Protein corona affects cellular uptake and intracellular trafficking of lipid nanoparticles

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Due to their intrinsic chemical-physical properties, lipid nanoparticles (NPs) are increasingly being employed as nanocarriers of therapeutic agents. Indeed, their high cytocompatibility, ease of functionalization and peculiar structure of closed bilayer vesicle, make them the most promising class of organic NPs for the treatment of many diseases.[1] Despite the advances in biomedical applications of NPs and numerous publications, few NPs have made it to clinical trials and even fewer have reached clinical practice.[2] This wide gap between bench discoveries and clinical applications is mainly because of our limited understanding of the biological identity of NPs. Under *in vivo* conditions lipid NPs get covered by an outer protein layer, which is formed when they come in contact with biological media (e.g. blood or plasma) and is commonly referred to as protein corona (PC).[3,4] To precisely predict the biological response to NPs, a deeper understanding of their in vivo biological identity (i.e., the PC structure) is needed. Thus, the PC is emerging as the 'game-changer' for the clinical application of NPs. However, so far it is not clearly understood how the PC impacts at cellular and sub-cellular levels and this is a crucial aspect for *in vivo* applications of biomaterials, especially for drug and gene delivery. To tackle this issue, we explored cellular uptake and intracellular dynamics of multicomponent lipid NPs in HeLa cells, by means of confocal fluorescence microscopy. Cellular uptake of lipid NPs was investigated by colocalization of fluorescence signals arising from lipid NPs and endocytic vesicles (Figure 1, panels A-C). According to previous findings, we focused on macropinocytosis, clathrin- and caveolin-mediated endocytosis (CME). Colocalization analysis of confocal images (Figure 1, panels D-F) showed that lipid NPs are mainly internalized by macropinosomes, while in presence of the PC clathrin-mediated endocytosis is the principal internalization pathway. Intracellular dynamics of lipid NPs was investigated by fluorescence correlation spectroscopy (FCS). In detail, we employed a fluorescence-based spatiotemporal fluctuation analysis method that makes possible to detect the mode of motion of vesicles from imaging, in the form of a mean square displacement (MSD) versus time-delay plot (image-derived MSD, hereafter referred to as *i*MSD).[5,6] The intracellular dynamics of lipid NPs and NP-PC complexes was compared to those of clathrin-coated endocytic vesicles, caveolae and micropinosomes. As Figure 2 clearly shows, the dynamical behaviour of lipid NPs closely resembled that of micropinosomes. On the other hand, dynamics of NP-PC complexes was more similar to that of clathrin-coated endocytic vesicles. By coupling results from colocalization studies and intracellular dynamics experiments, we claim that the PC is responsible for a switch in the internalization processes of lipid NPs, which, in turn, affects their intracellular trafficking mechanism.
These effects could be particularly relevant for a clinical use of NP-based delivery systems, whose cellular uptake and intracellular mode of motion determine their biological outcome. We predict that precise understanding of the role of NP-PC will yield fundamental insights and novel opportunities for accelerating the clinical translation of NPs from bench to bedside.

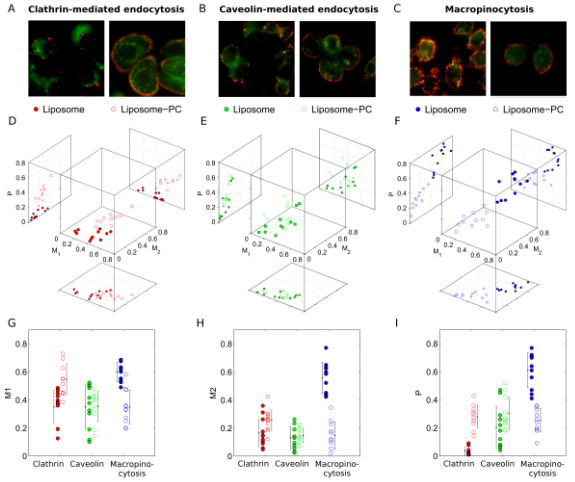

Figure 1: Colocalization images of red-labelled lipid NPs with with green-labelled endocytic vesicles, for (A) clathrin-mediated endocytosis, (B) caveolin-mediated endocytosis and (C) macropinocytosis. Results are expressed in terms of Manders coeffients (M1, M2) and Pearson's correlation coefficient, separately for each of uptake processes (D, E, F, respectively). (G, H, I) Projections of the measured values along the coordinate axes. Circles and circumferences correspond to liposome and liposome-PC complexes, respectively.

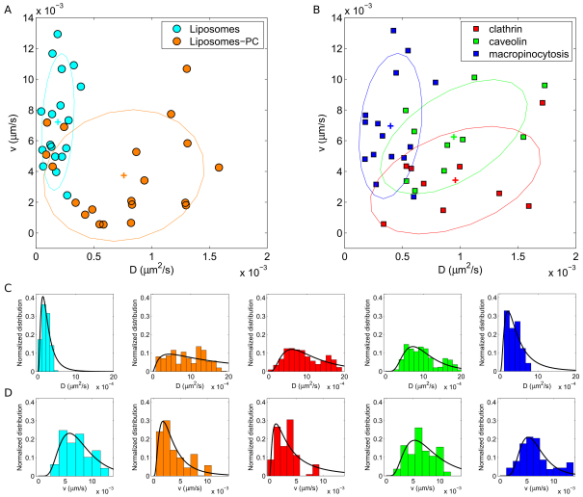

Figure 2: Dynamic parameters of (A) liposomes, liposome-PC complexes and (B) endocytic vesicles. Projections along the coordinate axes to obtain the corresponding distributions of (C) diffusion coefficients and (D) speed of the investigated objects. Distributions are weighted on the number of spots acquired in each time-series and take into account the experimental errors arising from the iMSD processing.

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