Electronic Supplementary Material (ESI) for Nanoscale Advances.

Biomolecular Interactions of Ultrasmall Metallic Nanoparticles and Nanoclusters

Alioscka A. Sousa[1,*], Peter Schuck[2], Sergio A. Hassan[3,*]

[1]Department of Biochemistry, Federal University of São Paulo, São Paulo, SP 04044, Brazil
[2]National Institute of Biomedical Imaging and Bioengineering, Bethesda, MD 20892, USA
[3]BCBB, National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892, USA

Supplemental Information

Nanoparticle interactions in aqueous solutions (Supplement to Section 3)

When two surfaces in an aqueous solution are separated by a distance below ~1-5 nm, depending on the nature of the interacting surfaces, traditional treatments of colloidal suspensions are inadequate. In this case, a more detailed description of the system is needed, as discussed in Section 3. Such systems are not amenable to simplified analytical approximations and are usually dealt with numerically, mainly through computer simulations, where the atomic details at the interfaces and the surface-specific interactions with the solution components can be incorporated. As the particles increase in size, beyond the ultrasmall regime, such specific effects become less dominant and traditional approximations more physically meaningful. In the limit of large particles, beyond the ultrasmall and classical NPs length scale, well-established frameworks have been developed over a century in colloidal, polymer, and surface science. Some fundamental notions have emerged that form the cornerstone of our current understanding of surface adsorption and colloidal stability.

Because of their practical relevance, the multidisciplinary nature of nanomedicine, and the fact that such concepts are often invoked to explain the behavior of classical NPs as well, both in vitro and in vivo, an overview is presented in this section. It may help bridge conceptually the ultrasmall, classical, and colloidal regimes and put in perspective the physical effects that dominate each. An effort is made to draw a parallel with the discussion in Section 3. The main textbooks to get acquainted with the basic concepts and how they are used in practice by experimentalists in a range of disciplines are [1-5] and references therein.

A general expression for the interaction energy between soft particles in an aqueous solution containing proteins and other polymers and smaller species can be written as

$$V = V_{\mathrm{elec}} + V_{\mathrm{vdW}} + V_s + V_c + V_d + V_x \qquad \text{(S1)}$$

where V_{elec} and V_{vdW} are the electrostatic and van der Waals terms, V_s is the contribution from solvent-mediated forces, V_c are the entropic effects of the coating layers, and V_d and V_x are the depletion and crowding effects of the solution components. In turn, each of these terms is divided into different physical contributions, sometimes acting in opposite directions. Analytical expressions for each of these terms can be (and have been) derived for simple geometries and idealized systems. This contrast to the situation for usNP. Thus, the discussion throughout this section is for two interacting spheres and a center-to-center separation r. However, the same qualitative partition holds for other systems (e.g., sphere vs. wall, two flat surfaces opposing each other, and so forth). The similarities and differences with respect to usNPs interacting with proteins or membranes should be kept in mind.

Electrostatics and van der Waals forces

The term V_{elec} arises from the net force on a particle due to the force from the other particle and the forces generated by the ions in the solution (counterions and co-ions) at a given temperature. The spatial distribution of ions, higher between the particles, create a pressure imbalance contributing to the interparticle repulsion and an electrostatic (Coulombic) force contributing to attraction. When the particles are far apart, these forces cancel out; as r decreases, the ion distribution changes, increasing the concentration in the interparticle space and changing the force balance. For like charges, repulsion beats attraction, and the net result is interparticle repulsion, the strength of which increases with decreasing r. These effects are usually visualized in terms of electric double layers and related macroscopic notions (Fig. S1A). If additional forces between the ion are introduced, e.g., dispersion, the balance of forces is affected. Such ion-correlation effects can be significant for multivalent ions, leading to a weakening of the net interparticle repulsion and even attraction.

The term V_{vdW} is determined by the density and excess polarizability of the composing atoms, properties embedded in the Hamaker constant (A), and by the particles' shape and size

(Fig. S1A). If $A > 0$ (the case for metallic NPs in aqueous media), the dispersion term is attractive for all r. For some NP shapes, the functional dependence on the surface-to-surface separations h can be obtained analytically for all h. For two interacting spheres, it is proportional to A/h for small separations; it decays as A/h^6 (London dispersion) at large separations, independently of the particles' shape. Retardation effects, likely inconsequential for usNPs in biological media, weakens the attraction at all r.

