



Supporting Information

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Mechanistic Studies of an Automated Lipid Nanoparticle Reveal Critical Pharmaceutical Properties Associated with Enhanced mRNA Functional Delivery In Vitro and In Vivo

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Title

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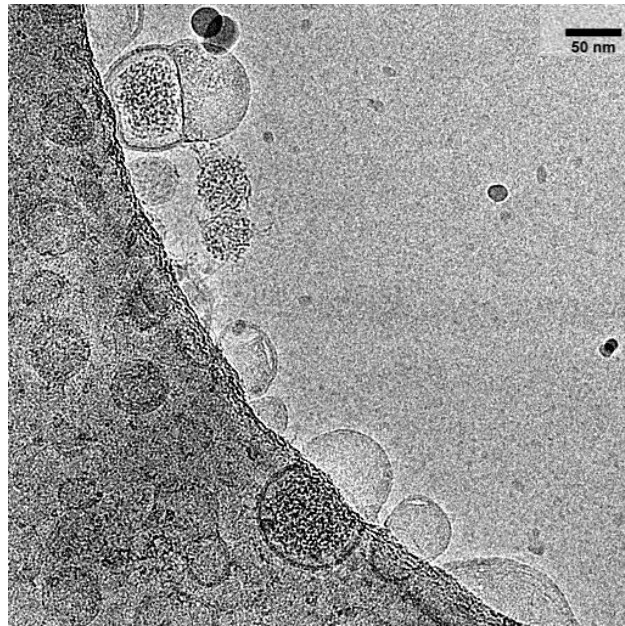


Figure S1. Morphology of large automated mRNA LNPs imaged by cryoTEM.

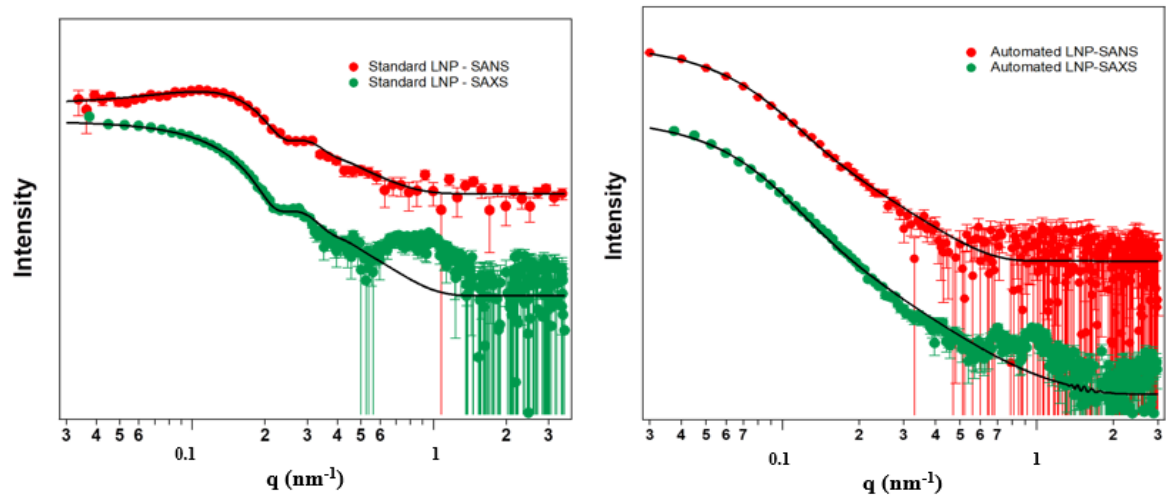


Figure S2. Structural characterisation of mRNA LNPs prepared from the standard platform (Standard LNPs, a) and the automated platform (Automated LNPs, b) using SANS and SAXS. The solid lines represent the best fitting using polydisperse core with N shells model.

