via hydrogen bonds and van der Waals attraction, which are operational in this range of pH.

394 The different stabilizing mechanisms are summarized in Figure 6. These mechanisms have been shown
395 to stabilize natural colloids¹⁷ as well as engineered nanomaterials⁷³⁻⁷⁷. When studying the stability of
396 fullerene, Espinasse et al. (2007) also noted the different mechanisms of stabilization of HA and SA⁷⁴.
397 Fullerene were more water-soluble in the presence of HA. HA's combination of hydrophobic regions
398 and ionizable functional groups allows the former to sorb to hydrophobic particles and the latter to
399 increase the particle's hydrophilicity. The amount of HA sorption onto carbon nanotubes was
400 proportional to the HA aromaticity⁷⁵. So, HA increases particles' stability by steric and charge
401 stabilization. However, SA's large size promoted the aggregation of stable, polar fullerenes by bridging
402 and encapsulating them⁷⁴. Indeed, in NaCl, SA coats positively charged titanium dioxide and hematite
403 nanoparticles which then confers electrostatic stability^{76,77}. SA also coats and stabilizes negatively
404 charged, carbonaceous nanomaterials, such as single-walled carbon nanotubes⁷³.

405 In aqueous media, we might expect that SA will stabilize NPs and increase their dispersal. At
406 the same time, it will induce retention when it is attached to a solid interface (i.e., soils, sediments,
407 etc.)^{69,74}. Furthermore, Cunha et al. (2020) showed that nanoplastics and microplastics' presence
408 enhanced the production of EPS carbohydrates by freshwater *Cyanothece sp.*, suggesting a feedback-
409 loop may occur⁷⁸. The presence of NOM will impact nanoplastics environmental fate and affect the
410 potential for co-transport of contaminants^{79,80}. For example, compared to a matrix containing only a
411 nanoplastic model and hydrophobic organic compounds (HOCs), the addition of HA to the matrix
412 increased *Daphnia magna's* rate of uptake of the HOCs by ingestion⁸¹.

413



414

415 *Figure 6: Summary of the mechanisms of stabilization of NPT-P by humic acid (HA) and sodium alginate (SA) in NaCl*

416

417

418 **CONCLUSION**

419 This study investigated the mechanisms of stabilization of nanoplastics by natural organic matter (NOM)
420 according to the media's ionic strength. The interaction of the nanoplastics with NOMs was determined
421 by characterizing the size distributions and shapes using asymmetrical flow field-flow fractionation
422 coupled to static light scattering (A4F-SLS) and confirmed by dynamic light scattering (DLS).
423 According to their origin, the different NOM models, i.e., sodium alginate (SA) for marine environments
424 and humic acid (HA) for terrestrial ones, present different stabilization mechanisms. Humic acid
425 stabilizes the nanoplastic dispersion by electrostatic repulsion between particles, while larger molecules
426 may sorb onto nanoplastics and provide a steric hindrance. However, sodium alginate adsorbs onto the
427 nanoplastics' surface and bridges particles to form small aggregates that remain stable by steric
428 hindrance against the increase in ionic strength. This study highlights the need to consider NOM's
429 physico-chemical properties when assessing nanoplastics behavior in the aqueous environment.

430 **SUPPORTING INFORMATION.**

431 Images and size description of the nanoplastics models (Figure S1 and S2, pages S2-S3); Description of
432 the asymmetrical field flow fractionation method (Section S1, page S4); Aggregation rates and Critical
433 Coagulation Concentrations of the nanoplastics in NaCl (Figure S3 and S4); Results of the Extended
434 Derjaguin Landau Verwey Overbeek (XDLVO) model (Table S1 and Figure S5, page S7,);
435 Characterization of the sodium alginate (Figure S6, text page S8); Images of nanoplastics in the presence
436 of natural organic matter (Figure S7 and text page S9); Evolution of nanoplastics concentration
437 according to time calculated from static light scattering (Figure S8, text page S10 and S11); Aggregation
438 kinetics of nanoplastics models in the presence of natural organic matter (Figure S9, page S12). This
439 material is available free of charge via the internet at <http://pubs.acs.org>.

440

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443

444

445 **REFERENCES**

- 446 (1) Geyer, R.; Jambeck, J. R.; Law, K. L. Production, Use, and Fate of All Plastics Ever Made.
447 *Science Advances* **2017**, *3* (7), e1700782. <https://doi.org/10.1126/sciadv.1700782>.
- 448 (2) Jambeck, J. R.; Geyer, R.; Wilcox, C.; Siegler, T. R.; Perryman, M.; Andrady, A.; Narayan, R.;
449 Law, K. L. Plastic Waste Inputs from Land into the Ocean. *Science* **2015**, *347* (6223), 768–771.
450 <https://doi.org/10.1126/science.1260352>.
- 451 (3) Gigault, J.; Pedrono, B.; Maxit, B.; Halle, A. T. Marine Plastic Litter: The Unanalyzed Nano-
452 Fraction. *Environ. Sci.: Nano* **2016**, *3* (2), 346–350. <https://doi.org/10.1039/C6EN00008H>.
- 453 (4) Lambert, S.; Wagner, M. Characterisation of Nanoplastics during the Degradation of
454 Polystyrene. *Chemosphere* **2016**, *145*, 265–268.
455 <https://doi.org/10.1016/j.chemosphere.2015.11.078>.
- 456 (5) Zhu, L.; Zhao, S.; Bittar, T. B.; Stubbins, A.; Li, D. Photochemical Dissolution of Buoyant
457 Microplastics to Dissolved Organic Carbon: Rates and Microbial Impacts. *Journal of Hazardous*
458 *Materials* **2019**, 121065. <https://doi.org/10.1016/j.jhazmat.2019.121065>.
- 459 (6) Koelmans, A. A.; Kooi, M.; Law, K. L.; van Sebille, E. All Is Not Lost: Deriving a Top-down Mass
460 Budget of Plastic at Sea. *Environmental Research Letters* **2017**, *12* (11), 114028.
461 <https://doi.org/10.1088/1748-9326/aa9500>.
- 462 (7) Ter Halle, A.; Jeanneau, L.; Martignac, M.; Jardé, E.; Pedrono, B.; Brach, L.; Gigault, J.
463 Nanoplastic in the North Atlantic Subtropical Gyre. *Environmental Science & Technology* **2017**, *51*
464 (23), 13689–13697. <https://doi.org/10.1021/acs.est.7b03667>.
- 465 (8) van Sebille, E.; Wilcox, C.; Lebreton, L.; Maximenko, N.; Hardesty, B. D.; van Franeker, J. A.;
466 Eriksen, M.; Siegel, D.; Galgani, F.; Law, K. L. A Global Inventory of Small Floating Plastic Debris.
467 *Environmental Research Letters* **2015**, *10* (12), 124006. [https://doi.org/10.1088/1748-](https://doi.org/10.1088/1748-9326/10/12/124006)
468 [9326/10/12/124006](https://doi.org/10.1088/1748-9326/10/12/124006).
- 469 (9) Allen, S.; Allen, D.; Phoenix, V. R.; Le Roux, G.; Durántez Jiménez, P.; Simonneau, A.; Binet, S.;
470 Galop, D. Atmospheric Transport and Deposition of Microplastics in a Remote Mountain Catchment.
471 *Nature Geoscience* **2019**. <https://doi.org/10.1038/s41561-019-0335-5>.
- 472 (10) Evangeliou, N.; Grythe, H.; Klimont, Z.; Heyes, C.; Eckhardt, S.; Lopez-Aparicio, S.; Stohl, A.
473 Atmospheric Transport Is a Major Pathway of Microplastics to Remote Regions. *Nat Commun* **2020**,
474 *11* (1), 3381. <https://doi.org/10.1038/s41467-020-17201-9>.
- 475 (11) GESAMP. Sources, Fate and Effects of Microplastics in the Marine Environment: Part Two of a
476 Global Assessment. **2016**, No. 93, 220.
- 477 (12) Horton, A. A.; Walton, A.; Spurgeon, D. J.; Lahive, E.; Svendsen, C. Microplastics in Freshwater
478 and Terrestrial Environments: Evaluating the Current Understanding to Identify the Knowledge Gaps
479 and Future Research Priorities. *Science of The Total Environment* **2017**, *586*, 127–141.
480 <https://doi.org/10.1016/j.scitotenv.2017.01.190>.
- 481 (13) Chamas, A.; Moon, H.; Zheng, J.; Qiu, Y.; Tabassum, T.; Jang, J. H.; Abu-Omar, M.; Scott, S. L.;
482 Suh, S. Degradation Rates of Plastics in the Environment. *ACS Sustainable Chem. Eng.* **2020**,
483 [acssuschemeng.9b06635](https://doi.org/10.1021/acssuschemeng.9b06635). <https://doi.org/10.1021/acssuschemeng.9b06635>.

