

Chapter 28

Nanothermodynamics Mediates Drug Delivery

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Abstract The efficiency of penetration of nanodrugs through cell membranes imposes further complexity due to nanothermodynamic and entropic potentials at interfaces. Action of nanodrugs is effective after cell membrane penetration. Contrary to diffusion of water diluted common molecular drugs, nanosize imposes an increasing transport complexity at boundaries and interfaces (e.g., cell membrane). Indeed, tiny dimensional systems brought the concept of “nanothermodynamic potential,” which is proportional to the number of nanoentities in a macroscopic

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system, from either the presence of surface and edge effects at the boundaries of nanoentities or the restriction of the translational and rotational degrees of freedom of molecules within them. The core element of nanothermodynamic theory is based on the assumption that the contribution of a nanosize ensemble to the free energy of a macroscopic system has its origin at the excess interaction energy between the nanostructured entities. As the size of a system is increasing, the contribution of the nanothermodynamic potential to the free energy of the system becomes negligible. Furthermore, concentration gradients at boundaries, morphological distribution of nanoentities, and restriction of the translational motion from trapping sites are the source of strong entropic potentials at the interfaces. It is evident therefore that nanothermodynamic and entropic potentials either prevent or allow enhanced concentration very close to interfaces and thus strongly modulate nanoparticle penetration within the intracellular region. In this work, it is shown that nano-sized polynuclear iron (III)-hydroxide in sucrose nanoparticles have a nonuniform concentration around the cell membrane of macrophages *in vivo*, compared to uniform concentration at hydrophobic prototype surfaces. The difference is attributed to the presence of entropic and nanothermodynamic potentials at interfaces.

28.1 Introduction

Nanomedicine is an innovating and a challenging scientific area of interest. It consists of the interdisciplinary combination [1] of nanotechnology and medicine. Nanomedicine includes the development of nanoparticles and techniques for diagnosis and treatment of many diseases at the nanoscale [2–5]. One of its major sectors is nanoparticle drug delivery [1, 3, 6]. Nanotechnology-based drug delivery systems have emerged as promising platforms for targeting and destroying mainly cancer cells [7–9] via photodynamic therapy (PDT) that uses a combination of light sources, photosensitizers such as phthalocyanines, and specific targeting molecules and nano-carriers for localized therapies at the micro/nanoscale. Following light activation of photosensitizers, reactive oxygen species are released and eventually destroy cancer cells [10], after successful intracellular internalization via endocytosis and subsequent accumulation in the endosome/lysosome compartments of the cell. Although that part of nanomedicine is highlighted as one of the most promising field, it faces with two crucial problems. Despite complex design of nano-carriers, such as synthesis of polyion complex micelles via electrostatic interactions between phthalocyanine complexes and various block copolymers [11], uncontrolled nanodrug aggregation and inefficient cell penetration is always present in most nanodrug delivery systems.

Almost all nanoparticles when come in touch with their target start to aggregate [12–15]. For example the aggregate size of SiO₂ nanoparticles increases when placed on colloidal solution. The aggregation of nanoparticles at interfaces

incommodates their entrance through the cellular membrane and thus into the internal part of the cell that is the aim of many drug delivery treatments. Indeed, the cellular energy unit, the mitochondrion, is the target of many nanoparticles synthesized for the treatment of well-known diseases such as Alzheimer's disease, cancer, and metabolic disorders [16, 17]. Hence, the thermodynamic aggregation of nanoparticles at the boundaries of the cells could lead to inefficacy arriving on their target (e.g. organelle, metabolic procedure, etc.) despite that they were designed appropriately.

The second constrain in a nanomedicine project is via to the presence of a large amount of electric charge, as most of the nanoparticles used for drug delivery treatment are highly negatively charged. For example, ZnPc is negatively charged [18, 19] as also some categories of dendrimers that could be used as carriers [20]. At this point, it must be mentioned that the cellular membrane is negatively charged because of its structure [21]. Indeed, the head groups of bilayer phospholipids consist of two anionic lipids, phosphatidylserine (PS) and phosphatidylinositol (PI), that are negatively charged [22]. Consequently, at the cellular interface a repulsive force is generated and then the negatively charged nanoparticles do not even reach the cellular membrane.

