Trapping, characterization and reactions of biocolloids in a salinity gradient

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Abstract. The properties of soft matter nanoparticles like exosomes are interesting for drug delivery and diagnostics applications. However, the simultaneous characterization of multiple properties, e.g., size and zeta potential, can only be done serially and is highly sensitive to the purification prior to characterization. Here we show how a salt gradient established in a nanofluidic channel induces opposing transport of particles and liquid that trap the particles. Particles are thus accumulated in the trap. We show how optical microscopy images of the particle positions in the salinity gradient provide a measurement of the size and surface charge. We demonstrate the method on a sample of exosomes and on individual particles. Finally, we show how biomolecular reactions at the surface of the nanoparticle can be detected from the optical microscopy analysis of the particles' trapping position.

Nanometer-sized biocolloids play an important role in many research fields, e.g., in drug delivery and diagnostics, and their size and surface charge are important parameters for their performance. The zeta potential, which depends on the surface charge, is an indicator of the biocolloids' stability in dispersions and is crucial for their initial adsorption on the cell membrane. Hence the zeta potential is both a key parameter when designing liposomes for drug delivery and for understanding the body's cell-to-cell communication with exosomes [1]. The particle size influences the endocytotic uptake rate after adsorption on the cell membrane, so the size is also important for their efficiency as drug delivery vehicles [2]. Current nanoparticle characterization techniques all have challenges. Measurements of size and zeta potential are routinely performed by dynamic light scattering (DLS) and laser Doppler electrophoresis (LDE), but they require multiple experiments and are challenged by polydisperse samples [3]. Alternatively, the size of individual particles can be measured by nano tracking analysis (NTA). Combined with refractive index measurements by interferometric imaging, this provides the two parameters simultaneously [4].

Here we present a nanofluidic-based method for determining the size and zeta potential from a single measurement with data acquisition times less than a minute [5]. In our method, we establish a salinity gradient across a funnel-shaped nanochannel. The nanochannel has a height of 240 nm, and the widths at the ends are 5 µm and 20 µm, see Figure 1a. The salinity gradient induces both a diffusiophoretic particle migration towards higher salt concentration and an oppositely directed difussioosmotic fluid flow in the nanochannel [5]. Particles in the nanochannel are trapped where the diffusiophoretic velocity $v_{ph}(x)$ and the diffusioosmotic fluid velocity $v_{os}(x)$ balance each other. The trapping position depends on the particles' diameter and zeta potential.

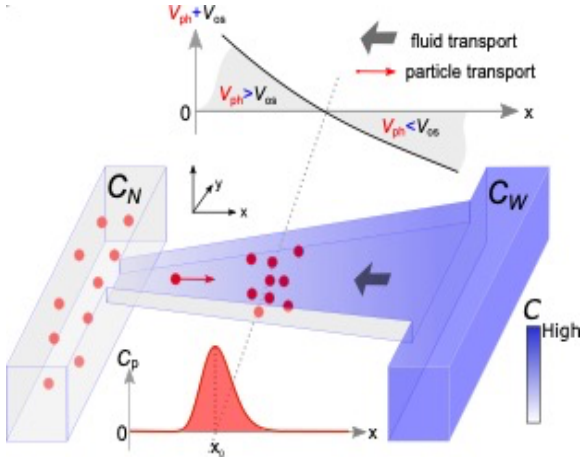

Fig. 1. Schematic of nanoparticle trapping in a funnel-shaped nanochannel by diffusioosmosis and diffusiophoresis. The salinity gradient is maintained by a continuous flow of solutions with PBS concentrations C_L and C_R (C_L<C_R) in the microchannels.

Figure 2a,b shows fluorescence microscopy images of trapped exosomes extracted from human blood serum and from a sample with two subpopulations of liposomes, respectively. The liposome subpopulations have different lipid compositions, and hence different zeta potentials, which causes a separation into two bands (Fig. 2b,c). The sizes and zeta potentials of both exosomes and liposomes are obtained from a fit of the fluorescence intensity along the nanochannel to a model that includes both the diffusion and the diffusio-phoretic and -osmotic velocities (Fig. 2c). The fitted values for the particle sizes and zeta

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potentials are consistent with results from standard methods (DLS and LDE).

The method is also able to characterize the diameter and zeta potential of individual liposomes by tracking their positions in the nanochannel over time (Fig. 3). So we can explore sample heterogeneity.