- 484 (14) Gewert, B.; Plassmann, M. M.; MacLeod, M. Pathways for Degradation of Plastic Polymers
485 Floating in the Marine Environment. *Environmental Science: Processes & Impacts* **2015**, *17* (9), 1513–
486 1521. <https://doi.org/10.1039/C5EM00207A>.
- 487 (15) Julienne, F.; Delorme, N.; Lagarde, F. From Macroplastics to Microplastics: Role of Water in
488 the Fragmentation of Polyethylene. *Chemosphere* **2019**, *236*, 124409.
489 <https://doi.org/10.1016/j.chemosphere.2019.124409>.
- 490 (16) Min, K.; Cuiffi, J. D.; Mathers, R. T. Ranking Environmental Degradation Trends of Plastic
491 Marine Debris Based on Physical Properties and Molecular Structure. *Nat Commun* **2020**, *11* (1), 727.
492 <https://doi.org/10.1038/s41467-020-14538-z>.
- 493 (17) Buffle, J.; Wilkinson, K. J.; Stoll, S.; Filella, M.; Zhang, J. A Generalized Description of Aquatic
494 Colloidal Interactions: The Three-Colloidal Component Approach. *Environ. Sci. Technol.* **1998**, *32* (19),
495 2887–2899. <https://doi.org/10.1021/es980217h>.
- 496 (18) Alimi, O. S.; Farnier Budariz, J.; Hernandez, L. M.; Tufenkji, N. Microplastics and Nanoplastics in
497 Aquatic Environments: Aggregation, Deposition, and Enhanced Contaminant Transport.
498 *Environmental Science & Technology* **2018**, *52* (4), 1704–1724.
499 <https://doi.org/10.1021/acs.est.7b05559>.
- 500 (19) Dong, Z.; Zhu, L.; Zhang, W.; Huang, R.; Lv, X.; Jing, X.; Yang, Z.; Wang, J.; Qiu, Y. Role of
501 Surface Functionalities of Nanoplastics on Their Transport in Seawater-Saturated Sea Sand.
502 *Environmental Pollution* **2019**, *255*, 113177. <https://doi.org/10.1016/j.envpol.2019.113177>.
- 503 (20) Keller, A. S.; Jimenez-Martinez, J.; Mitrano, D. M. Transport of Nano- and Microplastic
504 through Unsaturated Porous Media from Sewage Sludge Application. *Environ. Sci. Technol.* **2019**,
505 *acs.est.9b06483*. <https://doi.org/10.1021/acs.est.9b06483>.
- 506 (21) Liu, J.; Zhang, T.; Tian, L.; Liu, X.; Qi, Z.; Ma, Y.; Ji, R.; Chen, W. Aging Significantly Affects
507 Mobility and Contaminant-Mobilizing Ability of Nanoplastics in Saturated Loamy Sand. *Environ. Sci.*
508 *Technol.* **2019**, *acs.est.9b00787*. <https://doi.org/10.1021/acs.est.9b00787>.
- 509 (22) Li, Y.; Wang, X.; Fu, W.; Xia, X.; Liu, C.; Min, J.; Zhang, W.; Crittenden, J. C. Interactions
510 between Nano/Micro Plastics and Suspended Sediment in Water: Implications on Aggregation and
511 Settling. *Water Research* **2019**, *161*, 486–495. <https://doi.org/10.1016/j.watres.2019.06.018>.
- 512 (23) Oriekhova, O.; Stoll, S. Heteroaggregation of Nanoplastic Particles in the Presence of
513 Inorganic Colloids and Natural Organic Matter. *Environ. Sci.: Nano* **2018**, *5* (3), 792–799.
514 <https://doi.org/10.1039/C7EN01119A>.
- 515 (24) Bergami, E.; Krupinski Emerenciano, A.; González-Aravena, M.; Cárdenas, C. A.; Hernández,
516 P.; Silva, J. R. M. C.; Corsi, I. Polystyrene Nanoparticles Affect the Innate Immune System of the
517 Antarctic Sea Urchin *Sterechinus Neumayeri*. *Polar Biol* **2019**, *42* (4), 743–757.
518 <https://doi.org/10.1007/s00300-019-02468-6>.
- 519 (25) Chen, Z.; Liu, J.; Chen, C.; Huang, Z. Sedimentation of Nanoplastics from Water with Ca/Al
520 Dual Flocculants: Characterization, Interface Reaction, Effects of PH and Ion Ratios. *Chemosphere*
521 **2020**, 126450. <https://doi.org/10.1016/j.chemosphere.2020.126450>.
- 522 (26) Della Torre, C.; Bergami, E.; Salvati, A.; Faleri, C.; Cirino, P.; Dawson, K. A.; Corsi, I.
523 Accumulation and Embryotoxicity of Polystyrene Nanoparticles at Early Stage of Development of Sea

- 524 Urchin Embryos *Paracentrotus Lividus*. *Environ. Sci. Technol.* **2014**, *48* (20), 12302–12311.
525 <https://doi.org/10.1021/es502569w>.
- 526 (27) González-Fernández, C.; Toullec, J.; Lambert, C.; Le Goïc, N.; Seoane, M.; Moriceau, B.; Huvet,
527 A.; Berchel, M.; Vincent, D.; Courcot, L.; Soudant, P.; Paul-Pont, I. Do Transparent Exopolymeric
528 Particles (TEP) Affect the Toxicity of Nanoplastics on *Chaetoceros Neogracile*? *Environmental*
529 *Pollution* **2019**, *250*, 873–882. <https://doi.org/10.1016/j.envpol.2019.04.093>.
- 530 (28) Manfra, L.; Rotini, A.; Bergami, E.; Grassi, G.; Faleri, C.; Corsi, I. Comparative Ecotoxicity of
531 Polystyrene Nanoparticles in Natural Seawater and Reconstituted Seawater Using the Rotifer
532 *Brachionus Plicatilis*. *Ecotoxicology and Environmental Safety* **2017**, *145*, 557–563.
533 <https://doi.org/10.1016/j.ecoenv.2017.07.068>.
- 534 (29) Ramirez, L.; Ramseier Gentile, S.; Zimmermann, S.; Stoll, S. Behavior of TiO₂ and CeO₂
535 Nanoparticles and Polystyrene Nanoplastics in Bottled Mineral, Drinking and Lake Geneva Waters.
536 Impact of Water Hardness and Natural Organic Matter on Nanoparticle Surface Properties and
537 Aggregation. *Water* **2019**, *11* (4), 721. <https://doi.org/10.3390/w11040721>.
- 538 (30) Seoane, M.; González-Fernández, C.; Soudant, P.; Huvet, A.; Esperanza, M.; Cid, Á.; Paul-Pont,
539 I. Polystyrene Microbeads Modulate the Energy Metabolism of the Marine Diatom *Chaetoceros*
540 *Neogracile*. *Environmental Pollution* **2019**, *251*, 363–371.
541 <https://doi.org/10.1016/j.envpol.2019.04.142>.
- 542 (31) Singh, N.; Tiwari, E.; Khandelwal, N.; Darbha, G. K. Understanding the Stability of Nanoplastics
543 in Aqueous Environments: Effect of Ionic Strength, Temperature, Dissolved Organic Matter, Clay, and
544 Heavy Metals. *Environ. Sci.: Nano* **2019**, *6* (10), 2968–2976. <https://doi.org/10.1039/C9EN00557A>.
- 545 (32) Song, Z.; Yang, X.; Chen, F.; Zhao, F.; Zhao, Y.; Ruan, L.; Wang, Y.; Yang, Y. Fate and Transport
546 of Nanoplastics in Complex Natural Aquifer Media: Effect of Particle Size and Surface
547 Functionalization. *Science of The Total Environment* **2019**, *669*, 120–128.
548 <https://doi.org/10.1016/j.scitotenv.2019.03.102>.
- 549 (33) Tallec, K.; Blard, O.; González-Fernández, C.; Brotons, G.; Berchel, M.; Soudant, P.; Huvet, A.;
550 Paul-Pont, I. Surface Functionalization Determines Behavior of Nanoplastic Solutions in Model
551 Aquatic Environments. *Chemosphere* **2019**, *225*, 639–646.
552 <https://doi.org/10.1016/j.chemosphere.2019.03.077>.
- 553 (34) Cai, L.; Hu, L.; Shi, H.; Ye, J.; Zhang, Y.; Kim, H. Effects of Inorganic Ions and Natural Organic
554 Matter on the Aggregation of Nanoplastics. *Chemosphere* **2018**, *197*, 142–151.
555 <https://doi.org/10.1016/j.chemosphere.2018.01.052>.
- 556 (35) Dong, Z.; Zhang, W.; Qiu, Y.; Yang, Z.; Wang, J.; Zhang, Y. Cotransport of Nanoplastics (NPs)
557 with Fullerene (C₆₀) in Saturated Sand: Effect of NPs/C₆₀ Ratio and Seawater Salinity. *Water*
558 *Research* **2019**, *148*, 469–478. <https://doi.org/10.1016/j.watres.2018.10.071>.
- 559 (36) Magri, D.; Sánchez-Moreno, P.; Caputo, G.; Gatto, F.; Veronesi, M.; Bardi, G.; Catelani, T.;
560 Guarnieri, D.; Athanassiou, A.; Pompa, P. P.; Fragouli, D. Laser Ablation as a Versatile Tool To Mimic
561 Polyethylene Terephthalate Nanoplastic Pollutants: Characterization and Toxicology Assessment. *ACS*
562 *Nano* **2018**, *12* (8), 7690–7700. <https://doi.org/10.1021/acs.nano.8b01331>.
- 563 (37) Mao, Y.; Li, H.; Huangfu, X.; Liu, Y.; He, Q. Nanoplastics Display Strong Stability in Aqueous
564 Environments: Insights from Aggregation Behaviour and Theoretical Calculations. *Environmental*
565 *Pollution* **2020**, *258*, 113760. <https://doi.org/10.1016/j.envpol.2019.113760>.

