Towards a better understanding on agglomeration mechanisms and thermodynamic properties of TiO$_2$ nanoparticles interacting with natural organic matter

Frédéric Loosli $^a$, Letícia Vitorazi $^b$, Jean-François Berret $^b$ and Serge Stoll $^{a,*}$

Affiliations:

$^a$Group of Environmental Physical Chemistry, University of Geneva, F.-A. Forel Institute
Section des Sciences de la Terre et de l'Environnement, 10 route de Suisse, 1290 Versoix, Switzerland

$^b$Laboratoire Matière et Systèmes Complexes, UMR 7057 Université Paris-Diderot/CNRS,
Bâtiment Condorcet, 10 rue Alice Domon et Léonie Duquet, F-75205 Paris cedex 13, France

*Corresponding Author and Address:
Serge Stoll $^*$: Group of Environmental Physical Chemistry, University of Geneva, F.-A. Forel Institute,
Section des Sciences de la Terre et de l'Environnement, 10 route de Suisse, 1290 Versoix, Switzerland
Phone: +41 22 379 0333; Fax: +41 22 379 0302
email: serge.stoll@unige.ch

Abstract

Interaction between engineered nanoparticles and natural organic matter is investigated by measuring the exchanged heat during binding process with isothermal titration calorimetry. TiO$_2$ anatase nanoparticles and alginate are used as engineered nanoparticles and natural organic matter to get an insight into the thermodynamic association properties and mechanisms of adsorption and agglomeration. Changes of enthalpy, entropy and total free energy, reaction stoichiometry and affinity binding constant are determined or calculated at a pH value where the TiO$_2$ nanoparticles surface charge is positive and the alginate exhibits a negative structural charge. Our results indicate that strong TiO$_2$-alginate interactions are essentially entropy driven and enthalpically favorable with exothermic binding reactions. The reaction stoichiometry and entropy gain are also found dependent on the mixing order. Finally correlation is established between the binding enthalpy, the reaction stoichiometry and the zeta potential values determined by electrophoretic mobility measurements. From these results two types of agglomeration mechanisms are proposed depending on the mixing order. Addition of alginate in TiO$_2$ dispersions is found to form agglomerates due to polymer bridging whereas addition of TiO$_2$ in alginate promotes a more individually coating of the nanoparticles.

Keywords: TiO$_2$ nanoparticles, alginate, natural organic matter, isothermal titration calorimetry, metal oxide stability, aquatic systems
1. Introduction

Nanoparticles are produced in increasing quantities due to their unique surface properties (Auffan et al. 2009, Gottschalk et al. 2009) and are used in many domains and applications (Chen and Mao 2007, Lu et al. 2007, Luo et al. 2006). A non negligible amount of these engineered nanoparticles (ENPs) is thus entering aquatic systems as individual or agglomerated nanoparticles through industrial discharges, surface runoff from soils or from wastewater treatment effluent (Batley et al. 2013, Seitz et al. 2012). Once in aquatic environments, interactions with natural compounds will modify their stability, fate, bioavailability and toxic effects towards living organisms (Christian et al. 2008, Handy et al. 2008, Klaine et al. 2008, von Moos and Slaveykova 2014).

Among ENPs, TiO$_2$ nanoparticles are produced in high tonnage (Piccinno et al. 2012) and are present in many customer products such as food, cosmetic and painting industries (Chen and Mao 2007, Cozzoli et al. 2003, Gratzel 2004, Jimin et al. 2010, Khan and Dhayal 2008, Mahltig et al. 2005, Weir et al. 2012, Wongkalasin et al. 2011, Zhang and Sun 2004). TiO$_2$ ENPs production exceeds tons per year (Hendren et al. 2011, Piccinno et al. 2012, Schmid and Riediker 2008) and the expected concentration present in aquatic systems is in the ng L$^{-1}$ to µg L$^{-1}$ range (Batley et al. 2013, Gottschalk et al. 2009, Sani-Kast et al. 2015). Therefore TiO$_2$ is often considered to study the possible transformations of nanoparticles in aquatic systems. Most of the studies on TiO$_2$ ENPs transformation processes in presence of natural organic matter (NOM) demonstrate that humic substances and non humic substances such as extracellular polymeric substances are found to deeply modify the TiO$_2$ stability (Belen Romanello and Fidalgo de Cortalezzi 2013, Chae et al. 2012, Domingos et al. 2009, Erhayem and Sohn 2014, Gallego-Urrea et al. 2014, Shen et al. 2015, Zhang et al. 2013). Indeed recent findings considering the effect of NOM have shown that electrostatic repulsions and steric interactions between NOM molecules adsorbed onto the ENPs surface enhance the stability and thus the ENPs dispersion state (Hyung et al. 2007, Liu et al. 2010, Loosli et al. 2013, Louie et al. 2013, Palomino and Stoll 2013, Zhang et al. 2009). Moreover NOM complexation with ENPs is also found to induce the partial redispersion of already formed ENP agglomerates (Baalousha 2009, Loosli et al. 2014, Mohd Omar et al. 2014). All these studies are investigating the ENPs surface charge modification and give a special interest to the ENPs state of dispersion (resulting size, charge and stability of the ENPs) in presence of NOM. In the present study, a particular concern is given to the quantification of thermodynamic interaction parameters using isothermal titration calorimetry (ITC) for the investigation of interactions between anatase TiO$_2$ ENPs and alginate, an extracellular polymeric substance model (Bernhardt et al. 1985, Gregor et al. 1996). Indeed polysaccharides represent 10-30% of the NOM in lakes (Buffle et al. 1998, Wilkinson et al. 1999, Wilkinson et al. 1997) whereas in marine environment polysaccharide content may be as high as 50% of the dissolved organic carbon (Engel et al. 2004, Wells 1998). Alginate is a naturally occurring polysaccharide and is found in aquatic environments (Gombotz and Wee 1998). It is produced by brown algae species but also by some bacteria (Gombotz and Wee 1998, Saude et al. 2002). Alginate is a negatively charged linear block copolymer composing of 1→ 4 linked β-D-mannuronic acid and its C-5 epimer α-L-guluronic acid. Alginate is also used as a thickener
agent in food industry and as drug carrier in biomedicine (Helgerud 2009, Lee and Mooney 2012). ITC is a universal method which is suitable to follow the energies of association reactions. Indeed important parameters that are global properties of the systems such as the change of enthalpy $\Delta H$, the change of entropy $\Delta S$ and the change of the total free energy $\Delta G$ that occur during the binding processes can be extracted. ITC measurements also provide information on the interaction affinity by determination of the affinity binding constant $K_b$ and the interaction reaction stoichiometry. All these parameters are accessible through a single experiment. This method has been applied to a wide range of chemical and biochemical binding interactions and was especially used for protein-substrate interactions, structure-based drug design and supramolecular polymers self-associations (Arnaud and Bouteiller 2004, Brinatti et al. 2014, Cedervall et al. 2007, Chiappisi et al. 2013, Doyle 1997, Jelesarov and Bossard 1999, Kamiya et al. 1996, Kim et al. 2010, Ladbury 2001, Matulis et al. 2002, Vitorazi et al. 2014). Humic acid coverage on arsenic was shown to slightly reduce the binding interaction and the rate constant between arsenic-coated and ferrihydrite-kaolinite mixtures (Martin et al. 2009). Sheng et al. investigated the binding properties of copper ions with extracellular polymeric substances and showed that the process was mainly driven by entropy (Sheng et al. 2013). Another study on alginate-sodium dodecyl sulfate interaction shown that aggregation was due to hydrophobic interactions (Yang et al. 2008). Thermodynamic adsorption profile at a solvated organic-inorganic interface was done and gold ENPs interaction with carboxylic acid-terminated alkanethiols was found exothermic and enthalpy driven (Joshi et al. 2004, Ravi et al. 2013).

