Molecular orientation at biological interfaces: Water and lipids studied through surface-specific vibrational spectroscopy

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In this chapter we remain at the bio-nanointerface, now focusing on carbon nanotubes, and on the slow dynamics of their interaction with a monolayer. An important property of Single-Wall Carbon Nanotubes (SWNTs) is their ability to transgress the cell membrane. Most studies agree that functionalized nanotubes can be internalized into a cell by receptor-mediated endocytosis (RME, i.e. specific uptake) without damaging the membrane, while pristine nanotubes are likely to be cytotoxic. Although specific nanotube uptake has been studied extensively in in vivo systems, and simulations have covered the nonspecific case, a gap exists in our experimental knowledge of nonspecific interactions and the molecular processes involved. By means of a combination of tensiometry and surface-specific vibrational spectroscopy we elucidate the nonspecific interaction between a Langmuir lipid monolayer and DNA-covered carbon nanotubes and identify two separate stages in the interaction dynamics. In the first stage, the nanotubes bind to the lipid monolayer through electrostatic, nonspecific interactions mediated by the DNA molecules, which changes the interfacial water structure. The second stage comprises nanotubes being taken up into the hydrophobic region of the monolayer, reflected in changes in the lipid alkyl chain organization. A binding and dissociation model previously developed for RME describes our data well, revealing that the time constants of this nonspecific process are remarkably similar to those observed for the RME case. This similarity implies that cell-level observations are not necessarily sufficient to distinguish between different cell entry mechanisms, as is commonly assumed. Despite the apparently strong SWNT-monolayer interaction, vesicle leakage experiments reveal no rupture of lipid membranes of vesicles in the presence of SWNTs.
7.1 Introduction

Single-Wall Carbon Nanotubes (SWNTs) have been proposed as suitable candidates for transport of genetic material and drugs into cells in gene- and cancer therapy applications [62, 150, 151]. SWNTs are able to perform this task very efficiently because of their large surface area to which cargo molecules can be bonded noncovalently. The applicability of these functionalized SWNTs depends critically on their ability to cross the barrier formed by the cell membrane. As such, the interaction between SWNTs and the phospholipids and proteins that comprise the cell membrane is of great importance. Pristine nanotubes may aggregate and damage the cell membrane [149, 152], while functionalized SWNTs have been reported to enter cells spontaneously [153–155] and without any cytotoxic effects [119, 156, 157]. Particularly, for DNA-wrapped SWNTs, in vivo studies have shown the cell entry mechanism to be receptor-mediated endocytosis (RME) [158, 159]. Jin et al. have developed a model that includes the influence of particle size and shape on the endocytosis rate that accurately fits the observations from single particle tracking fluorescence microscopy [119]. Nonspecific interactions of SWNTs with the cell membrane, on the other hand, have been covered mostly by theory and simulations. SWNTs were shown to spontaneously insert into the hydrophobic alkyl chain region of a phospholipid bilayer [160], and this interaction, driven by unspecific van der Waals, steric, electrostatic and acid–base forces, was shown to be equal in strength to specific interactions [161]. Because specific and unspecific interaction strengths are comparable, it is important to recognize the role of processes other than RME, i.e. nonspecific interactions, in the SWNT-membrane interaction. While it may not significantly contribute to internalization of the SWNTs into cells, the nonspecific component could well play a significant role in SWNT cytotoxicity. In this study, we aim to experimentally address this issue by analyzing the nonspecific interaction between DNA-wrapped SWNTs and a single-lipid Langmuir monolayer acting as a model membrane. Using vibrational sum-frequency generation (SFG) spectroscopy, a powerful, non-invasive optical technique that is able to probe interfaces specifically, with a sensitivity to only a few molecular layers [25, 122, 162, 163], we characterized the dynamical behavior of the interfacial water and the lipids upon binding of SWNTs. Tensiometry provides additional information on the thermodynamics. For quantitative analysis, a binding-dissociation model was applied to describe the dynamics of the interaction. Finally, a membrane leakage experiment was performed to see if the strong observed interaction results in membrane rupture.

