Supplementary material: Impact of mixing and shaking on mRNA-LNP drug product quality characteristics

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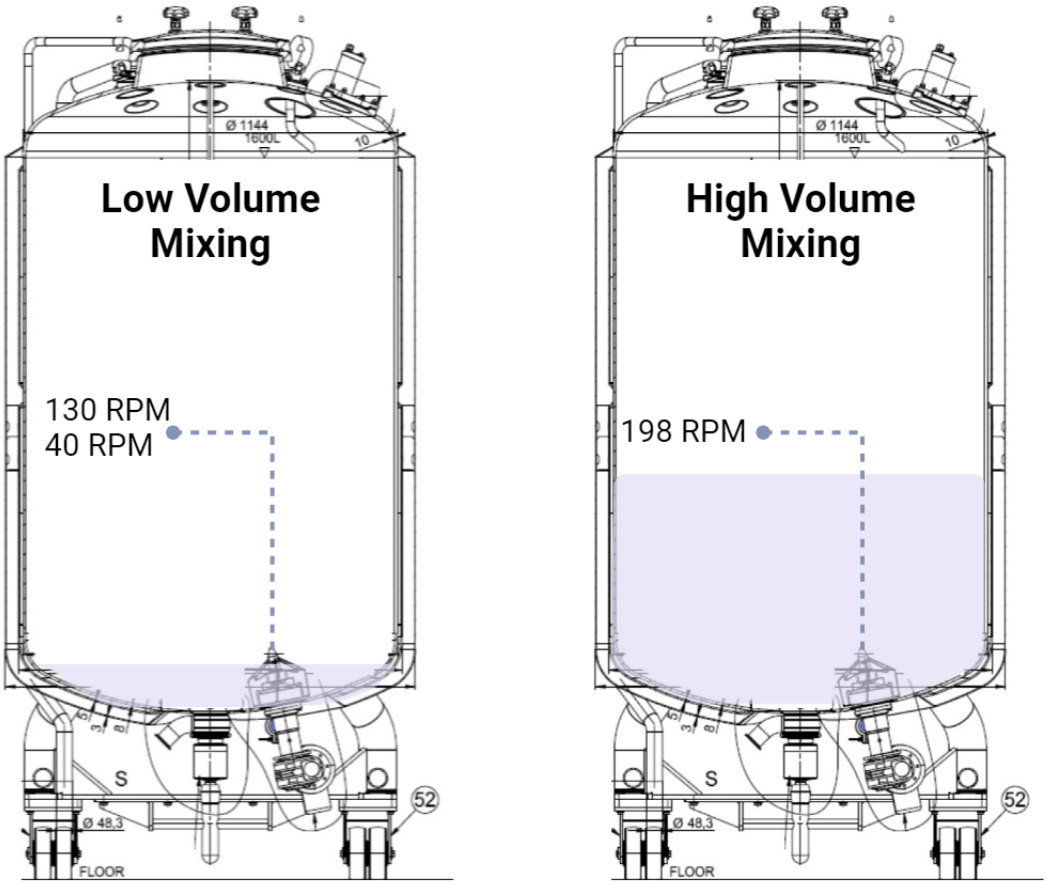

Figure S1: Image showing the liquid level, indicated in blue, for the low volume (135 L) and high volume (741 L) mixing setup discussed in the main manuscript. Note that in the high volume mixing set-up the mixer is completely covered.