The present work is dealing with a novel approach that has a high potential to contribute to a better understanding of the behavior of ENPs in the presence of NOM. A detailed description is achieved on the complex interaction phenomena which occur between ENPs and NOM by determining key thermodynamic parameters and their link with ENPs surface charge properties to understand agglomeration mechanisms. Such quantitative information is often missing when ENP interactions with aquagenic compounds have to be investigated.

2. Materials and methods
2.1. Materials
TiO$_2$ engineered nanoparticles were obtained from Nanostructured & Amorphous Material Inc (Houston, TX, USA) as a 15% wt suspension of 15 nm anatase nanoparticles in water (Loosli et al. 2013). This stock solution was then, after homogenization, diluted by adding Milli Q water (Millipore, Zoug, ZG, Switzerland, with R $>18$ M$\Omega$ cm, T.O.C. $<2$ ppb) to reach a final 5 g L$^{-1}$ TiO$_2$ mass concentration. For sodium alginate (A2158, Sigma Aldrich, Buchs, SG, Switzerland) a 10 mM solution in term of charge concentration was prepared by dissolving the low viscosity biopolymer in MilliQ water and by stirring it overnight. NaOH and HCl (1 M, Titrisol®, Merck, Zoug, ZG, Switzerland) were used after dilution to adjust the dispersions and solutions at pH 3.1 and 11.0. The compounds were dialyzed simultaneously into the same water buffer using separate 12-14 kDa cutoff dialysis membranes (Spectrum Laboratories, Inc., Rancho Dominguez, CA, USA) to minimize the change of enthalpy ($\Delta H$)
from dilution process during titration. The solvent from the dialysis was used to dilute the TiO$_2$ and alginate suspensions to experimental concentrations (from 0.1 to 1.4 g L$^{-1}$ for TiO$_2$ and from 0.05 to 2.5 mM for alginate). Finally, prior utilization they were filtered through a 0.45 µm cellulose acetate filter (VWR, Nyon, VD, Switzerland) to remove possible presence of laboratory air dust. No loss of TiO$_2$ and alginate was observed during the filtration process.

2.2. Isothermal titration calorimetry measurement

The heat exchange between TiO$_2$ ENPs and alginate was determined using a VP-ITC calorimeter (MicroCal Inc., Northampton, MA, USA) with a sample cell volume equal to 1.4643 mL. The working temperature was set to 298.15 K and, after a preliminary 2 µL ligand (L) injection, 28 injections of 10 µL of ligand into the sample cell containing 1.4643 mL (which is equal to the cell volume) of the macromolecule (M) were realized with an injection duration of 20 seconds and a 210 seconds delay between each successive injection. The agitation speed was set to 307 rpm. Equation (Eq. (1)) to fit the data representing the heat of exchange (dQ/dn$_L$) during the association process as a function of the molar charge ratio (Z = [L]/[M], where [L] and [M] are the ligand and macromolecule molar charge concentration) was derived from the Multiple Non-Interacting Sites (MNIS) model where binding sites are considered to be independent (Courtois and Berret 2010).

$$\frac{dQ}{dn_L}(Z) = \frac{1}{2} \Delta H_b \left[ 1 + \left( 1 - \frac{[L]}{n[M]} - \frac{1}{nK_b[M]} \right) \left( 1 + \frac{[L]}{n[M]} + \frac{1}{nK_b[M]} \right)^2 - 4 \left( \frac{[L]}{n[M]} \right)^{-\frac{1}{2}} \right]$$

In Eq. (1) the fitting parameters $\Delta H_b$ in kJ mol$^{-1}$, $K_b$ in M$^{-1}$ and $n$ represent the binding enthalpy, binding constant and reaction stoichiometry respectively. The system total free energy, $\Delta G$ in kJ mol$^{-1}$, and entropy, $\Delta S$ in kJ K$^{-1}$ mol$^{-1}$, are calculated from the fitting parameters ($K_b$ and $\Delta H_b$ values) with $\Delta G = -RT\ln K_b$ and $\Delta S = (\Delta H - \Delta G)/T$. The ligand and macromolecule charge numbers for the ith-injection are equal to:

$$L_i = L_{i-1} + V_i \cdot [L]_m \cdot N_A \quad \text{and} \quad M = [M]_m \cdot V_{cell} \cdot N_A$$

with $[TiO_2]_m = [TiO_2]_M \cdot S_A \cdot \sigma_{TiO_2} \cdot 10^{18} + N_A$ and

$$[Alginate]_m = [Alginate]_M \div MW \, (\text{monomer}) \cdot \alpha$$

In Eq. (2), $V_i$ and $V_{cell}$ represent the volume of ligand injected and the cell volume respectively, [L] or [M]$_m$ correspond to the mol of charge of ligand or macromolecule per unit of volume and $N_A$ to the Avogadro constant. The concentration in term of mol of charge per unit of volume for TiO$_2$ and alginate are expressed in Eq. (3) and (4) respectively. In Eq. (3), [TiO$_2$]$_m$ represent the TiO$_2$ mass concentration, $S_A$ the ENPs specific surface area and $\sigma_{TiO_2}$ the TiO$_2$ hydroxyl sites density. In Eq. (4) [Alginate]$_m$ represents the alginate mass concentration and $\alpha$ the degree of ionization.

TiO$_2$ charge concentrations were calculated based on manufacturer data of the primary TiO$_2$ diameter (equal to 15 nm and in good agreement with the mode value of the number size distribution determined by dynamic light scattering, Fig. S2b) to calculate $S_A$ and on the other
hand on a previous study to obtain $\sigma_{\text{TiO}_2}$ (Kominami et al. 2000). A $S_A$ and $\sigma_{\text{TiO}_2}$ equal to 100 m$^2$ g$^{-1}$ and 5 sites nm$^{-2}$ respectively were used to determine the TiO$_2$ charge concentration. The factor of conversion between the mass and charge concentration was thus set so that a 1 g L$^{-1}$ TiO$_2$ dispersion corresponds to a 0.83 mM charge concentration. The degree of ionization of alginate was set equal to 1 (completely deprotonated) due to the net polymer carboxylic acid moieties $pK_a$ decrease in presence of strongly oppositely charged particles (Carnal and Stoll 2011).

Furthermore the mixing order of the two compounds was investigated to better understand the TiO$_2$-alginate and alginate-TiO$_2$ interactions and the agglomeration process as illustrated in Fig. 1. In a first set of experiments (type I) alginate was playing the role of ligand and was added to the TiO$_2$ dispersion. In the second set of experiments realized (type II) TiO$_2$ ENPs (L) were added to alginate (M).