7.2 Methods

DNA-Wrapped SWNTs. Density gradient ultracentrifugation purified and (6,5) enriched DNA-SWNTs with an average length of 250 nm were used. (GT)$_{16}$ ssDNA is bound noncovalently to the SWNT surface [164]. A 20% surface coverage of ssDNA on the SWNTs and adsorption of ~3 ssDNA molecules
per tube are estimated. The energetically highly favorable adsorption of ss-DNA to the SWNT surface prevents aggregation of SWNTs, while minimizing the presence of free ssDNA in the SWNT sample [165]. DNA-SWNTs were solvated in PBS buffer.

**PBS Buffer.** Phosphate buffer was prepared by dissolving 0.12 g NaH$_2$PO$_4$ in 100 mL D$_2$O (Cambridge Isotope Laboratories, Inc, 99.93% purity). NaOH was added to a pH of 7.4.

**Tensiometry.** In tensiometry, the two-dimensional pressure along the circumference of a needle tip is measured. This surface pressure is indicative of the surface free energy, which changes upon the binding or insertion of new molecules to or into an existing monolayer [129, 130]. Tensiometry measurements were performed using the Wilhelmy plate method (Kibron Inc.).

**SFG Setup.** To obtain molecular-level insight into the interaction, we employ vibrational sum-frequency generation (SFG) spectroscopy. SFG spectroscopy is a technique that allows one to record the orientation and internal, conformational order of surface molecules [14]. SFG relies on the resonant enhancement of frequency mixing between visible (VIS) and infrared (IR) laser pulses when the infrared pulse is resonant with a surface vibration. Because it is an even-order ($\chi^{(2)}$) non-linear optical process, SFG is bulk-forbidden. The technique thus provides the vibrational spectrum of specifically the surface molecules with sensitivity generally unobtainable from conventional linear spectroscopies. As such, it has been used extensively to investigate bio-mimetic systems [25, 122, 133, 162, 163]. The setup and procedure to analyze the data are described in detail in previous publications [53, 54]. In brief, for our SFG experiments, we used a regeneratively amplified Ti:sapphire system (Legend, Coherent, Inc.) to produce pulses of 120 fs FWHM centered at 800 nm, with a 1 kHz repetition rate and a power of 2.5 W. 1 W of this output power was used to pump a tunable optical parametric amplifier system (TOPAS, Lights Conversion Inc.) to generate pulses at mid-infrared (IR) wavelengths. 0.5 W of the total 800 nm output was frequency-narrowed with an 25 cm$^{-1}$ etalon and used as the upconverting (VIS) beam in the SFG experiment. Polarizers in the VIS and IR beam path were used to ensure detection of SF spectra in the SSP polarization (SF S polarized, VIS S polarized, and IR P polarized). The SSP polarization scheme produces the most intense SF response for the studied system.

$\lambda/2$ plates placed before the polarizers were used to adjust the power of the VIS and IR beams in order to prevent heating of the sample. Experiments were performed at a VIS power of 10 $\mu$J per pulse and an IR power of 3.0-4.5 $\mu$J. The SF beam is focused onto a spectrograph (Princeton Instruments, Acton SP 2300) which is coupled to an Electron Multiplied Charge Coupled Device (EMCCD, Andor Technologies) for detection.

**SFG Procedure.** A home-built, Teflon coated 20 mL aluminum trough was used as a sample holder during the SFG experiments. A DPTAP monolayer
was prepared on the buffer subphase by dropcasting a solution of DPTAP in chloroform up to a surface pressure of 19 mN/m. DNA-wrapped SWNTs in PBS buffer were injected underneath the DPTAP monolayer through a hole in the side of the trough to prevent disruption of the lipids. The substrate was stirred by a magnetic microstir during and after injection. SFG spectra were recorded before and at specific times after SWNT injection.

The frequency region of the SFG spectra is restricted by the bandwidth of the IR-pulse. In our setup the IR-pulse has a FWHM of $\sim 200 \text{ cm}^{-1}$. Since the region of interest ($\sim 2100-3100 \text{ cm}^{-1}$) is wider than the width of the IR-pulse the output frequency of the TOPAS was automatically tuned to produce IR pulses to cover the whole range. Spectra were recorded for 6 minutes each.