- 566 (38) Wu, J.; Jiang, R.; Lin, W.; Ouyang, G. Effect of Salinity and Humic Acid on the Aggregation and
567 Toxicity of Polystyrene Nanoplastics with Different Functional Groups and Charges. *Environmental*
568 *Pollution* **2019**, *245*, 836–843. <https://doi.org/10.1016/j.envpol.2018.11.055>.
- 569 (39) Yu, S.; Shen, M.; Li, S.; Fu, Y.; Zhang, D.; Liu, H.; Liu, J. Aggregation Kinetics of Different
570 Surface-Modified Polystyrene Nanoparticles in Monovalent and Divalent Electrolytes. *Environmental*
571 *Pollution* **2019**, *255*, 113302. <https://doi.org/10.1016/j.envpol.2019.113302>.
- 572 (40) Zhang, F.; Wang, Z.; Wang, S.; Fang, H.; Wang, D. Aquatic Behavior and Toxicity of
573 Polystyrene Nanoplastic Particles with Different Functional Groups: Complex Roles of PH, Dissolved
574 Organic Carbon and Divalent Cations. *Chemosphere* **2019**, *228*, 195–203.
575 <https://doi.org/10.1016/j.chemosphere.2019.04.115>.
- 576 (41) Saavedra, J.; Stoll, S.; Slaveykova, V. I. Influence of Nanoplastic Surface Charge on Eco-Corona
577 Formation, Aggregation and Toxicity to Freshwater Zooplankton. *Environmental Pollution* **2019**, *252*,
578 715–722. <https://doi.org/10.1016/j.envpol.2019.05.135>.
- 579 (42) Summers, S.; Henry, T.; Gutierrez, T. Agglomeration of Nano- and Microplastic Particles in
580 Seawater by Autochthonous and de Novo-Produced Sources of Exopolymeric Substances. *Marine*
581 *Pollution Bulletin* **2018**, *130*, 258–267. <https://doi.org/10.1016/j.marpolbul.2018.03.039>.
- 582 (43) Dong, S.; Cai, W.; Xia, J.; Sheng, L.; Wang, W.; Liu, H. Aggregation Kinetics of Fragmental PET
583 Nanoplastics in Aqueous Environment: Complex Roles of Electrolytes, PH and Humic Acid.
584 *Environmental Pollution* **2020**, 115828. <https://doi.org/10.1016/j.envpol.2020.115828>.
- 585 (44) Liu, Y.; Hu, Y.; Yang, C.; Chen, C.; Huang, W.; Dang, Z. Aggregation Kinetics of UV Irradiated
586 Nanoplastics in Aquatic Environments. *Water Research* **2019**, *163*, 114870.
587 <https://doi.org/10.1016/j.watres.2019.114870>.
- 588 (45) Okshevsky, M.; Gautier, E.; Farner, J. M.; Schreiber, L.; Tufenkji, N. Biofilm Formation by
589 Marine Bacteria Is Impacted by Concentration and Surface Functionalization of Polystyrene
590 Nanoparticles in a Species-specific Manner. *Environmental Microbiology Reports* **2020**, *12* (2), 203–
591 213. <https://doi.org/10.1111/1758-2229.12824>.
- 592 (46) Flemming, H.-C.; Wingender, J. The Biofilm Matrix. *Nat Rev Microbiol* **2010**, *8* (9), 623–633.
593 <https://doi.org/10.1038/nrmicro2415>.
- 594 (47) Stumm, W.; Morgan, J. J. *Aquatic Chemistry : Chemical Equilibria and Rates in Natural*
595 *Waters*; Wiley: New York, 1996.
- 596 (48) El Hadri, H.; Gigault, J.; Maxit, B.; Grassl, B.; Reynaud, S. Nanoplastic from Mechanically
597 Degraded Primary and Secondary Microplastics for Environmental Assessments. *NanoImpact* **2020**,
598 100206. <https://doi.org/10.1016/j.impact.2019.100206>.
- 599 (49) Holthoff, H.; Egelhaaf, S. U.; Borkovec, M.; Schurtenberger, P.; Sticher, H. Coagulation Rate
600 Measurements of Colloidal Particles by Simultaneous Static and Dynamic Light Scattering. *Langmuir*
601 **1996**, *12* (23), 5541–5549. <https://doi.org/10.1021/la960326e>.
- 602 (50) Gigault, J.; El Hadri, H.; Reynaud, S.; Deniau, E.; Grassl, B. Asymmetrical Flow Field Flow
603 Fractionation Methods to Characterize Submicron Particles: Application to Carbon-Based Aggregates
604 and Nanoplastics. *Analytical and Bioanalytical Chemistry* **2017**, *409* (29), 6761–6769.
605 <https://doi.org/10.1007/s00216-017-0629-7>.

- 606 (51) Gregory, J. Approximate Expressions for Retarded van Der Waals Interaction. *Journal of*
607 *Colloid and Interface Science* **1981**, *83* (1), 138–145. [https://doi.org/10.1016/0021-9797\(81\)90018-7](https://doi.org/10.1016/0021-9797(81)90018-7).
- 608 (52) *Particle Deposition and Aggregation: Measurement, Modelling and Simulation*; Elimelech, M.,
609 Ed.; Colloid and surface engineering series; Butterworth-Heinemann: Oxford, 1998.
- 610 (53) Hogg, R.; Healy, T. W.; Fuerstenau, D. W. Mutual Coagulation of Colloidal Dispersions. *Trans.*
611 *Faraday Soc.* **1966**, *62*, 1638. <https://doi.org/10.1039/tf9666201638>.
- 612 (54) van Oss, C. J. Acid–Base Interfacial Interactions in Aqueous Media. *Colloids and Surfaces A:*
613 *Physicochemical and Engineering Aspects* **1993**, *78*, 1–49. [https://doi.org/10.1016/0927-](https://doi.org/10.1016/0927-7757(93)80308-2)
614 [7757\(93\)80308-2](https://doi.org/10.1016/0927-7757(93)80308-2).
- 615 (55) Valsesia, A.; Desmet, C.; Ojea-Jiménez, I.; Oddo, A.; Capomaccio, R.; Rossi, F.; Colpo, P. Direct
616 Quantification of Nanoparticle Surface Hydrophobicity. *Commun Chem* **2018**, *1* (1), 53.
617 <https://doi.org/10.1038/s42004-018-0054-7>.
- 618 (56) Vold, M. J. Van Der Waals' Attraction between Anisometric Particles. *Journal of Colloid*
619 *Science* **1954**, *9* (5), 451–459. [https://doi.org/10.1016/0095-8522\(54\)90032-X](https://doi.org/10.1016/0095-8522(54)90032-X).
- 620 (57) Baalousha, M. Effect of Nanomaterial and Media Physicochemical Properties on
621 Nanomaterial Aggregation Kinetics. *NanoImpact* **2017**, *6*, 55–68.
622 <https://doi.org/10.1016/j.impact.2016.10.005>.
- 623 (58) Zhou, D.; Keller, A. A. Role of Morphology in the Aggregation Kinetics of ZnO Nanoparticles.
624 *Water Research* **2010**, *44* (9), 2948–2956. <https://doi.org/10.1016/j.watres.2010.02.025>.
- 625 (59) Wu, W.; Giese, R. F.; van Oss, C. J. Stability versus Flocculation of Particle Suspensions in
626 Water—Correlation with the Extended DLVO Approach for Aqueous Systems, Compared with
627 Classical DLVO Theory. *Colloids and Surfaces B: Biointerfaces* **1999**, *14* (1–4), 47–55.
628 [https://doi.org/10.1016/S0927-7765\(99\)00023-5](https://doi.org/10.1016/S0927-7765(99)00023-5).
- 629 (60) Lan, Y.; Caciagli, A.; Guidetti, G.; Yu, Z.; Liu, J.; Johansen, V. E.; Kamp, M.; Abell, C.; Vignolini,
630 S.; Scherman, O. A.; Eiser, E. Unexpected Stability of Aqueous Dispersions of Raspberry-like Colloids.
631 *Nat Commun* **2018**, *9* (1), 3614. <https://doi.org/10.1038/s41467-018-05560-3>.
- 632 (61) DelRio, F. W.; de Boer, M. P.; Knapp, J. A.; David Reedy, E.; Clews, P. J.; Dunn, M. L. The Role
633 of van Der Waals Forces in Adhesion of Micromachined Surfaces. *Nature Materials* **2005**, *4* (8), 629–
634 634. <https://doi.org/10.1038/nmat1431>.
- 635 (62) Huang, X.; Bhattacharjee, S.; Hoek, E. M. V. Is Surface Roughness a "Scapegoat" or a Primary
636 Factor When Defining Particle–Substrate Interactions? *Langmuir* **2010**, *26* (4), 2528–2537.
637 <https://doi.org/10.1021/la9028113>.
- 638 (63) Petosa, A. R.; Jaisi, D. P.; Quevedo, I. R.; Elimelech, M.; Tufenkji, N. Aggregation and
639 Deposition of Engineered Nanomaterials in Aquatic Environments: Role of Physicochemical
640 Interactions. *Environmental Science & Technology* **2010**, *44* (17), 6532–6549.
641 <https://doi.org/10.1021/es100598h>.
- 642 (64) Goodwin, J. W. *Colloids and Interfaces with Surfactants and Polymers: An Introduction*,
643 Wiley.; 2004.
- 644 (65) Messaud, F. A.; Sanderson, R. D.; Runyon, J. R.; Otte, T.; Pasch, H.; Williams, S. K. R. An
645 Overview on Field-Flow Fractionation Techniques and Their Applications in the Separation and