Experiments were realized at pH 3.1 (and at pH 11.0 for negative controls) without addition of electrolyte. Such a pH value was utilized to address the interaction between isolated, dispersed ENPs and alginate. It is important to note that results presented here remains valid as long as $pH < p\text{H}_{PCN,\text{TiO}_2}$. Moreover this pH corresponds to the commercial dispersion pH (after dilution to a 5 g L$^{-1}$ concentration) and was chosen to avoid any pH surface charge modification effects. The domain of concentration investigated was from 0.5 mM alginate in 0.1 g L$^{-1}$ TiO$_2$ to 2.5 mM alginate in 0.5 g L$^{-1}$ TiO$_2$ for Type I experiments and from 1 g L$^{-1}$ TiO$_2$ in 0.05 mM alginate to 5 g L$^{-1}$ TiO$_2$ in 0.25 mM alginate for Type II experiments. Such TiO$_2$ concentration are higher than the expected environmental concentration, which are in the ng to $\mu$g L$^{-1}$ range (Batley et al. 2013, Gottschalk et al. 2009, Sani-Kast et al. 2015), because...
of the importance to obtain an optimum signal with the calorimeter. Also it should be noted that concentrations could be significantly higher than the expected environmental concentration in case of local pollution events.

2.3. Zeta potential and size distribution measurements
Zeta (ζ) potential values and z-average hydrodynamic diameters of TiO$_2$ dispersion and alginate solution as a function of pH as well as TiO$_2$ in presence of alginate (for Type I and II titrations) as a function of charge ratio were determined by measuring the particles velocity using laser doppler velocimetry with phase analysis light scattering (M3-PALS) and diffusion coefficient using dynamic light scattering (DLS) (Zetasizer Nano ZS instrument, Malvern Instruments, Worcestershire, UK). The instrument was operating at 298.15 K with a 4 mW He-Ne laser working at 633 nm and the detection angle for DLS measurement was 173° (back scattering). For ζ potential values determination the Smoluchowski approximation model was applied according to the formation of large agglomerates (Baalousha 2009). All polydispersity indexes were found below 0.6.

3. Results and discussion
To determine the binding properties between TiO$_2$ ENPs and alginate, ITC experiments were realized at pH < pH$_{PCN,TiO_2}$ where strong electrostatic interactions occur between the negatively charged alginate and positively charged TiO$_2$ ENPs. As shown in Fig. 2, in which the ζ potential values of TiO$_2$ and alginate are represented as a function of pH, at pH 3.1 (large gray vertical line) the ENPs exhibit a positive surface charge (ζ potential = +40.9 ± 1.4 mV (mean ± standard deviation on mean of triplicates)) and the biopolymer structural charge is negative with a ζ potential value found equal to -13.0 ± 0.1 mV. At pH < pH$_{PCN,TiO_2}$ the TiO$_2$ ENPs are dispersed with a z-average diameter equal to 47 ± 1 nm (Figs. S1 and S2 in Supporting information) and alginate z-average diameter is equal to 178 ± 21 nm (Figs. S3 and S4).

![Zeta potential values of TiO$_2$ (open squares) and alginate (open circles) as a function of pH. TiO$_2$ isoelectric point is found here equal to 6.2 ± 0.1 whereas alginate exhibit a negative structural charge in the full pH range. At pH 3.1 TiO$_2$ and alginate have ζ potential values equal to +40.9 ± 1.4 mV and -13.0 ± 0.1 mV respectively (large gray vertical line). At pH 11.0 both compounds are negatively charged (narrow gray vertical line). [TiO$_2$] = 50 mg L$^{-1}$, [Alginate] = 100 mg L$^{-1}$ and [NaCl] = 0.001 M.](image-url)

Fig. 2 - Zeta potential values of TiO$_2$ (open squares) and alginate (open circles) as a function of pH. TiO$_2$ isoelectric point is found here equal to 6.2 ± 0.1 whereas alginate exhibit a negative structural charge in the full pH range. At pH 3.1 TiO$_2$ and alginate have ζ potential values equal to +40.9 ± 1.4 mV and -13.0 ± 0.1 mV respectively (large gray vertical line). At pH 11.0 both compounds are negatively charged (narrow gray vertical line). [TiO$_2$] = 50 mg L$^{-1}$, [Alginate] = 100 mg L$^{-1}$ and [NaCl] = 0.001 M.
3.1. Determination of TiO$_2$-Alginate thermodynamic binding parameters by ITC

Titration of TiO$_2$ dispersion with alginate (Type I) When alginate is added to TiO$_2$ ENPs strong interactions are observed as shown in Fig. 3a which represents a thermogram of the heat of exchange as a function of time for a 100 mg L$^{-1}$ TiO$_2$ dispersion titrated by a 0.5 mM alginate charge concentration. Generally when adding alginate to the TiO$_2$ dispersion the instrument responds so as to compensate the binding reaction exchange energy to maintain a small difference of temperature between the reference cell and the "reaction" cell constant. Both cells are isolated in an adiabatic jacket. The heat compensation is recorded and in the present case "negative" peaks reveal an exothermic process. Each peak corresponds to a consecutive alginate addition. In the thermogram the first response (first peak) is of lower intensity than the following owing to the smaller alginate volume injected (2 µL instead of the 10 µL "normal" injection volume). For the next ten injections the peak amplitudes are constant. It means that the number of TiO$_2$ free sites available for random alginate adsorption is high enough to undergo adsorption of a maximum and identical alginate amount. Then the peak intensity rapidly decreases as less positively TiO$_2$ sites are available and finally only low heat exchange corresponding to dilution effect is observed for the last injections due to sites saturation (exchange energy identical to alginate titration in water at pH 3.1, as shown in Fig. S5, with dilution giving rise to low exothermic interaction).

From the data presented in Fig. 3a, the variation of the exchange energy as a function of alginate over TiO$_2$ charge ratio ($Z = [\text{Alginate}]/[\text{TiO}_2]$) is plotted in Fig. 3b. The values of interaction heat of exchange ($\frac{dQ}{dn}$) for each injection are equal to the corresponding peak area integration from the real-time thermogram. For the 100 mg L$^{-1}$ TiO$_2$ titration with 0.5 mM alginate the binding enthalpy is found equal to -8.7 kJ mol$^{-1}$ which indicates an exothermic interaction process. $K_b$ is equal to $3.5 \times 10^7$ M$^{-1}$ which points out a high binding affinity between TiO$_2$ and alginate in this electrostatic scenario (positively charged TiO$_2$ ENPs in the presence of negatively charged alginate). The reaction stoichiometry is found equal to 0.56. All these fitting parameters (derived from Eq. (1)) allow the calculation of the free energy $\Delta G$ and entropy $\Delta S$ changes during the interaction process. These energy changes are equal to $-4.3 \times 10^4$ kJ mol$^{-1}$ and $+115.3$ J mol$^{-1}$ K$^{-1}$ respectively. Two other experiments with different concentrations but with the same [L]/[M] ratio have been done to evaluate the effect of relative concentration on binding processes. The real-time thermograms and respective integrated heat data fitted with the MNIS model for these experiments are represented in Figs. S6 and S7 and all the fitting and calculated parameters derived from the type I titrations are listed in Table 1.
Fig. 3 - a) Real-time thermogram for TiO$_2$ 0.1 g L$^{-1}$ titration with alginate 0.5 mM at pH < pH$_{PCN,TiO_2}$ and at 298.15 K. The heat flow refers to the thermal compensation of the calorimeter to keep the sample at a constant temperature. Here negative peaks indicate an exothermic reaction. After about fifteen injections sites saturation occurs and only dilution effect is observed (small negative peaks). b) The respective integrated heat data (dQ/dn$_L$) as a function of molar charge ratio ([Alginate]/[TiO$_2$]) is fitted with the multiple non-interacting sites (MNIS) model. The binding enthalpy, the binding constant and the reaction stoichiometry where found here equal to -8.7 kJ mol$^{-1}$, $3.5 \times 10^7$ M$^{-1}$ and 0.56, respectively.

