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- 7 References: 101
- 8 Tables: 0
- 9 Figure legends: 4

**A semi-empirical model for transport of
inorganic nanoparticles across a lipid bilayer –
Implications for uptake by living cells.**

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Abstract

Due to increasing application, release of nanoparticles (NPs) and nanomaterials (NMs) into the environment becomes likely. Knowledge about NP uptake in organisms is crucial for risk assessment including estimations on the behavior of NPs based on their physicochemical properties. In this study, we have applied the current scientific knowledge to construct a mathematical model which estimates the transport of NPs through a model biological membrane. The semi-empirical model developed showed all parameters studied to substantially affect the agglomeration of the NPs in suspension, thereby also affecting passive transport. We quantified the effects of pH, ionic strength and organic matter (OM) concentration of the medium and NP size of several inorganic NPs on the permeation through the lipid membrane. Model outcomes and experimental results described in literature were strongly correlated for several metal oxide NPs. With caution, the model may be used to explain some of the existing variance in nano-uptake and toxicity experiments.

Keywords: Nanoparticles; (size-dependent) uptake; passive uptake; agglomeration; model membrane

Introduction

Many classes of nanoparticles (NPs) and nanomaterials (NMs) have been synthesized and characterized over the last decades. Examples include gold [1], metal oxide [2], semiconductor [3], and organic NPs [4]. These materials are promising for many kinds of applications including catalysis [5], medical diagnosis and therapy [6], sensors [7], cosmetics [8], and coatings [9]. It is inevitable that this abundant use of NPs will lead to some release into the environment. Therefore, concern has been expressed about possible adverse effects of synthetic NPs [10, 11]. In spite of the rapidly increasing number of products containing NPs [12], quantitative knowledge about impacts on human health and ecosystems remains limited. There is ample evidence [13, 14] that exposure to synthetic NPs may result in toxic effects on various organisms.

Understanding of the mechanisms by which NPs exert toxic effects is increasing: toxicity is thought to be related to specific physicochemical properties like size, surface area, surface charge and surface chemistry; due to the large specific surface area of NPs relative to bulk-sized particles, release of NP associated toxicants to the surrounding medium is high. Additionally, it is hypothesized [16, 17] that a NP expresses the largest amount of toxicity when it has crossed the biological membrane and has free access to internal cellular matrices like structural proteins such as actin and microtubules, oligonucleotides (DNA and RNA), as well as larger constructions such as mitochondria or Golgi apparatus, i.e. the Trojan Horse effect. The direct interaction of NPs with biological membranes remains a key part of identifying the potential hazard of NPs because it can hamper biological responses or cause the biological membrane to disintegrate altogether. The combination of observations suggests that the overall cellular damage can be assessed when the ability of a NP to attach and breach the plasma membrane and enter the living cell is known. This suggestion is in agreement with the results acquired from toxicity assessments on molecular xenobiotics in which bilayer permeation models [18, 19] are used: the absence of a toxic effect can be attributed to a low uptake. These models on xenobiotics use empirical parameters like the n-octanol/water partitioning coefficient (K_{ow}) and molecular weight (Mw), descriptors which have demonstrated good predictive power when applying to small molecules. When

assessing cellular uptake of NPs, one may consider the application of the same models. However, this application raises theoretical and practical difficulties: Can M_w and K_{ow} serve to predict the effects of the nanoforms of chemical substances? And if so, can substance properties of NPs be determined by the commonly used assessment methods? Answering these questions involves determining the differences in behaviors and effects on cellular matrices between xenobiotics and their larger nanoforms.

A notable difference between the two is the large size of NPs relative to conventional chemicals. Diffusion through a lipid bilayer, or passive transport, has been relatively well described for small organic molecules in the last decades [20, 21]. Accordingly, passive uptake of NPs by living cells was assumed possible only for relatively small sizes [22, 23]. In contrast, recent studies show that classes of particles of certain size and hydrophobicity [24-28] can passively translocate a lipid membrane. Apart from its effect on diffusion, the relatively large size of NPs also induces colloidal interactions with the biological membrane exterior. Although most NPs express adsorptive behavior when in contact with biological materials like proteins and membranes, due to these colloidal interactions, the rate and state of adsorption may be heavily reduced. Experiments [29-32] show that NP charge, membrane charge and surface coverage are often the rate-determining properties of the system. This effect along with colloidal agglomeration, which greatly alters the permeate volume, makes distinguishing between ad- and absorption (in)to the biological membrane difficult. Also, reaching an experimental thermodynamic equilibrium state between water and a membrane is often not practical. Most NP suspensions have been shown to increase their effective size through agglomeration upon entering natural aqueous media, which has a significant effect on the overall bioavailability of nanoparticulates [33-38]. Several studies have addressed the agglomeration of different NPs in aqueous solutions, including the effect of increasing ionic strength (IS), different pH levels and the interaction of NPs with natural organic matter (NOM) [39-45], i.e. heteroaggregation, on the size of agglomerates. These processes and system properties affect the balance between attractive and repulsive forces and ultimately control the rate and state of agglomeration of the NPs in solution. Both experimental and theoretical methods exist for determining the colloidal stability of a NP suspension [40, 41, 46, 47]. Classical DLVO theory (the theory on interaction forces

between charged surfaces in aqueous media according to Derjaguin, Landau, Verwey and Overbeek) [48, 49] uses the combination of electrostatic and Van der Waals interaction to construct energetic interaction profiles for charged bodies. However, it has been proven insufficient for predicting the agglomeration kinetics of NPs in complex environmental media [50, 51]. Among a variety of additional interactions, one may also consider steric and bridging interactions due to the adsorption of NOM as well as Lewis acid-base (polar), hydration and magnetic [52, 53] interactions, because these interactions have shown to play a significant role in the agglomeration kinetics of NPs.

To the best of our knowledge, to date there is no integrated model for NP membrane permeation accounting for the many processes on which it depends. The aim of this study was to use the current scientific knowledge to quantify how processes and physicochemical properties determine the passive uptake of NPs by cells. To this end, we developed a simple mathematical model using conventional system parameters to make a quantitative estimation of passive transport of NPs across lipid bilayers. To test the plausibility of our model we correlated model output with experimental observations using living cells from literature.