The first two terms of Eq. S1 comprise the DLVO theory; the other terms are, by definition, non-DLVO contributions, but this name is usually given to V_s. Depending on the conditions used to solve the Poisson-Boltzmann equation, different forms of the DLVO potential are obtained. If the particles have radii R_i (core plus coating layer) and charges q_i, $V_{\mathrm{DLVO}} \equiv V_{\mathrm{elec}} + V_{\mathrm{vdW}}$ can be written as (Fig. S1A)

$$V_{\mathrm{DLVO}}(r) = \frac{q_1 q_2}{\varepsilon} \frac{e^{\kappa R_1}}{(1+\kappa R_1)} \frac{e^{\kappa R_2}}{(1+\kappa R_2)} \frac{e^{-\kappa r}}{r} - \frac{A}{6} \frac{R_1 R_2}{(R_1+R_2)} \frac{1}{(r-R_1-R_2)} \qquad \text{(S2)}$$

where $1/\kappa$ is the Debye length and ε the static dielectric permittivity of bulk water (the short-range repulsion of the van der Waals is omitted). The electrostatic term emphasizes the parallels with a screened Coulomb potential between point charges. This model has been thoroughly validated and used to explain many experimental observations. Depending on the conditions of the solution and the particles design, the competition between repulsive electrostatics and attractive dispersion can lead to the presence of a primary or a secondary minimum (Fig. S1A) that determine the thermodynamic or kinetic stability of the suspension and the coagulation/flocculation kinetics, the critical electrolyte concentration and its dependence on valency (Schulze-Hardy rule), and other observed trends. However, persistent discrepancies at decreasing size have led to corrections and alternative formalism.

Solvent-mediated forces

Because of its simplicity and track record of success, the DLVO theory is often invoked to explain the behavior of NPs in vitro and in vivo, a questionable assumption for usNPs; a lower size limit for the applicability of Eq. S2, or any of its corrections within the continuum approximation, can be estimated from the effective area of contact (Fig. S1A). For particles

smaller than $\sim$ 15 nm in at least one dimension, the forces contributing to V_S are expected to make significant contributions and even dominate the ultrasmall regime. There is no simple analytical expression for this term that can be used as successfully in practice as V_{DLVO}. A few general statements on water-induced forces (one component of solvent-induced forces) can nonetheless be made that are supported by experiments and simulations. These forces originate in the rearrangement of the hydrogen-bond (HB) network as two particles approach one another. Figure S1B shows the potentials generated by these forces in two very different systems: two small molecules in water and two highly charged usNPs in a cell culture. The physical origin of the forces is the same in both systems despite their contrasting sizes and complexities. As the solutes approach one another, the forces are generally strong and repulsive between hydrophilic surfaces and weaker and attractive between hydrophobic ones. A desolvation barrier is seen in all cases, the height of which reflects the water's resistance to removal from the space between the particles, which requires a disruption of its HB network. Once this resistance is overcome, swift or moderate attraction occurs as the removed water molecules find new favorable interactions in the bulk. The strength and exact dependence of these forces on r depends on many factors but can be modeled phenomenologically as $V_+ \exp(-h/h_+)$ (long-rage) and $-V_- \exp(-h/h_-)$ (short-range), respectively; $V_\pm > 0$ and $h_\pm$ are characteristic decay lengths.

The contributions of ions to V_S is a different matter altogether and, at this point, more difficult to ascertain. Their distributions are determined by the water structure and dynamics, and mainly by the usNP surface chemistry. Understanding and quantifying the forces elicited by the ionic atmosphere and its role in nano-bio interactions require suitable techniques, as discussed in Sections 2 (experiments) and 5 (simulations).

Surface-layer forces

The term V_C contains coating-specific entropic contributions originating in the overlapping and compression of layer molecules as the particles associate (Fig. S1C). These two independent effects play a significant role in the steric stabilization of colloidal solutions against aggregation and deposition. For conceptual clarity, the discussion is for two particles coated with long molecular chains, e.g., grafted or adsorbed polymers. As r decreases and the layers begin to

overlap, there is an increase of osmotic pressure in the overlapping zone due to unfavorable entropy of mixing of the layer molecules. The higher concentration decreases the water chemical potential relative to the bulk, so water is sucked in to dilute the region and re-establish equilibrium, separating the particles (this mixing interaction can become attractive under certain conditions; see Eq. S3 below). As r decreases farther, the layer molecules become more restricted in their movement as they are compressed against the other particle's core. The loss of configurational entropy further contributes to the interparticle repulsion (elastic interaction).