On the other hand, Tiny dimensional systems introduce the concept of "nanothermodynamic potential" [23] which is proportional to the number of nanoentities in a macroscopic system, from either the presence of surface and edge effects at the interfaces or the restriction of the translational and rotational degrees of freedom of molecules within them that specify an entropic change. In the case of nanothermodynamic potentials, the core element of the thermodynamic potential is based on the assumption that the contribution of a nanosize ensemble to the free energy of a macroscopic system has its origin at the excess interaction energy between the nanostructured entities. As the size of a system is increasing, the contribution of the nanothermodynamic potential to the free energy of the system becomes negligible. Contrary to stronger interactions, which are independent of system's size, weak physical interactions are dependent on the local morphological features and the various constrains that might restrict the molecular translational motion at the nanoscale. However, the experimental demonstration of nanothermodynamic potentials is difficult to be implemented as sophisticated experimental approaches are required [24].

The nanothermodynamic potential is directly correlated to internal stress, which is responsible for a plurality of morphological structures and shapes at the nano/microscale and therefore it plays a major role in Natural evolution. Indeed, a sound example is a spontaneous generation of complexity in the form of self-assembled structures, the outcome of overstressing during a dynamical transformation of a system attempting to attain a state of *minimum* energy and *maximum* entropy [25].

Particularly, the stress-strain response at interfaces is specified, besides the chemical composition and the specific geometrical confrontations, by both the nature of the molecular forces between the boundaries and the difference of the internal, chemical, entropic, and surface components of the free energy of the interface system [26]. The challenging situation in the case of weak interactions

within a specific system, e.g., hydrogen polar interactions, where the order of magnitude of free energy difference from physisorption in thin layers is in the pJ range and the associated strained field does not exceed a few Ångströms, is the theoretical description of the energy transfer and the experimental verification of the internal energy differences. Any description should take into account both the molecular interactions and the conformational configuration of the combined specific system, as well as any additional constraint that might be important, such as the size and the concentration of nanocomposites. Finally, besides thermodynamic potential, an additional key theme is the interplay between the combinatorial entropic changes, including any possible restriction of the translational symmetry of molecules at the nanoscale.

In this work, nano-sized particles of polynuclear iron (III)-hydroxide in sucrose are deposited on macrophages' cells *in vivo* as also on the surface of hydrophobic silicon wafers and then the size and concentration of the created nano-aggregations on the interfaces between the two substances in the two cases are studied using Atomic Force Microscopy (AFM). The results emphasize the role of thermodynamics in intracellular nanodrug delivery.

28.2 Experimental

Here, THP-1 cell line was used. Cells of that cell line are monocyte-like and they were derived from a 1-year-old boy with leukemia (ATCC[®]TIB-202). Cell culture was performed in an incubator at a 95 % w/w ambient air, 5 % w/w CO₂ gaseous environment, and the temperature is maintained at 37 °C. The medium was RPMI 1640 serum with 2 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, and 10 mM HEPES. Generally, the THP-1 cell line presents a quite quick rate of proliferation and it is suspended. Subsequently, the cells are deposited on silicon wafers coated with Poly-L-Lysine (PLL) to make them adherent. Next, silicon wafers were poured with 5 μ L of solution polynuclear iron (III) hydroxide in sucrose (Venofer[®], 20 mg Fe/mL in 30 % w/v sucrose solution at a pH of about 10.5–11.1). For the AFM measurements, wafers are left to dry and then fixed with 4 % paraformaldehyde (PFA).

An AFM (Bruker di Innova) was used to evaluate the morphology and roughness of the samples that contain polynuclear iron (III) hydroxide in sucrose diluted on macrophages. The AFM images were acquired in ambient conditions, in tapping mode by a phosphorus-(n)-doped silicon cantilever (Bruker, RTESPA) with a nominal spring constant of 40 N/m at 300 kHz resonance frequency and nominal radius of 8 nm. The AFM images were obtained at different scanning areas at a maximum scanning rate of 0.5 Hz and with an image resolution of 512 \times 512 pixels. The AFM was placed on a vibration isolation table and inside an acoustic isolation chamber.