- 646 Characterization of Polymers. *Progress in Polymer Science* **2009**, *34* (4), 351–368.
647 <https://doi.org/10.1016/j.progpolymsci.2008.11.001>.
- 648 (66) Beckett, Ronald.; Jue, Zhang.; Giddings, J. Calvin. Determination of Molecular Weight
649 Distributions of Fulvic and Humic Acids Using Flow Field-Flow Fractionation. *Environ. Sci. Technol.*
650 **1987**, *21* (3), 289–295. <https://doi.org/10.1021/es00157a010>.
- 651 (67) Yu, S.; Liu, J.; Yin, Y.; Shen, M. Interactions between Engineered Nanoparticles and Dissolved
652 Organic Matter: A Review on Mechanisms and Environmental Effects. *Journal of Environmental*
653 *Sciences* **2018**, *63*, 198–217. <https://doi.org/10.1016/j.jes.2017.06.021>.
- 654 (68) Chen, C.-S.; Le, C.; Chiu, M.-H.; Chin, W.-C. The Impact of Nanoplastics on Marine Dissolved
655 Organic Matter Assembly. *Science of The Total Environment* **2018**, *634*, 316–320.
656 <https://doi.org/10.1016/j.scitotenv.2018.03.269>.
- 657 (69) He, L.; Rong, H.; Wu, D.; Li, M.; Wang, C.; Tong, M. Influence of Biofilm on the Transport and
658 Deposition Behaviors of Nano- and Micro-Plastic Particles in Quartz Sand. *Water Research* **2020**,
659 115808. <https://doi.org/10.1016/j.watres.2020.115808>.
- 660 (70) Bhattacharya, P.; Lin, S.; Turner, J. P.; Ke, P. C. Physical Adsorption of Charged Plastic
661 Nanoparticles Affects Algal Photosynthesis. *The Journal of Physical Chemistry C* **2010**, *114* (39),
662 16556–16561. <https://doi.org/10.1021/jp1054759>.
- 663 (71) Xu, R. *Particle Characterization: Light Scattering Methods*; Springer Netherlands, 2006.
- 664 (72) Lodeiro, P.; Achterberg, E. P.; Pampín, J.; Affatati, A.; El-Shahawi, M. S. Silver Nanoparticles
665 Coated with Natural Polysaccharides as Models to Study AgNP Aggregation Kinetics Using UV-Visible
666 Spectrophotometry upon Discharge in Complex Environments. *Science of The Total Environment*
667 **2016**, *539*, 7–16. <https://doi.org/10.1016/j.scitotenv.2015.08.115>.
- 668 (73) Saleh, N. B.; Pfefferle, L. D.; Elimelech, M. Influence of Biomacromolecules and Humic Acid on
669 the Aggregation Kinetics of Single-Walled Carbon Nanotubes. *Environ. Sci. Technol.* **2010**, *44* (7),
670 2412–2418. <https://doi.org/10.1021/es903059t>.
- 671 (74) Espinasse, B.; Hotze, E. M.; Wiesner, M. R. Transport and Retention of Colloidal Aggregates of
672 C₆₀ in Porous Media: Effects of Organic Macromolecules, Ionic Composition, and Preparation
673 Method. *Environ. Sci. Technol.* **2007**, *41* (21), 7396–7402. <https://doi.org/10.1021/es0708767>.
- 674 (75) Hyung, H.; Kim, J.-H. Natural Organic Matter (NOM) Adsorption to Multi-Walled Carbon
675 Nanotubes: Effect of NOM Characteristics and Water Quality Parameters. *Environmental Science &*
676 *Technology* **6**. <https://doi.org/10.1021/es702916h>.
- 677 (76) Loosli, F.; Vitorazi, L.; Berret, J.-F.; Stoll, S. Towards a Better Understanding on Agglomeration
678 Mechanisms and Thermodynamic Properties of TiO₂ Nanoparticles Interacting with Natural Organic
679 Matter. *Water Research* **2015**, *80*, 139–148. <https://doi.org/10.1016/j.watres.2015.05.009>.
- 680 (77) Chen, K. L.; Mylon, S. E.; Elimelech, M. Aggregation Kinetics of Alginate-Coated Hematite
681 Nanoparticles in Monovalent and Divalent Electrolytes. *Environ. Sci. Technol.* **2006**, *40* (5), 1516–
682 1523. <https://doi.org/10.1021/es0518068>.
- 683 (78) Cunha, C.; Silva, L.; Paulo, J.; Faria, M.; Nogueira, N.; Cordeiro, N. Microalgal-Based
684 Biopolymer for Nano- and Microplastic Removal: A Possible Biosolution for Wastewater Treatment.
685 *Environmental Pollution* **2020**, *263*, 114385. <https://doi.org/10.1016/j.envpol.2020.114385>.

- 686 (79) Liu, J.; Ma, Y.; Zhu, D.; Xia, T.; Qi, Y.; Yao, Y.; Guo, X.; Ji, R.; Chen, W. Polystyrene Nanoplastics-
687 Enhanced Contaminant Transport: Role of Irreversible Adsorption in Glassy Polymeric Domain.
688 *Environmental Science & Technology* **2018**, *52* (5), 2677–2685.
689 <https://doi.org/10.1021/acs.est.7b05211>.
- 690 (80) Velzeboer, I.; Kwadijk, C. J. A. F.; Koelmans, A. A. Strong Sorption of PCBs to Nanoplastics,
691 Microplastics, Carbon Nanotubes, and Fullerenes. *Environ. Sci. Technol.* **2014**, *48* (9), 4869–4876.
692 <https://doi.org/10.1021/es405721v>.
- 693 (81) Lin, W.; Jiang, R.; Xiao, X.; Wu, J.; Wei, S.; Liu, Y.; Muir, D. C. G.; Ouyang, G. Joint Effect of
694 Nanoplastics and Humic Acid on the Uptake of PAHs for *Daphnia Magna*: A Model Study. *Journal of*
695 *Hazardous Materials* **2020**, *391*, 122195. <https://doi.org/10.1016/j.jhazmat.2020.122195>.
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Supporting Information for:

Stabilization of fragmental polystyrene nanoplastic by natural organic matter:

Insight into mechanisms

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The Supporting Information contains 9 figures, 1 table, 2 sections of text (SI1 and SI2), 9 references, 13 pages.