The mixing and elastic effects are easy to conceptualize but challenging to describe theoretically. Thermodynamic arguments and mean-field approximations of polymers provide working analytical expressions under certain solvation conditions and coverage densities. Equations are cumbersome for the general case, but for identical spherical particles, the mixing contribution can be written in its simplest form as

$$V_c = \frac{\gamma KT}{16}\left(\frac{1}{2} - \chi\right)(4R + r)(2R - r)^2\theta + \nu\Gamma KT \exp(-h/\lambda) \qquad \text{(S3)}$$

where R is the radius of the particles (core plus layer thickness δ), χ is the Flory-Huggins interaction parameter, $\gamma > 0$ depends on the molar volume of the layer molecules and their surface density Γ, $\nu > 0$ is a constant, $h = r - 2\delta$ is the core surface-to-surface distance, and λ is a characteristic radius of the layer molecules (the radius of gyration for low Γ). The step function θ defines the mixing regime ($r < 2R$), whereas the second term becomes effective only at the onset of compression ($r < 2R - \delta$; the exponential decay result from the mean-field approximation used).

The conceptual differences with the entropic forces discussed in Section 3 in the context of usNPs are apparent. However, the effects just discussed can start playing a role in NP-NP interactions of classical NPs if the layer molecules are long enough (e.g., polymeric) for the molecules to effectively intermingle or experience a measurable elastic restriction of movement upon association.

Depletion Forces

The term V_d is also entropic and stems from changes in the space accessible to the depletants as the particles approach one another (Fig. S1D). Depletants are constituents of the solution, smaller than the particles, that do not restrict their particles' movement. Their sizes and concentrations determine the range and strength of the attractive interparticle force. At large r, the volume excluded by the particles is maximal; as r decreases below a certain threshold (onset of depletion), the volume of the excluded region between the particle begins to drop, and the configurational entropy of the depletants rises accordingly. The associated thermodynamic reward drives interparticle attraction.

The simplest formulation of depletion potential (Asakura-Oosawa) for two hard particles of radius R in a medium with depletants of radius a and bulk concentration c yields

$$V_d = -\frac{\pi cKT}{12}(2B^3 - 3B^2r + r^3) \qquad \text{(S4)}$$

for $2R < r < B \equiv 2(R + a)$, i.e., between the onset of depletion and close contact; note that cKT is the (osmotic) pressure in the bulk. Several improvements have been made to the model (e.g., soft particles, penetrable depletants) and formalism itself, mainly with liquid-structure theories, including integral equation and density functional methods.

Perhaps because intuition suggests that this effect should be small compared to the other terms in Eq. S1, the theoretical prediction of its existence was ignored for almost thirty years. Today, the role of depletion forces is well established and used to drive and control a variety of physicochemical processes, including phase separation and self-assembly, and the properties of soft materials, e.g., nanocomposite and polymer-colloid mixtures. Besides these practical applications, depletion forces can be used to modulate the interaction between NPs in vitro, e.g., by systematically varying the size ratio (Eq. S4). The role of depletion in cell biology has only recently begun to be recognized as a possible player in the assembly of subcellular structures, such as the cytoskeleton and chromosomes.

Crowding forces

The term V_x contains both entropic and enthalpic contributions. Like the solvation term V_s, some effects can be estimated theoretically using simplified models, but a detailed atom-level treatment is ultimately necessary, especially to understand the associated enthalpic effects. Unlike depletants, crowders are comparable in size to the particles, or larger, and restrict the particles' mobility (Fig. S1E); confinement is an extreme case. The size and shape of both particles and crowders, and the crowders concentration, determine the range and strength of the effective interparticle force.

Like depletion, the entropic component can induce attraction for similar volume-excluded reasons (Fig. S1E). As r decreases, more space is available to the crowders, which increases their configurational entropy resulting in attraction (repulsion, less likely, may happen if the shapes of the crowders or particles are such that the system's entropy decreases upon particle association). The dependence of the attractive force on the shape of particles and crowders affects the binding modes and affinities of complexes, and this can be easily understood on entropic basis (Fig. S1E).

