# Quantitative colocalization between cationic liposomes and DNA revealed by Fluorescence Cross-Correlation Spectroscopy

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COVID-19 mRNA-based vaccines have exhibited the importance of lipid-nucleic acid nanoparticles. One critical aspect in the development of such formulations is quantifying the extent of association (i.e. co-localization) between lipids and DNA, which thus far has been difficult to obtain quantitatively. Here we propose an approach based on fluorescence cross-correlation spectroscopy (FCCS) [1] to overcome this limitation [2]. The method consists of following the dynamics of lipids and DNA fluorescently labeled with two distinct dyes (red and green, respectively). By following the correlations between the motions of lipids and DNA the method is able to distinguish the cases where the lipid and DNA move together in the same particles, from those where non-complexed lipid and DNA move freely and independently (Figure 1). Hence, the co-localization between lipids and DNA can be determined. Importantly, the number of DNA molecules per lipid nanoparticle, which is an important parameter difficult to determine experimentally, can also be extracted.
FCCS therefore becomes a powerful methodology to understand the interactions between lipids and DNA/mRNA; understand their phase behavior and formulations; and to accelerate mRNA vaccine development. The methodology can also be extended to other colloidal systems involving association/dissociation between two nanosized species as long as they can be fluorescently labelled.

## REFERENCES

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[2] A.I. Gómez-Varela, R. Gaspar, A. Miranda, J.L. Assis, R.R.H.F. Valverde, M. Einicker-Lamas, B.F.B. Silva, P.A.A. De Beule, J. Biophotonics 14 (2021) e202000200.

## FIGURES

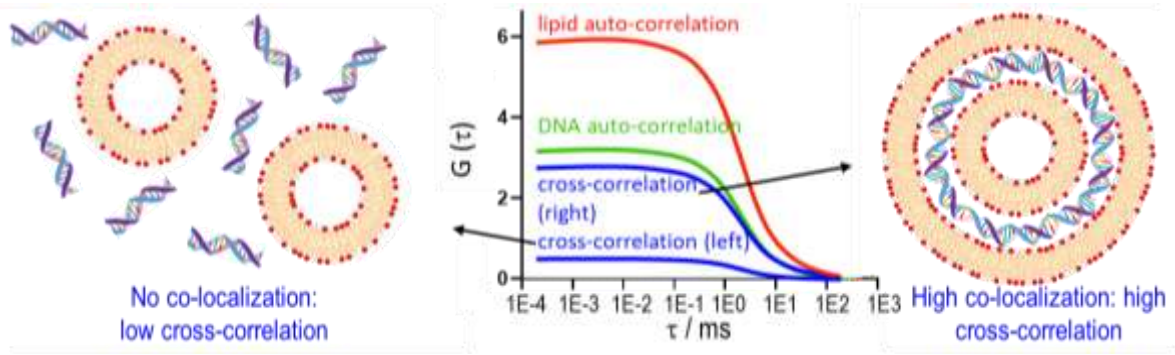


**Figure 1:** Illustration of the use of FCCS to monitor the formation of cationic liposome – DNA complexes. Liposomes and DNA are labelled with two spectrally-resolved dyes and their motions are analyzed simultaneously, which allows determining the individual auto- and cross-correlation functions. From the amplitude of the cross-correlation a quantitative measure of the co-localization between liposomes and DNA can be obtained. Non-bound liposomes and DNA have very low cross-correlation (left). Lipid-DNA complexes show high cross-correlation amplitudes (right)