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Figure S9: Aggregation rate of NPT-P with a) 30 mg L⁻¹ humic acid b) 57 mg L⁻¹ sodium alginate, as a function of ionic strength and pH (Error bars = standard deviation)

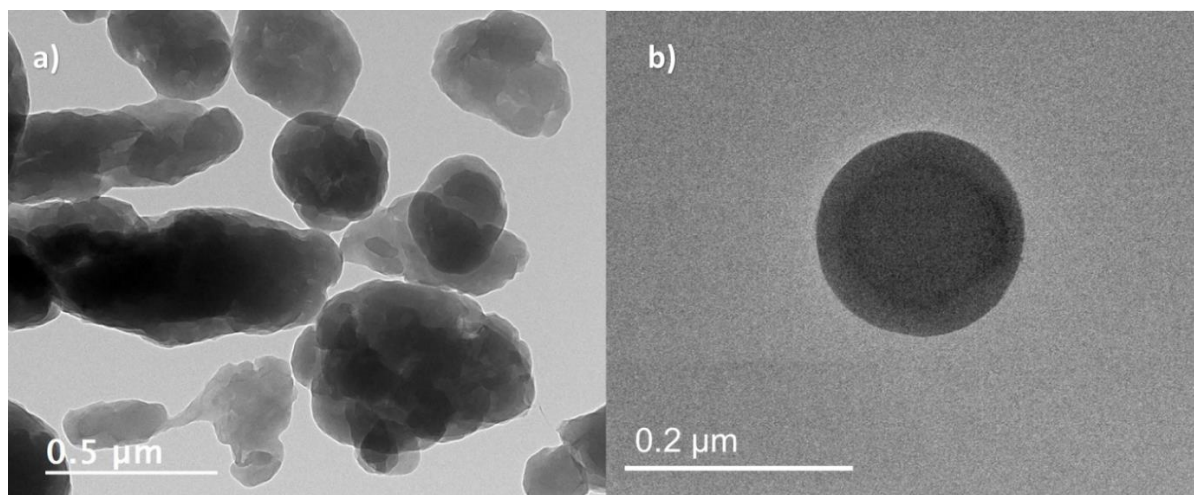


Figure S1: Transmission Electron Microscopy Images of a) NPT-P and b) PSL COOH

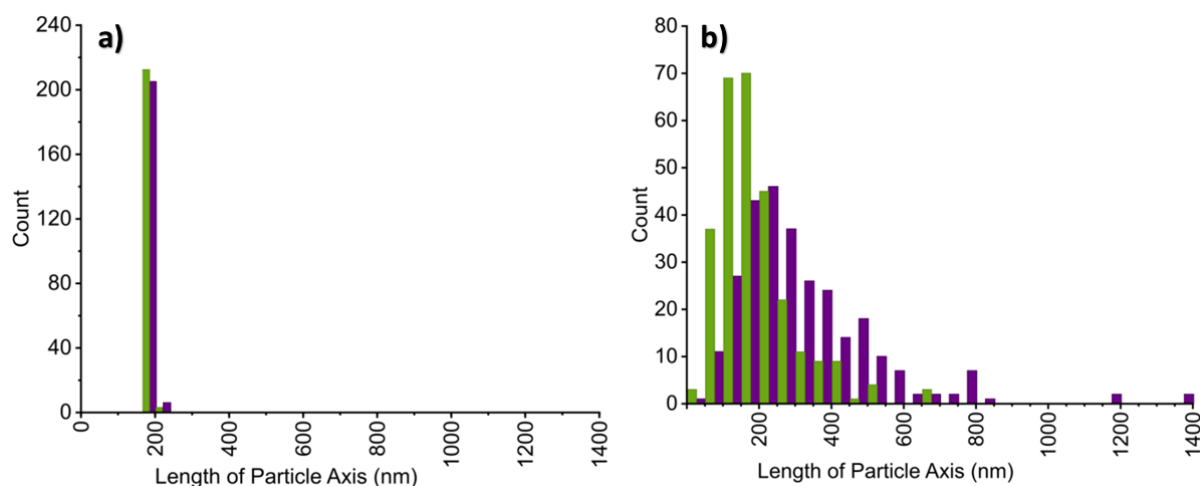


Figure S2: Histogram of short axis (green) and long axis (purple), as determined by TEM images of a) PSL COOH and b) NPT-P nanoplastic models in linear distribution (PSL COOH: $n = 212$ and NPT-P: $n = 283$)

TEM images were obtained by a Jeol JEM 2100 HR (200kV) with an LaB₆ filament. The camera was a Gatan Orius SC 200 D. A 4 μL drop was deposited on a full carbon grid and allowed to air dry. Data was analyzed with ImageJ software and the NanoDefine plugin, using the watershed fitting mode (Verleysen et al., 2019). The length of the major axis and minor axis were the longest and shortest lengths of the minimum bounding rectangle. Based on these images and assuming the NPT-P particles is either a sphere or an ellipsoid, the specific surface area was determined to be 30.2 ± 16.4 and 34.2 ± 18.9 $\text{m}^2 \text{g}^{-1}$, respectively. The specific surface area was determined to be 29.6 ± 0.6 $\text{m}^2 \text{g}^{-1}$ for PSL COOH.

The NPT-P minor and major axis followed a log-normal distribution, as determined with the orthogonal distance regression method. Equation (S1) was used to fit the data, with μ the average of distribution and σ the standard deviation of distribution.

$$y = \frac{A}{\sigma x \sqrt{2\pi}} \exp \frac{-\ln(\frac{x}{\mu})^2}{2\sigma^2} \quad (\text{S1})$$

The following parameters were determined:

- For the NPT-P major axis:
 - $\mu = 395.70 \pm 30.65$
 - $\sigma = 380.52 \pm 48.34$
 - $A = 20734.02 \pm 6774.76$
 - R^2 (COD) = 0.99994
- For the NPT-P minor axis :
 - $\mu = 213.54 \pm 3.93$
 - $\sigma = 461.88 \pm 13.53$
 - $A = 9986.26 \pm 260.98$
 - R^2 (COD) = 0.98101

Supplemental Information 1:

Method for Asymmetrical Flow Field Flow Fractionation (A4F) coupled to Static Light Scattering (SLS)

The mobile phase flow was generated by a 1200 series high-performance liquid chromatography (HPLC) pump (Agilent Technologies, Les Ulis, France). The Asymmetrical Flow Field Flow Fractionation (A4F) system was an Eclipse 3+ (Wyatt Technology, Dernbach, Germany). Injections were performed with an Agilent Technologies 1200 series autosampler. At the outlet, the detectors were a 1200 series UV-vis absorbance detector (Agilent Technologies, Les Ulis, France) and a DAWN HELEOS multi-angle laser, static light scattering (SLS) detector (Wyatt Technology). For UV-Vis detection, the selected wavelength was 254nm.

The A4F channel height was established using a spacer (Mylar film) of 250 μm . The dimensions of the spacer were 26.5 cm length and narrowing width from 2.1 to 0.6 cm. The accumulation wall was composed of Polyethersulfone (PES) 10 kDa membranes (Wyatt Technology). The A4F method was based on the general (fast) method O described by Gigault et al. (2017). The elution flow rate was fixed at 0.5 mL min^{-1} . The injection flow rate was fixed at 0.2 mL min^{-1} . The focus-flow during the relaxation was 0.5 $\text{mL}\cdot\text{min}^{-1}$ and the cross-flow rate during elution (V_c), was a function of time (t) $V_c = 2e^{-0.27t}$.

The mobile phase was composed of 0.5 mmol L^{-1} NaNO_3 (>99% purity Reagent Plus, Sigma Aldrich), which was filtered on polyethersulfone (PES) filters (0.1 μm , Pall®), purchased from VWR (Fontenay-sous-Bois, France). The injection volume was 100 μL . First, the effects of the duration of the focus period and the ionic strength of the injected dispersion, on the quality of detection were studied. Subsequently, a focus time of 5 minutes and an ionic strength of 600 mmol L^{-1} was selected. The injected dispersion was prepared in the same way as for the aggregation kinetic study, described below.

Data from a minimum of 14 out of 18 SLS detectors were collected and processed using Astra software, version 6 (Wyatt Technology). The radius of gyration (R_g) was determined using the Berry formalism using SLS signal at different angles.