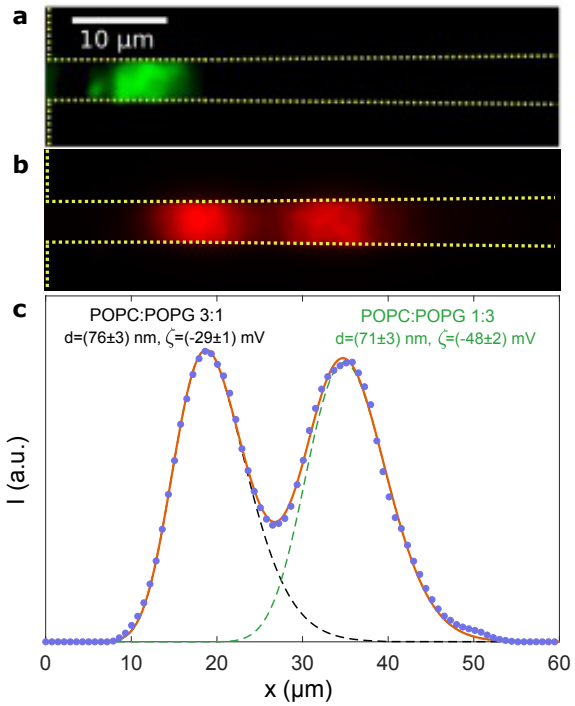

Fig 2. (a) Fluorescence image of a band of exosomes trapped in a nanochannel by a salinity gradient. From the analysis we obtain an exosome diameter of (78±7) nm and a zeta potential of (-18±1) mV. (b) Fluorescence image of trapped liposomes from a mixture. Two bands appear due to the different zeta potential of the two subpopulations. (c) Fluorescence intensity of the liposomes shown in panel c and a corresponding fit. The values of the fitted parameters are shown on the figure.

Finally, we use the chip as a label-free sensor for real-time sensing of ligands binding to liposomes [6]. In this experiment, the nanofluidic trap is populated with liposomes that have surfaces decorated with DNA probes. The DNA probes are partially double-stranded and carry a double cholesterol anchor at one end. The cholesterol anchors are inserted in the lipid bilayer. The other end is single-stranded and 15 bases long. The trapped liposomes are then exposed to a solution containing 15 bases long single-stranded targets complementary to the single-stranded probes on the liposomes. The hybridization of the targets on the liposomes' surfaces yields a change of their zeta potentials and results in a detectable shift of the trapping position. This is readily seen on the fluorescence images of the trap.

The concentration of nanoparticles by diffusiophoretic trapping has potential applications within exosome characterization, where biorecognition of, e.g., surface DNA, proteins, and antibodies, is a promising candidate for early-stage cancer diagnostics.

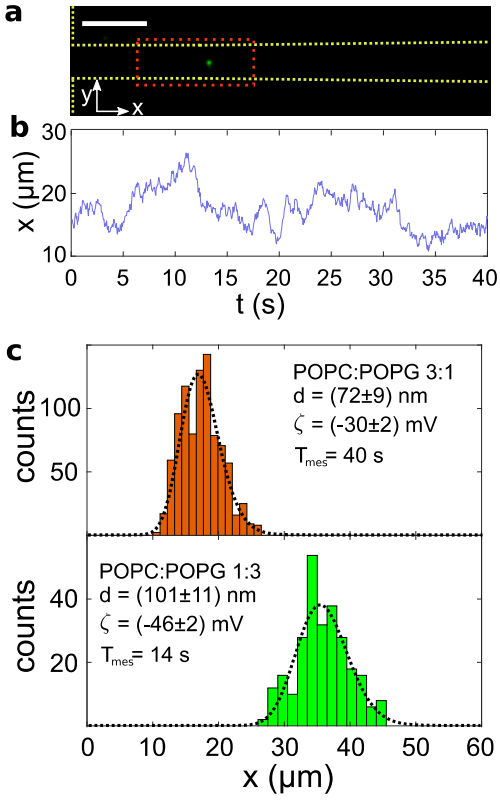

Fig. 3 (a) Fluorescence microscopy image of a single liposome trapped by a salinity gradient in a nanochannel. (b) The x-coordinates of a single, trapped liposome in a nanochannel. (c) Histogram of the x-coordinates shown in panel b and corresponding fit (top), and a similar histogram and corresponding fit for a liposome with a different size and zeta potential (bottom).

References

1. S Honary, & F Zahir, Tropical Journal of Pharmaceutical Research, **12**(2), 255–264 (2013).
2. E. Blanco, H. Shen, M. Ferrari, Nature Biotechnology **33**, 941 (2015).
3. Tomaszewska, E., Soliwoda, K., Kadziola, K., Tkacz-Szczesna, B., Celichowski, G., Cichomski, M., Szmaja, W., Grobelny, J, Journal of Nanomaterials, 313081 (2013).
4. Kashkanova, A. D., Blessing, M., Gemeinhardt, A., Soulat, D., & Sandoghdar, V., Nature Methods, **19**(5), 586–593 (2022).
5. M. K. Rasmussen, J. N. Pedersen, R. Marie, Nature communications, **11**, 1-8 (2020).
6. M. K. Rasmussen, J. N. Pedersen, R. Marie, ACS Sens. **5**, 12, 4057–4063 (2020)