Phase-State Dependent Silica Nanoparticle Uptake of Giant Unilamellar Vesicles

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Phase-state dependent Silica Nanoparticle Uptake of Giant Unilamellar Vesicles

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Abstract

We quantify endocytosis-like nanoparticle uptake of model membranes as a function of temperature and therefore phase state. As model membranes, we use giant unilamellar vesicles consisting of 1,2-dipentadecanoyl-sn-glycero-3-phosphocholine (15:0 PC). Time-series micrographs of the vesicle shrinkage show uptake rates that are a highly non-linear function of temperature. A global maximum appears close to the main structural phase transition at $T = T_m + 3$ K, and a minor peak at the pretransition $T = T_p = 22$ °C. The quality of the kinetics linear fits reveals a deviation from the linear trend at the vesicle shrinkage peaks. To further elucidate the origin of the shrinkage peak, we performed force spectroscopy on a supported lipid bilayer. The results indicate a collapse of the adhesion energy at the structural phase transition. Further using literature results on the bending modulus as function of temperature and Helfrich's model, this allows us to make qualitative conclusions on the membrane tension as function of temperature.
Introduction

Cell exposure to nanoparticles has been extensively studied for potential medical benefits, like coatings for implant materials [1]–[3], carrier material for drug delivery [4]–[7], clinical trials to image tumors [8], antibacterial agents [9], and nanomedicine [10]–[12]. However, many studies have documented the potential harms, as nanoparticles are widespread in the environment such as in cosmetics, paints, and printer toners [13]–[15]. Undesirable effects have been reported, such as toxic and coagulatory effects [16] and pregnancy complications [17]. In general, these studies can be categorized into three groups. Firstly, applied studies have been done in vivo to obtain a macroscopic picture, such as determining LD_{50} values [18], or quantifying the biodistribution [19]. Secondly, in vitro studies focusing on a microscopic picture have shown no nanoparticle intake at 4 °C, in contrast to intake at 37 °C incubation temperature [20] [21] [22]. According to a machine-learning based metadata study, the main toxicity is connected to material chemistry [23]. Thirdly, focusing on the fundamental side, artificial model membranes give insight into the forces governing the nanoparticle-cell interaction [24]–[26].

Helfrich published a physical model of nanoparticle engulfment based on three major energetic contributions, adhesion energy (E_{adh}) as the driving force, bending energy (\kappa) and tension energy (\sigma) as the opposing forces [27], [28]. Based on the interplay of these forces, three different regimes of nanoparticle-membrane interaction were identified: Repulsion, partial engulfment, and full engulfment. Repulsion appears, when the adhesion energy is below a threshold given by the bending modulus of the membrane and the particle radius. For partial engulfment, the adhesion energy exceeds this lower limit, but does not reach an upper limit \tau_{c,\sigma} = \frac{2\kappa}{E_{adh} - \sigma}, above which full engulfment appears. When fully engulfed, the nanoparticle separates from the membrane by fission of the membrane neck and creates a membrane defect, a transient pore, which heals after its characteristic lifetime. Typical lifetimes vary in the range of t \approx 100 ms [29], but can be largely increased to the order of seconds by applying tension e.g., via micropipette aspiration [30]. Pore lifetimes in cells are longer than in artificial membranes, as commonly reported in the seconds to minutes regime [31], [32].

The amount of work to form a pore is proportional to the isothermal compressibility, which is minimal at the structural phase transition. Thus in this regime pore formation is facilitated [33]. Pores give opportunity to fluid flow, which reduce membrane tension in vesicles. A good estimate whether the bending (\lambda > 1) or the tension (\lambda < 1) is the dominating opposing force is given by the relation \lambda = \frac{2\kappa}{\sigma} [34].

The first key parameter, the bending modulus, for a fluid membrane, e.g. DOPC at room temperature, can be estimated to \kappa = 10^{19} J [35]. However, close to a structural phase transition, the bending modulus, just as all other susceptibilities not constant, instead, it is highly non-linear [36]–[38]. Heimburg calculated the bending modulus and found a dip in the bending modulus as function of temperature in the melting transition [36]. Some experimental studies using DMPC and DPPC GUV, measure the bending modulus in the melting regime and above, but not at lower temperatures [37], [38]. In contrast to Heimburg, they observe an anomaly within T_m < T \leq T_m + 6 K. Within T \leq T_m + 3 K the bending modulus first rises to a prominent maximum, until it decreases until for T \leq T_m + 6 K to a temperature independent limit for both DMPC and DPPC [37]. Interestingly, cholesterol, well known to suppress first order lipid phase transitions, increases the bending modulus and diminishes the prominent maximum [38].