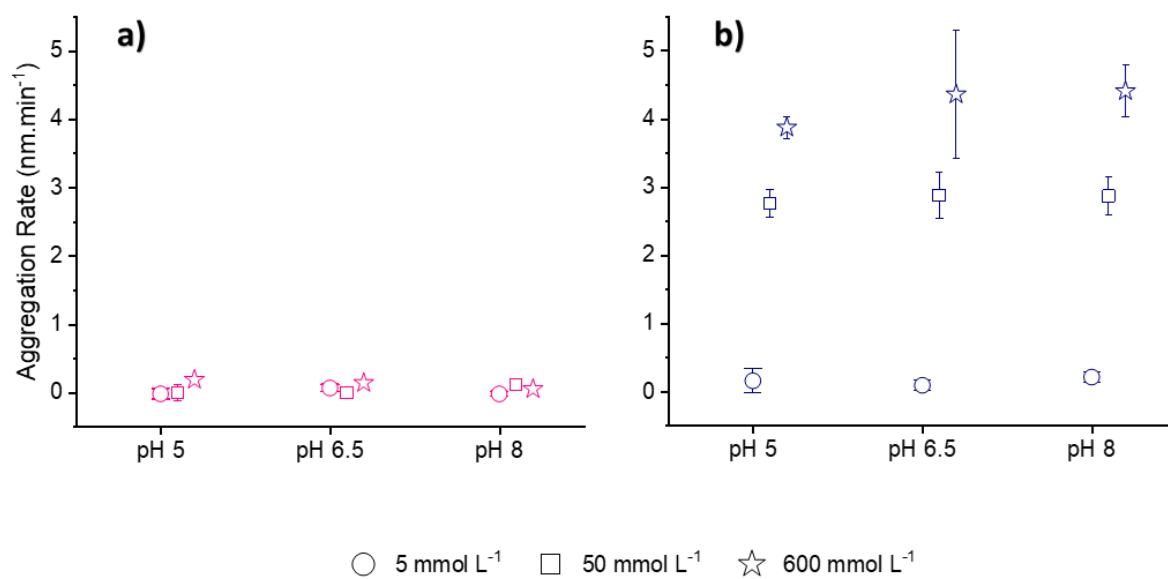


Figure S3: Aggregation rate of a) PSL COOH and b) NPT-P models, as a function of NaCl concentration and pH (Error bars = standard deviation)

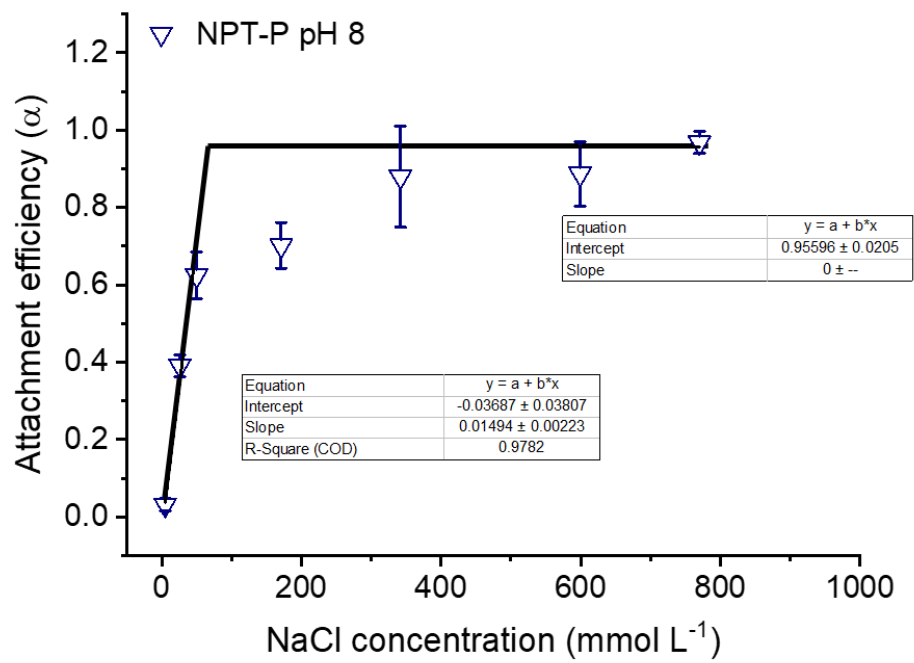
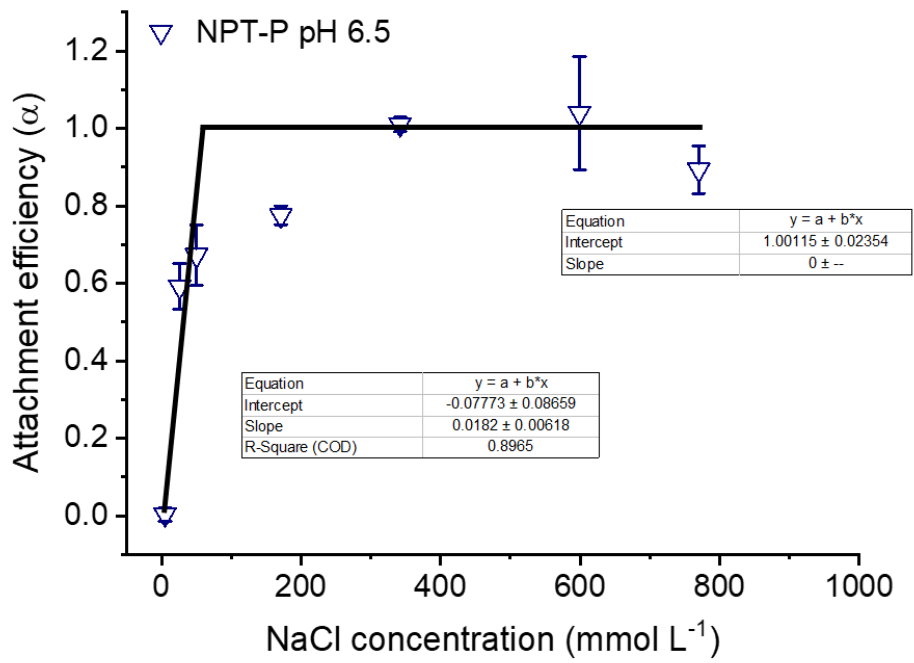


Figure S4: Determination of the Critical Coagulation Concentration (CCC) of NPT-P particles in NaCl at pH 6.5 and pH 8

Table S1: Energy barrier between particles according to DLVO and XDLVO theories, scaled to $k_B T$

| Particle 1 | PSL COOH | NPT-P | | |
|--------------|----------|-------|----------------------------------|-----------------------------------|
| Particle 2 | PSL COOH | NPT-P | small (50 nm) NPT-P asperity* | large (100 nm) NPT-P asperity* |
| DLVO theory | 70 | 76 | 21 | 38 |
| XDLVO theory | 28 | 24 | 7 | 12 |

* Assuming the NPT-P particles are ellipsoids, the smallest radius of curvature, defined as the square of the short axis divided by the long axis, is on average 64nm.

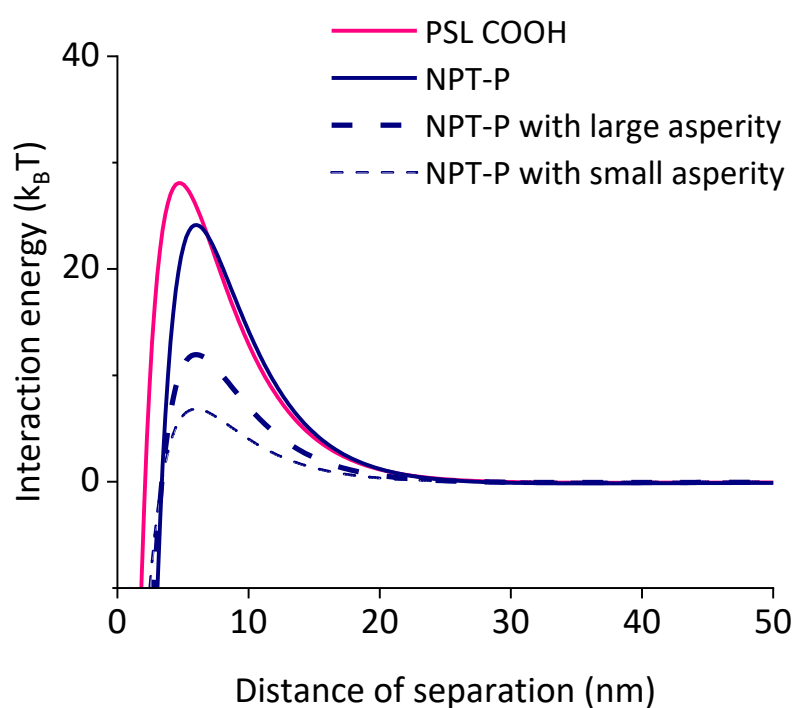


Figure S5: Interaction energy, scaled to $k_B T$, between different nanoplastic models, according to XDLVO theory