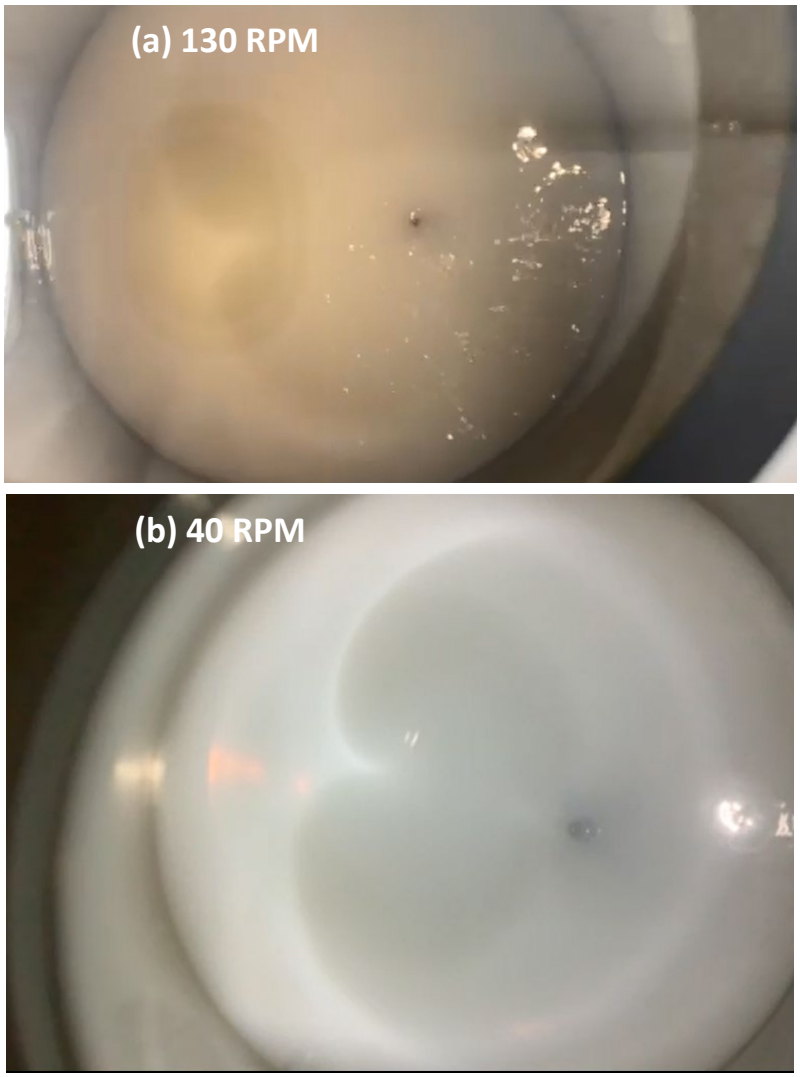

Figure S2: Image of the inside of the vessel during low volume mixing at (a) 130 RPM and (b) 40 RPM. The mixer can be seen as the dot right central of the image. Notice how mixing at 130 RPM disturbs the liquid surface significantly while the 40 RPM mixing speed does not seem to perturb the liquid surface.