Newer measurements, spanning the whole relevant temperature axis, find for the gel state a bending modulus one order of magnitude higher than in the fluid state [39]. In terms of the bending modulus as function of temperature, this study presents a complete picture. Concerning the limits, they find a
constant \( \kappa = 10^{-18} \) J for DPPC GUV deep in the gel phase, as well as a constant \( \kappa = 10^{-19} \) J in the deep fluid phase. However, at the structural phase transitions, as well as in the ripple phase in-between, the bending energy is non-linear. At the main structural phase transition, as well as at the pretransition, \( \kappa \) has a dip, as calculated by Heimburg. In the ripple phase, which is confined by the main structural phase transition and the pretransition, \( \kappa \) rises linearly from \( \kappa = 10^{-19} \) J to \( \kappa = 10^{-18} \) J. It is likely, that these results are transferable to cells, as a slight variation in membrane composition does not result in a strong change in bending modulus, according to a good agreement between the bending modulus of red blood cell membrane and vesicles [40].

However, structural phase transitions in artificial lipid bilayers are similar to, but not identical to phase transitions of cells. The existence of phase transitions in organisms, like *Escherichia Coli*, lung surfactant, or fibroblast cells has been well known since the 1970’s [41]. A recent study by our group has revealed, that the width of the phase transition in biological membranes is much higher [42]. Consequently, many more cell types might display structural phase transitions than reported in literature, as commonly a narrow window around \( T = 37 \) °C is investigated. Despite the broadness of the transition on the scale of a cell population, the width is expected to be sharper on the single-cell scale, and even sharper for local lipid domains, which has been visualized by Yamamoto et al. [43].

The dependence of the bending modulus is controlled by the hydrophobic part of the lipid bilayer [44]. The quantitative behavior of the bending modulus has been found to be a power law of the packing parameters [45]. As the melting transition consists of structural changes, such as change in area and volume per lipid molecule [46], a change in the bending modulus is expected.

A great simplification and model view for bending is the compression and expansion of outer and inner monolayer, respectively. Expansion and compression result in a change in lateral pressure \( \pi \), which the famous Clausius-Clapeyron relation \( \frac{dT_m}{d\pi} = T_m \frac{\Delta A}{\Delta H} \) directly links to a change in the melting temperature, where \( \Delta H \) is the enthalpy and \( \Delta A \) is the change in area [47]. Consequently, all susceptibilities change in phase, as all thermodynamic susceptibilities are coupled [48], [49].

The second key parameter, membrane tension is characterized by two regimes. In the low-tension regime, the tension rises exponentially with increasing area dilation, at the same time thermal fluctuations become restricted. In the high-tension regime, the dependence is linear, as the area-per-molecule expands directly [50]. Membrane tension has been experimentally proven to exist for example in red blood cells [40] and changes drastically, e.g. in diameter changing blood vessels, like capillaries, or during the adhesion process of any type of molecule or nanoparticles.

The third key parameter, the adhesion, strongly depends on the material of the NP. Here, we use silica, one of the universally used materials for nanoparticles as additive to food (E 551, E 558 Silicon dioxide and Silicate\textsuperscript{TM}), drug delivery vehicles, and others. Regarding the adhesion energy between silica and lipid membranes – several reports in literature exist as summarized in the following paragraphs.

Anderson et al. measured force-distance curves between silica and phospholipid membranes in two different environments [51]. In ultrapure water, they found the repulsion regime of the membrane and silica. In buffered physiological salt concentrations, the silica-membrane interaction is attractive, as repelling charges are shielded by the ions. The adhesion energy of silica to DMPC in the fluid phase here is reported to be in the order of about \( E_{adh} = 0.5 - 1 \) mJ/m\(^2\). However, adhesion energies ranging from \( 10^{-5} \) mJ/m\(^2\) - 1 mJ/m\(^2\) are well-known for different conditions [51], [52].