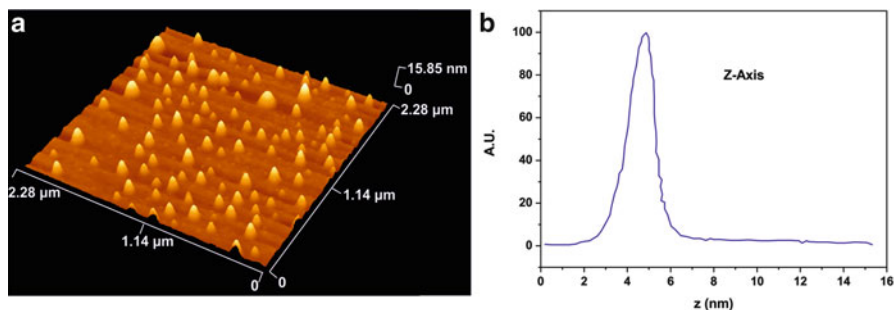
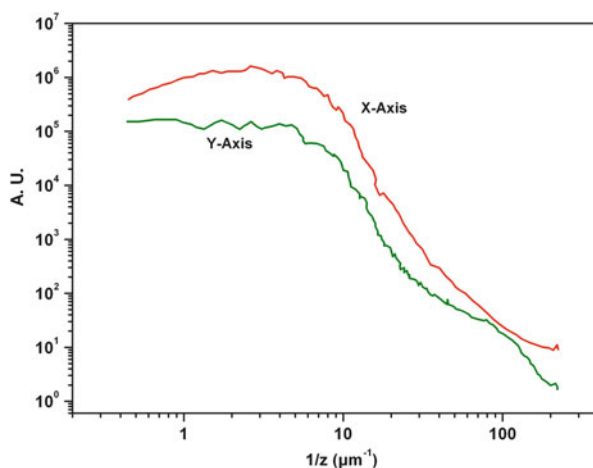


Fig. 28.1 (a) 3-D AFM image of agglomeration of polynuclear iron (III)-hydroxide nanoparticles in sucrose on Si wafers. (b) Size distribution of polynuclear iron (III)-hydroxide nanoparticles in sucrose along the z axis showing a Gaussian distribution

Fig. 28.2 Fast Fourier transform of polynuclear iron (III)-hydroxide particles in sucrose along the x and y axes. The Gaussian and FFT distributions indicate homogeneous thermodynamic potentials at the interface between the Si surface and the nanoparticles



28.3 Results

In the AFM image of Fig. 28.1a spherical nanoparticles of the deposited polynuclear iron (III)-hydroxide are evenly distributed on the Si surface having an average size in the $X - Y$ plane of 70 nm. The size distribution of polynuclear iron (III)-hydroxide nanoparticles in sucrose along the z axis follows the Gaussian distribution as it is shown in the histogram of Fig. 28.1b. It is obvious that there is a uniform spatial distribution of thermodynamic potential gradients between the Si surface and the nanoparticles. Additionally, analyzing with Fast Fourier transform the polynuclear iron (III)-hydroxide particles in sucrose on Si along the x and y axes homogeneous thermodynamic potentials at the interface between the Si surface and the nanoparticles were indicated via the Gaussian and FFT distributions, Fig. 28.2. On the contrary, distribution of nanoparticles among macrophage cells (THP-1 cell line) is uneven, Fig. 28.3a, suggesting the presence of thermodynamical potential at

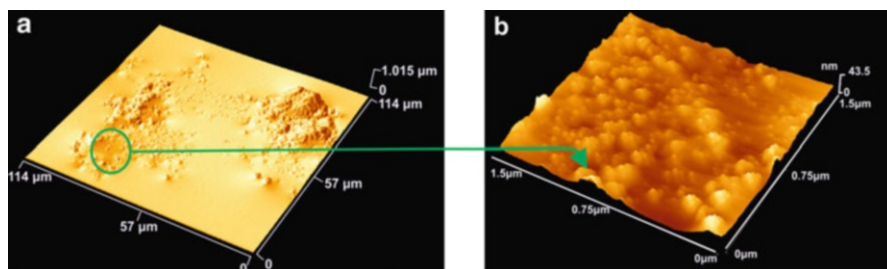


Fig. 28.3 (a) Accumulation of iron (III)-hydroxide nanoparticles in sucrose near the boundaries of macrophage cell in vivo that indicates the presence of nonuniform thermodynamic gradients at the cell's external wall. (b) A detail ($1.5 \times 1.5 \mu\text{m}$) for a low roughness area of Fig. 28.3a on the macrophage cell