Methods

The processes underlying passive transport of NPs through a membrane depend on NP and bilayer characteristics. A selection of several important processes and characteristics was based on the available general scientific knowledge of the colloidal behavior of NPs, their interaction with plasma membranes, and to which extent these processes affect the overall and separate stages of transport across plasma membranes. The hydrodynamic size, charge, hydrophobicity and lipophilicity of the NP along with the charge and permeability of the membrane are found to be very influential and most effective for describing the different stages of passive transport. Here we present a simple mathematical model in which the processes and different stages of passive uptake of NPs in model cells are formulated as a function of these properties.

Model formulations

For the determination of hydrodynamic size as function of several system properties we chose to

apply a semi-empirical expression originally developed by Li et al. [40, 41]:

$$Eq. 1. \quad R_H = 2r \left\{ 1 + \frac{4k_B T N_b}{3\mu W} t \right\}^{\frac{1}{d_F}}$$

where R_H is the average hydrodynamic radius of the NP agglomerate (m), r is the primary particle radius (m), k_B the Boltzmann constant ($1.38 \times 10^{-23} \text{ JK}^{-1}$), T the temperature (K), N_b is the number concentration of primary particles in the bulk solution (m^{-3}), μ the dynamic viscosity of the medium (taken to be $8.9 \times 10^{-3} \text{ Nsm}^{-2}$ for aqueous solutions at 25°C), d_F the fractal dimension of agglomerates for diffusion-limited agglomeration (1.8) [41] and t represents time (s). High agglomeration kinetics, expressed by a low value for the dimensionless colloid stability ratio W , gives rise to an increase of the average fractal agglomerate radius over time. W is defined as the reciprocal ratio of successful collisions and collision frequency of the particles and therefore is a function of both inter-particle attractive and repulsive forces [34, 46]:

$$Eq. 2. \quad W = \left[\int_0^\infty \lambda(h) \frac{\exp(V_T(h)/(k_B T))}{(2+h)^2} dh \right] \left[\int_0^\infty \lambda(h) \frac{\exp(V_A(h)/(k_B T))}{(2+h)^2} \right]^{-1}$$

where λ is a correction factor for the diffusion coefficient related to the separation distance h which is the normalized surface-to-surface distance between two particles (m) [34] and V_T and V_A are the sums of total and attractive energies, respectively (J). For the purpose of this study, energy profiles were constructed for smooth, spherical and non-magnetic NPs using four separate expressions for interaction energies: Van de Waals forces, electrostatic forces, Lewis acid-base (polar) forces and organic matter (OM) repulsion/bridging. However, adsorption of OM distorts the electrostatic forces, Van de Waals and Lewis acid-base interactions. The extent of this distortion could be estimated using the specific physicochemical properties of the OM [41, 54-56] (SI2). In our simulation, the OM had properties similar to that of standard Suwannee River Fulvic Acid (SRFA), the physicochemical characterization of the OM is described in detail in the supporting information, sections SI2 and SI6. The stability ratio, and consequently average agglomerate size, was thereby determined by dividing the total energetic integral (fast agglomeration) over the attractive integral (slow agglomeration), Eq. 2. Detailed statements on the

interaction energies and default values for the physicochemical properties used as input parameters for NPs can be found in SI1 and SI2, respectively.

Electrostatic effects in nanoparticle-bilayer adsorption

We considered the exchange of NPs between a stationary bulk solution and adsorbent layer, a system in which hydrodynamic forces are neglected. In our model, the fluxes between these compartments were approximated following Fick's first law for diffusion: $\frac{dN_a}{dt} = kD(t)[N_b - N_a]$. Where N_b and N_a represent the particle number concentrations in the bulk solution and adsorbent layer, respectively. We thereby applied a mechanism of reversible adsorption with desorption dependent on the particle number concentration in the adsorbent layer. Diffusion coefficients D were calculated via the average hydrodynamic radius, as determined by eq. 1, using the Stokes-Einstein equation. Here, we consider monodispersity with the hydrodynamic radius representing the real-time size of all agglomerates to be a reasonable simplification, as a measured change in the average hydrodynamic radius provides useful information about the apparent diffusion coefficient and levels of molecular adsorption [56-60]. Adsorption fluxes as part of the overall passive transport are graphically represented by Fig. 1. In our simulation the membrane exterior had a surface potential of -40 mV. Fluxes were therefore adjusted by using rate-limiting factors k so that EDLVO (an extension to DLVO theory) interactions between the NPs and the negatively charged bilayer were accounted for. Distortion of k due to surface coverage of the membrane can be accounted for via the application of partition ratios K that account for variations in the equilibrium states of ad- and absorption due to the screening and agglomeration behaviour of adsorbed particles (see section on bilayer diffusivity). Further statements on k and the interaction energies can be found in SI3.

Bilayer diffusivity

We applied an empirical relationship between both the size and lipophilicity of NPs on the one hand and the permeability of bilayers to NPs on the other as reported by Fiedler and Violi [26], derived from the expression of the intrinsic resistance of biological membranes [61, 62]:

$$\text{Eq. 3. } \frac{dN_p}{dt} = \frac{D_m K}{V(t)^s \delta} [N_a - N_p]$$

With $\frac{dN_p}{dt}$ representing the number of translocation events of NPs per time unit through a membrane region with length δ (taken to be 6.8×10^{-9} m). The diffusion coefficient in the membrane region is represented as a quotient of the average diffusivity of the membrane D_m (as calculated to be 2.0×10^{-27} ms⁻¹ for spherical fullerene NPs with an approximate radius r of 5×10^{-10} m [26]) and the scaled permeate hydrodynamic volume V^s (m³), as calculated from the hydrodynamic radius R_H (Eq. 1, SI4). Here it should be noted that our method of conversion of the hydrodynamic radius to volume is only valid for spherically shaped agglomerates, i.e. for the reaction limited agglomeration regime (RLA). The concentration of particles in the adsorbent layer N_a (m⁻³) is determined by the process descriptions described in the previous section. K is a partitioning function that describes the ratio of NPs between water and lipid bilayer phases that arises from the Gibbs free energy change upon NP-bilayer interaction. For the purpose of this study, we assumed that eq. 3 provides an estimation on the permeability of lipid bilayers for NPs and that transport is governed primarily by a distinct and uniform hydrophobic barrier region, indicated by the membrane layer in Fig. 1, through which the permeability of small, lipophilic NPs or agglomerates is increased. Further details on the algorithm can be found in the SI: parameters for permeation, SI4. For the construction of the mathematical model, we used Matlab (R2012a), symbolic and curve fitting toolboxes from Maple (version 15).