Adhesion of a lipid bilayer to silica exists in two equilibrium distances [53]. One distance is 2.5 nm separation between the bilayer and the silica substrate, with a water layer in between. In the other distance, the bilayer is closely attached to the silica substrate, resulting in a highly distorted bilayer.
due to substrate roughness. Other distances are instable due to hydration forces, where a distortion of orientation of water molecules is energetically unfavorable. This is in contradiction to a schematic in the study of Anderson et al. [51].

A study of our group regarding the ion concentration switching on and off the adhesion energy, was reported by Wittman et al. [54]. For a supported lipid bilayer in the fluid phase (DOPC) and a micrometer sized silica bead, we found a salt dependent attraction threshold value, at about $c_{crit} = 15$ mM NaCl. At higher salt concentrations, the adhesion energy saturates at about $E_{adh} = 60 \mu J/m^2$, being significantly smaller than the one cited above. The threshold ion concentration of the adhesion energy between silica and lipid membranes obviously should play a role in NP uptake of GUV from model membranes. Indeed, such a threshold value of the salt concentration for the uptake of nanoparticles as a function of particle size has been identified by Strobl et al.: For $d = 60$ nm in diameter particles and DOPC GUV, nanoparticle uptake occurs only above a threshold of about $c_{crit} = 15$ mM. This suggests an all-or-nothing behavior [24]. After all, to the best of our knowledge, the adhesion energy has so far not been measured in the gel state at non-zero salt conditions.

Another important thermodynamic interaction aspect has been reported by us earlier. For attractive nanoparticle-membrane interaction, we found that silica nanoparticle directly shift the melting transition temperature of the wrapping membrane [26]. For the case of a supported lipid bilayer, this has even been reported to asymmetrically affect the two bilayer leaflets, causing an individual leaflet melting [55]. Strobl et al. applied this to direct nanoparticle uptake measurements and expanded this mechanistic model by adding a thermodynamic point of view [25]. Surprisingly, not only nanoparticle-membrane interactions are altered, but membrane-membrane adhesion forces, too. Membrane-membrane adhesion forces include membrane undulation forces, which are tension dependent. During nanoparticle uptake tension rises, as membrane is consumed and water incompressibility keeps the vesicle volume constant. However, pores allow fluid to escape the vesicle and relieve tension. The trade-off of these two processes highly depends on the pore lifetime, which again is strongly dependent on the membrane state and is enlarged close to the main phase transition of the membranes.

Strobl et al. extended the model of Deserno et al. and simulation results identified an additional regime, where nearly unlimited nanoparticle uptake appears. This study also gave a first glimpse experimentally into nanoparticle uptake of gel state vesicles and observed qualitative differences for different nanoparticle sizes. However, there is a lack of systematic studies on experimentally determined nanoparticle uptake as function of the phase state. Here, we focus on variation of the phase state along the temperature axis using 60 nm silica nanoparticles and 15:0PC GUVs.

The structure of the paper is as follows: Firstly, we present a typical vesicle shrinkage kinetic, which we measured for various temperatures and fitted linearly. Secondly, we identify the phase states of the giant unilamellar vesicles via calorimetry. Thirdly, we regard the quality of the linear fits to identify linear and non-linear uptake regimes. This is put under perspective of the main outcome of this study, which is the vesicle shrinkage rate as function of temperature. Finally, we used force spectroscopy to determine the adhesion energy of silica to a supported lipid bilayer as function of temperature.
Results and Discussion

Figure 1a shows the experimental setup to measure shrinkage kinetics of giant unilamellar vesicles after addition of nanoparticles. One well of a 96-well plate served as the experimental chamber, whereas an identical adjacent well was used to measure and adjust the temperature using a thermocouple. Once the temperature was equilibrated, we added the nanoparticle solution, mixed gently, and started recording fluorescence micrographs. The time series in Figure 1c exemplarily shows the shrinkage of a giant unilamellar vesicle with ongoing time. We calculated the normalized surface area shrinkage by assuming a spherical shape of the vesicle, as shown in Figure 1b. We fitted the very first data points linearly to quantify the initial shrinkage rate.