**LUV preparation.** Large unilamellar vesicles (LUVs) were produced using the protocol described by Engel et al. [166] Briefly, 12.5 $\mu$mol DOPC was dissolved in 800 $\mu$L chloroform in a 10 mL glass tube. The solvent was evaporated by flushing with dry nitrogen and putting the tube in a vacuum desiccator for 20 minutes. The lipid film created in this process was dissolved in 0.5 mL PBS buffer pH 7.4 (prepared as described above) containing 50 mM calcein. The solution was left for at least 30 minutes to ensure complete hydration of the lipids, then frozen in liquid nitrogen and unfrozen in a water bath of $\sim 40^{\circ} \text{C}$. The latter step was repeated ten times. The solution was extruded through a filter holder (Liposofast, Avestin, Inc.) for at least eleven times, using filters with a pore diameter of 200 nm. Free calcein was extracted from the produced vesicles by using size-exclusion column chromatography (Sephadex G-50 fine) and elution with PBS buffer containing 100 mM NaCl.

**Fluorometer.** A plate reader (Perkin Elmer VICTOR$^3$ Multilabel Counter) was used to measure fluorescence of the vesicles. 6 $\mu$L of the vesicle solution was added to 100 mM NaCl PBS buffer in wells of a 96 wells plate. After measuring fluorescence of the untreated vesicles, SWNTs in PBS buffer were added up to a final well volume of 200 $\mu$L and fluorescence was measured as a function of time. The plate was shaken just before each fluorescence measurement to mix the suspension. At the end of the experiment 15 $\mu$L 10% Triton X-100 (Sigma-Aldrich) was added to disintegrate the vesicles.

**7.3 Results and discussion**

Here, we use a bottom-up approach in which lipid monolayers of 1,2-dipalmitoyl-3-trimethylammonium-propane (DPTAP) and large unilamellar vesicles (LUVs) of 1,2-Dioleoyl-sn-Glycero-3-Phosphocholine (DOPC) lipid bilayers serve as simplified models for the complex multilipid bilayer of the cell membrane. (6,5)-SWNTs with an average length of 250 nm were made water-soluble by adhering single-stranded DNA (ssDNA). We employ vibrational sum-frequency generation (SFG) spectroscopy to directly probe the conformation and orientation of the lipids and water, and the changes therein upon nanotube adsorption. Ad-
Additionally, we performed surface tension measurements to investigate the thermodynamics of the lipid monolayer-SWNT interaction and membrane leakage experiments to investigate the occurrence of membrane rupture resulting from SWNT-phospholipid bilayer interaction.

![Graph showing surface pressure of DPTAP monolayers as a function of time.](image)

**Figure 7.1.** Surface pressure of DPTAP monolayers as a function of time. Trace 1: DPTAP is added to a buffered water-air interface to reach a surface pressure of 19 mN/m at t=~5 minutes. Trace 2: DPTAP monolayer, DNA-SWNT solution is injected underneath the monolayer at the arrow-marked point to reach a SWNT concentration of the subphase of 40 pM. Trace 3: DPTAP monolayer, DNA-SWNT solution is injected at the arrow marked point to reach a SWNT concentration of the subphase of 160 pM.

**Tensiometry.** All measurements were performed at a temperature of 23±1°C, and an initial monolayer surface pressure of 19 mN/m. Figure 7.1 shows the time evolution of the surface pressure of a DPTAP monolayer during and after the injection of DNA-SWNTs into the sub-phase containing PBS buffer. The reference measurement (no SWNTs added; marked 1), shows a slow decrease in surface pressure after adding the lipids to the water surface. This decrease is due to evaporation of water from the surface. The behavior is different when DNA-SWNTs are injected beneath the lipid monolayer (marked by arrows in Figure 7.1): minutes after injection of SWNTs underneath the monolayer, a small, concentration dependent dip in the surface pressure of the monolayer occurs. This dip is not observed after the injection of an equal amount of pure PBS buffer underneath a DPTAP monolayer (data not shown), indicating that it is due to the interaction between the lipid monolayer and the DNA-SWNTs. Moreover, at longer times the pressure increases, starting ~100 minutes after SWNTs are added to the subphase.