Parameter selection

We used points of zero charge $pH_{p.z.c.}$, Lifshitz-van de Waals energies ΔG^{LW} , acid-base (polar) energies ΔG^{AB} and primary particle radius r as NP properties and ionic strength IS , pH and OM concentration C_{OM} as medium properties for the physicochemical parameterization of the model system, as these were considered to be the most important characteristics. We focused on spherical metal oxide NPs because surface potentials can be approximated using the Nernst equation and EDLVO interactions are well described. Because we found few quantitative experimental data on the lipophilicity of NPs, we

used results from contact angle measurements and molecular modeling studies to estimate K (SI2, SI4). We focused on the results from molecular modeling because these studies were able to quantify water-to-bilayer Gibbs free energy changes with limited effects of colloidal interactions and agglomeration. We set the scaling factor s constant at 0.8 [26-28, 63] and K by default to 1×10^5 for hydrophilic NPs and NP agglomerates (SI4). The number concentration of NPs in the bulk solution N_b was set constant, whereas concentrations in the adsorbent layer N_a and permeated area N_p were zero at $t = 0$, respectively. Also, there was no agglomeration at $t = 0$. We used total permeated primary particle surface area per bilayer area (m^2/m^2) as an endpoint value to quantify the total passive transport of NPs and NP agglomerates because surface area can effectively be related to interaction with cellular matrices, cellular uptake and toxicity. Also, data on uptake [16, 64-67] as well as toxicity [68] for a range of different NPs and NP characteristics were used to test the plausibility and quality of the model. To this end, system characteristics from several studies were implemented in the mathematical model and outcomes were tested for a correlation with experimental data and observations.

Results

Using classical colloid chemistry and the relationships found in literature (eq. 1-3), we simulated the evolution of hydrodynamic size over time and determined its effect on adsorption and permeation through a model biological membrane. The outcome shows the amount of surface area to have permeated the membrane to be around $10^{14} \text{ m}^2/\text{m}^2$ for a range of metal and metal oxide NPs (Fig. 2). This similarity in permeation for different NPs is expected because colloidal interactions are enhanced upon increasing NP size, and thus permeation of a small NP is primarily governed by its initial state of agglomeration [27, 28, 37].

Effectiveness of passive uptake for different NP species

At standard conditions the oxide NPs $\gamma\text{-Al}_2\text{O}_3$, ZnO, MgO and CoO are able to passively cross over a lipid membrane most effectively (Fig. 2). The large difference in permeation between semi-hydrophobic $\alpha\text{-Al}_2\text{O}_3$ and $\gamma\text{-Al}_2\text{O}_3$ was due to the differences in the surface energy components for the

polymorphs. The effect of increased charge density on NPs was noticeable: the large charge on SiO₂ and WO₃ particles results in unfavorable EDL overlap with the lipid bilayer causing inhibition of adsorption. In contrast, a small NP surface potential facilitates permeation through the lipid bilayer because the particles acquire relatively small permeate volumes due to electrostatic stabilization. Although the effect of EDL interaction and agglomeration on bilayer permeation can be quantified, large differences in permeation between hydrophilic and hydrophobic NPs are expected due to variations in NP lipid affinity [27, 28, 69-71]. Additional calculations (SI4) show that for hydrophobic fullerene C60, gold, silver, copper oxide and other rare earth oxide NPs with radii 0.5-2 nm uptake may be increased up to several orders of magnitude.

Influence of NP charge

We have further explored the model by investigating its response to changes in NP charge, by varying the pH in the simulation. The outcome shows the combined effects of agglomeration and NP-bilayer EDL interaction on NP permeation: around the point of zero charge $pH_{p.z.c.}$, NP agglomerates acquire a large volume inhibiting permeation through the bilayer, whereas EDL interaction becomes dominant at extreme pH (Fig. 3a). We also noted a large difference in the predicted bilayer permeability for silica particles in freshwater ($IS \approx 1$ mM) and marine waters ($100 < IS < 1000$ mM). An effect that is reversed for titanium dioxide particles, although around ten orders of magnitude weaker (Fig. 3b).

Influence of permeate size and OM

The competing effects of permeate volume and EDL interaction is visible upon varying the NP size in our simulation: uptake of γ -Al₂O₃ particles increases towards a maximum around a diameter of 22 nm, whereas silica and zinc oxide uptake show maxima around 10 and 40 nm, respectively (Fig. 3c). These maxima arise from the combined effects of unfavorable geometry of the NP-bilayer EDL interaction and decreased agglomeration upon increasing the NP size; such maxima have been observed experimentally [72, 73]. The model also predicts a ~100-fold increase in passive uptake for certain types of NPs in media with high OM concentrations (Fig. 3d). An increase arising from steric and electrostatic stabilization of the NPs suspension through OM coating.

Association between passive uptake and toxicity

Comparing model calculations for several metal oxide NPs to toxicity data for human keratinocytes (HaCaT) and adenocarcinomic alveolar basal epithelial cells (A549) revealed a correlation: the cells showed relatively high sensitivity for zinc oxide and nickel oxide NPs for which our model predicts high uptake. Accordingly, tungsten oxide shows no significant toxic effects [68] (Fig. 4):

Discussion

Model plausibility and limitations

Based on current knowledge, we constructed a simple mathematical model to estimate the transport of NPs through lipid bilayers. Literature has shown that the combination of eq. 1 and 2 possesses good predictive power for the stability of NPs in clean and well-defined model natural aqueous media [41, 44]. However, predictive power of DLVO theory and its extensions remains limited as research still aims to determine the factors that influence the extent of several specific interaction mechanisms, e.g. OM and NP cation complexation and bridging [74]. Moreover, our model is only relevant for spherical NPs because statements on EDLVO interactions are not applicable to other geometries. Here, an extension to our model is possible via the selection of appropriate statements on the interaction energies of NPs of multiple geometries [75-77]. This study disregarded heteroaggregation with relatively large particulate (organic) matter because it is found that this process causes the suspension to lose its nano-specific properties as its new (fractal) dimensions exceed the ‘nano-range’, >100 nm. Though, due to the variety and variability in the character of the environmental medium the stability, and consequently bioavailability, of NP suspensions should always be re-determined for alternative experimental settings. Additionally, the application of a more real-life situation of polydisperse NP suspensions, in sharp contrast with our assumption of monodispersity, may enhance diffusion and/or adsorption processes [100].