To compare nanoparticle uptake rates and membrane state, it is crucial to measure the excess heat capacity of the used vesicle samples via calorimetry to identify the main phase transition region. Figure 2 shows the measured excess heat capacity $C_p(T)$ of giant unilamellar vesicles (orange line) and multilamellar vesicles (blue line) of the used lipids. The excess heat capacity of giant unilamellar vesicles shows a pretransition at $T_p = 22$ °C and the main transition at $T_m = 34$ °C. The broadness of the peaks are $\text{FWHM}_p = 1$ K and $\text{FWHM}_m = 1.5$ K, respectively. This is very different to the well-known transition behavior of MLVs, which show a more cooperative phase transition. These MLV show a pre and main transition at $T_p = 21.8$ °C and $T_m = 33.9$ °C with a width of $\text{FWHM}_p = 2.3$ K and $\text{FWHM}_m = 0.3$ K, respectively, in accordance with literature [26].
Figure 2 Linear fit quality (blue squares) as a function of temperature and therefore phase state. The phase state is characterized by the excess heat capacity (orange line). Here, two phase transitions can be identified, a pretransition at $T_p = 22 \, ^\circ C$ and the main transition at $T_m = 34 \, ^\circ C$. The insets show respectively three time-series of the nanoparticle uptake process represented by the reduction in vesicle surface area, visualized above as fluorescent micrographs.

Figure 2 furthermore shows the adjusted $R^2$ value of the linear fits to the shrinkage kinetics as function of temperature. By far, most data points are close to 1, implying a good accuracy for the linear fit. The linear trend suggests further shrinkage for longer observation time.

Few adjusted $R^2$ values deviate from 1 in figure 2, implying a worse accuracy of a linear fit. The inset time-series in figure 2 show a non-zero curvature indicating an asymptotic, saturating trend. Here, better fitting quality was obtained by fitting exponential functions. However, due to the already relatively high absolute adjusted $R^2$ value and due to an additional fitting parameter in the exponential function, a comparison is obsolete. Note, that the nanoparticle uptake kinetics with saturating trends are in proximity of the phase transition regions on the temperature axis, indicating a correlation of phase transition and NP uptake.
To gain a deeper understanding of the kinetic process, we present the time evolution of the vesicle area shrinkage rate as function of time for four exemplary temperatures close to the pre and main transition in Figure 3a. The results reveal that no vesicle area shrinkage rate time evolution is perfectly constant, as expected from the slope of a linear fit function. Moreover, the shrinkage rate time evolution at $T = 37\,^\circ C$ is the only one, which reaches a saturation at around 0.2 $\%$ per second, as it is constant over 10 min and having therefore reached a steady state. This contradicts the previously presumed saturating trend suggested by the inset in figure 2 and supports an unlimited nanoparticle uptake for at least moderately increased observation times. However, for all other exemplary temperatures, the vesicle area shrinkage rates decline steadily, possibly collapsing entirely for slightly longer observation time. Figure 3b supports this conclusion, as all shrinkage rates approach 0, except at $T = 37\,^\circ C$, which saturates at a value significantly greater than 0.

Figure 4 shows the vesicle area shrinkage rates as function of temperature (blue squares). The values for vesicle area shrinkage rates range from practically 0 to 1 $\%$ per second. The vesicle area shrinkage rate as function of temperature displays a qualitatively similar shape to the excess heat capacity. The vesicle area shrinkage rates are elevated in proximity to both phase transitions. In both cases, the temperatures, where maximal shrinkage rates appear, are slightly shifted to higher temperature. The local maximal rate close to the pretransition is observed slightly above $T_p$, and the local maximal rate

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**Figure 3** Vesicle surface area shrinkage rate a) kinetic and b) as function of total vesicle area shrinkage for characteristic temperatures.

**Figure 4** Vesicle area shrinkage rate (blue squares) as function of temperature and therefore phase state. The phase state characterization by the excess heat capacity has been underlaid for orientation (orange line).
for the main transition is observed at \( T_m' = T_m + 3 \text{ K} \). Besides the obvious correlation of uptake maxima and transition temperatures, it is interesting, that no direct correlation of the magnitudes of heat capacity and shrinkage rate are observed. Consequently, the vesicle area shrinkage rate here close to \( T_p \) is more sensitive to structural lipid phase transitions than the excess heat capacity. This suggests that even for broad transitions in synthetic lipid mixtures or even broader transitions in biological membranes [42] phase transitions could strongly influence uptake rates.