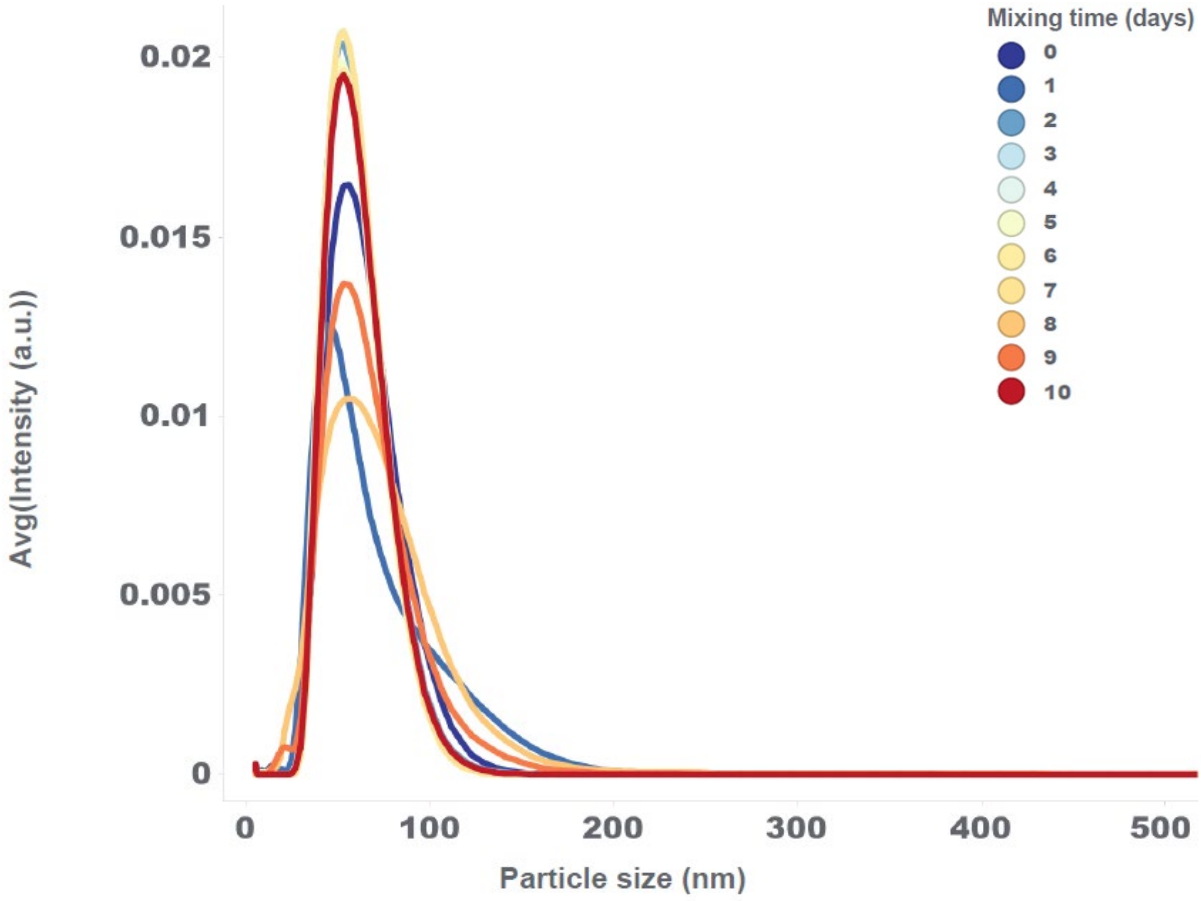

Figure S3: Particle size distributions for low volume mixing at 40 RPM for 10 days. All particle size distributions remain unimodal.