Eq. 3 was originally developed to determine the permeation of small NMs (~1 nm) through lipid bilayers, but may also be applicable for larger NPs and NP agglomerates. This consideration is supported by the results acquired from simulation studies [27, 28] and experiments [24, 25, 78-80] which indicate

that NP agglomerates are able to translocate across membranes at low rates, inversely proportional to V^s . Although studies on the permeation of organic solutes and NPs give similar scaling factors for permeate volume, $\frac{\Delta N_p}{\Delta t} \sim V^{-0.8}$ [26, 63], variations between predictions based on experiments and predictions based on simple geometric considerations may have to be explained by specific membrane-colloid interactions.

The behaviour of NP suspensions, as described through all theoretical and empirical relationships cited in this study, is highly influenced by several key physicochemical properties [40, 41, 43-45] and are all suitable for describing the different stages that underlie passive transport. For these reasons, the combination of these relationships suggests some degree of plausibility of the mathematical model proposed in this study.

Effectiveness of passive uptake for different NP species

Our model suggests that among the metal oxide NPs studied, γ -alumina, zinc oxide, magnesium oxide and cobalt oxide NPs are most effectively taken up by passive transport. This has been confirmed by experiments on cellular uptake of oxides of alumina, zinc and cobalt [16, 64-67]. We interpret this agreement as an indication of plausibility of our model: the uptake of these types of NPs may be partially explained by a mechanism of passive uptake. The model also suggests that permeation of anionic NPs is slightly faster than for cationic NPs. Ceria and iron oxide NPs uptake by model lung cells (A549) and non-phagocytotic HeLa cells was increased when the NPs were negatively charged [81, 82]. These observations may therefore be partially explained by NP-bilayer EDL overlap. However, an alternative explanation may be the ability of anionic NPs to increase the local density of lipids by increasing the tilt angle of PC headgroups [83]. This may result in the loss of integrity in biological membranes and increased permeability.

Influence of permeate size

The model also suggests maximum uptake efficiency at diameters of 10, 22 and 40 nm, whereas in literature the optimum is often reported to be around 50 nm [72, 73]. This observed difference may be

partially attributed to error replication, though this type of error is not likely a result of the sensitivity of the model as relatively large variations for input parameters gave rise to relatively small variations in the calculated values for passive transport: Fig 2; Fig 3; Table SI2.1. However, we present two possible mechanistic explanations for the observed differences: 1) The predominant mechanism in a specific nano-uptake assay is a form of endocytosis, not part of our diffusion model, which may increase uptake by several orders of magnitude [25], and 2) the laboratory experiment is unable to quantitatively determine the effect of the initial state of agglomeration of the applied NP suspension on cellular uptake. To our knowledge no studies have been able to determine the effect of size or geometry of agglomerates on long-range electric double layer (EDL) interactions with biological membranes. Still, one may expect distortion of the electrostatic interaction terms due to variations in the fractal dimension d_F . This distortion may become significant at relatively large fractal dimensions as agglomerates are more densely packed thereby magnifying the local electrostatic potential. Since the fractal dimensions of agglomerates are relatively low ($1.8 < d_F < 2.5$ for $\log(W) < 3$), and the rotational diffusion time of an agglomerate with an average hydrodynamic radius of 50 nm is similar to uninhibited diffusion over a typical EDL length κ of 10 nm, we know that a charged agglomerate with an EDL will have sufficient time to approach the membrane in the energetically most favourable orientation. Thus, when the primary particle size is in the same order as κ , the profile of the energetic barrier for adsorption of agglomerates reduces to that of primary particles.

Influence of NP surface functionality

The effect of NP surface functionality on permeation has been studied by several groups [28, 69, 84]; large differences in bilayer permeability for hydrophobic and hydrophilic NPs are often observed: Qiao et al. performed a molecular dynamics simulation [69] to calculate that the translocation time of hydrophilic $C_{60}(OH)_{20}$ is approximately 9 orders of magnitude larger compared to that for pristine C_{60} . Other hydrophobic NPs like gold, silver and polystyrene NPs have shown to preferentially absorb into the hydrocarbon center of lipid bilayers [84-87]. Correlation between these simulations and *in vitro* studies is possible: An increase in hydrophilicity due to the adsorption of polyethylene glycol (PEG) led to

decreased particle uptake by macrophages [70, 71], as well as in non-phagocytic HeLa cells [88]. The combination of these simulations and experiments thus shows that the effective uptake of NPs is proportional to the lipid affinity of the NP, which suggests that our model calculations for hydrophobic NPs can only be compared with calculations for hydrophilic NPs by applying partitioning ratios K which may differ by several orders of magnitude. Difficulties remain because of large differences between penetration rates, which have been reported [26] for the same type of NPs. For example, carbon NMs show varying bilayer solvation energies as function of NP metric surface area, an effect that has been verified by Lin et al. [89]. Variations in K are also expected upon NP screening and/or agglomeration. Screening, through inter-particle repulsion, may significantly affect K in the case of highly charged particles. Though, when an primary particle shows no preference for either the water- or the lipid bilayer phase ($K = 1$), the partition function for a NP suspension does not include a term for inter-particle repulsion because there is no site at which NPs accumulate. In the case of highly lipophilic NPs, i.e. when the bilayer-NP interaction energy is significantly larger than the NP-NP interaction energy, the bilayer may eventually disintegrate an agglomerate into an equilibrium state of primary particles. In such case, the total free energy change may be approximated as the summation of solvation energies over all particles in the agglomerate. Because in this scenario all particles in the agglomerate experience similar driving forces, partition ratios for primary particles and agglomerates will also be similar. In the case of agglomerates of low lipophilicity, the partition ratio is less well defined: the change in total free energy cannot be approximated as a summation over all primary particles. This is because not all particles in the agglomerate will interact with lipid molecules. For this reason, one may expect a higher value for K for non-lipophilic agglomerates compared to non-lipophilic primary particles. We hypothesize that this variation in K may be estimated through relating the accessible surface area of agglomerates to the metric surface area of primary particles. However, the determination of these properties still invokes theoretic and experimental limitations [90]. Though the application of theoretical partitioning ratios for NPs looks promising, careful use is advised as molecular simulations often make assumptions in contrast with the

dynamic behavior of the biological membrane [91]. For these reasons, additional experiments are needed to assess the relative affinities of different types of NPs to lipids.

