Supplementary information for: Expanding the optical trapping range of lipid vesicles to the nano-meter regime

Calculation of vesicle deformation

We calculate the degree of optically induced deformation of an optically trapped sucrose loaded vesicle based on elastic theory of shells and argue that the trapped sucrose core prevents the vesicle from bending and converts the optical energy into stretching of the bilayer which is several orders of magnitude more costly than buckling of the bilayer.

We consider a vesicle having a radius of R and loaded with 1M or sucrose corresponding to an index of refraction of $n_{suc}(1M)=1.39$ (S1). The lipid bilayer surrounding the sucrose core is impermeable to sucrose on the time scale of the experiments and any severe deformation of the vesicle causes efflux of water, an effect which is counteracted by the osmotic pressure. However, at high power densities optical stretching of the vesicle might still occur due to oppositely pointing surface stresses imposed on the vesicle, an effect exploited in optical stretching of cells (S2). To model the effect of the optical stresses we therefore consider the average optical stretching force acting on an elastic shell enclosing a constant volume having $n_{suc}=1.39$ which is given by (S3)

$$F_{opt} = \frac{n_w P}{c} Q,$$

where P is the total power hitting the shell and c is the speed of light in vacuum. $Q = Q_{back} + Q_{front}$ and Q_{back} and Q_{front} are the fractions of momentum transferred at the back and front interfaces, respectively:

$$Q_{front} = 1 + R - n(1-R)$$
$$Q_{back} = (1-R)[n + Rn - (1-R)]$$

where R is the reflection coefficient given by $R=(n_{suc}-n_w)^2/(n_{suc}+n_w)^2$ and $n=n_{suc}/n_w$. The optical force is balanced by an elastic restoring force in the vesicle membrane (S3)

$$F_{shell} = -\pi Y T \Delta b$$

where T is membrane thickness (5nm) and Y is Young's modulus (10^7N/m for bilayers (S4)). Δb is the deformation along the optical axis which is assumed to be small compared to the size of the shell. Under

equilibrium conditions the total force is zero, $\boldsymbol{F}_{opt} + \boldsymbol{F}_{shell} = 0$, which allows the deformation to be expressed as a function of laser power

$$\Delta b = \frac{n_w PQ}{\pi c Y T}.$$

For small changes in the shape of the vesicle the deformation is linearly proportional to the total power density hitting the vesicle. For typical high powers used in this work, P = 50-100mW, this leads to deformations of on the order of 1nm of the optically trapped sucrose loaded vesicle. Smaller vesicles are exposed to higher stresses at the same laser power but since the cross section of a small vesicles is significantly smaller than the laser focus the power passing through the vesicle becomes much lower than the total power in the focus.

Image analysis based 'Stokes-drag' calibration of optical trap using image analysis

Single particle tracking of a fluorescently labeled vesicle (as shown in Supplementary Figure S2) is performed by calculating the first moment of the intensity distribution, the so-called centroid of the intensity in every image: $(x_c,y_c) = (\Sigma_{xy} I(x,y)*x, \Sigma_{xy} I(x,y)*y) / \Sigma_{xy} I(x,y)$, where I is pixel intensity. This results in particle trajectories as shown in Supplementary Figures S2 and S3b. The amplitude of the periodic excursion of the vesicle is related to the strength of the trap by the force balance between the trapping force, $F_{trap}=\kappa\Delta x$, and the fluid drag, $F_{drag}=\gamma v$, leading to the following relation: $\kappa = \gamma v/\Delta x$ where v is the velocity of the surrounding fluid and Δx is the measured amplitude.

Protocol for glass substrate passivation

Passivation of the glass substrate was achieved by forming a supported lipid bilayer by allowing small unilamellar vesicles (DOPC, Avanti Polar Lipids) to fuse to a hydrophilic glass surface for 2 h at room temperature. Lipids suspended in chloroform were dried on glass under nitrogen flow and further dried in vacuum for 2 h. The lipid film was hydrated at room temperature in PBS buffer at 100 mM NaCl, pH = 7.4. To form small unilamellar vesicles, we extruded the hydrated lipid mass through 50nm. The vesicle composition was DOPC and a small fraction of biotinylated DPPE lipids (0.1%). After incubation with vesicles the surface was thoroughly washed using Millipore water. Glass surfaces used for supported bilayers were oxidized by etching in piranha solution (3:1 sulfuric acid and hydrogen peroxide) for 30 min. For trapping small unilamellar vesicles (extruded at 50nm) we passivated the glass

surfaces using 1mg/mL BSA in PBS for 10 min due to possible interactions between the strong laser irradiation needed in these experiments with a supported bilayer.