The SWNT-induced decrease in surface pressure can be understood by the
local induction of gelled patches in the lipid monolayer through interactions with the negatively charged DNA covering the SWNT. The formation of gelled patches as a result of charged polystyrene nanoparticles interacting with DPPC and DOPC vesicles was recently shown by Wang et al. [46] The presence of high-density, immobilized patches will leave the monolayer area between the patches less condensed, thereby decreasing the overall surface pressure. The subsequent increase in surface pressure occurring after one or two hours (depending on the precise SWNT concentration) must indicate a new stage in the interaction of the DNA-SWNTs with the monolayer. As demonstrated below, a likely explanation is the partial insertion of SWNTs into the monolayer.

Vibrational sum-frequency generation spectroscopy. SFG spectra, normalized to a quartz reference, obtained after injecting DNA-wrapped SWNTs in the buffered heavy water (D$_2$O) subphase are shown in figure 6.2a. Heavy water was used for clearer separation of the different OD and CH resonances, i.e. to circumvent complications in analysis due to overlap of the OH and CH-stretch resonances.

The most prominent change in the spectra of figure 7.2a after adding SWNTs is the decrease of the OD stretch intensity between 2000-2700 cm$^{-1}$, for convenience further referred to as two resonances OD$\nu$1 centered at $\sim$2350 cm$^{-1}$ and OD$\nu$2 centered at $\sim$2500 cm$^{-1}$. Furthermore, the CH stretch responses are changing in time as well: first the CH$_3$ symmetric stretch (2880 cm$^{-1}$) and the CH$_3$ Fermi Resonance (2940 cm$^{-1}$) intensities grow, while at later times these decrease again. Simultaneously and in parallel the CH$_2$ symmetric stretch (2850 cm$^{-1}$) intensity increases. Finally, around 2720 cm$^{-1}$ a broad, low intensity peak emerges.

To quantify the observed spectral changes, the SFG spectra were fitted by assigning Lorentzian lineshapes to the CH modes and Voigt profiles to the water bands [25, 63]. The relative intensity $I_{SF,n}$ of the $n^{th}$ Lorentzian resonance is proportional to the square of the peak area $A_n$. Figure 7.2b shows this quantity for the OD$\nu$1 and OD$\nu$2 OD stretch resonances of interfacial water at the DPTAP monolayer. After adding DNA-SWNTs, the OD stretch resonances decrease in time. Two effects may contribute to this decrease. Firstly, the presence of the negative charge at the interface disrupts the collective alignment of resonant transition dipole moments induced by the positive headgroups of DPTAP [34, 53, 167]. Such alignment increases the SFG response, as the SFG selection rule requires that symmetry must be broken. The alignment caused by this electrostatic interaction can be cancelled by the presence of the negative charges on the ssDNA. Secondly, DNA-SWNTs at the interface displace water molecules just underneath the monolayer, thereby effectively reducing the number of water molecules contributing to the SFG response. While one might expect the SWNT volume concentration at the interface to be low (given the low bulk concentration), the observation that both the intensity and the shape of the OD stretch resonances change in time (figure 7.2a) shows that both effects are operative here, since an overall decrease in alignment would only affect
the intensity. The shape change is reflected most notably in the appearance of a new OD peak around 2720 cm\(^{-1}\) after addition of DNA-SWNTs, as well as a blueshift of the OD\(_{\nu1}\) stretch peak. These results are reminiscent of earlier SFG studies concerning the interaction of lambda-phage DNA with DPTAP monolayers [53,54] and therefore illustrate the direct contact of DNA adsorbed on the SWNT surface with the lipid headgroups.