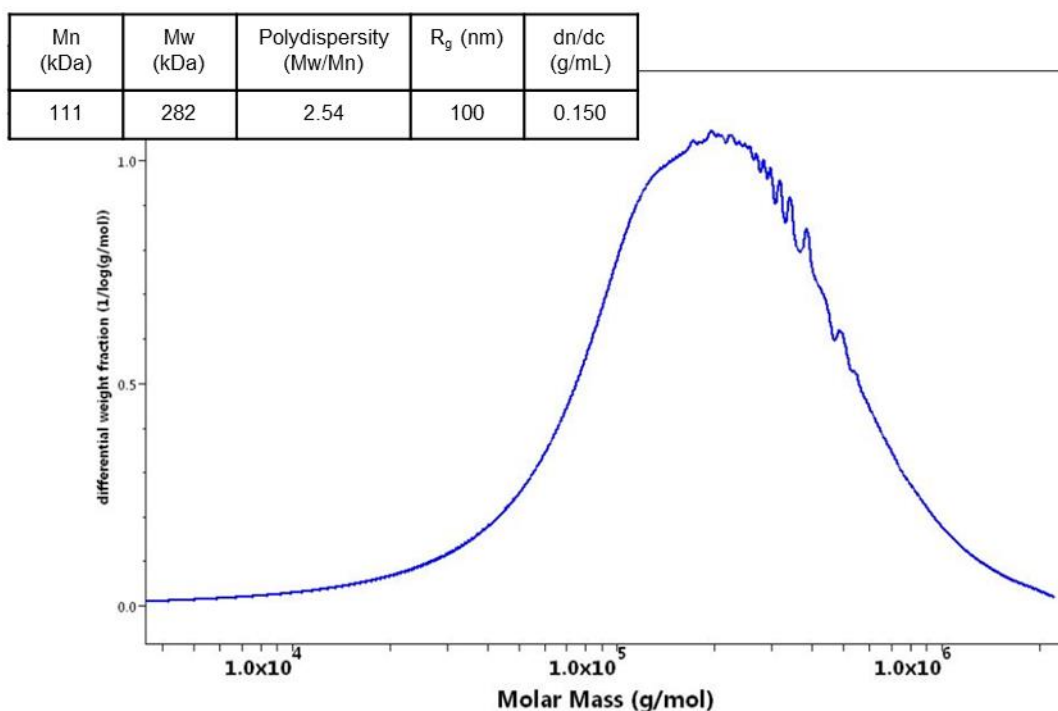


Figure S6: Molar mass distribution of sodium alginate, as determined by SEC coupled to SLS and RI

The source and extraction method of sodium alginate are not standardized, which results in wide variations in molar mass (Masuelli and Illanes, 2014). So, the weight-averaged molar mass of this macromolecule was measured by SEC coupled to SLS and refractive index (RI) measurement. On-line purified mobile phase ($0.1 \text{ mmol L}^{-1} \text{ NaNO}_3$) was delivered with an 1200 series HPLC pump (Agilent Technologies) at a flow rate of 5 mL min^{-1} to a chromatographic pre-column SB-807G, followed by four columns, SB-805HQ, SB-807HQ, SB-802HQ and SB-803HQ (Shodex, Munich, Germany). The temperature was kept at 30°C . Sodium alginate was injected at a concentration of 500 mg L^{-1} in $600 \text{ mmol L}^{-1} \text{ NaCl}$. At the outlet, sodium alginate was characterized by the DAWN HELEOS SLS detector (Wyatt Technology) and RI was measured by an Optilab T-rEX (Wyatt Technology).

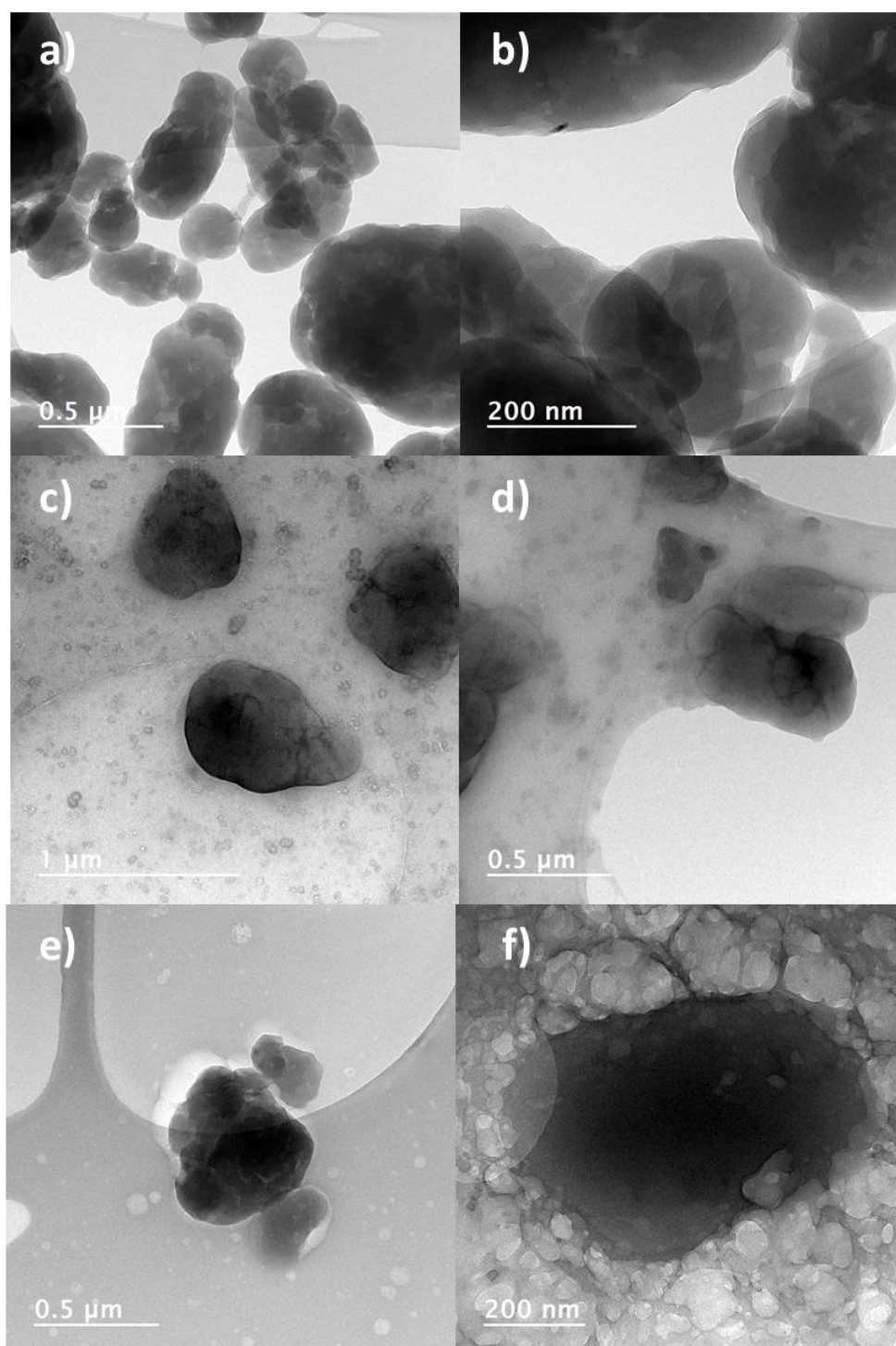


Figure S7: Transmission Electron Microscopy Images of a) b) NPT-P without organic matter c) d) NPT-P with humic acid (HA) and e) f) NPT-P with sodium alginate (SA)

Images a, c and e, show NPT-P at a lower magnification, to illustrate the matrix of NOM (c and e) or lack thereof. Images b, d, and f were taken at a higher magnification to observe the interface between NOM and NPT-P. The particles appear to be embedded in SA (e and f). In image e, the lighter halo around the particle is probably caused by the displacement of the. Figures c and d show that while NPT-P is also embedded in HA (c), at closer magnification, they seem less closely associated (d).