Table 1: Fitting parameters $\Delta H_b$, $K_b$ and $n$ from ITC analysis of the integrated heats with MNIS model and calculated $\Delta G$ and $\Delta S$ from $K_b$ and $\Delta H_b$ values. The formation of complexes between TiO$_2$ ENPs and alginate is found spontaneous, mainly driven by entropic effects and enthalpically favorable.

| Alginate in TiO$_2$ | $\Delta H_b$ [kJ mol$^{-1}$] | $K_b$ [M$^{-1}$] | $n$ | $\Delta G$ [kJ mol$^{-1}$] | $\Delta S$ [J K$^{-1}$ mol$^{-1}$] |
|---------------------|----------------------------|----------------|-----|-----------------------------|----------------------------------|
| 0.5 mM in 0.1 g L$^{-1}$ | -8.7 | $3.5 \times 10^7$ | 0.56 | -43.0 | 115.3 |
| 0.7 mM in 0.14 g L$^{-1}$ | -9.0 | $2.4 \times 10^7$ | 0.56 | -42.2 | 111.3 |
| 2.5 mM in 0.5 g L$^{-1}$ | -8.7 | $1.3 \times 10^7$ | 0.61 | -40.6 | 107.2 |
Alginate interaction with TiO$_2$ ENPs is found to be a spontaneous process with high values of Gibbs free adsorption energy ($\Delta G < -40$ kJ mol$^{-1}$). The binding enthalpy is favorable to the formation of complexes ($\Delta H_b < 0$) and found independent on the compounds concentration. The main interaction driving process is due to an important gain in entropy ($-T\Delta S < \Delta H_b$) with $T\Delta S$ value of about $+30$ kJ mol$^{-1}$ in our experimental conditions. The binding constant is found to decrease when increasing concentration as $K_b \sim c^{-2}$. It implicates the decrease of the calculated Gibbs free energy and entropy (Courtois and Berret 2010) as the binding enthalpy change is constant and independent on concentration ($-8.8 \pm 0.2$ kJ mol$^{-1}$). The charge stoichiometry was found constant with $n = 0.58 \pm 0.04$.

**Titration of alginate with TiO$_2$ (Type II)** The ITC thermogram and the respective integrated heat data for a TiO$_2$ 1 g L$^{-1}$ and 0.05 mM alginate reaction are shown in Fig. 4. The interaction is also found exothermic and for a charge ratio around $Z = 1$ no further interaction between TiO$_2$ and alginate occurs and only dilution effect is observed (same exchange heat than in Fig. S8 for TiO$_2$ titration in water). In Table 2 thermodynamic parameters are listed for experiments at different concentrations (Figs. S9 and S10). The binding enthalpy is found independent of the concentration and equal to $-8.7 \pm 0.4$ kJ mol$^{-1}$ and the complexation process is also driven by an important entropy gain.

**Table 2:** Fitting parameters $\Delta H_b$, $K_b$ and $n$ from ITC analysis of the integrated heats with MNIS model and calculated $\Delta G$ and $\Delta S$ from $K_b$ and $\Delta H_b$ values. The formation of complexes between TiO$_2$ ENPs and alginate is found spontaneous, mainly driven by entropic effects and enthalpically favorable.

| TiO$_2$ in Alginate | $\Delta H_b$ [kJ mol$^{-1}$] | $K_b$ [M$^{-1}$] | $n$ | $\Delta G$ [kJ mol$^{-1}$] | $\Delta S$ [J K$^{-1}$ mol$^{-1}$] |
|---------------------|-----------------------------|-----------------|-----|-----------------------------|---------------------------------|
| 1 g L$^{-1}$ in 0.05 mM | -8.2 | $1.6 \times 10^7$ | 0.82 | -41.1 | 110.3 |
| 1.4 g L$^{-1}$ in 0.07 mM | -9.0 | $1.4 \times 10^7$ | 0.96 | -40.8 | 106.6 |
| 5 g L$^{-1}$ in 0.25 mM | -8.8 | $7.9 \times 10^6$ | 0.93 | -39.4 | 102.6 |
Fig. 4 - a) Real-time thermogram for alginate 0.05 mM titration with TiO$_2$ 1 g L$^{-1}$ at pH < pH$_{PCN, TiO_2}$ and at 298.15 K. Negative peaks indicate an exothermic reaction and after about ten injections sites saturation occurs and only dilution effect is observed (small positive peaks). b) The respective integrated heat data as a function of TiO$_2$ over alginate molar charge ratio is fitted with the MNIS model. The binding enthalpy, the binding constant and the reaction stoichiometry are found here equal to -8.2 kJ mol$^{-1}$, $1.6 \times 10^7$ M$^{-1}$ and 0.82, respectively.

When comparing the two titration types similar binding enthalpy value are observed. Both types of interaction are enthalpically favorable but mainly driven by entropic gain arising from the alginate and ENP counter-ions and water molecules release during adsorption processes. Similar total free energy changes were observed during the association process between ZnO NPs with lysozyme, as well as between proteins and amino acid functionalized gold NPs (Chakraborti et al. 2010, Chatterjee et al. 2010, De et al. 2007). However it should be noted here that the interaction process is mainly driven by an important gain of entropy whereas in the other studies (Chakraborti et al. 2010, Chatterjee et al. 2010) the strong interactions ($K_b = 0.9 \times 10^6$ M$^{-1}$) were enthalpically favorable but entropically unfavorable due to conformational restriction of proteins. Reactions stoichiometry close to unity clearly indicates that the interaction is electrostatic. When working in conditions where electrostatic
interactions are favorable (positively charged ENPs and negatively charged polysaccharides) a stoichiometry close to unity in term of molar charge ratio denotes an electrostatic driven process for compounds in which charges are not sterically hindered (which is the case here for both TiO$_2$ and alginate) and where other functional groups (such as alcohol) are not playing main roles. Nevertheless the stoichiometry of the two titration types is different and explained by different mechanisms of interaction depending on the mixing order. For type I, when alginate is added to TiO$_2$, the polysaccharide is prompt to facilitate bridging between the ENPs which restrict the access to positive TiO$_2$ charges due to conformational hindrance during bridging agglomeration process. Consequently the stoichiometry is smaller than unity ($n = 0.58 \pm 0.04$).