Model evaluation

The general trends of our model are in agreement with literature, which shows that non-phagocytic cells favor the uptake of small-sized particles [25, 81, 82, 88]. Because of this agreement, we find our model able to screen a NP for its potential of cellular uptake by diffusion. We hypothesize that the model focusing on relatively small spherical metal oxide NPs ($r < 10$ nm) provides the best predictions on uptake by cells as a large NP size enhances colloidal interactions, thereby inhibiting passive transport (Fig. 3c). Additionally, larger particles are often observed to enter cells by endocytosis, possibly linked to increased receptor activation [92]. Specific cellular properties have also been shown to affect the uptake of NPs, i.e. uptake is related to differences between cell types e.g., phagocytic vs. non-phagocytic, cancer vs. normal cells and monocytes vs. macrophages [93-95]. However, the metabolic activity of the cells used may affect particle uptake as has been shown with opposing results [73, 93, 96]. These studies collectively show that active uptake mechanisms are cell-line specific and consequently are relatively little predictive of bio-accumulation and toxicity. As an alternative, experimental models using ‘barren’ giant unilamellar vesicles (GUVs), supported lipid bilayers (SLBs) [29-31] and molecular simulations using self-consistent field theory (SCF) [97, 98] have successfully been used to predict the interactions between NPs and cell membranes [99, 100]. These studies show that membrane deformation and wrapping are induced by NP adhesion, effects that strongly reminds of cellular endocytosis processes observed in nature. This demonstrates that the complicated mechanisms of endocytosis can be explained by simple physical effects, indicating the potential of passive transport in nanotoxicity assessments as well as of nano drug delivery applications.

Results from toxicity studies and their correlation with output from our mathematical model (Fig. 4) also suggest that cellular uptake via a (partial) passive mode is significant for NPs of limited sizes ($r < 20$ nm): adverse cellular effects due to NP exposure that have been explained by 1) the inhibition of membrane-bound receptor function and 2) in- and extracellular signaling pathways as well as 3) the

physical disruption of the cellular plasma membrane, are processes which can easily be incorporated as extensions to our model. We recognize that various active modes of cellular uptake and intrinsic modes of NP toxicity like the –UV induced– production of reactive oxygen species (ROS) and dissolution of heavy metals (Cu^{2+} , Ag^+) play key roles in NP toxicity. However, intrinsic modes of toxicity may be expressed as a function of the rate of intracellular toxicant release, which can be estimated by determining the effective agglomerated surface area and stability in the cell culture media. Here, more experimental difficulties arise because processes like dissolution, agglomeration and sedimentation are inherently inter-related, because all are dependent on surface area [101]. Moreover, it remains difficult to distinguish between the toxic effects of the particles themselves and an increased toxic environment due to NP solubilization. Due to the high degree of complexity additional research is necessary to investigate when, how and where a NP exerts its toxicity: directly or indirectly, extra-cellular or intra-cellular.

Perspectives

The results from our literature and modeling study pinpoints many areas of uncertainty, however also provides a framework for the screening of NPs by demonstrating that multiple scientific insights in the behavior of NP suspensions can help to understand the physical-chemical factors in control of passive transport. It is much too early to generalize this understanding to *a priori* prediction of NP toxicity, based on particle characteristics. However, we do feel that the results from this study explain how particle size and other particle characteristics can affect NP toxicity by their influence on passive uptake across biological membranes.

Acknowledgements

We thank Mieke Kleijn and Harke Pera for proofreading the manuscript, their helpful discussions and support.

Figures

Fig. 1. Graphic representation of the model. Reversible diffusion of NPs, or agglomerates, between the bulk and an adsorbent layer is followed by reversible diffusion across a membrane region. NPs are indicated by the white spheres. The system is described with pH , ionic strength IS , NP radius r and OM concentration C_{OM} as adjustable parameters.

Fig. 2. Estimated permeation of NPs through a model membrane in NP surface area per bilayer area (m^2/m^2). Uptake simulation over 24 hours, NP concentration in the bulk $N_b=50$ mg/L, $pH=7.5$, ionic strength, $IS=50$ mM, NP radius, $r=2$ nm (red) and $r=10$ nm (blue). We applied no initial agglomeration or organic matter (OM) to distinguish between the effects of the properties of NPs rather than those of OM-coated NPs or agglomerates. Values for all partitioning ratios K were set constant at 1×10^5 .

Fig. 3. Estimated permeation of NPs through a model membrane in NP surface area per bilayer area (m^2/m^2) as function of pH (a), ionic strength (b), NP radius (c) and NOM concentration (d). In the separate simulations, parameters were set constant at: NP concentration $N_b=50$ mg/L, NP radius $r=10$ nm, $pH=7.5$, ionic strength $IS=50$ mM and OM concentration $C_{OM}=0$ mg/L. Values for all partitioning ratios K were set constant at 1×10^5 .

Fig. 4. Estimated permeation of NPs through a model membrane in NP surface area per bilayer area (m^2/m^2) vs. measured

toxicity endpoints for human HaCaT and A549 cells exposed to WO_3 , La_2O_3 , MgO , NiO and CuO NPs (data shown from left to right). The left axis denotes cell viability (%) and the right axis the intracellular production of reactive oxygen species (ROS), relative to control (1). Data taken from [68].

Associated content

Supporting Information. Detailed information and equations for the formulations of the model system is provided as Supporting Information (SI). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Supporting information

for

**A semi-empirical model for passive transport of
inorganic nanoparticles across lipid bilayers –
Implications for uptake by living cells.**

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690 The supporting information contains the following sections:

691

692 SI1. Calculation of NP-NP interaction energies and energy profiles

693 SI2. Physicochemical characterization of NPs

694 SI3. Parameters for adsorption

695 SI4. Parameters for permeation

696 SI5. Characterization of OM and OM adsorption

SII. Calculation of NP-NP interaction energies and energy profiles

The energy profile was constructed using four expressions for interaction energies: Van de Waals forces, electrostatic forces, Lewis acid-base (polar) forces and steric repulsion/bridging due to the coating by organic matter:

$$(1) \ V_{tot} = V_{VdW} + V_E + V_{AB} + V_{OM}$$

The Van de Waals attractive energy V_{VdW} between two identical spherical particles can be computed using equation 2, which incorporates the retardation effect [1, 2]:

$$(2) \ V_{VdW}(h) = \frac{\pi h_0^2 \Delta G^{LW} r}{h(1 + 11.12h/\lambda_c)}$$

Where ΔG^{LW} is the Lifshitz-van de Waals free energy of interaction between NPs in water at distance h_0 , which is the minimum equilibrium distance due to Born repulsion, 0.157 nm (see SI2 for the physicochemical characterization of NPs). r is the particle radius. h is the separation distance between the interacting surfaces and λ_c is the characteristic wavelength of the interaction, which is often assumed to be 100 nm [3].