(S1) Refractive index of solutions at high concentrations. W. M. M. Yunus.; Rahman, A. A. *Appl. Opt.* **1988**, 27 (16), 3341-3343.

(S2) Optical Deformability of Soft Biological Dielectrics. Guck, J.; Ananthakrishnan, R.; Moon, T. J.; Cunningham, C. C.; Käs. *Phys. Rev. Lett.* **2000**, 84 (23), 5451-5454.

(S3) Quantification of droplet deformation by electromagnetic trapping. Møller, P. C. F.; Oddershede, L. B. *Eur. Phys. Lett.* **2009**, 88, 48005.

(S4) Physical Properties of Surfactant Bilayer Membranes: Thermal Transitions, Elasticity, Rigidity, Cohesion, and Colloidal Interactions. Evans, E.; Needham, D. *J. Phys. Chem.* **1987**, 91 (16), 4219-4228.

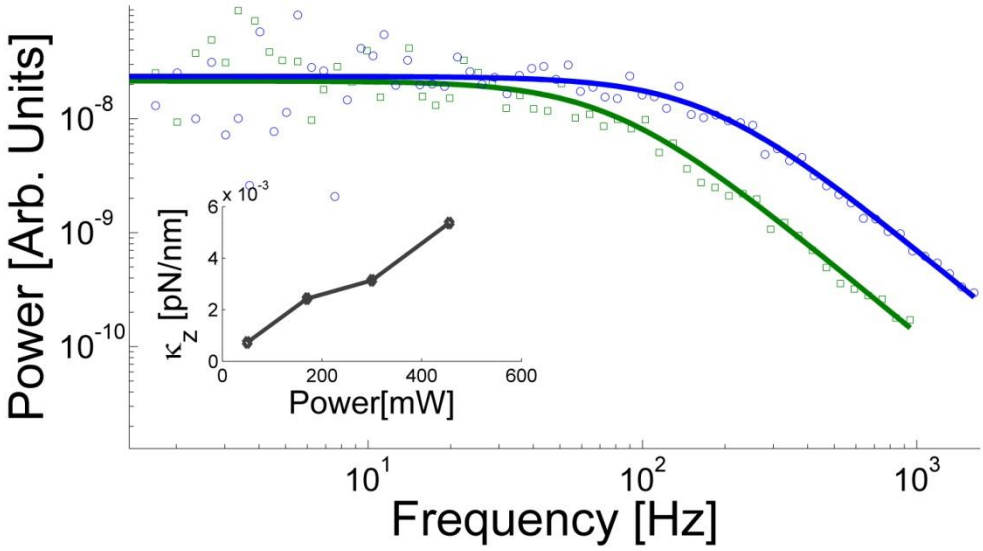

Figure S1. Axial calibration of an optical trap.. A d = 500 nm lipid vesicle containing 1M sucrose is trapped by two different laser powers, P = 170 mW and P = 455 mW, and the corresponding two power spectra are plotted. The inset the corresponding axial trapstiffnesses plotted in for four different laser powers.