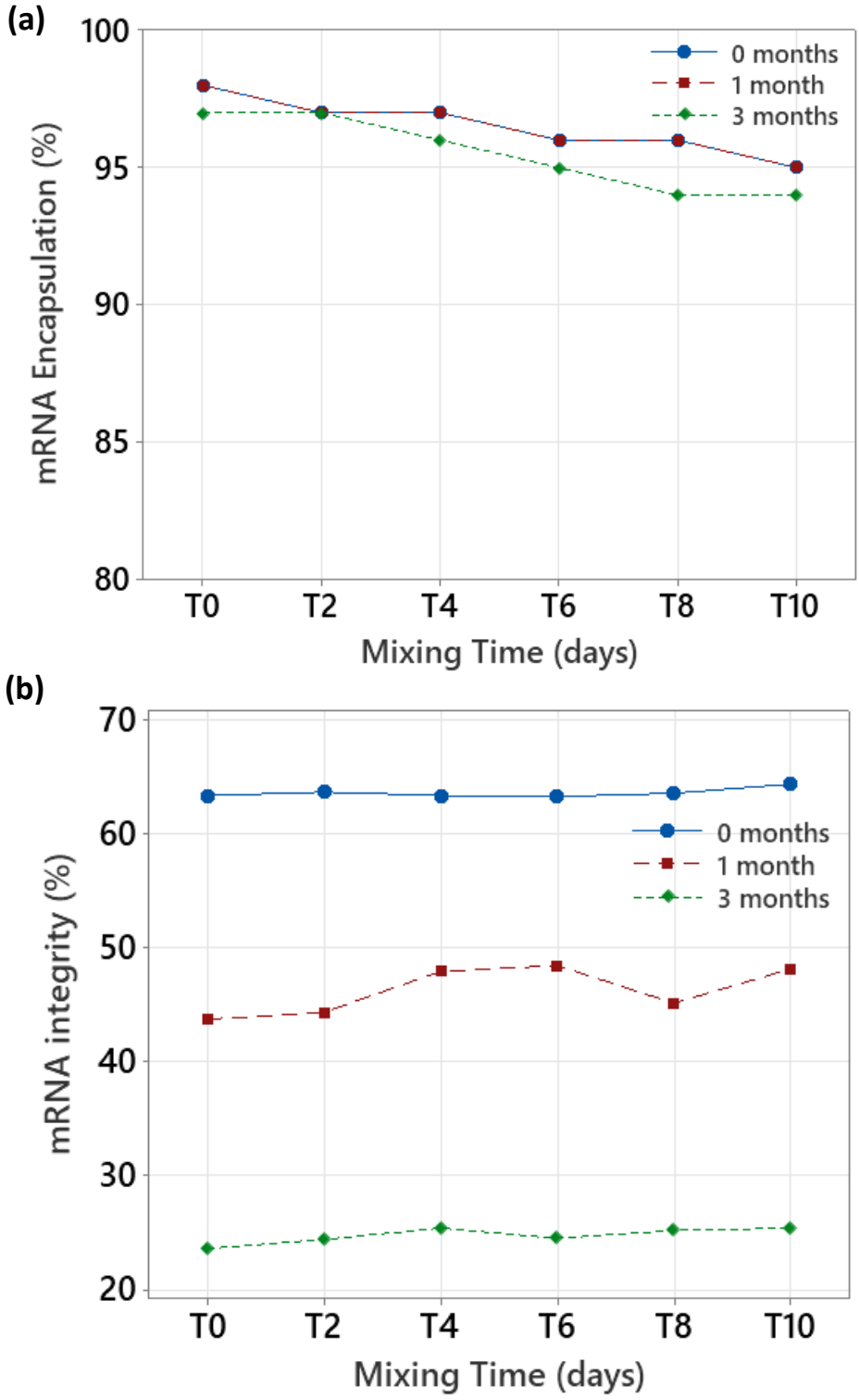

Figure S4: (a) mRNA encapsulation and (b) mRNA integrity, both as function of the mixing time in days. After reaching mixing time point samples are stored at room temperature up to 3 months, showing start of stability study (blue), one month (red) and 3 months (green).

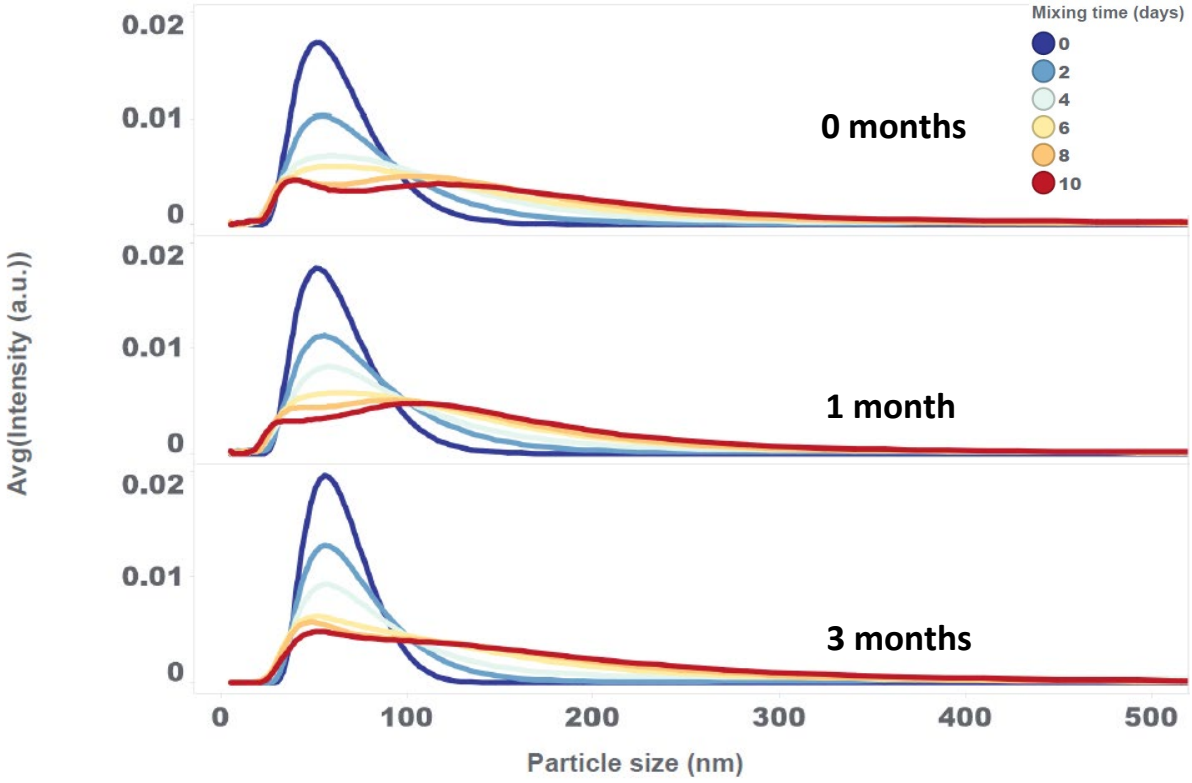

Figure S5: Particle size distributions measured with the Nanoflowsizer for each mixing time point. Results before stability, after one month stability at room temperature and after three months stability at room temperature are shown.