The conformation of lipids in the monolayer can be inferred from the CH peak intensity ratio in the SFG spectrum. A fully ordered monolayer, with all-trans CH\(_2\) groups, has a practically centrosymmetric region of CH\(_2\) bonds, and therefore these vibrations are not SFG active. However, the CH\(_3\) end groups of the lipid tails in an ordered monolayer form a non-symmetric layer
along the surface normal, and, when the CH₃ groups are collectively aligned, these are highly SFG active. The ratio of the symmetric stretch intensities \( \frac{I_{CH_3}}{I_{CH_2}} \) therefore directly reflects the order in the alkyl chain of the lipid monolayer [18]. This order parameter is plotted in figure 7.2b as a function of time. Adding SWNTs underneath a DPTAP monolayer leads to an increase in order of the DPTAP monolayer during the first stage of the interaction. Remarkably, ~100 minutes after the injection of DNA-SWNTs the lipid order decreases again, eventually to a value that is even lower than that before the addition of the nanotubes.

The initial increase in the order parameter implies that the interaction with the SWNTs cause the monolayer to become more condensed, without adding more lipids to the surface. This observation is in agreement with the formation of local condensed patches concluded from tensiometry measurements. Since the relative contribution of well-ordered lipids to the SFG signal is high (since the lipid molecules in these ordered patched are better mutually aligned), the existence of more condensed patches raises the overall order parameter. The monolayer was found to be spatially homogeneous, suggesting that condensed patches are small compared to the ~100 μm diameter of the laser beam spot on the sample surface. This is in line with the notion that the condensed patches are only present at the DNA-SWNT-lipid interaction site, as postulated by Wang et al. [46]

The subsequent \((t \geq 100 \text{ min})\) decrease of the order parameter \(\frac{I_{CH_3}}{I_{CH_2}}\) towards a value lower than the original value (figure 7.2b) implies that the lipid monolayer becomes less ordered, despite the observed increase in surface pressure at these interaction times. The increase in surface pressure may be traced to either electrostatic interactions of the DNA-SWNTs with the lipid head groups or to DNA-SWNTs (partly) inserting into the hydrophobic region of the monolayer [130]. The observation that the OD stretch intensities ODν1 and ODν2 remain small and unchanged on this timescale (figure 7.2b), shows that the electrostatic interaction of the lipid head group with the DNA remains unchanged. Hence the likely explanation for the slow changes in both lipid structure and surface pressure are insertion of the SWNTs into the monolayer. This insertion into the environment of hydrophobic lipid tails requires the strongly hydrophobic surface of the SWNTs to be exposed. The long timescale is therefore attributed to the process of DNA uncovering the SWNTs. Every hydrophilic \((GT)_{16}\) DNA strand possesses 32 sites to attach to the SWNT’s surface through π-stacking interactions. Because of the numerous binding sides of DNA strands to the nanotube, the loosening of DNA molecules from the SWNT’s surface is a slow process, even on the long timescales of the current study [168]. However, uncovering the SWNT surface does not require desorption of DNA from the SWNT. Given that only about 20% of the SWNTs is covered by DNA [160], a non-directional diffusive motion of DNA along the SWNT surface will be a much faster process, given the shallow potential energy surface at such an interface. We therefore suggest that an irreversible, stepwise
insertion into the hydrophobic alkyl chain region of the lipid monolayer occurs, with the SWNTs inserting a bit further every time a part their surface is bared by the random movement of the DNA strands. The observed interruption of the ordering of the monolayer through its CH vibrations in the SFG spectra is then likely explained by direct contact between the bare SWNT surface and the alkyl chains. The decrease in free energy that drives this process can be observed from the increase in surface pressure in the tensiometric measurements. In this picture loose DNA and parts of the SWNTs remain present just underneath the monolayer, thereby still suppressing the OD stretch intensities OD\nu_1 and OD\nu_2.

![Figure 7.3](image_url)

**Figure 7.3.** Schematic representation of the interaction of DNA-SWNTs (black rods covered with grey dashes) with a lipid monolayer (grey circles and chains). Two stages can be identified: first the interaction is mediated through DNA (upper panel), while at later times diffusion of DNA bares more of the SNWTs’ surface which allows them to partially nestle within the lipids’ hydrophobic alkyl chain region (lower panel).