The electrostatic repulsive energy V_E between two identical spheres of radii r was approximated with equation 3 [2, 4]:

$$(3) \ V_E(h) = \frac{\varepsilon \varepsilon_0 (k_B T)^2 r}{4e^2} (\gamma^d)^2 \left[\ln \left(\frac{1 + \exp(-\kappa h)}{1 - \exp(-\kappa h)} \right) + \ln (1 - \exp(-2\kappa h)) \right]$$

Where y^d represents the reduced potential at the Stern layer: $y^d = e \frac{\psi^d}{k_B T}$, ψ^d is the potential (V) in the diffuse layer, as approximated via $\psi^d = \psi^s \left(\frac{r}{r+z} \right) e^{-\kappa z}$, where $z = 0.5$ nm [4] and the inverse Debye length $\kappa^{-1} = \sqrt{\frac{\epsilon \epsilon_0 k_B T}{2 N_A I e^2}}$. k_B is the Boltzmann constant; 1.38×10^{-23} J/K; T is absolute temperature, 298 K; e is unit charge, 1.602×10^{-19} C; ϵ_0 is the vacuum permittivity, 8.854×10^{-12} CV⁻¹m⁻¹; ϵ is the relative permittivity of water, 78.5; N_A is Avogadro's number, 6.02×10^{23} mol⁻¹; I is the ionic strength (M); ψ^s represents the surface potential of NPs with OM coating.

The acid-base energy V_{AB} between two identical spheres is expressed in equation 4:

$$(4) \quad V_{AB} = \pi r \lambda \Delta G^{AB} \exp \left(\frac{h_0 - h}{\lambda} \right)$$

Where λ is the correlation length or decay length of the molecules of the liquid medium (for pure water, this value is estimated to be 1 nm [5]); ΔG^{AB} is the polar or acid-base free energy of interaction between NPs at the distance h_0 (SI2).

The forces contributed by an adsorbed layer of organic matter can be computed with scaling theory [6–8], which is based on minimizing the surface free energy under the constraint that total amount of adsorbed OM is fixed in the region between two interacting surfaces. The interaction energy due to the OM layers was computed with equation 5:

$$(5) \quad V_{OM}(h) = \pi r \left(\frac{a_{sc} k_B T}{(2r)^3} \right) \Phi_{s0}^{9/4} D_{Sc} \left\{ -\frac{16 \Gamma D_{Sc}}{\Gamma_0} \ln \left(\frac{2\delta}{h} \right) + \frac{4 D_{Sc}^{5/4}}{2^{5/4}} \left(\frac{8\Gamma}{\Gamma_0} \right)^{9/4} \left[\frac{1}{h^{1/4}} - \frac{1}{(2\delta)^{1/4}} \right] \right\}$$

Where a_{sc} is a numerical constant relatable to the experimental osmotic pressure of semidilute solutions,

taken to be 2.6×10^2 nm; Φ_{s0} is polymer concentration at a single saturated surface, taken to be 0.3; D_{sc} is the scaling length, 1 nm [4]; Γ is total amount of OM adsorbed on a single surface; Γ_0 is the adsorbed amount at saturation; δ represents the thickness of the adsorbed OM layer, taken to be 1 nm (SI5). The first and the second terms within the brace in equation 5 represent bridging attraction and steric repulsion, respectively. All equations on the interaction energies give the interaction energy in J.

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SI2. Physicochemical characterization of NPs

NPs were characterised using three physicochemical properties: points of zero charge $pH_{p.z.c.}$, Lifshitz-van de Waals energies ΔG^{LW} and Lewis acid-base energies ΔG^{AB} .

Surface potentials ψ_c^s of the bare NPs were estimated using the Nernst equation: $\psi_c^s = \frac{2.303k_B T}{e} (pH_{p.z.c.} - pH)$, where $pH_{p.z.c.}$ is the pH of the medium at which the bare NPs have no net charge (table SI2.1).

Lifshitz-van de Waals free energies ΔG^{LW} were determined via the relationship $\Delta G^{LW} = 2 \left(\sqrt{\gamma_l^{LW}} - \sqrt{\gamma^{LW}} \right) \left(\sqrt{\gamma^{LW}} - \sqrt{\gamma_l^{LW}} \right)$. Here γ^{LW} and γ_l^{LW} are the Lifshitz-van de Waals surface tensions of the NPs (table SI2.1) and water, the latter being 21.8 mJ/m² [1].

The Lewis acid-base energies ΔG^{AB} were determined via $\Delta G^{AB} = -4 \left[\sqrt{(\gamma_l^+ \gamma_l^-)} + \sqrt{(\gamma^+ \gamma^-)} - \sqrt{(\gamma^+ \gamma_l^-)} - \sqrt{(\gamma_l^+ \gamma^-)} \right]$ for interaction in water, where γ_l^+ and γ_l^- are the electron acceptor and donor terms of water, $\gamma_l^+ = \gamma_l^- = 25.5$ mJ/m² [1], and γ^+ and γ^- the electron acceptor and donor terms of the NPs (table SI2.1).

Short-range interaction forces between NPs have shown to depend on the amount of adsorbed OM molecules, which distort the electronic properties of the bare NP surface. In case of OM coating, we assumed that the ionized groups of the polyelectrolyte molecule are uniformly distributed over the NP surfaces and that the polyelectrolyte molecule completely screens the dispersive and electrostatic forces exerted by the underlying NP surface. Also the polyelectrolyte layer is rigid, i.e. there are no mobile charge carriers. Harding and Berg [2] characterized the dispersion force component of the surface energy

of silica particles treated with coupling agents to varying degrees of coverage. Following this work, we assumed that the distorted surface energy components could be determined from the inferred fractional coverage as follows:

$$(2) \quad \gamma^{LW} = \gamma_c^{LW} - \frac{\Gamma}{\Gamma_0} (\gamma_c^{LW} - \gamma_{OM}^{LW})$$

$$(3) \quad \gamma^+ = \gamma_c^+ - \frac{\Gamma}{\Gamma_0} (\gamma_c^+ - \gamma_{OM}^+)$$

$$(4) \quad \gamma^- = \gamma_c^- - \frac{\Gamma}{\Gamma_0} (\gamma_c^- - \gamma_{OM}^-)$$

$$(5) \quad \psi^s = \psi_c^s - \frac{\Gamma}{\Gamma_0} (\psi_c^s - \psi_{OM}^s)$$

Where γ_c^{LW} , γ_c^+ and γ_c^- are the surface tension components of the bare surface of the NPs (table SI2.1). γ_{OM}^{LW} , γ_{OM}^+ and γ_{OM}^- are the surface tension components of the OM, taken to be 45 mJ/m², 0.3 mJ/m² and 45 mJ/m² as input values as are common for humic acid-covered particles, celluloses, lignins, plasma proteins and other polymers [3-7]. ψ_{OM}^s is the surface potential of NPs with complete OM coverage, taken to be -40 mV [3]. For statements on the approximation of the partial coverage $\frac{\Gamma}{\Gamma_0}$ by the adsorbed OM layer see section SI5.