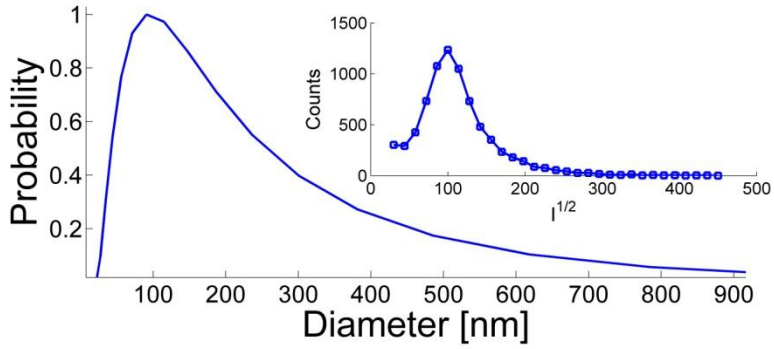

Figure S2. Size distribution of vesicles extruded through a 50nm polycarbonate filter. Data in the main graph is obtained by dynamic light scattering, the peak position corresponding to a diameter of *d*=89nm. The inset shows a histogram of fluorescence intensities from the same population of vesicles immobilized on a surface and recorded by a confocal microscope. The intensity profile for each vesicle (N=7603) was fitted to a 2D Gaussian to obtain the intensity threshold which separates the signal from the background (Matlab software available upon request). Overall, the curves are relatively similar, but the directly measured intensity distribution shows somewhat fewer counts for larger intensity values than the DLS curve. This is probably caused by that fact that the larger vesicles saturate the detector and that intensities for vesicles comparable to or larger than the confocal volume will be underestimated.

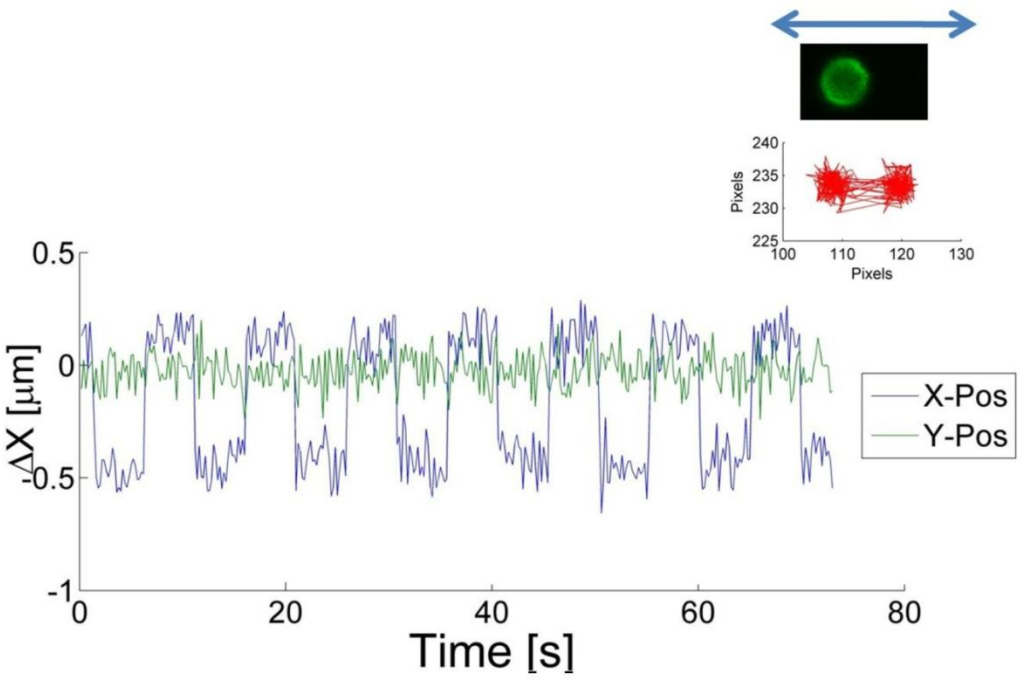

Figure S3. Stokes-drag calibration of an optically trapped vesicle. The position of a trapped vesicle is tracked by image analysis while the sample is oscillated with a triangular waveform (f=0.1Hz, peak to peak ampliude = 50μm). Upper part of inset shows a typical picture of the vesicle (labeled by membrane bound TR-DHPE), lower part of inset shows a scatterplot of positions visited by the trapped vesicle.