Another effect of crowding is the reduced translational and rotational diffusion of particles, which affect all associated processes. Thus, if a reaction is diffusion-controlled, the reaction rate will decrease; if reaction-controlled, it will increase because the entropic attraction lowers the energy barrier. The result of these opposing effects is difficult to predict, especially if the path to the final complex involves multiple intermediates, as seems to be the case of usNP-protein associations; in this case, each reaction and metastable binding mode may be affected separately.

The configurational entropy of large or immobilized crowders does not change with the interparticle separation, so the only remaining effects of crowding are arrested diffusion and the forces induced by the confining surfaces. These forces become increasingly dominant in the limit of confinement and have entropic and enthalpic components as well. The former stems from a restriction in configurational entropy of the confined particles, including roto-translational degrees of freedom and mobility of the layer molecules, akin to elastic compression. These entropic effects can change association equilibria and protein folding.

The enthalpic contributions are more difficult to conceptualize as they are closely related to the solvent-induced effects V_S. One effect stems from removing large amounts of polar/polarizable medium (water) from the space occupied by the crowders or confining structures. Other properties of interfacial water are more intriguing. For example, it has been shown that two microspheres with like charges that repel each other in an electrolyte solution as predicted by the DLVO theory, begin to attract one another as they become trapped between two planar surfaces; upon further confinement, repulsion is re-established, but with non-DLVO interparticle behavior. Similar effects have been observed close to a single surface.

Systematic experiments on usNP confinement and surface proximity may be more challenging than experiments on crowding but could provide valuable information on their in vivo behaviors. Indeed, the intracellular environment contains many surfaces and crevices, and a large proportion of intracellular water is interfacial (see review in). Because usNP interactions within such regions can differ from those in the bulk, an account of these effects is needed to understand and quantify usNP behavior in the living cell or their interactions with cells. Unless steps are taken to mimic realistic intracellular environments in the test tube, essential features of the usNP-protein interactions can be lost. This problem has been recognized in the study of protein function. For example, enzyme catalytic activity can change one order of magnitude between the infinite dilution limit (a common situation in vitro) and in crowded environments. Proteins enclosed in inverse micelles of varying diameters (~5-25 nm) have been used to study the effects of confinement; surface-proximity effects have been studied with proteins linked by short molecular chains (~1-6 nm) to the surface of liposomes.

1 Israelachvili, J. N. *Intermolecular and Surfaces Forces*. 3rd edn, (Academic Press, 2011).
2 Lyklema, J. in *Fundamentals of Interface and Colloid Science* Vol. 1 (Academic Press, Oxford, UK, 1991).
3 Lyklema, J. in *Fundamentals of Interface and Colloid Science* Vol. 2 (Academic Press, Oxford, UK, 1995).
4 Lyklema, J. in *Fundamentals of Interface and Colloid Science* Vol. 5 (Academic Press, Oxford, UK, 2005).
5 Tadros, T. F. in *Colloids and Interfaces* Vol. 1 (Wiley-VCH, Weinheim, 2007).
6 Hassan, S. A. & Mehler, E. L. in *Comprehensive Biophysics* (ed E. Egelman) (Academic Press, 2012).
7 Hassan, S. A. Computational Study of the Forces Driving Aggregation of Ultrasmall Nanoparticles in Biological Fluids. *ACS Nano* **11**, 4145 (2017).