Fig. 5 - a) Integrated heat data and $\zeta$ potential values as a function of alginate over TiO$_2$ charge ratio for TiO$_2$ 0.1 g L$^{-1}$ titration with alginate 0.5 mM at pH $< \text{pH}_{\text{PCN, TiO}_2}$. For a ratio up to 0.5, the binding enthalpy and the $\zeta$ potential values are constant and found equal to - 8.8 ± 0.3 kJ mol$^{-1}$ and +37.6 ± 0.3 mV. Then charge inversion ($\zeta$ potential = -16.4 ± 0.2 mV) is observed for $Z = 0.54$ and for $Z > 0.70$ sites saturation occurs and no more interaction is observed. b) z-average diameter as a function of molar charge. Strong TiO$_2$ ENPs destabilization occurs for $Z > 0.5$ whereas, below this ratio, z-average diameter increase is linear indicating ENPs bridging.

For type II titration, when TiO$_2$ is added to alginate the ENPs are more closely individually coated and the charge stoichiometry is closer to the unity ($n = 0.90 \pm 0.07$). These different
mechanisms are related to the initial predominant compound concentration and physico-
chemical properties in the reaction cell. The bridging mechanism in type I is favorable to
higher entropy gain (ΔS columns in Tables 1 and 2). Indeed, in addition to entropy gain due to
the polymer counter-ions and water molecules release, alginate is expected to be less
collapsed onto the ENPs surface and thus lower conformational entropy loss is observed than
in the type II titration. This conformational entropy gain is nevertheless negligible in
comparison to the entropy gain due to counter-ions and water molecule release. Another
similarity between the type I and type II titrations is the heat exchange signature on real-time
thermogram for the injection just prior binding sites saturation which is the consequence of an
important system physical change (as discussed below).

To ensure that electrostatic interactions are governing the complexation process a negative
control was done at pH 11.0. At this pH both the TiO$_2$ and the alginate exhibit negative
surface and structural charges (Fig. 2), respectively, and no interaction was observed due to
electrostatic repulsions between the two compounds (Fig. S11).

### 3.2. Influence of TiO$_2$-alginate complexes structural charge on binding heat of
exchange

Determination of the ζ potential and hydrodynamic diameter values for the type I and ζ
potential values for type II titrations are done here to evaluate charge modification during
titration process and correlate it with the heat exchange values determined from ITC
measurements. The time between each successive ligand addition was equal to 210 seconds
similarly to the ITC titrations. Hydrodynamic diameters values for type II titration were not
determined owing to the insufficient DLS signal until the 8th injection (sites saturation).

**Type I titration:** The binding enthalpy and ζ potential values as a function of molar charge
ratio for type I titration, alginate 0.5 mM in TiO$_2$ 0.1 g L$^{-1}$ at pH 3.1, are represented in Fig. 5
. For an alginate over TiO$_2$ charge ratio up to 0.50, binding enthalpy per mol of injectant as
well as the ζ potential are constant and equal to $-8.8 \pm 0.3$ kJ mol$^{-1}$ and $+37.6 \pm 0.3$ mV
respectively. High ζ potential values in this molar charge ratio domain indicates that TiO$_2$
surface coverage is far to be complete and enough TiO$_2$ positively charged binding sites are
available for further alginate adsorption which is confirmed by constant and maximum
interaction enthalpy values. Then for a ratio of 0.54 TiO$_2$ charge inversion is observed (ζ
potential = $-16.4 \pm 0.2$ mV) and the corresponding binding enthalpy is decreasing to $-1.6$ kJ
mol$^{-1}$. For the three next alginate injections the ζ potential values and the binding enthalpy
slightly decrease and, for a charge ratio greater than 0.70, sites saturation occurs and no
interaction between alginate and TiO$_2$ is recorded, due to electrostatic repulsions and steric
effects at high alginate concentration as shown by Lin et al. (Lin et al. 2012), with a
corresponding ζ potential plateau value at $-30.4 \pm 1.8$ mV. The z-average diameter of the TiO$_2$
ENPs as a function of the molar charge ratio Z is represented in Fig. 5b. Two domains in term
of size variation are observed. In the first one, for a charge ratio below 0.5, the TiO$_2$ z-average
diameter increases linearly when alginate is added. This is due to the alginate polymer
bridging between the ENPs. Then a second domain is reached for further alginate addition
were stronger destabilization of TiO$_2$ is observed due to surface charge neutralization and inversion which result in particle precipitation.

Fig. 6 - Integrated heat data and ζ potential values as a function of TiO$_2$ over alginate charge ratio for an alginate 0.05 mM titration with TiO$_2$ 1 g L$^{-1}$ at pH < pH$_{PCN,TiO_2}$. For a charge ratio equal to 1, charge inversion is observed (ζ potential = +19.3 ± 1.5 mV) and interactions are no longer occurring.

**Type II titration:** In Fig. 6 is presented the variation of ζ potential and binding enthalpy as a function of TiO$_2$ over alginate molar charge ratio for the titration of a 0.05 mM alginate solution with a 1 g L$^{-1}$ TiO$_2$ dispersion at pH < pH$_{PCN,TiO_2}$. Net decrease of TiO$_2$-alginate interaction takes place after six ENPs injections and, for a molar ratio equal to 1, charge inversion is observed with ζ potential values found equal to +19.3 ± 1.5 mV. Then by further increasing the TiO$_2$ concentration thermodynamic interactions are no longer observed.

A good agreement is found between the ζ potential and the interaction enthalpy values for both titration mode (type I and type II) as well as with the z-average diameter values for the type I titration. For the type I and II titrations TiO$_2$-alginate thermodynamic interactions are not occurring when charge inversion and sites saturation are observed (for Z ≥ 0.7 and 1, respectively) due to electrostatic repulsions and steric effects between the TiO$_2$-alginate agglomerates and the titrant. Hence simple dilution effect is observed. The main difference between the two types of titration is the molar charge ratio needed to achieve charge inversion (Z ≈ 0.5 for type I whereas Z ≈ 1 for type II). When alginate is added to the TiO$_2$ dispersion, alginate is bridging the TiO$_2$ as shown by z-average diameter values increase for Z ≤ 0.5 which confirms the bridging mechanism of agglomeration. Dynamic light scattering permits to assign the real-time thermogram signature occurring prior non-interaction domain to an important precipitation domain in agreement with z-average diameter and ζ potential values.

**4. Conclusion**

The association process between TiO$_2$ nanoparticles and alginate is found here mainly driven by an important gain of entropy due to the release of alginate counter-ions and water
molecules. Our results also suggest that the change of binding enthalpy, via electrostatic interactions, is favorable for the binding process and that this thermodynamic value is independent of concentration and mixing order. The mixing order is nevertheless found to play a key role on the reaction stoichiometry and for the molar charge ratio needed to fully saturate the binding sites available for complexation. This is due to different mechanisms of association (charge neutralization when TiO\textsubscript{2} is added to alginate, bridging when alginate is added to TiO\textsubscript{2}). ITC measurements allows to determine and explicitly quantify important thermodynamic parameters ($\Delta H_b$, $K_b$, $\Delta G$ and $\Delta S$) and to propose, when associated with light scattering techniques, different mechanisms of interactions depending on the mixing order between engineered nanoparticles and natural organic matter. It should be noted that thermodynamic properties of adsorption could also be dependent on the particle size and type as well as surface site distribution.