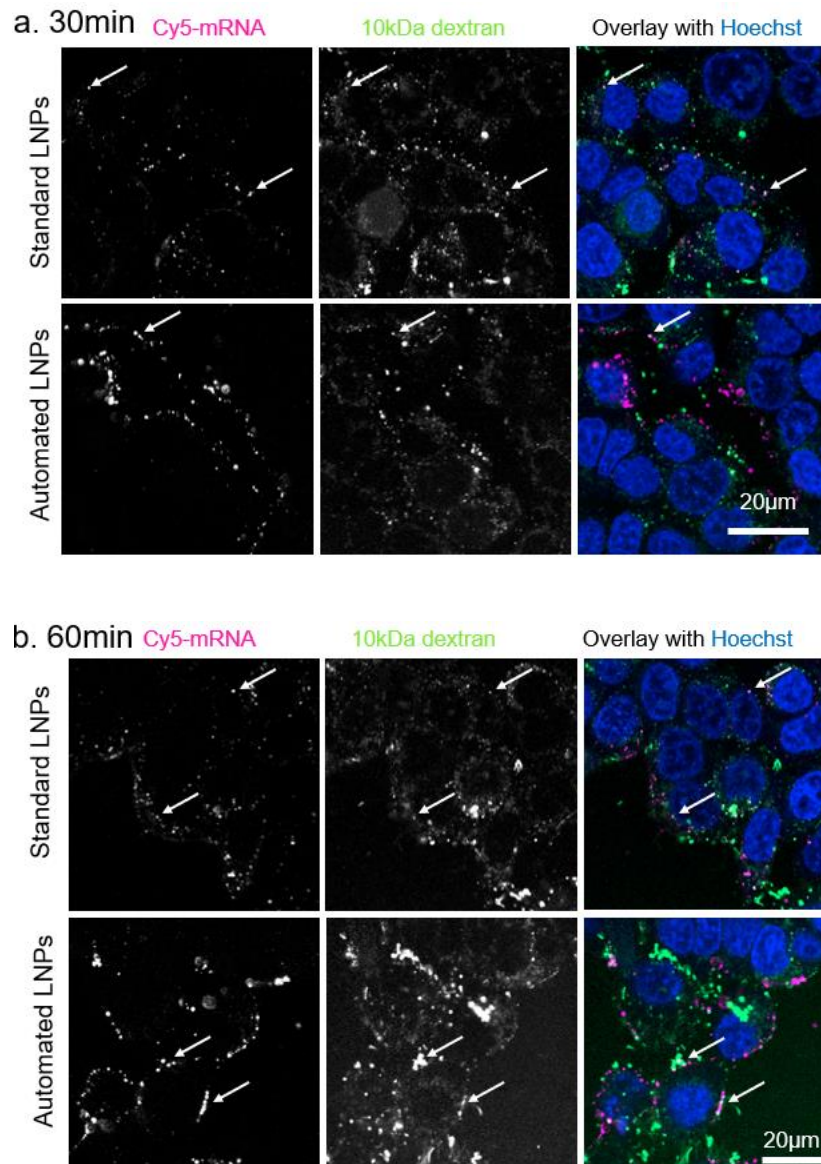
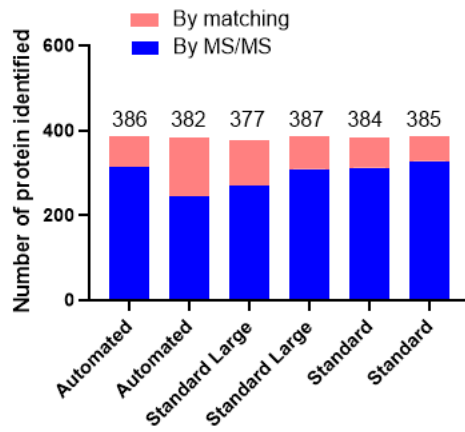


Figure S3. Colocalisation of LNPs with 10 kDa dextran when incubating for 30 min (a) and 60 min (b) in H358 cell lines. Blue, nucleus. Pink, Cy5-mRNA. Green, 10kDa dextran. White arrow indicates colocalisation.

a.



b.

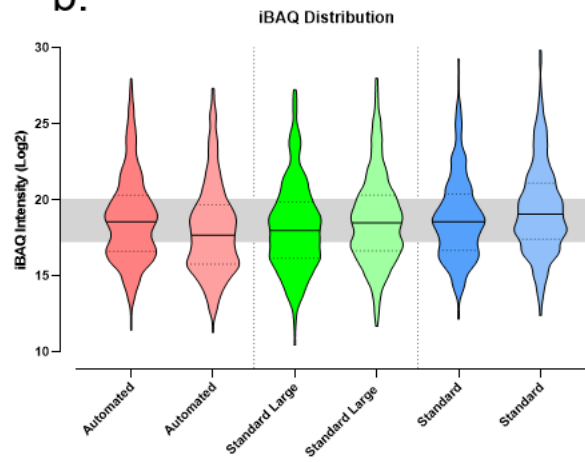


Figure S4. Characterisation of protein corona coating of LNP nanoparticles. a, the overall number of protein corona species identified by MS/MS sequencing and spectra matching. b, the overall intensity distribution of all identified corona protein species. Two biological repeats.

Table S1. Fitting parameters of SAXS and SANS using polydisperse core with 2 shells model

Fitting Parameters	SANS	SANS	SAXS	SAXS
	standard	automated	standard	automated
Scale	0.0679±0.0006	0.018±0.0001	0.0587±0.0003	0.00586±0.00002
Core (R. nm)	19.16±0.03	22.45±0.07	19.16±0.03	22.45±0.07
Polydispersity	0.162±0.002	0.402±0.002	0.162±0.002	0.402±0.002
SLD of core (nm ⁻²)	1.80E-04±1.18E-07	2.30E-04±2.38E-07	9.42E-04±1.89E-08	9.81E-04±3.19E-07
Shell 1 thickness (nm)	1.96±0.08	2.32±0.02	1.96±0.08	2.32±0.02
SLD of shell 1 (nm ⁻²)	1.60E-06±6.63E-07	1.21E-04±2.02E-06	9.45E-04±1.10E-07	9.14E-04±4.15E-07
Shell 2 thickness (nm)	4.12±0.02	4.10±0.02	4.12±0.02	4.10±0.02
SLD of shell 2 (nm ⁻²)	1.20E-04±2.68E-07	1.06E-04±9.32E-07	9.53E-04±4.54E-08	9.49E-04±1.71E-07
SLD of solvent (nm ⁻²)	1.50E-04	1.50E-04	9.50E-04	9.50E-04
Background	0.015	0.025	9.00E-05	0.0002

Table S2. SLD used for SAXS and SANS data fitting

Components	SLD (10^{-4} nm^{-2})	SLD (10^{-4} nm^{-2})
	neutron	X-ray
H ₂ O/D ₂ O	-0.56/6.4	9.5
mRNA	3.6	16
MC3	0.09	7.9
D7-Cholesterol	1.318	9.06
D83-DSPC	6.258	8.5
DMG-PEG	0.112	9.5
Core components		
27% D ₂ O+73% H ₂ O	1.5	9.5
mRNA	3.6	16
MC3+Cholesterol	0.42	8.2

Note: molecular volume cited from reference ²⁶