This shift of a response function maximum – which we observe in vesicle area shrinkage rate – in respect to the membrane phase transition temperature of about \( \Delta T = +3 \text{ K} \) with respect to \( T_m \) has been observed before in the key model quantity bending energy, down to the exact same \( T_{\text{max, bending}} = T_m + 3 \text{ K} \) [37], [39]. However, a high bending modulus impedes nanoparticle uptake, contradicting the experimental data. Consequently, the bending energy maximum cannot be the origin of the vesicle area shrinkage rate elevation in the observed shrinkage regime. Note that the bending modulus exhibits dips locally at the phase transition temperatures [39].

This contradiction can only be resolved by regarding further changes in the physical situation, which is the nanoparticle-membrane interaction. Earlier studies have shown that the chemical potential of the lipid molecules in the membrane is altered when in contact with silica. This interaction shifts the phase transition temperature to \( T_{m'} = T_m - 2.5 \text{ K} \) for 60 nm silica-nanoparticles, being nanoparticle size dependent [26], [56]. However, as the nanoparticle interaction stabilizes the fluid phase, the nanoparticle uptake peaks should be shifted to lower temperatures, ergo this does not resolve the contradiction, yet.

To resolve the contradiction, we conducted atomic force spectroscopy to quantify the silica-membrane adhesion as function of temperature and therefore phase state. Figure 5a shows the experimental setup. We glued a silica microsphere to a cantilever and fabricated a supported lipid bilayer on mica. Due to technical reasons, we used DMPC, which is practically equivalent to 15:0 PC but a one unit longer acyl-chain, shifting its melting transition to \( T_m = 24 \text{ °C} \). Figure 5b shows typical force distance curves. While we observed the characteristic adhesion jumps (green line) during retraction, surprisingly, for many force curves recorded close to \( T_m \), we did not observe any adhesion (pink line). The occurrence of adhesive retractions and non-adhesive retractions is shown in figure 5c. The heatmap shows the adhesion force as function of temperature, whereas the frequency of adhesive events is color-coded. Clearly, the frequency of adhesive events is a function of temperature, and therefore a function of phase state. This finding is supported by the conductance of the measurements. The experiment was always conducted in one sitting, using the same bilayer specimen at the same positions, switching back and forth between temperatures inside the phase transition temperature regime and further away. Thus, we concluded, that close to the bilayers structural phase transition temperature, the adhesivity collapses.

Another quantity strongly implicating the adhesion collapsing at the phase transition is the adhesion force, presented in figure 5d. Whereas the absolute values of the adhesion energy being around \( E_{\text{adh}} = 0.5 \text{ mJ/m}^2 \), the adhesion energy of relatively few measured adhesive events range close to \( E_{\text{adh}} = 0.1 \text{ mJ/m}^2 \). However, for a distribution of the adhesion energy around zero, the subjective curve filtering process presumably introduces a bias into this evaluation, as adhesive very close to zero are filtered out. For higher temperatures in the fluid phase, the adhesion energy recovers quickly to similar values as the gel phase.
In summary, the interaction of three key parameters govern nanoparticle uptake: adhesion as the driving force, bending and tension as impeding forces. This results in peaks in uptake at $T_p$ and $T_{m'} = T_m + 3$ K for the absolute vesicle area shrinkage.

Our results on the adhesion energy complement the knowledge about this interaction. First, the phase state dependent bending energy is well-known from literature and exhibits a dip at both the pretransition and the main transition, subsequently followed by local maxima at $T_p$ and $T_{m'} = T_m + 3$ K. Second, the adhesion energy is roughly comparable below and above the structural phase transition and actually collapses at the phase transition temperature. Therefore, membrane tension is left as the only unknown variable and using Helfrich’s model, it has to be non-linear to fit the data. More concrete, the tension must exhibit an antisymmetric shape to the bending energy, but with a dip spanning over the peak regions of our vesicle area shrinkage data.

Regarding the time evolution of membrane tension, it is strongly dependent on the excess area of the vesicle, the uptake amount and kinetics, as well as the relaxation of tension via volume flux through pores [57]. At the start of our experiments, we can assume zero membrane tension. With increasing uptake, this tension increases as shown earlier [25]. Water can only quickly escape through membrane pores. In addition, it is well known that close to the main transition the area compressibility is maximal and the pore forming energy is known to collapse at the phase transition region, due to the inverse proportionality to the isothermal compressibility [33], resulting in decreased membrane tension.
around $T_m$. Moreover, for a constant decrease of membrane area due to engulfment of NP, while the vesicle volume is roughly constant, the rise in tension is proportional to the area compressibility. But in turn, the main transition is sensitive to membrane tension [58], [59]. Thus, a dip of the membrane tension around the phase transition region is plausible, but directly and fully entangling the role of membrane tension in the here shown state dependent NP uptake is a separate extensive study itself.