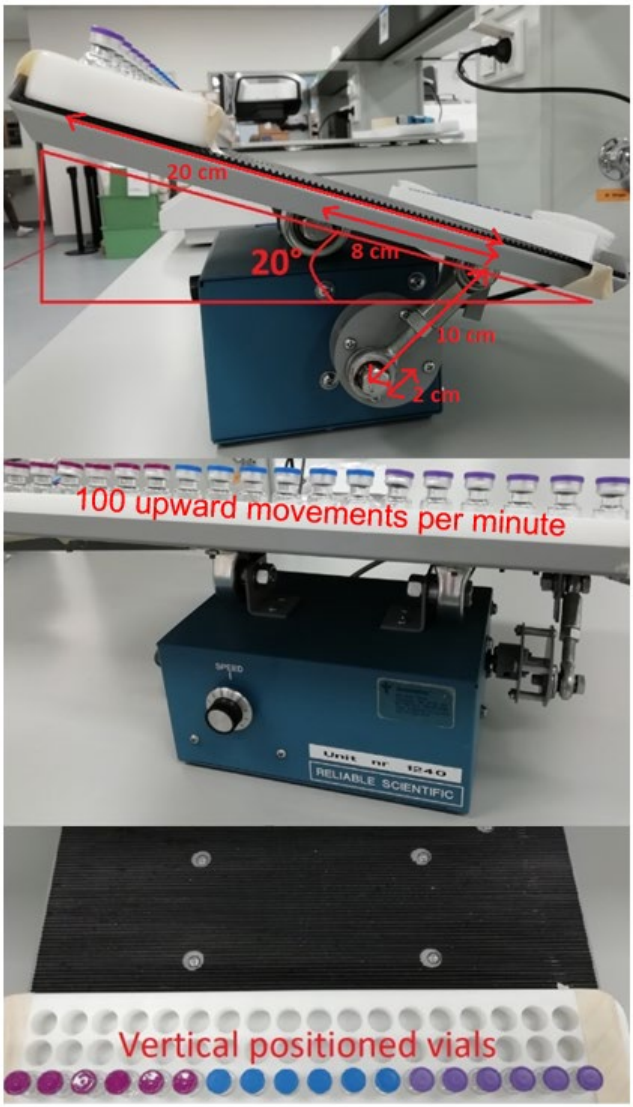

Figure S6: Single platform laboratory shaker used for the shaking experiments, set at 100 upward movements per minute.