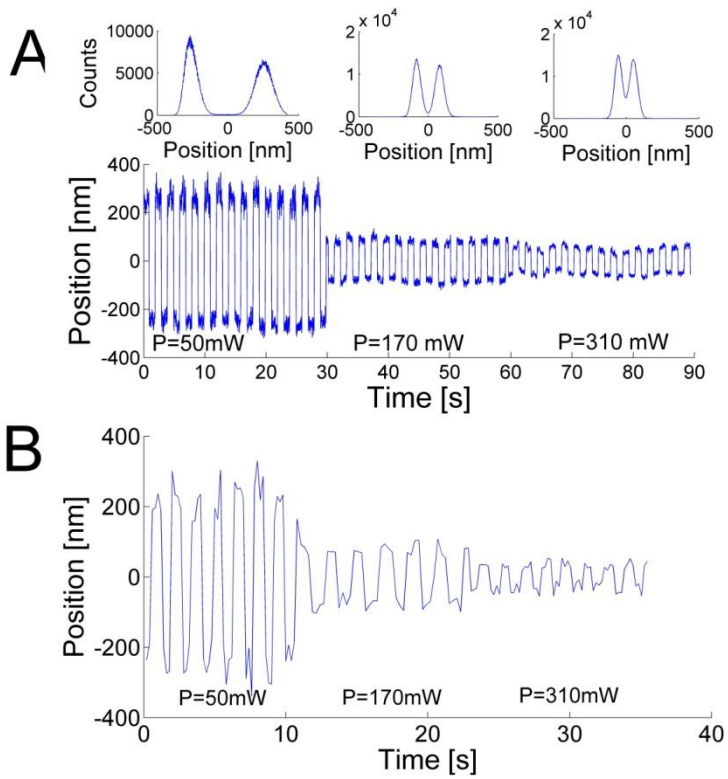

Figure S4. Comparison of quadrant photodiode based and image analysis based position determination. The measurements were performed on the same vesicle using identical trapping powers. During the experiments the laser power of the trapping laser was increased from 50mW to 170mW and finally to 310mW. A) Positions visited by the trapped vesicle as determined by quadrant photodiode detection of the forward scattered laser light. Upper insets show position histograms at the three laser powers. B) Positions visited by the trapped vesicle as determined by image analysis (centroid tracking).

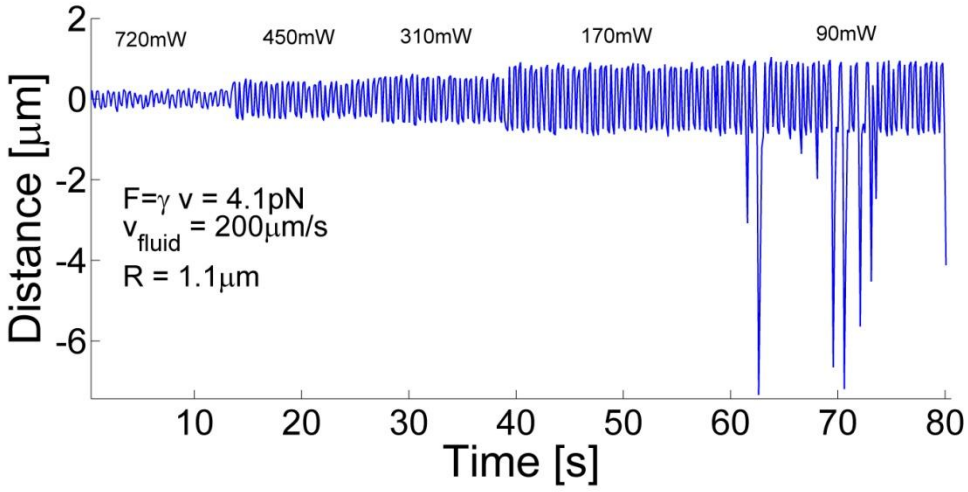

Figure S5. Measurements of maximum optical force on trapped vesicle. The sample chamber was moved with respect to a d = 2.2 μm trapped vesicle at constant speed in a periodic fashion (f=2Hz, A= 50μm). The fluid drag force is always F=4.1pN. At high laser powers this force is not sufficient to push the vesicle out of the trap. At P=90mW the vesicle occasionally escapes the trap. Hence, the maximum force exerted on the vesicle using a laser power of 90 mW is ~ 4.1 pN. As trapping strength is proportional to laser power, the maximum force using a laser power of ~ 900 mW would probably be ~ 40 pN.