**Conclusion**

In summary, we here showed a strong non-linear relation between NP uptake rates and temperature for synthetic phospholipid membranes close to the main transition. At temperatures slightly above the pre and main transition uptake maxima have been observed. This is understood best by interpreting the mechanical membrane properties as state dependent, non-linear properties. Thus, membrane properties have to be considered as thermodynamic properties that depend on the membrane state. As in turn, the membrane state close to a main transition can be significantly altered due to direct particle membrane interaction or indirect particle membrane interaction via induced membrane tension, highly non-linear uptake rates can appear. In addition, these structural transitions are highly sensitive towards changes of the environmental conditions like temperature, shear stress, pH and other ion concentrations. In turn, in pathophysiological scenarios like inflammation such pronounced non-linear changes in membrane properties could result in pronounced changes in NP uptake. Moreover, it would be highly interesting to pursue the question whether these phenomena are conserved for biological cell membranes as well or if the non-linear variations of the membrane properties appear over a much wider temperature range and thus in the physiological range appear linear again.
Experimental

Nanoparticles

Monodisperse, non-porous silica nanoparticles with silanol groups on the nanoparticles surface were purchased from nanoComposix (Prague, Czech Republic). The nanoparticles were diluted to a final surface area concentration of \( C = C \frac{4\pi r^2}{4} = 1 \text{ m}^2/l \).

Giant unilamellar vesicles

1,2-dipentadecanoyl-sn-glycero-3-phosphocholine (15:0 PC) dissolved in chloroform was purchased from Avanti Polar Lipids (Alabaster, Alabama, USA). Membrane fluorescent marker 3,3'-ditetradecyloxacarbocyanine (Texas Red™ DHPE) was purchased (Thermo Fisher Scientific Inc., USA). Buffer ingredients Na2HPO4, NaH2PO4, sucrose, and D-(+)-glucose monohydrate was purchased from Merck (Darmstadt, Germany). For all aqueous solutions, ultrapure water (pure Aqua, Germany) with a specific resistance \( \rho \geq 18 \text{ M} \Omega \text{cm} \) was used.

Giant unilamellar vesicles were produced by electroformation technique [60]. Briefly, lipids were mixed with \( c = 0.05 \text{ mol}\% \) of the fluorescent marker DiOC14 and diluted in chloroform to a total concentration of \( c_m = 10 \text{ mg/ml} \). The lipid solution was spread onto fluorine tin oxide (FTO)-coated glass slides. A thin lipid film formed after solvent evaporation through vacuum extraction. Using the lipid film on the FTO-coated glass slides, a chamber was built, using Teflon spacers. Two holes in in the spacers enabled filling the chamber with vesicle interior medium. Finally, a rectangular AC voltage was applied in two steps, first for \( t_1 = 15 \text{ min} \) (\( f = 10 \text{ Hz}, E_{\text{eff}} = 0.6 \text{ V/mm} \)), then for \( t_2 = 4 \text{ h} \) (\( f = 10 \text{ Hz}, E_{\text{eff}} = 2.4 \text{ V/mm} \)) at 10 Kelvin above phase transition temperature. The GUV were transitioned into vials and stored for weeks in the fridge without loss of quality.

At the stage of GUV preparation, the vesicle interior and exterior medium consisted of saccharose solution. The pH was adjusted to \( \text{pH} = 7 \). Osmolarity was measured (Osmomat 030, Gonotec GmbH, Germany) and eventually adjusted to \( c_n = 150 \text{ mM} \).

Nanoparticle uptake setup

A transparent flat bottom polystyrene 96 well plate was used to prevent unspecific adhesion of the GUV and the substrate. The well plate was mounted on a fluorescence microscope (Axiovert 200M, Carl Zeiss Microscopy, Jena, Germany).