Figure S1: Nano-bio interactions in complex solutions; some forces become more dominant than others depending on particle size (ultrasmall or classical, or colloids). The examples are for particle-particle interactions: (A) **Electrostatic and van der Waals forces**: Simplest description based on a continuum view of water and point ions. Water is characterized by a constant, spatially homogeneous static dielectric permittivity ε, while ions are represented by point charges spatially distributed according to the PB equation. The ion distributions $\rho_i(\mathbf{r})$ (counterions and co-ions) exert an osmotic force F_p that causes interparticle repulsion and a Coulomb force F_C that induces attraction; the direct interparticle force F_0 is repulsive for like charges. The net result is interparticle repulsion, the strength of which is determined by the temperature T and the ionic strength $I = \sum_i z_i^2 c_i /2$ of the solution through the Debye length $\lambda = 1/\kappa$ in Eq. S2. The ions distribution around each particle is commonly divided into layers, e.g., the Stern layer (with potential V_S), the diffuse layer with effective length λ, and a slipping plane (with zeta-potential V_ζ). When the effective van der Waals interaction, with attractive dispersion force F_v, is added, the resulting DLVO (after Derjaguin-Landau-Verweay-Overbeek) potential generally yields two minima (right, top); their depths (V_c and V_f) and the height (V_b) of the separating barrier can be controlled through T, ε, c_i, and z_i, and the particles design parameters. This continuum view starts to break down at length scales of 1-5 nm, depending on several factors (see text), and alternative formalism has been proposed (e.g., the Sogami-Ise theory). Assuming a threshold L $\sim$ 2 nm (right, bottom) for sphere-wall adsorption, and a departure of no more than 10 % from the continuum (estimated as $\mathcal{A}/4\pi R^2$, with $\mathcal{A}$ being the area of the gray cap; $\delta \sim 5$ Å is the characteristic length-scale of the water structure), a rough estimate suggests that non-DLVO forces become important for NPs smaller than ~15 nm in diameter. The behavior of water and ions in the outer (Ω_o) and inner (Ω_i) interfacial region can differ significantly from that of a structureless medium, and water-water and water-ion hydrogen bonds create effects known as solvent-induced forces. (B) **Solvent-induced forces**: Departures from the DLVO theory arise from several sources; one of them, the forces generated by water and ions that cannot be captured in a continuum representation. A water-structure force F_w is controlled by the water dynamics and by its hydrogen-bond network, and the specific interactions between water and the NPs and between water and the ions. In turn, ions moving in such non-bulk regions are affected, which changes both their osmotic force F_p' and the Coulombic force F_C' acting on the particle. Invoking the DLVO theory presumes $F_w = F_p' = F_C' = 0$. The strength and direction of these forces are difficult to predict. In general, water-induced forces between hydrophilic surfaces (upper panel) generate interparticle repulsion because water molecules are strongly bound to the surfaces, and work is needed to remove them from the interparticle space (vertical arrow) and bring the particles at close contact; by contrast, water-induced forces between hydrophobic surfaces (lower panel) generate interparticle attraction, because work is needed to prevent water from leaving the interparticle space (vertical arrow). This behavior was observed in dynamics simulations in two quite different systems (potentials not drawn to scale; see [6,7] for details): interacting amino acid pairs in pure water (right, top) and interacting usNP pairs in a cell culture (right, bottom). In the first case, amino acids with different

charges/polarity were considered to study the changes in water structure and dynamics responsible for the induced forces. Thus, charge-charge (thick solid line), charge-polar (dotted), polar-polar (dashed), nonpolar-nonpolar (thin solid) interactions were considered to mimic varying degrees of hydrophilicity/hydrophobicity. In the second case, two highly charged AuNPs with diameters of 1.4 nm and 2.5 nm covered with anionic coating layers were considered, showing a more complex behavior but reminiscent of the smaller hydrophilic solutes. (C) **Surface-layer forces**: Mixing interactions (top panel) can be attractive or repulsive depending on the nature of the solvent relative to the coating molecules (cf. Eq. S3; $\chi < 1/2$ for NPs designed for biological applications). The interparticle repulsive force F_m stems from the water's chemical potential (μ'_w) in the overlapping volume, which becomes smaller than that in the bulk (μ_w), forcing water to be sucked in and expand the region. Elastic compression (bottom panel) always induces a repulsive force F_m; it stems from the reduced configurational entropy (S'_c) of the compressed layer molecules relative to the uncompressed ones (S_c). Mixing interaction is thought to be more important for colloidal stabilization. (D) **Depletion forces**: When particles are surrounded by smaller solutes (depletants; in blue), there is a space around each particle (shown in white) inaccessible to the solutes. The size of this excluded volume depends on the depletants' size and is maximal when the particles are far from each other. When the interparticle separation decreases, the excluded regions begin to overlap, freeing space for the depletants to move and increasing their configurational entropy. The resulting interparticle force F_m is thus attractive. The strength of this force depends on the amount of entropy gained in the process, which depends on the concentration of depletants and the particle/depletant size ratio. These parameters can be modified to modulate the effective interparticle interactions in vitro. Depletion forces are always present regardless of the specific particle-depletant or depletant-depletant interactions. Any added potential beyond the idealized hard-sphere potential minimally required to exclude volume further modulates this purely thermodynamic force. The mechanistic view of depletion, as an imbalance of osmotic pressure due to a drop of depletants bombardment in the depleted zone, is more intuitive and can be estimated from dynamics simulations if a short-range particle-depletant repulsion is added (the hard-sphere potential being a mathematical limit). The entropic picture allows a conceptual connection with the entropic effects of crowding. (E) **Crowding forces**: Macromolecular crowding, and the extreme case of confinement, have enthalpic and entropic effects, as well as dynamic and kinetic effects stemming from changes in the solvent viscosity and particle diffusion. The entropic component (depicted here) is also related to volume exclusion. Depending on the size and shape of both particles and crowders, the system's configurational entropy changes with the interparticle separation. If the overall entropy increases when the particles approach one another, attraction will be favored; otherwise, crowding will induce repulsion. In most cases, the entropic component of crowding induces an interparticle attractive force F_χ. This purely thermodynamic force is commonly studied by liquid-structure theories, especially hard-sphere models, to provide a theoretical framework for experimental observations. The dependence of this force on the particles' size and shape affects the product of a reaction. This feature is easy to understand from the configurational entropy perspective and can be formalized using hard-sphere arguments,