Material (density, g/ml)	$pH_{p.z.c.}$	γ_c^{LW} (mJ/m ²)	γ_c^+, γ_c^- (mJ/m ²)	references
SiO ₂ (2.648)	2.5±0.5	39.2±2.4	0.8±0.3, 41.4±3.0	8, 9
TiO ₂ (4.23)	5.5±1.5	42.1±1.2	0.6±1.2, 46.3±5.1	8, 9
CeO ₂ (7.65)	7.3	49.84	0.1225, 65.61	8, 10
α -Al ₂ O ₃ (4)	8.5±0.5	31.6±3.5	0.6±0.4,	8, 9

			27.2±2.4	
γ -Al ₂ O ₃ (4.0)	8.1	41.0	0.1, 71.8	11
ZnO (5.61)	9.5±0.8	48.6	0.8, 43.4	12, 13
CuO (6.315)	9.5	22.97	0.27, 1.23	12, 14
α -Fe ₂ O ₃ (5.242)	8.1±1.1	45.6±1.8	0.3±0.4, 50.4±3.4	8, 9
Fe ₃ O ₄ (5.18)	7	48.9±1.0	0.08±0.03, 39±5	8, 15
ZrO ₂ (5.68)	5.5±2.7	34.8±2.5 35.45	1.3±0.9, 3.6±1.7	8, 9, 16
			1.97 6.51	
MgO (3.65)	10.75	34.68	2.30, 16.44	8, 16
Y ₂ O ₃ (5.01)	9.5±1.6	34.95	1.87, 9.24	8, 16
WO ₃ (7.16)	0.5	60*	^a	17, 18
SnO ₂ (6.95)	4.8±0.8	31.1±3.2	2.9±1.2, 8.5±2.5	9, 12
NiO (6.67)	10.6±0.7	60*	^a	12
Cr ₂ O ₃ (5.22)	7.2±1.0	38.5	0.740, 9.70	12, 19
CoO (6.44)	9.9±1.6?	0.21 45 239	^a	18, 20
La ₂ O ₃ (6.51)	10	60?*	^a	12

Au (19.3)	-	42.1	0.160, 5.64	19
ZnS (4.09)	2	48.9	0.0, 38.0	13, 21
fullerene C60 (1.72)	1	40.6±5.8	1.0±1.7, 5.9±8.0	22, 23
Polystyrene (C8H8)n (1.05)	-	37.54	0.57, 5.27	1

Table SI2.1: Physicochemical properties for various NMs and polymorphs. Points of zero charge, $pH_{p.z.c.}$ and surface tension components γ_c^{LW} , γ_c^+ and γ_c^- were used as input parameters in this modelling study. ^aWhen no data could be found in literature, γ_c^+ and γ_c^- were set equal to the values for NPs with comparable hydrophobicity.

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SI3. Parameters for adsorption

The NP number concentration in the adsorbent layer was determined via the expression $\frac{dN_a}{dt} = kD(t)[N_b - N_a]$. For the determination of the time-dependent diffusion constants D the Stokes-Einstein relation was used: $D(t) = \frac{k_B T}{6\pi\eta R_H(t)}$, where R_H represents the hydrodynamic radius as determined via agglomeration kinetics. Employing a general and simplistic case, the rate-limiting factor k for transport across a diffuse double layer was estimated via $k = \left(\int_{h=h(V_{tot}^m=0)}^{h=\kappa} \left(1 + \frac{r}{h}\right) \exp\left(\frac{V_{tot}^m}{k_B T}\right) - 1 dh \right)^{-1}$ for an energetic barrier with the shape of a truncated parabola [1, 2]. V_{tot}^m is the energy profile constructed by the summation of Van de Waals, electrostatic and acid-base energies between the NP and the membrane (in sphere-plate geometry): $V_{tot}^m = V_{vdW}^m + V_E^m + V_{AB}^m$. These expressions are analogous to the expressions in section SI1 and are given by [3, 4]:

$$(1) \quad V_{vdW}^m(h) = -\frac{\pi h_0^2 \Delta G_m^{LW} r}{12h(1 + 14h/\lambda_c)}$$

$$(2) \quad V_e^m(h) = \frac{\varepsilon \varepsilon_0 (k_B T)^2 a}{4e^2} \left[2y^d y_m^d \ln \left(\frac{1 + \exp(-\kappa h)}{1 - \exp(-\kappa h)} \right) + \left((y^d)^2 + (y_m^d)^2 \right) \ln(1 - \exp(-2\kappa h)) \right]$$

$$(3) \quad V_{AB}^m(h) = \pi r \lambda \Delta G_m^{AB} \exp\left(\frac{h_0 - h}{\lambda}\right)$$

Where the Lifshitz-van de Waals and Lewis acid-base interaction energies were calculated from $\Delta G_{LW}^m = 2 \left(\sqrt{\gamma_l^{LW}} - \sqrt{\gamma_m^{LW}} \right) \left(\sqrt{\gamma^{LW}} - \sqrt{\gamma_l^{LW}} \right)$ and $\Delta G_{AB}^m = 2\sqrt{\gamma_l^+}(\sqrt{\gamma_m^-} + \sqrt{\gamma^-} - \sqrt{\gamma_l^-}) + 2\sqrt{\gamma_l^-}(\sqrt{\gamma_m^+} + \sqrt{\gamma^+} - \sqrt{\gamma_l^+}) - 2(\sqrt{\gamma_m^+ \gamma^-} + \sqrt{\gamma_m^- \gamma^+})$, where $\gamma_m^{LW} = 30 \text{ mJ/m}^2$; $\gamma_m^+ = 0.3 \text{ mJ/m}^2$; $\gamma_m^- = 30$

mJ/m² are the surface tension components of the model membrane, as are common for many types of biological materials [5, 6]. The reduced potential at the Stern layer of the membrane y_m^d and corresponding potential in the diffuse layer were determined analogously to NPs as described in SI1. We used -40 mV as the surface potential of the membrane as is common for a range of cell lines and experimental conditions. Here, we disregarded OM steric and bridging interactions because we did not consider the OM-coating of the negatively charged model membrane.