To dilute the GUV and to change the exterior medium, \( V = 2 \mu l \) GUV solution were pipetted into a well filled with \( V = 70 \mu l \) consisting of \( c = 56 \text{ mM NaCl, 20 mM glucose, 18 mM PBS buffer and deionized water} \). GUV are gently mixed and they sediment due to a higher density of the incapsulated sucrose solution.

The temperature was controlled using a micro controller and a Peltier element. The temperature was measured and monitored in an equivalent adjacent well by a thermocouple. Preheating was applied for 30 min before each experiment to ensure an equilibrated temperature in the well plate.

The following addition of nanoparticles via gentle pipette aspiration mixing initiated the kinetic uptake process. To quantify for the uptake, we measured the vesicle surface area through fluorescent imaging with time steps \( t_{\text{step}} = 5 \text{ min} \) over an overall time period of \( t_{\text{total}} = 30 \text{ min} \) in case of saturation and \( t_{\text{total}} = 2 \text{ h} \) in case of no saturation. For each single independent experiment, the shrinkage of a single GUV is recorded.

Data evaluation
Using an elliptical fit and the isotropic assumption, we determined the surface area for each vesicle at every time step. Preliminary work has shown a transition from linear to exponential decay in shrinkage as function of time for the present nanoparticle size and surface area concentration [24]. We fitted the data linearly but added the adjusted \( r^2 \) data to show deviation from linearity. The main parameters are absolute shrinkage, which is a function of temperature.

Once the nanoparticles are added to the vesicles, they start interacting. The initial interaction is attractive and leads endocytosis – a full wrapping of the nanoparticle with membrane and complete engulfment into the vesicle. As a consequence, the vesicle's diameter shrinks over time, as can be seen in fig. 1.

**Differential Scanning Calorimetry**

All liquids were degassed prior to the calorimetry (MicroCal VP-DSC, MicroCal Inc., now Malvern Panalytical Ltd, UK) and adjusted to \( p = 15 \) psi pressure. A scan rate \( \beta = 15 \) K/h was chosen to ensure a quasi-static process. From three subsequent up and down cycles, the last up scan was evaluated. The baseline was determined and subtracted as described previously [61]. Concentrated GUV stock solution was used as the DSC sample, for the reference, pure saccharose solution was used.

**Supported Lipid Bilayer Fabrication**

To fabricate the supported lipid bilayer, the vesicle fusion technique was used. 95% DMPC or 15:0 PC and 5% DOPC with a concentration of 25 mg/ml, dissolved in chloroform, was purchased from Avanti Polar Lipids and used without further purification. Out of the total lipid mass of \( m = 1 \) mg, 0.05% were fluorescence dye (DIOC14(3), Biotium Inc., USA), for a quality control using continuous bleaching. The lipid solution was dried under a gentle stream of \( N_2 \), after a subsequent thorough elimination of the organic solvent in a desiccator overnight. The lipid cake was hydrated using \( V = 1 \) ml HEPES buffer containing \( C_{\text{HEPES}} = 15 \) mM, \( C_{\text{NaCl}} = 75 \) mM. To form the precursor multilamellar vesicles, the lipid cake was sonicated for \( t = 30 \) min at 20 K above the lipid phase transition temperature. Subsequently, small unilamellar vesicles were fabricated, by further, more powerful sonication of the multilamellar vesicles for another \( t = 30 \) min using a VialTweeter (VialTweeter UIS250v, Hielscher Ultrasonics GmbH, Germany) at power \( p = 75\% \) and cycle duration \( t = 75\% \).

Freshly cleaved mica is incubated with the small unilamellar vesicles suspension overnight at 20 K above the lipids phase temperature. Subsequently, after thorough rinsing with ionized buffer solution to eliminate loosely adhered vesicles, the supported lipid bilayer is checked for the characteristic bleaching at the rim using continuous bleaching.

**Atomic Force Microscopy Setup**

The supported lipid bilayer was inserted into a BioCell™ (JPK BioAFM Business, Germany) and mounted on the optical microscope (ZEISS Axiovert 200, Carl Zeiss Microscopy Deutschland GmbH, Germany) and the atomic force microscope (JPK NanoWizard II, JPK BioAFM Business, Germany). We used MLCT-O10 cantilevers and glued a 7,38 \( \mu \)m sized silica bead (microParticles GmbH, Germany) to the cantilever following a JPK technical protocol [62].

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