Lipid affinity to nanoparticles indicates possible nanoparticle toxicity

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Motivation

The problem of determining the possible mechanisms of **nanoparticle** (NP) toxicity has recently been addressed in [1]. In Fig. 1, initial experiments to investigate these mechanisms with super-resolution fluorescent microscope are presented. From the overlapping signal of cell membranes and NPs we can conclude that NPs very likely wrap themselves in constituents of **cell membranes** - lipids and proteins [2,3]. Additional experiments have confirmed this **interaction** to be **non-specific**. From these experiments we postulate that NP toxicity is closely connected to the strength of interactions between NPs and lipids/proteins (also called affinity). To enable ***in vitro*** **testing of NPs affinity to lipids (toxicity)**, we have devised two novel approaches. DLS is used for measuring the rate of NP effect on vesicle size. With EPR we are able to sense NP-induced changes to lipid ordering in the membrane. With both methods we have been able to confirm proof of concept and are still working on experiment refinement.

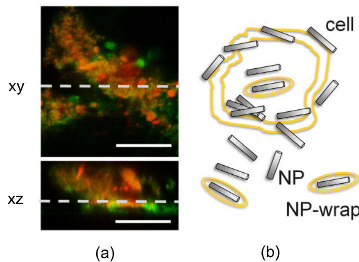
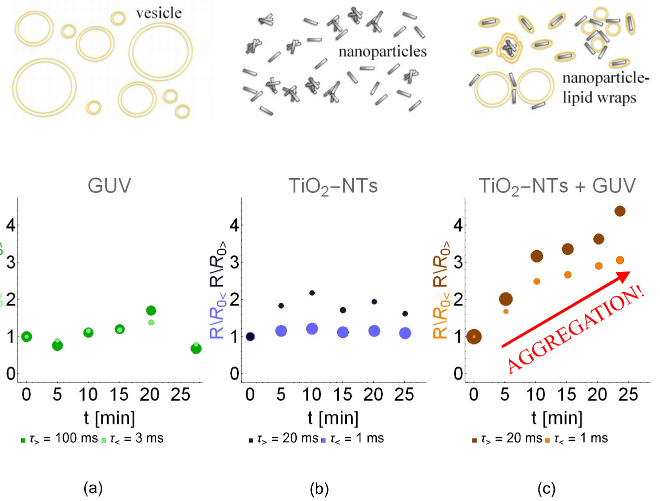


Figure 1: (a) Example of fluorescence superresolution image of cells incubated with NPs for one day. Cells were labeled with CellMask (green) and nanoparticles with Alexa 647 (red). Scale bar is 10 µm. (b) Schematics of expected situation on image (a).

EPR an DLS methods both show measurable nanoparticle-lipid affinity.

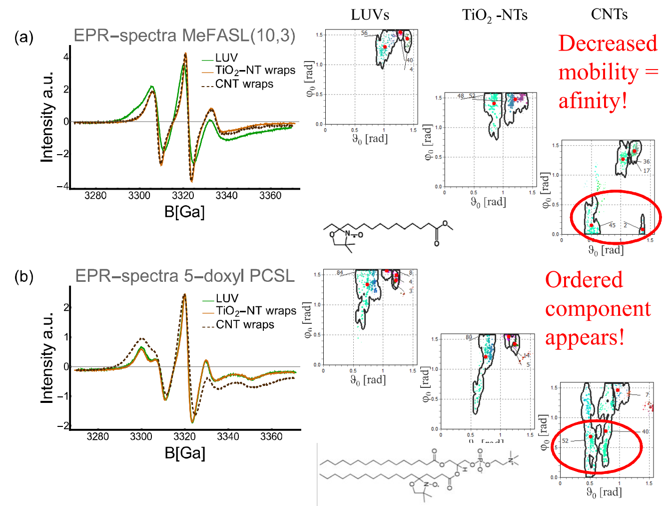
Dynamic Light Scattering (DLS)

Figure 2: Parameters acquired by fitting DLS autocorrelation function (Fig 4a). Two average sizes of population are shown: big particles – dark color, smaller particles – light color. Two controls (a) giant unilamellar vesicles (GUV) and (b) titanium dioxide nanotubes (TiO_2-NTs) are show together with (c) the experiment: TiO2-NTs mixed with GUVs. Schematics above represent situations in the sample. Note that only in (c) the size of particles substantially increases implying **NT-lipid affinity of medium-strength**.



Electron Paramagnetic Resonance(EPR)

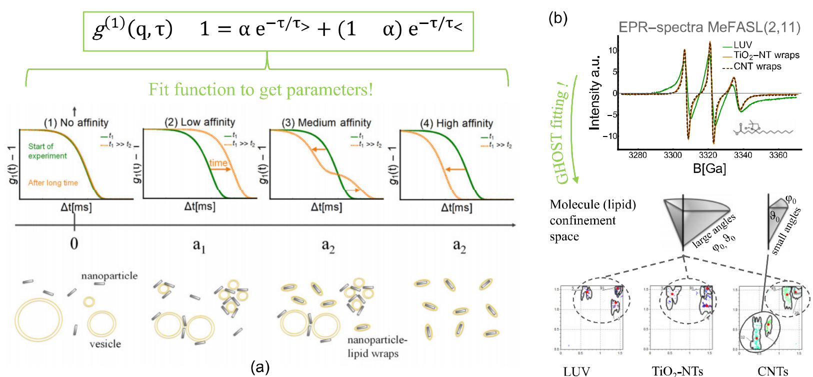
Figure 3: EPR-spectra (left) and results of EPR spectra fitted with GHOST method (right) [4]. Parameters obtained are φ_0 and ϑ_0 (schematics in Fig. 4b, below), which define the mobility of spin-probe in the membrane. **Bigger the number, greater the mobility of probe.** Measurements shown were done with two probes with spin-probe positioned (a) at the end of the tail of fatty acid (a) and (b) right below the lipid head group. Note that the **mobility of both probes** is significantly **reduced for** carbon nanotubes (**CNTs**).



Materials and methods

- DLS data was acquired with a red laser, avalanche photodiode and *ALV Corelator Software - v. 3.0*.
- Acquired data was fitted with a sum of two exponentials to obtain parameters in Fig. 2 (Fig. 4a).
- DLS sample preparation: suspension of TiO_2-NTs and GUVs is mixed right before the measurement.
- EPR data acquisition: standard Bruker spectrometer
- EPR sample preparation: addition of suspension of TiO_2-NTs in bicarbonate buffer (pH = 10) during preparation of LUVs with reverse phase evaporation

Figure 4: (a) Presentation of expected autocorrelation functions for different experimental situations (schematic below) in our DLS experiments. Above graphs is a two-parameter function which characterizes **two populations** in our system (big and small particles) that contribute to most of our signal. (b) Example of acquired EPR spectra with example of fitting parameters for three different experiments. Schematics explain how to read plot of two **confinement parameters φ_0 and ϑ_0**.



Literature

[1] Schmid, O., Stoeger T., Strancar J. et al. Smart Nano Tox – Deliverable 1.1: Position paper on predictive *in vitro* models (2016)

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[4] Štrancar, J. et al. Spin Label EPR-Based Characterization of Biosystem Complexity. J. Chem. Inf. Model. 45, 394–406 (2005).

Acknowledgments

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