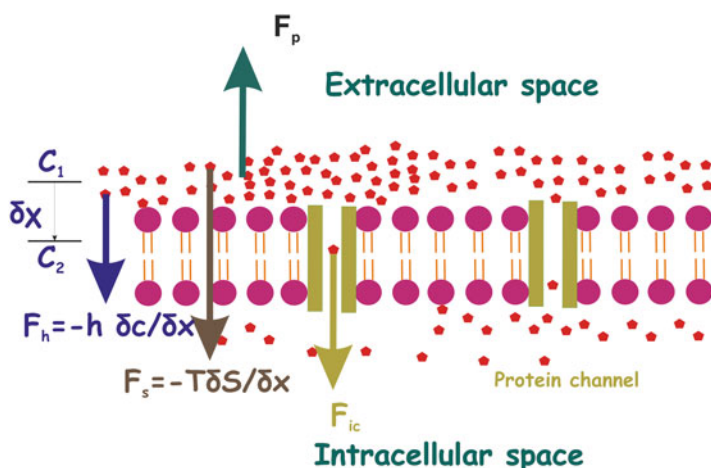


Fig. 28.4 Schematic layout of thermodynamic potential gradients near the interface of a cell. The accumulation of nanoparticles at the interface creates an entropic (F_s) and a nanothermodynamic force (F_h) across the interface. Nanoparticles are then forced inwards. A polar force (F_p) from negatively charged nanoparticles diverts them far away from the membrane

the interfaces. In Fig. 28.3b the same trend is displayed in an area of the macrophage cell even though the roughness of the aggregated nanodrugs is much lower.

Besides the chemical potential gradient near interfaces that forces particle motion from high to low concentration areas, nanoparticles brought the concept of “nanothermodynamic potential,” which is proportional to the number of nanoentities in a macroscopic system or the restriction of the translational and rotational degrees of freedom of molecules, which is the source of a nanothermodynamic entropic potential. The free energy change δG at the interface between the membrane of a cell and the extra cellular medium, Fig. 28.4, under isobaric isothermal conditions is given by the relation:

$$\delta G = \delta U - T\delta(S) - h_i\delta(c_j) - \mu_i\delta(c_i) \quad (28.1)$$

where $\delta(U)$ is the internal energy change from polar interactions, $\delta(S)$ is the entropic energy change from trapping of nanoparticles on the external wall of the cell [27], h_i and c_i are the Hill's potential and the concentration of nano-sized particles, μ_i and c_i are the chemical potential and the concentration of macro-sized particles. The spatial variation of free energy $-\frac{\delta G}{\delta x}$ is the total force F exerted on the nanoparticles at the boundary of membrane and in the absence of macro-sized particles, Fig. 28.4, and is given by the equation,

$$F = -\frac{\delta G}{\delta x} = -\frac{\delta U}{\delta x} + T\frac{\delta S}{\delta x} + h\frac{\delta c}{\delta x} = F_p - F_s - F_h \quad (28.2)$$

where F_p, F_s, F_h are the electrostatic force (repulsive or attractive) between the nanoparticles and the membrane, the entropic force due to immobilization of nanoparticles at the interface, and the nanothermodynamic force due to the concentration of nanoparticles at the interface respectively, Fig. 28.4. The index j is omitted from c and h as Eq. (28.2) contains only terms which are related to nano-sized particles. The entropic and nanothermodynamic forces are directed inwards the membrane wall, while the electrostatic force of both negatively charged nanoparticles and the membrane is directed outwards the wall. Therefore both the entropic and nanothermodynamic potentials mediate nanoparticle intracellular transport within living cells.

Conclusions

Nano-sized particles of polynuclear iron (III)-hydroxide in sucrose have an increased concentration at the boundaries of the cell membrane of macrophages in vivo, compared to a uniform concentration on hydrophobic silicon wafers. The difference is attributed to the presence of entropic and nanothermodynamic potentials at interfaces that enhances aggregation of nanoparticles at interfaces.

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