Summarizing the long term effects, injection of DNA-SWNTs underneath a DPTAP monolayer leads to an increased surface pressure after several hours. The order of the lipid tails in the monolayer is disturbed and no significantly aligned water layer is detectable underneath the monolayer. A picture in which the gradual baring of the SWNT’s surface of adsorbed DNA strands allows it to partially penetrate the monolayer explains all of these observations. The charged DNA strands are left below the hydrophobic alkyl chains, electrostatically bound to the monolayer in a manner similar to that previously described by Campen et al. [54] Figure 7.3 shows a schematic representation of the two stages of the interaction.

**Binding and dissociation model.** For further quantitative analysis, a model to describe the dynamical behavior observed in the SFG measurements is applied. To this end, we use the model that was originally developed for endocytosis of DNA-SWNTs [119]. While this may appear to be an entirely different
The process, the dynamics can be described by equivalent parameters, as shown in the following. The binding of the SWNTs to the membrane, promoted by electrostatics in our experiments, is given by the rate $k_a$. Some of this binding may be reversed at a dissociation rate $k_r$. At the same time, the endocytosis model describes that the number of bound DNA-SWNT-receptor compounds disappears and reappears through endocytosis and cellular expulsion at a rate $k_e$ and $k_{rec}$, respectively. While in our Langmuir monolayer system no endocytosis can take place, the analogy is that nanotubes insert into the alkyl chain region of the monolayer in a process that can be expected to be similar [160]. Hence, the uptake of the bared SWNTs into hydrophobic region of the monolayer and the possible expulsion from the hydrophobic region is described by the parameters $k_e$ and $k_{rec}$, respectively. We may then compose the following set of differential equations for the concentration of SWNTs in the subphase $L(t)$, the number of SWNTs bound to the monolayer $C_s(t)$, and the number of SWNTs embedded in the monolayer $C_i(t)$:

$$\frac{dL(t)}{dt} = -k_a L(t) + k_r C_s(t)$$ (7.1)

$$\frac{dC_s(t)}{dt} = k_a L(t) - (k_r + k_e) C_s(t) + k_{rec} C_i(t)$$ (7.2)

$$\frac{dC_i(t)}{dt} = k_e C_s(t) - k_{rec} C_i(t)$$ (7.3)

We simplify the system by assigning an ingrowing constant concentration to $L(t)$, which is reasonable given a) the observation from the SFG data that the system reaches a steady state and b) the assumption that only a fraction of the SWNTs interacts with the lipid membrane, as is known from similar studies. [119, 159] A more detailed description can be found in the Supporting Information. The parameters in this model can be related directly to the results of the SFG measurements: firstly, the change in the total water signal ($\text{OD} \nu_1 + \text{OD} \nu_2$) should be proportional to the square of the sum of the SWNT density at the monolayer the density of bound SWNTs $C_s(t)$ and the number of SWNTs embedded in the monolayer $C_i(t)$ since the DNA from these SWNTs is located at the interface (note that, due to the coherent nature of the SFG approach, the SFG intensity scales with the square of the interfacial number density). Secondly, the order parameter ($I_{CH_3}/I_{CH_2}$) should increase with the square of $C_s(t)$ because of lipid templating, but drop with the square of $C_i(t)$ because of the disordering effect caused by SWNTs nestled in the alkyl chains. Equation 2 and 3 were solved numerically and fitted to our SFG data. Figure 7.4 shows that this simple model provides a good description of the time-dependent SFG intensities for the water and the methyl/methylene order parameter, with values for the rate parameters $k_a$, $k_r$, $k_e$ and $k_{rec}$ of $7.00 \times 10^{-4}$, 0.340, 0.100, and $9.68 \times 10^{-2}$ per minute, respectively. For the first two of these parameters the exact same values were used as in the studies by Jin et al., while the other two were within a factor 3 to those used to describe endocytosis for DNA-SWNTs.
of equal size at similar concentration [119].

![Diagram](image)

**Figure 7.4.** The data points of figure 7.2 fitted by the binding-dissociation model. Grey squares give the CH order parameter, black squares the water ODv1 + ODv2 peak.

