Synchrotron SAXS and Confocal Microscopy: A Synergic Interplay Towards The Rational Design of Lipid Nanocarriers with Superior Efficiency

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Gene-based therapeutic approaches are based upon the concept that, if a disease is caused by a mutation in a gene, then adding back the wild-type gene should restore regular function and attenuate the disease phenotype. To deliver the gene of interest, both viral and nonviral vectors are used. Viruses are efficient, but their application is hindered by detrimental side effects. Among nonviral vectors, cationic liposomes are the most promising candidates for gene delivery and form stable complexes with nucleic acids (lipoplexes) [1]. Despite several advantages over viral vectors, the transfection efficiency (TE) of lipoplexes is too low compared with those of engineered viral vectors. This is due to lack of knowledge about the interactions between complexes and cellular components. Rational design of efficient lipoplexes requires deeper comprehension of the interactions between the vector and the DNA as well as the cellular pathways and mechanisms involved [2, 3]. The importance of the lipoplex structure in biological function is revealed in the application of synchrotron small-angle X-ray scattering in combination with functional TE measurements and fluorescence confocal microscopy. After showing how the nanostructure of lipoplexes can change upon interaction with cellular membranes and how such changes affect the delivery efficiency, we will discuss the case of cholesterol-containing lipoplexes with superior TE [4, 5]. We prove that cholesterol-containing lipoplexes enter the cells using different endocytosis pathways that are strictly related to their nanostructure. Formulations with high cholesterol content efficiently escape from endosomes and exhibit a lamellar-nonlamellar phase transitions in mixture with biomembrane mimicking lipid formulations. These studies highlight the enrichment in cholesterol as a decisive factor for transfection and will contribute to the rational design of lipid nanocarriers with superior TE. In the second part of the talk, we will introduce an emerging concept in lipid-mediated gene delivery, i.e. the lipoplex restructuring upon interaction with biological fluids. During both in vitro and in vivo transfection, lipoplexes interact with media containing proteins that compete for the nanoparticle surface and modify their biological identity. The interaction with proteins, besides giving novel interfacial properties to the vectors, can easily affect their inner structure, where lipid and genetic material co-exist. After the interaction with plasma, the inner structure of the investigated nanovectors is altered, depending on lipid composition and amount of plasma, still keeping their payload. Very interestingly, different phase alterations are observed by synchrotron SAXS involving the rearrangement of the supramolecular structure into large and small cubic phases. Those findings are of high biological relevance, as the lipid phase arrangement in cubic symmetry is one of the factors associated both to the vector fusogenic attitude, and, on the other hand, to its readiness to payload release to the final target.

1. Caracciolo, G.; Amenitsch, H. Cationic liposome/DNA complexes: From structure to interactions with cellular membranes. *European Biophysics Journal* **2012,** *41* (10), 815-829.
2. Pozzi, D.; Marchini, C.; Cardarelli, F.; Rossetta, A.; Colapicchioni, V.; Amici, A.; Montani, M.; Motta, S.; Brocca, P.; Cantù, L. Mechanistic understanding of gene delivery mediated by highly efficient multicomponent envelope-type nanoparticle systems. *Molecular pharmaceutics* **2013,** *10* (12), 4654-4665.
3. Pozzi, D.; Marchini, C.; Cardarelli, F.; Salomone, F.; Coppola, S.; Montani, M.; Zabaleta, M. E.; Digman, M.; Gratton, E.; Colapicchioni, V. Mechanistic evaluation of the transfection barriers involved in lipid-mediated gene delivery: Interplay between nanostructure and composition. *Biochimica et Biophysica Acta (BBA)-Biomembranes* **2014,** *1838* (3), 957-967.
4. Pozzi, D.; Marchini, C.; Cardarelli, F.; Amenitsch, H.; Garulli, C.; Bifone, A.; Caracciolo, G. Transfection efficiency boost of cholesterol-containing lipoplexes. *Biochimica et Biophysica Acta - Biomembranes* **2012,** *1818* (9), 2335-2343.
5. Pozzi, D.; Cardarelli, F.; Salomone, F.; Marchini, C.; Amenitsch, H.; La Barbera, G.; Caracciolo, G. Role of cholesterol on the transfection barriers of cationic lipid/DNA complexes. *Applied Physics Letters* **2014,** *105* (7), 073701.