**Acknowledgments**

The authors are grateful to the financial support received from the Swiss National Foundation (200020_152847 and 200021_135240). The work leading to these results also received funding from the European Union Seventh Framework Programme (FP7/2007-20013) under agreement no NMP4-LA-2013-310451. L.V. also thanks the CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) in Brazil for postdoctoral fellowship.

**References**

Arnaud, A. and Bouteiller, L. (2004) Isothermal titration calorimetry of supramolecular polymers. Langmuir 20(16), 6858-6863.

Auffan, M., Rose, J., Bottero, J.-Y., Lowry, G.V., Jolivet, J.-P. and Wiesner, M.R. (2009) Towards a definition of inorganic nanoparticles from an environmental, health and safety perspective. Nature Nanotechnology 4(10), 634-641.

Baalousha, M. (2009) Aggregation and disaggregation of iron oxide nanoparticles: Influence of particle concentration, pH and natural organic matter. Science of the Total Environment 407(6), 2093-2101.

Batley, G.E., Kirby, J.K. and McLaughlin, M.J. (2013) Fate and risks of nanomaterials in aquatic and terrestrial environments. Accounts of Chemical Research 46(3), 854-862.

Belen Romanello, M. and Fidalgo de Cortalezzi, M.M. (2013) An experimental study on the aggregation of TiO\textsubscript{2} nanoparticles under environmentally relevant conditions. Water Research 47(12), 3887-3898.

Bernhardt, H., Hoyer, O., Schell, H. and Lusse, B. (1985) Reaction-mechanisms involved In the influence of algogenic organic-matter on flocculation. Zeitschrift Fur Wasser Und Abwasser Forschung-Journal for Water and Wastewater Research 18(1), 18-30.

Brinatti, C., Mello, L.B. and Loh, W. (2014) Thermodynamic Study of the Micellization of Zwitterionic Surfactants and Their Interaction with Polymers in Water by Isothermal Titration Calorimetry. Langmuir 30(21), 6002-6010.
Buffle, J., Wilkinson, K.J., Stoll, S., Filella, M. and Zhang, J.W. (1998) A generalized description of aquatic colloidal interactions: The three-colloidal component approach. Environmental Science & Technology 32(19), 2887-2899.

Carnal, F. and Stoll, S. (2011) Adsorption of Weak Polyelectrolytes on Charged Nanoparticles. Impact of Salt Valency, pH, and Nanoparticle Charge Density. Monte Carlo Simulations. Journal of Physical Chemistry B 115(42), 12007-12018.

Cedervall, T., Lynch, I., Lindman, S., Berggard, T., Thulin, E., Nilsson, H., Dawson, K.A. and Linse, S. (2007) Understanding the nanoparticle-protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles. Proceedings of the National Academy of Sciences of the United States of America 104(7), 2050-2055.

Chae, S.-R., Xiao, Y., Lin, S., Noeiaghaei, T., Kim, J.-O. and Wiesner, M.R. (2012) Effects of humic acid and electrolytes on photocatalytic reactivity and transport of carbon nanoparticle aggregates in water. Water Research 46(13), 4053-4062.

Chakraborti, S., Chatterjee, T., Joshi, P., Poddar, A., Bhattacharyya, B., Singh, S.P., Gupta, V. and Chakrabarti, P. (2010) Structure and Activity of Lysozyme on Binding to ZnO Nanoparticles. Langmuir 26(5), 3506-3513.

Chatterjee, T., Chakraborti, S., Joshi, P., Singh, S.P., Gupta, V. and Chakrabarti, P. (2010) The effect of zinc oxide nanoparticles on the structure of the periplasmic domain of the Vibrio cholerae ToxR protein. Febs Journal 277(20), 4184-4194.

Chen, X. and Mao, S.S. (2007) Titanium dioxide nanomaterials: Synthesis, properties, modifications, and applications. Chemical Reviews 107(7), 2891-2959.

Chiappisi, L., Hoffmann, I. and Gradzielski, M. (2013) Complexes of oppositely charged polyelectrolytes and surfactants - recent developments in the field of biologically derived polyelectrolytes. Soft Matter 9(15), 3896-3909.

Christian, P., Von der Kammer, F., Baalousha, M. and Hofmann, T. (2008) Nanoparticles: structure, properties, preparation and behaviour in environmental media. Ecotoxicology 17(5), 326-343.

Courtois, J. and Berret, J.F. (2010) Probing Oppositely Charged Surfactant and Copolymer Interactions by Isothermal Titration Microcalorimetry. Langmuir 26(14), 11750-11758.

Cozzoli, P.D., Comparelli, R., Fanizza, E., Curri, M.L. and Agostiano, A. (2003) Photocatalytic activity of organic-capped anatase TiO2 nanocrystals in homogeneous organic solutions. Materials Science & Engineering C-Biomimetic and Supramolecular Systems 23(6-8), 707-713.

De, M., You, C.-C., Srivastava, S. and Rotello, V.M. (2007) Biomimetic interactions of proteins with functionalized nanoparticles: A thermodynamic study. Journal of the American Chemical Society 129(35), 10747-10753.

Domingos, R.F., Tufenkji, N. and Wilkinson, K.J. (2009) Aggregation of titanium dioxide nanoparticles: role of a fulvic acid. Environmental Science & Technology 43(5), 1282-1286.
Doyle, M.L. (1997) Characterization of binding interactions by isothermal titration calorimetry. Current Opinion in Biotechnology 8(1), 31-35.

Engel, A., Thoms, S., Riebesell, U., Rochelle-Newall, E. and Zondervan, I. (2004) Polysaccharide aggregation as a potential sink of marine dissolved organic carbon. Nature 428(6986), 929-932.

Erhayem, M. and Sohn, M. (2014) Stability studies for titanium dioxide nanoparticles upon adsorption of Suwannee River humic and fulvic acids and natural organic matter. Science of the Total Environment 468–469(0), 249-257.

Gallego-Urrea, J.A., Perez Holmberg, J. and Hassellov, M. (2014) Influence of different types of natural organic matter on titania nanoparticle stability: effects of counter ion concentration and pH. Environmental Science: Nano 1(2), 181-189.

Gombotz, W.R. and Wee, S.F. (1998) Protein release from alginate matrices. Advanced Drug Delivery Reviews 31(3), 267-285.

Gottschalk, F., Sonderer, T., Scholz, R.W. and Nowack, B. (2009) Modeled Environmental Concentrations of Engineered Nanomaterials (TiO2, ZnO, Ag, CNT, Fullerenes) for Different Regions. Environmental Science & Technology 43(24), 9216-9222.

Gratzel, M. (2004) Conversion of sunlight to electric power by nanocrystalline dye-sensitized solar cells (vol 164, pg 3, 2004). Journal of Photochemistry and Photobiology a-Chemistry 168(3), 235-235.

Gregor, J.E., Fenton, E., Brokenshire, G., vandenBrink, P. and Osullivan, B. (1996) Interactions of calcium and aluminium ions with alginate. Water Research 30(6), 1319-1324.

Handy, R.D., Owen, R. and Valsami-Jones, E. (2008) The ecotoxicology of nanoparticles and nanomaterials: current status, knowledge gaps, challenges, and future needs. Ecotoxicology 17(5), 315-325.

Helgerud, T., Gåserød, O., Fjæreide, T., Andersen, P. O. and Larsen, C. K. (2009) Food Stabilisers, Thickeners and Gelling Agents. Imeson, A. (ed), pp. 50-72, Wiley-Blackwell, Oxford, UK.

