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Small Angle Neutron Scattering for Investigation of Next-Generation Delivery Systems for RNA

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After the licensing of lipid nanoparticles (LNPs) comprising messenger RNA (mRNA) for vaccination against COVID-19, nanoparticles comprising mRNA for pharmaceutical application have been gaining increasing attention in the scientific community.

mRNA is a single stranded RNA, which is the template for the synthesis of proteins by the cell. For its use as the active ingredient in pharmaceutical products, the molecular properties of mRNA have been optimized in terms of stability and activity. Several different mRNA formats, such as modified RNA (mRNA), which was used in the first COVID vaccines, self-amplifying RNA (saRNA), or circular RNA (circRNA) are by now considered for pharmaceutical applications. Virtually any protein can be expressed by mRNA, and various types of therapeutic intervention, including cancer therapy, protein replacement, gene editing, and vaccination against infectious diseases.

LNPs as used in the COVID-19 vaccines consist of four lipid components, namely an ionizable lipid, a phospholipid, cholesterol and a polyethylenimine-functionalized lipid (PEG-lipid) in a relatively narrow range of molar composition. LNPs have been intensely investigated, including by use of small angle X-ray scattering (SAXS) and small angle neutron scattering (SANS), leading to some common consensus on their key structural features. While for vaccines against viral infections, LNPs in combination with modified mRNA (modRNA) have proven to be successful, for other applications, and other mRNA formats, other types of delivery systems may be more appropriate.

Here we give examples where SANS, in combination with SAXS, light scattering, and other techniques, was used for extended characterization of potential next-generation delivery systems for mRNA. Different types of lipid- and polymer-based nanoparticles and different mRNA formats were investigated. The structure and molecular organization could be accurately resolved and correlated with biological activity. Such insight can be the basis for assembly of tailored delivery systems for future applications, when certain structural features are required of the intended targeting and release characteristics.

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