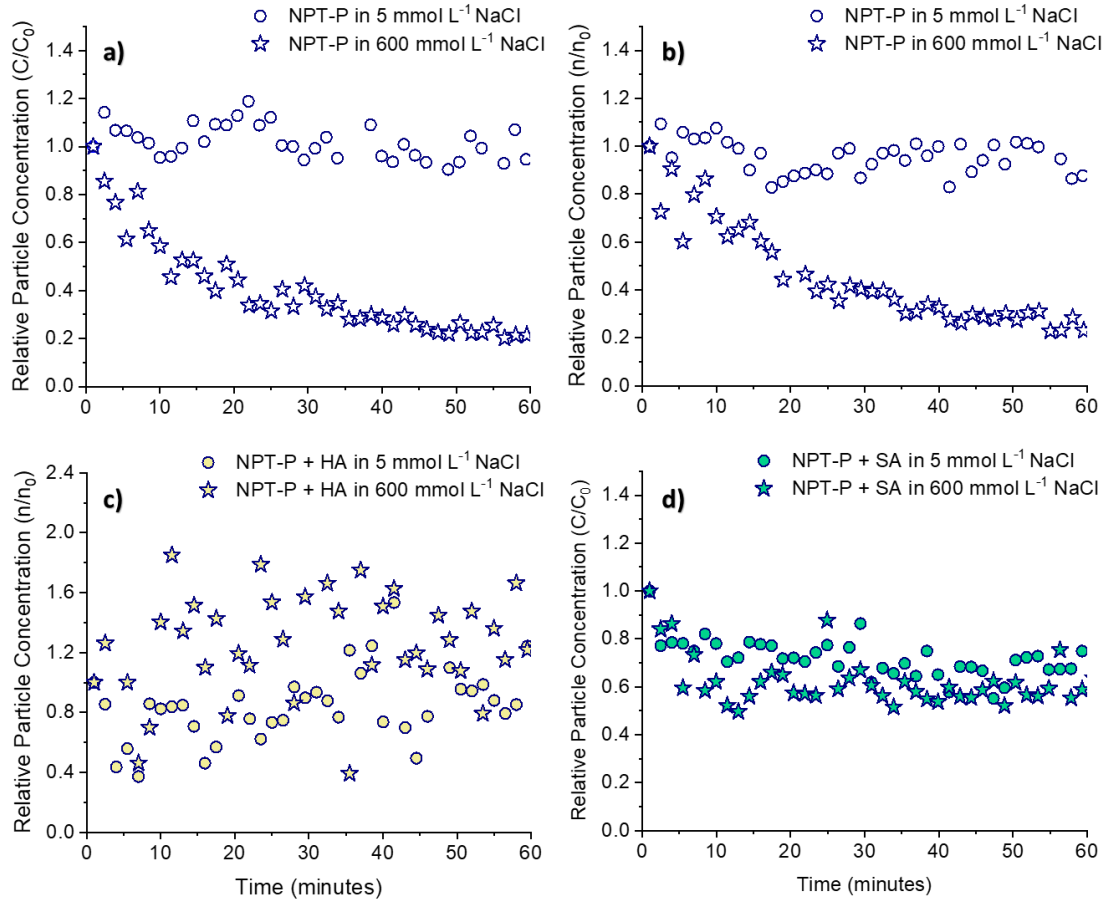


Figure S8: Relative particle concentration of NPT-P at 5 and 600 mmol L⁻¹ NaCl at a) pH 6.5, b) pH 8, c) with 30 mg L⁻¹ humic acid (HA) at pH 6.5 and d) with 50 mg L⁻¹ sodium alginate (SA) at pH 8

The relative particle concentration (n/n_0) was determined according to the relation that links the intensity of scattered light and the particle size. This relationship is only valid assuming that all particles are spherical (Wyatt, 1993). The intensity of scattered light, at a give scattering angle and particle concentration $I_{(\theta,c)}$, is given by the following equation:

$$I_{(\theta,c)} = KcM_W P_{(\theta)} \quad (S7)$$

with θ , the angle at which light is scattered, which is equal to 170 degrees and c the concentration in g L⁻¹, K the instrument constant, M_W the molar mass in g mol⁻¹ and P_{θ} the form factor. For a sphere, the form factor P_{θ} is given by:

$$P_{(\theta)} = \left[\frac{3}{u^3} (\sin(u) - u \cos(u)) \right]^2 \quad (S8)$$

For a sphere, u is defined as:

$$u = q * r_p \quad (S9)$$

with r_p the sphere's radius and q the wave vector, defined as:

$$q = \frac{4\pi}{\lambda} \sin\left(\frac{\theta}{2}\right) \quad (\text{S10})$$

with λ the wavelength of scattered light, which is 658 nm. So we have:

$$u = \frac{2\pi D_{zH} \sin\left(\frac{\theta}{2}\right)}{\lambda} \quad (\text{S11})$$

For a sphere the M_w is proportional to the sphere volume:

$$M_w \propto \left(\frac{D_{zH}}{2}\right)^3 \quad (\text{S12})$$

So, equation S7 can be written as:

$$I_{(\theta,c)} \propto Kc \left(\frac{D_{zH}}{2}\right)^3 \left[\frac{3}{u^3} (\sin(u) - u\cos(u))\right]^2 \quad (\text{S13})$$

Since, c is proportional to the particle concentration n and K and Θ are held constant, so:

$$n \propto \frac{I_{(\theta,c)}}{\left(\frac{D_{zH}}{2}\right)^3 \left[\frac{3}{u^3} (\sin(u) - u\cos(u))\right]^2} \quad (\text{S14})$$

Equation S14 can be simplified to:

$$n \propto \frac{I_{(\theta,c)}}{\left(\frac{9}{q^6 r_p^3}\right) (\sin(u) - u\cos(u))^2} \quad (\text{S15})$$

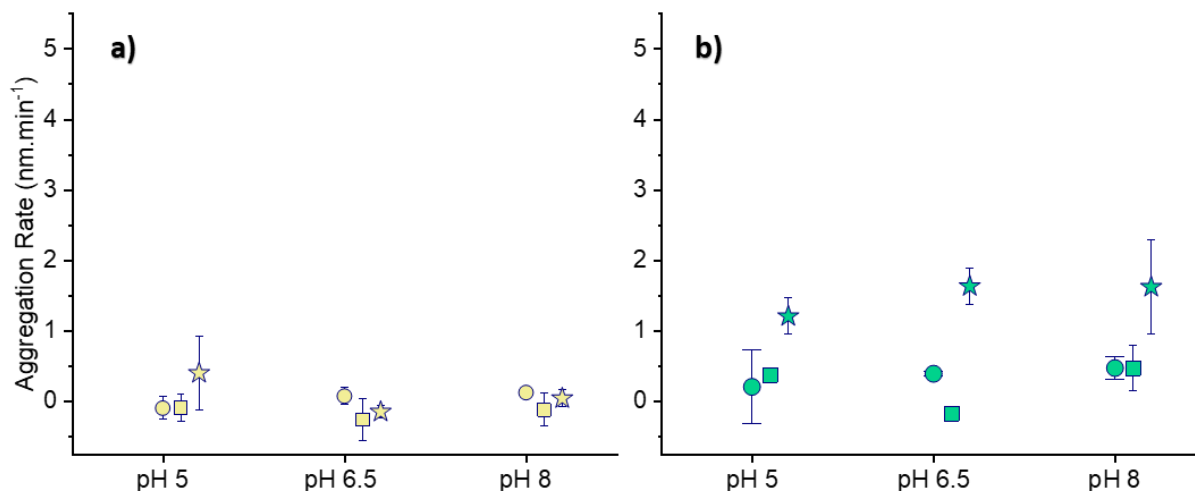


Figure S9: Aggregation rate of NPT-P with a) 30 mg L⁻¹ humic acid b) 57 mg L⁻¹ sodium alginate, as a function of ionic strength and pH (Error bars = standard deviation)

References :

- Elimelech, M. (Ed.), 1998. Particle deposition and aggregation: measurement, modelling and simulation, Colloid and surface engineering series. Butterworth-Heinemann, Oxford.
- Gigault, J., El Hadri, H., Reynaud, S., Deniau, E., Grassl, B., 2017. Asymmetrical flow field flow fractionation methods to characterize submicron particles: application to carbon-based aggregates and nanoplastics. *Analytical and Bioanalytical Chemistry* 409, 6761–6769. <https://doi.org/10.1007/s00216-017-0629-7>
- Gregory, J., 1981. Approximate expressions for retarded van der waals interaction. *Journal of Colloid and Interface Science* 83, 138–145. [https://doi.org/10.1016/0021-9797\(81\)90018-7](https://doi.org/10.1016/0021-9797(81)90018-7)
- Hogg, R., Healy, T.W., Fuerstenau, D.W., 1966. Mutual coagulation of colloidal dispersions. *Trans. Faraday Soc.* 62, 1638. <https://doi.org/10.1039/tf9666201638>
- Masuelli, M.A., Illanes, C.O., 2014. Review of the characterization of sodium alginate by intrinsic viscosity measurements. *Comparative analysis between conventional and single point methods.* *International Journal of BioMaterials Science and Engineering* 1, 11.
- Valsesia, A., Desmet, C., Ojea-Jiménez, I., Oddo, A., Capomaccio, R., Rossi, F., Colpo, P., 2018. Direct quantification of nanoparticle surface hydrophobicity. *Commun Chem* 1, 53. <https://doi.org/10.1038/s42004-018-0054-7>
- van Oss, C.J., 1993. Acid—base interfacial interactions in aqueous media. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 78, 1–49. [https://doi.org/10.1016/0927-7757\(93\)80308-2](https://doi.org/10.1016/0927-7757(93)80308-2)
- Verleysen, E., Wagner, T., Lipinski, H.-G., Kägi, R., Koeber, R., Boix-Sanfelieu, A., De Temmerman, P.-J., Mast, J., 2019. Evaluation of a TEM based Approach for Size Measurement of Particulate (Nano)materials. *Materials* 12, 2274. <https://doi.org/10.3390/ma12142274>
- Wyatt, P.J., 1993. Light scattering and the absolute characterization of macromolecules. *Analytica Chimica Acta* 272, 1–40. [https://doi.org/10.1016/0003-2670\(93\)80373-S](https://doi.org/10.1016/0003-2670(93)80373-S)