Hendren, C.O., Mesnard, X., Droegge, J. and Wiesner, M.R. (2011) Estimating production data for five engineered nanomaterials as a basis for exposure assessment. Environmental Science & Technology 45(7), 2562-2569.

Hyung, H., Fortner, J.D., Hughes, J.B. and Kim, J.-H. (2007) Natural organic matter stabilizes carbon nanotubes in the aqueous phase. Environmental Science & Technology 41(1), 179-184.

Jelesarov, I. and Bosshard, H.R. (1999) Isothermal titration calorimetry and differential scanning calorimetry as complementary tools to investigate the energetics of biomolecular recognition. Journal of Molecular Recognition 12(1), 3-18.

Jimin, X., Deli, J., Min, C., Di, L., Jianjun, Z., Xiaomeng, L. and Changhao, Y. (2010) Preparation and characterization of monodisperse Ce-doped TiO₂ microspheres with visible light photocatalytic activity. Colloids and Surfaces A: Physicochemical and Engineering Aspects 372(1-3).
Joshi, H., Shirude, P.S., Bansal, V., Ganesh, K.N. and Sastry, M. (2004) Isothermal titration calorimetry studies on the binding of amino acids to gold nanoparticles. Journal of Physical Chemistry B 108(31), 11535-11540.

Kamiya, M., Torigoe, H., Shindo, H. and Sarai, A. (1996) Temperature dependence and sequence specificity of DNA triplex formation: An analysis using isothermal titration calorimetry. Journal of the American Chemical Society 118(19), 4532-4538.

Khan, R. and Dhayal, M. (2008) Electrochemical studies of novel chitosan/TiO2 bioactive electrode for biosensing application. Electrochemistry Communications 10(2), 263-267.

Kim, W., Yamasaki, Y., Jang, W.-D. and Kataoka, K. (2010) Thermodynamics of DNA condensation induced by poly(ethylene glycol)-block-polyslysin through polyion complex micelle formation. Biomacromolecules 11(5), 1180-1186.

Klaine, S.J., Alvarez, P.J.J., Batley, G.E., Fernandes, T.F., Handy, R.D., Lyon, D.Y., Mahendra, S., McLaughlin, M.J. and Lead, J.R. (2008) Nanomaterials in the environment: Behavior, fate, bioavailability, and effects. Environmental Toxicology and Chemistry 27(9), 1825-1851.

Kominami, H., Itonaga, M., Shinonaga, A., Kagawa, K., Konishi, S. and Kera, Y. (2000) Studies in Surface Science and Catalysis. E. Gaigneaux, D.D., P. Grange, P. A. Jacobs, J. A. Martens, P. Ruiz, and Poncelet, G. (eds), pp. 1089-1096, Elsevier.

Ladbury, J.E. (2001) Isothermal titration calorimetry: application to structure-based drug design. Thermochimica Acta 380(2), 209-215.

Lee, K.Y. and Mooney, D.J. (2012) Alginate: Properties and biomedical applications. Progress in Polymer Science 37(1), 106-126.

Lin, S., Cheng, Y., Liu, J. and Wiesner, M.R. (2012) Polymeric coatings on silver nanoparticles hinder autoaggregation but enhance attachment to uncoated surfaces. Langmuir 28(9), 4178-4186.

Liu, X., Wazne, M., Han, Y., Christodoulatos, C. and Jasinkiewicz, K.L. (2010) Effects of natural organic matter on aggregation kinetics of boron nanoparticles in monovalent and divalent electrolytes. Journal of Colloid and Interface Science 348(1), 101-107.

Loosli, F., Le Coustumer, P. and Stoll, S. (2013) TiO2 nanoparticles aggregation and disaggregation in presence of alginate and Suwannee River humic acids. pH and concentration effects on nanoparticle stability. Water Research 47(16), 6052-6063.

Loosli, F., Le Coustumer, P. and Stoll, S. (2014) Effect of natural organic matter on the disagglomeration of manufactured TiO2 nanoparticles. Environmental Science: Nano 1, 154-160.

Louie, S.M., Tilton, R.D. and Lowry, G.V. (2013) Effects of Molecular Weight Distribution and Chemical Properties of Natural Organic Matter on Gold Nanoparticle Aggregation. Environmental Science & Technology 47(9), 4245-4254.
Lu, A.-H., Salabas, E.L. and Schueth, F. (2007) Magnetic nanoparticles: Synthesis, protection, functionalization, and application. Angewandte Chemie-International Edition 46(8), 1222-1244.

Luo, X.L., Morrin, A., Killard, A.J. and Smyth, M.R. (2006) Application of nanoparticles in electrochemical sensors and biosensors. Electroanalysis 18(4), 319-326.

Mahltig, B., Bottcher, H., Rauch, K., Dieckmann, U., Nitsche, R. and Fritz, T. (2005) Optimized UV protecting coatings by combination of organic and inorganic UV absorbers. Thin Solid Films 485(1-2), 108-114.

Martin, M., Celi, L., Barberis, E., Violante, A., Kozak, L.M. and Huang, P.M. (2009) Effect of humic acid coating on arsenic adsorption on ferricydrite-kaolinite mixed systems. Canadian Journal of Soil Science 89(4), 421-434.

Matulis, D., Rouzina, I. and Bloomfield, V.A. (2002) Thermodynamics of cationic lipid binding to DNA and DNA condensation: Roles of electrostatics and hydrophobicity. Journal of the American Chemical Society 124(25), 7331-7342.

Mohd Omar, F., Abdul Aziz, H. and Stoll, S. (2014) Aggregation and disaggregation of ZnO nanoparticles: Influence of pH and adsorption of Suwannee River humic acid. Science of the Total Environment 468-469(0), 195-201.

Palomino, D. and Stoll, S. (2013) Fulvic acids concentration and pH influence on the stability of hematite nanoparticles in aquatic systems. Journal of Nanoparticle Research 15(2), 1-8.

Piccinno, F., Gottschalk, F., Seeger, S. and Nowack, B. (2012) Industrial production quantities and uses of ten engineered nanomaterials in Europe and the world. Journal of Nanoparticle Research 14(9).

Ravi, V., Binz, J.M. and Rioux, R.M. (2013) Thermodynamic profiles at the solvated inorganic-organic interface: The case of gold-thiolate monolayers. Nano Letters 13(9), 4442-4448.

Sani-Kast, N., Scheringer, M., Slomberg, D., Labille, J., Praetorius, A., Ollivier, P. and Hungerbühler, K. (2015) Addressing the complexity of water chemistry in environmental fate modeling for engineered nanoparticles. Science of the Total Environment, In Press, doi:10.1016/j.scitotenv.2014.1012.1025.

Saude, N., Cheze-Lange, H., Beunard, D., Dhubler, P., Guillochon, D., Caze, A.M., Morcellet, M. and Junter, G.A. (2002) Alginate production by Azotobacter vinelandii in a membrane bioreactor. Process Biochemistry 38(2), 273-278.

Schmid, K. and Riediker, M. (2008) Use of nanoparticles in Swiss industry: A targeted survey. Environmental Science & Technology 42(7), 2253-2260.