From the model, further quantitative conclusions can be drawn. For instance, it predicts that $C_s(t)$ and $C_i(t)$ become equal at long time scales. This is consistent with the baring of the SWNT surface of DNA, since in that case the binding factor $C_s(t)$ would describe the DNA bound to the lipid head groups, and $C_i(t)$ a corresponding number of SWNTs nestled in the alkyl chain region. Furthermore, the model predicts that at this concentration 0.2 percent of the total DNA-SWNTs interact with the monolayer.

**Membrane leakage experiment.** The proposed insertion of SWNTs into the lipid monolayer can be expected to severely compromise the model membrane’s integrity. Extrapolating this result to an in vivo system, such an interaction may damage the cell membrane to a point of rupture and leakage, as has been demonstrated before for antimicrobial peptides [130].

To check whether rearrangement of the lipid membrane by DNA-SWNTs disturbs it severely, a membrane leakage experiment was performed. Ideally, the vesicles used in this experiment would have been made of DPTAP for maximal consistency between experiments. However, due to the gel to liquid phase transition temperature of DPTAP of ~45°C [169] it is not possible to form vesicles at 23°C. To circumvent this complication, vesicles were prepared from DOPC, which has a comparable alkyl chain size to DPTAP, while its gel to liquid phase transition temperature of -12°C [170] enables the formation of stable vesicles at room temperature. While DOPC is zwitterionic and DPTAP
cationic, the resulting decreased electrostatic interaction between DNA-SWNTs and the DOPC bilayer can be compensated by using a higher concentration of SWNTs [54].

DNA-wrapped SWNTs were added to a suspension of 200 nm diameter large unilamellar vesicles (LUVs) containing calcein, a fluorescent dye. Large unilamellar vesicles (LUVs) were produced through the protocol described by Engel et al. [166] Inside the vesicles, the calcein concentration is high, thereby quenching the fluorescence intensity [171]. If DNA-SWNTs induce membrane rupture, calcein molecules leak out of the vesicle’s interior. The resulting dilution of the calcein solution then reduces self-quenching, and the observed macroscopic fluorescence increases.

Rather than finding the fluorescence increase associated with membrane rupture, the first observation after adding SWNTs to the suspension of DOPC vesicles is actually a two-step decrease of the fluorescence. An instantaneous, trivial decrease is present due to the linear absorption of (both exciting and fluorescence) radiation by the SWNTs. This factor is readily corrected for by calibrating the total fluorescence intensity to that observed after adding the surfactant Triton X-100 at the end of each measurement. This surfactant efficiently dissolves all LUVs, negating the self-quenching and causing a sharp increase in fluorescence. The correction factors are quantitatively consistent with a Lambert-Beer-like extinction behavior expected for a homogeneous suspension of nanotubes.

![Figure 7.5](image)

**Figure 7.5.** Results of membrane leakage experiment, normalized to maximal fluorescence = 1 for each sample. Arrows mark the addition of vesicles, SWNTs and Triton X-100. Solid lines are added for clarity.

More interesting is the slow decrease over ~100’s of minutes, which is faster and more pronounced at higher SWNT concentrations (7.5). This decrease in
fluorescence has to be caused by an interaction between LUVs and SWNTs, and can be explained in two ways. Firstly, SWNTs attaching to the vesicles will cause quenching of the excitation light due to co-localization of the semiconducting SWNTs and the trapped calcein. Energy transfer between carbon nanotubes and calcein could take place radiatively or through other intermolecular energy transfer pathways, given the nanometer-scale distance of donor and acceptor molecules. Secondly, the decrease in fluorescence may be explained by the transfer of phospholipids from the vesicle’s membrane onto the SWNT surface. Lipids diffusing away from the vesicles’ membrane along the SWNT would cause the vesicles to shrink, thus increasing the self-quenching effect and lowering the overall fluorescence. In either case, the SWNT induced decrease in fluorescence is testimony of the interaction of the SWNTs with the vesicle’s membrane. It is evident that, despite this interaction, the LUVs remain intact, since the large fluorescence intensity increase associated with membrane rupture is clearly absent.