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SI4. Parameters for permeation

We used the following formula to estimate the permeability $\frac{dN_p}{dt}$ of the model membranes for NPs and agglomerates as function of permeate size and lipophilicity:

$$(1, 2) \quad \frac{dN_p}{dt} = \frac{D_m K}{V(t)^s \delta_m} [N_a - N_p] \quad \text{with } K = \exp\left(-\frac{\Delta G_{w \rightarrow m}}{k_B T}\right)$$

Where D_m is the diffusivity of the membrane, $2.0 \times 10^{-27} \text{ ms}^{-1}$; $V(t)$ is the time-dependent permeate hydrodynamic volume, as approximated via $V(t) = \frac{4}{3} \pi R_H(t)^3$; N_a is the particle number concentration in the adsorbent layer and δ_m is the thickness of the membrane, taken to be 6.8 nm. From literature [2, 3] size scaling factors s for hydrophobic and semi-hydrophobic NP agglomerates could be derived: 0.8 for fullerene agglomerates and 0.1-1.1 for agglomerates with variable hydrophobicity (relative hydrophobicity $H = 0.56-0.75$). These values were in agreement with scaling factors for organic molecules. Consequently, we set the scaling factor s constant at 0.8.

The partition coefficient K was computed from the free energy of transfer from water to the membrane region $\Delta G_{w \rightarrow m}$. From contact angle measurements (table SI2.1) it was observed that hydrophilicity was similar for a range of metal oxides. For this reason we extrapolated the value for K , as found to be $\sim 1 \times 10^5$ for fullerenol and silica (see table SI5.1) to all hydrophilic NPs. As a better indicator for relative lipophilicity, values for $\Delta G_{w \rightarrow m}$ were standardized to accompany the effect of NP size via its relationship with surface area: $A = 4\pi \left(\frac{d}{2}\right)^2$ (table SI5.1). To the opinion of the authors this standardization without including bilayer curving energy is justified because of the small size of the NP relative to bilayer thickness.

	NP diameter d (nm)	Membrane constituent	$\Delta G_{w \rightarrow m}$ ($k_B T$)	$\Delta G_{w \rightarrow m}$ (J/m ²)	Log(K)	reference
Au	2.0	DMPC	-1.8×10^2	5.9×10^{-2}	78	4
(C ₈ H ₈) _n	1.3	DPPC	-1.5×10^2	1.1×10^{-1}	63	5
C ₆₀	~1.0	DPPC	-3.3×10^1	$\sim 4.3 \times 10^{-2}$	14	6
C ₆₀ (OH) ₂₀	~1.0	DPPC	-1.2×10^1	$\sim 4.0 \times 10^{-2}$	5.2	7
(SiO _{1.5}) ₈	~0.6	DPPC	-1.1×10^1	$\sim 1.6 \times 10^{-2}$	4.8	6

Table S15.1: Energies of transfer from water to membrane and corresponding theoretical partitioning ratios.

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SI5. Characterization of OM and OM adsorption

NOM in most natural waters consists for about 40% fulvic acids (FA), 10% humic acids (HA), 10% polysaccharides (PS) and 40% low molecular weight acids (LMWA), fig. S7.1 [1-4]. Upon release of a NP suspension in a body of natural water rapid organic matter adsorption is often observed [5-7]; it is assumed that NOM characteristics can be related to the adsorption kinetics [8, 9]. Adsorption mechanisms have been extensively debated: Belessi et al. [10] proposed that limiting adsorption kinetics may be due to electrostatic interaction between deprotonated carboxylic acid and phenolic acid groups and the NP surface. Additionally, hydrophobic binding forces have shown to depend on the number of hydrophobic derivatives in the NOM and its structural conformation. Apart from ligand exchange interactions and hydrophobic forces that may contribute to about 30% and 10% of the NOM binding forces, multivalent cation bridging (40%) and Van de Waals forces (20%) have shown to affect the sorbate-sorbent binding for natural clays [11]. Moreover, specific binding mechanisms, i.e. thiol-bridging for gold, may be (de-)activated by the application of specific ionic strength or pH regimes, thereby causing inter-particle variations in adsorption affinities.

Fig. SI7.1. General composition (a) and concentrations (b) of NOM in natural aqueous matrices. After Thurman, 1985 [1]. HAS are commonly the largest natural organic molecules in natural waters (2-6 nm), FAs are smaller (0.5-2 nm). FAs normally contain a higher number of carboxylic and phenolic acid groups (6-13 meq/g) than HAs (1-9 meq/g) [12-14]. Most natural organic molecules show charging, dependent of environmental pH [15-17]. Commonly the larger the organic matter species, the larger the fraction of hydrophobic derivatives in the NOM substance.

Despite the large amount of factors and interrelated processes influencing NOM adsorption, general kinetic models have been used successfully. Following simple first order kinetics and monolayer limited random sequential adsorption (RSA), we used a Langmuir isotherm to estimate the fractional coverage of NPs [2, 18, 23]:

$$(1) \quad \frac{\Gamma}{\Gamma_0} = \frac{k_L C_{OM}}{1 + k_L C_{OM}}$$

Where C_{OM} is the OM concentration in the bulk medium. For our modeling purpose we approximated the characteristic Langmuir adsorption affinity k_L to be constant as it is relatively unaffected by the origin of the OM, i.e. proteins, natural organic acids or synthetic polymers, or experimental conditions IS , pH and particle sizes r (table SI7.1).

Substrate	k_L	OM species	Reference
Silica, SiO_2	3×10^{-3}	BSA, HA	20, 21
Alumina, $\gamma-Al_2O_3$	2×10^0	BSA, four HA fractions	20, 22
Hematite, $\alpha-Fe_2O_3$	7×10^{-1}	HA, microcystin-LR	21, 23
Titanium dioxide, TiO_2	4×10^{-2}	BSA, AHA, HA, adipic acid, oxalic acid	20, 24, 25
Zinc oxide, ZnO	3×10^{-1}	SRNOM, BSA	26, 27
Ceria, CeO_2	3×10^{-1}	benzoic acid, phthalic acid, HA, Phosphonated-PEG	28, 29, 30, 31

Table SI7.1: Langmuir adsorption affinities k_L of several metal oxides for organic matter used in the simulation. BSA: Bovine Serum Albumin, HA: Humic Acid, AHA: Aldrich Humic Acid, SRNOM: Suwannee River Natural Organic Matter.

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