Seitz, F., Bundschuh, M., Darbcunz, A., Bandow, N., Schaumann, G.E. and Schulz, R. (2012) Titanium dioxide nanoparticles detoxify pirimicarb under UV irradiation at ambient intensities. Environmental Toxicology and Chemistry 31(3), 518-523.

Shen, M.-H., Yin, Y.-G., Booth, A. and Liu, J.-F. (2015) Effects of molecular weight-dependent physicochemical heterogeneity of natural organic matter on the aggregation of fullerene nanoparticles in mono- and di-valent electrolyte solutions. Water Research 71(0), 11-20.
Sheng, G.-P., Xu, J., Luo, H.-W., Li, W.-W., Li, W.-H., Yu, H.-Q., Xie, Z., Wei, S.-Q. and Hu, F.-C. (2013) Thermodynamic analysis on the binding of heavy metals onto extracellular polymeric substances (EPS) of activated sludge. Water Research 47(2), 607-614.

Vitorazi, L., Ould-Moussa, N., Sekar, S., Fresnais, J., Loh, W., Chapel, J.P. and Berret, J.F. (2014) Evidence of a two-step process and pathway dependency in the thermodynamics of poly(diallyldimethylammonium chloride)/poly(sodium acrylate) complexation. Soft Matter 10(47), 9496-9505.

von Moos, N. and Slaveykova, V.I. (2014) Oxidative stress induced by inorganic nanoparticles in bacteria and aquatic microalgae - state of the art and knowledge gaps. Nanotoxicology 8(6), 605-630.

Weir, A., Westerhoff, P., Fabricius, L., Hristovski, K. and von Goetz, N. (2012) Titanium dioxide nanoparticles in food and personal care products. Environmental Science & Technology 46(4), 2242-2250.

Wells, M.L. (1998) Marine colloids - A neglected dimension. Nature 391(6667), 530-531.

Wilkinson, K.J., Balnois, E., Leppard, G.G. and Buffle, J. (1999) Characteristic features of the major components of freshwater colloidal organic matter revealed by transmission electron and atomic force microscopy. Colloids and Surfaces a-Physicochemical and Engineering Aspects 155(2-3), 287-310.

Wilkinson, K.J., Joz-Roland, A. and Buffle, J. (1997) Different roles of pedogenic fulvic acids and aquagenic biopolymers on colloid aggregation and stability in freshwaters. Limnology and Oceanography 42(8), 1714-1724.

Wongkalasin, P., Chavadej, S. and Sreethawong, T. (2011) Photocatalytic degradation of mixed azo dyes in aqueous wastewater using mesoporous-assembled TiO2 nanocrystal synthesized by a modified sol-gel process. Colloids and Surfaces a-Physicochemical and Engineering Aspects 384(1-3), 519-528.

Yang, J., Zhao, J. and Fang, Y. (2008) Calorimetric studies of the interaction between sodium alginate and sodium dodecyl sulfate in dilute solutions at different pH values. Carbohydrate Research 343(4), 719-725.

Zhang, A.-P. and Sun, Y.-P. (2004) Photocatalytic killing effect of TiO2 nanoparticles on Ls-174-t human colon carcinoma cells. World Journal of Gastroenterology 10(21), 3191-3193.

Zhang, W., Rattanaudompol, U.s., Li, H. and Bouchard, D. (2013) Effects of humic and fulvic acids on aggregation of aqu/nC60 nanoparticles. Water Research 47(5), 1793-1802.

Zhang, Y., Chen, Y., Westerhoff, P. and Crittenden, J. (2009) Impact of natural organic matter and divalent cations on the stability of aqueous nanoparticles. Water Research 43(17), 4249-4257.
Fig. S1 - TiO$_2$ z-average diameter values as a function of pH. At pH 3.1 (large gray vertical line) the TiO$_2$ ENPs are dispersed with a z-average diameter value found equal to $47 \pm 1$ nm. At pH 11.0 (narrow gray line), the ENPs are also stable with diameter value equal to $53 \pm 1$ nm. [TiO$_2$] = 50 mg L$^{-1}$ and [NaCl] = 0.001 M.
**Fig. S2** - TiO$_2$ ENPs intensity and number size distribution at pH < pH$_{PCN,ENPs}$. [TiO$_2$] = 50 mg L$^{-1}$. 
Fig. S3 - Alginate z-average diameter values as a function of pH. Alginate z-average diameter is constant with diameter value equal to 178 ± 21 nm. [Alginate] = 100 mg L$^{-1}$. 
Fig. S4 - Alginate intensity and number size distribution at pH < pH_{PCN,ENPs}. [Alginate] = 100 mg L$^{-1}$. 
**Fig. S5** - a) Real-time thermogram representing the heat exchange for a 2.5 mM alginate charge concentration titration in water at $\text{pH} < \text{pH}_{\text{PCN, TiO}_2}$. b) Corresponding interaction binding enthalpy as a function of injection number. Only dilution effect is observed.
**Fig. S6** - a) Real-time thermogram for TiO$_2$ 0.14 g L$^{-1}$ titration with alginate 0.7 mM at pH $< \text{pH}_{\text{PCN, TiO}_2}$ at 298.15 K. Negative peaks indicate an exothermic reaction. After about fifteen injections sites saturation occurs and only dilution effect is observed (small negative peaks).

b) Corresponding integrated heat data as a function of molar charge ratio.
Fig. S7 - a) Real-time thermogram for TiO$_2$ 0.5 g L$^{-1}$ titration with alginate 2.5 mM at pH < pH$_{PCN,TiO_2}$ at 298.15 K. Negative peaks indicate an exothermic reaction. After about fifteen injections sites saturation occurs and only dilution effect is observed (small negative peaks). b) Corresponding integrated heat data as a function of molar charge ratio.
Fig. S8 - a) Real-time thermogram and b) the corresponding integrated heat data as a function of injection number for a 5 g L\(^{-1}\) TiO\(_2\) dispersion titration in water at pH < pH\(_{\text{PCN,TiO}_2}\) and at 298.15 K. Dilution effect is observed. The dilution of the TiO\(_2\) ENPs in water is an endothermic process.
Fig. S9 - a) Real-time thermogram for alginate 0.07 mM titration with TiO$_2$ 1.4 g L$^{-1}$ at pH < pH$_{PCN,TiO_2}$ and at 298.15 K. Negative peaks indicate an exothermic reaction. After about ten injections sites saturation occurs and only dilution effect is observed (small positive peaks). b) Corresponding integrated heat data as a function of molar charge ratio.
Fig. S10 - a) Real-time thermogram for alginate 0.25 mM titration with TiO$_2$ 5 g L$^{-1}$ at pH < pH$_{PCN,TiO_2}$ and at 298.15 K. Negative peaks indicate an exothermic reaction. After about ten injections sites saturation occurs and only dilution effect is observed (small positive peaks). b) Corresponding integrated heat data as a function of molar charge ratio
Fig. S11 - a) Real-time thermogram representing the heat exchange for a 0.1 g L$^{-1}$ TiO$_2$ titration with a 0.5 mM charge concentration alginate at pH 11.0. b) Corresponding integrated heat exchange data as a function of alginate over TiO$_2$. No interaction is observed in agreement with electrostatic repulsions between the negatively charged compounds (Fig. 2).