including scaled particle theory. Binding modes and affinities may change because of changes in the entropic/enthalpic balance of each mode: e.g., crowding may favor binding to one site, but enthalpy may favor binding to another (right panel). The kinetics of the reaction can be affected as well (see text).

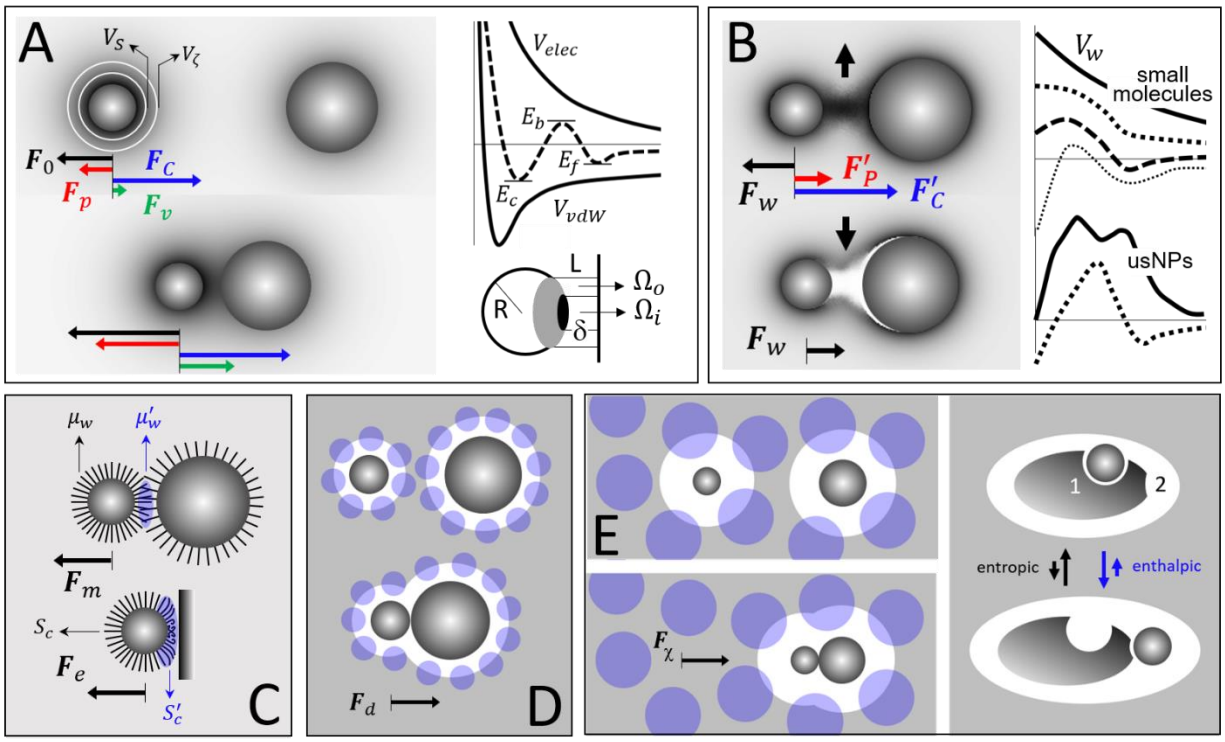

Figure S1