Making sense of structure in complex gene-delivery formulations using Fluorescence Cross-Correlation Spectroscopy (FCCS) and Small-Angle X-ray Scattering (SAXS)

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New advanced materials in life sciences, such as lipid-based nucleic acid nanoparticles for gene therapeutics, are growing in complexity and employing an ever-growing number of components for improved functionality. Understanding how the different material components interact with each other, as well as the emerging formulation nanostructures, is crucial, but it also poses a new challenge in characterization. Here we present two examples of advanced characterization using Fluorescence Cross-Correlation Spectroscopy (FCCS) [1,2] and Small-Angle X-ray Scattering (SAXS) [3] to address both these challenges. In the first case, we use FCCS to quantify the coating of polymer-DNA cores with lipid shells [2]. By labelling the polymer cores and liposome shells with green and red fluorescent dyes, respectively, we can follow the correlations between the motions of lipids and polymers. Hence, we can distinguish the cases where the cores and shells move together as a single entity (hence, indicative of successful coating), from those where cores and shells move freely and independently (Figure 1A). In the second case, we use SAXS to unravel the internal structure of lipid-DNA nanoparticles. While the scattering pattern identifies a lamellar structure composed of lipid bilayers with DNA sandwiched in between them, analysis of the lamellar peak shape allows an estimation of the number of lamellar-DNA layers (Figure 1B) [3].

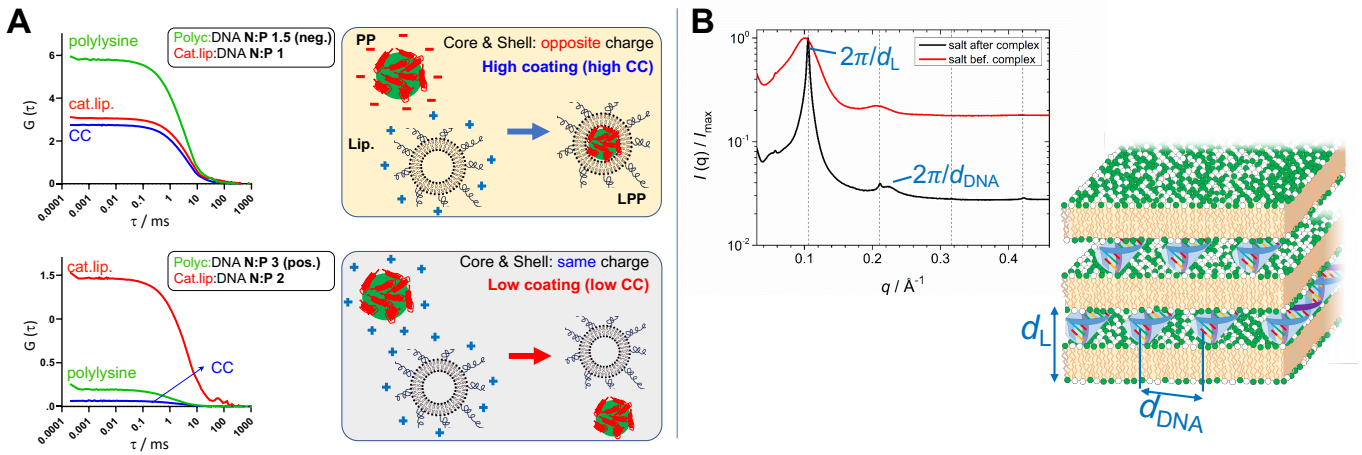

Figure 1. (A) FCCS results for lipid-polymer-DNA core-shell nanoparticles. A high amplitude of the cross-correlation (CC) function is indicative of high colocalization between liposomes and polycation-DNA cores. This happens predominantly when the cores and liposomes have opposite charges (top). When cores and liposomes have identical charge signs, nanoparticle coating was not observed (bottom). (B) SAXS results for two PEGylated lipid-DNA nanoparticle systems prepared in different salt conditions. Although both structures show a lamellar-type structure, particles prepared in high salt conditions have broader Bragg peaks, hinting at a smaller number of lamellar layers.

[1] Gómez-Varela, A.I. et al., *J. Biophotonics*, **2021**, *14*, e202000200.
[2] Paris, J.L., et al., *bioRxiv preprint*, **2022**, https://doi.org/10.1101/2022.02.06.479316.
[3] Silva, B. F. B. *et al., Biochim. Biophys. Acta-Biomembr*, **2014**, *1838*, 398–412