7.4 Conclusion

Adsorption of DNA-wrapped SWNTs to the surface of a DPTAP monolayer on a time scale of minutes was shown by tensiometry and Vibrational Sum Frequency spectroscopy. This interaction initiates the formation of local condensed patches within the DPTAP monolayer, as is shown by a decrease in surface pressure and an increase in the order of the lipids’ alkyl chains through the order parameter extracted from the SFG spectra. Additionally, the interaction is accompanied by a strong perturbation of water molecules directly underneath the monolayer, indicating the presence of the DNA-SWNTs within the top few molecular layers of water.

On a longer timescale (hours), the injected SWNTs increase the surface pressure, while SFG shows a disordering of the alkyl chains. These observations are explained by (partial) penetration of SWNTs into the DPTAP monolayer. The results suggest that the DNA strands on the SWNTs are rearranged or loosened in a slow thermodynamic equilibration process that bares more and more of the SWNTs hydrophobic surface. The total free energy at the interfacial system decreases when parts of SWNTs’ surface interact with the lipids’ alkyl chains. The negatively charged DNA remains electrostatically bound to the positively charged lipid headgroup, as can be inferred from the persisting lowered water signal in the SFG spectra. A simple model of reversible adsorption and insertion into the lipid hydrophobic region reproduces the observed kinetics very well, with parameters that are remarkably similar to those inferred previously for specific interactions. This result shows that for this system specific interactions are not necessarily stronger than unspecific ones.

The possible rupture of a model membrane through this interaction was probed by adsorbing DNA-SWNTs to DOPC vesicles containing fluorescent molecules. While significant interaction between the DNA-SWNTs and the vesicles was apparent by the SWNT-induced quenching of fluorescence, the
membrane remained intact.

Our results provide a solid base for further quantitative research regarding the interaction of SWNTs and lipids, focusing both on the molecular-scale interactions and their emerging macroscopic phenomena.
7.5 Supporting Information

The model described by solving equations 1–3 describes the process of DNA-SWNTs binding to the lipid membrane, and the subsequent uptake of SWNTs into the membrane, each governed by rate constants $k$ that represent the probability of binding or dissociation taking place in. As such, $L(t)$, $C_s(t)$, and $C_i(t)$ are dimensionless while the rate constants are given in units of min$^{-1}$. In the study by Jin et al. where this model was applied to describe the endocytosis of DNA-SWNTs, $L(t)$ was chosen to have a Gaussian time profile, corresponding for the growth and decay of the SWNT concentration in a perfusion experiment. However, in this study the SWNTs are injected into a non-flowing subphase and stirred, an ingrowing constant, i.e. sigmoid function is more suited. To describe the ingrowth, which is governed by both non-directional diffusion and electrostatic attraction of the DNA-SWNTs towards the cationic lipid monolayer, we choose a Gauss error function that is commonly applied in statistical distributions:

$$L(t) = L_0 \left(1 + \text{erf}(Dt)\right) \quad (7.4)$$

with $L_0$ the equilibrium concentration and the prefactor $D = 0.033$ a diffusion constant found through fitting determining the ingrowth rate, and with

$$\text{erf}(x) = \frac{2}{\sqrt{\pi}} \int_0^x e^{-y^2} dy \quad (7.5)$$

Having thus defined $L(t)$, we can numerically solve equations 2 and 3 to find $C_s(t)$ and $C_i(t)$ with boundary conditions $C_s(0) = C_i(0) = 0$.

In this study, the model is used to described the dynamics of the order parameter $R$ deduced from the fitted SFG intensities. Since these intensities are observed to change in time, we can define $R(t)$ as

$$R(t) = \frac{I_{CH_3}(t)}{I_{CH_2}(t)} \quad (7.6)$$

As discussed in the main text, the binding and dissociation model describes the dynamics of $R(t)$ through the relation

$$R(t) = R_0 + A\sqrt{C_s(t)} - B\sqrt{C_i(t)}, \quad (7.7)$$

Where $R_0$ is the order parameter of the lipid monolayer before adding SWNTs, and $A$ and $B$ are scaling factors describing the relative effect of DNA-SWNT binding and SWNT nestling on the order parameter $R(t)$. We found the model to fit the data for $A = B$, implying that binding and nestling of a single nanotube have an equal but opposite effect in the order of the alkyl chains.