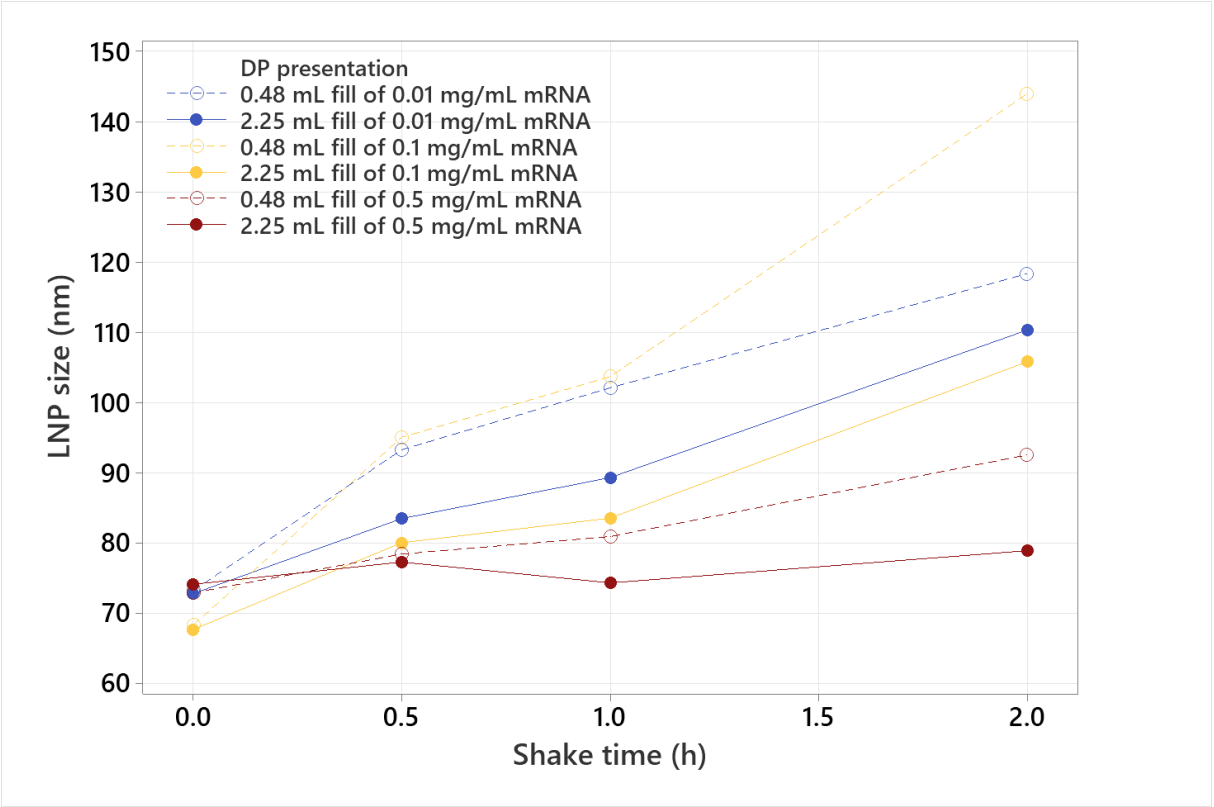

Figure S7: LNP size (measured with Zetasizer) as function of shake time. Different concentrations and fill volumes were tested. 2-hour shake time point for presentation with 0,48 mL fill and 0,1 mg/mL mRNA is considered an outlier. mRNA strain with double strain length compared to 16-hour shake tests was used.

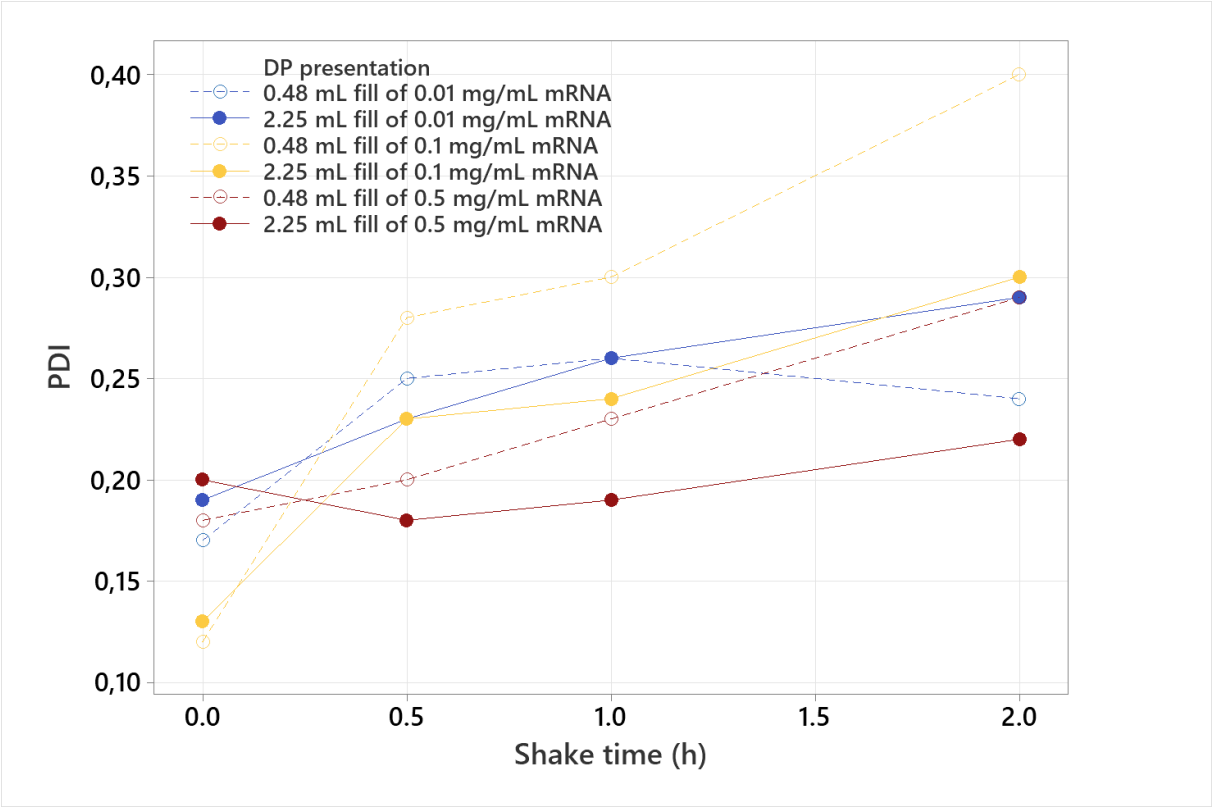

Figure S8: PDI (measured with Zetasizer) as function of shake time. Different concentrations and fill volumes were tested. 2-hour shake time point for presentation with 0,48 mL fill and 0,1 mg/mL mRNA is considered an outlier. mRNA strain with double strain length compared to 16-hour shake tests was used.

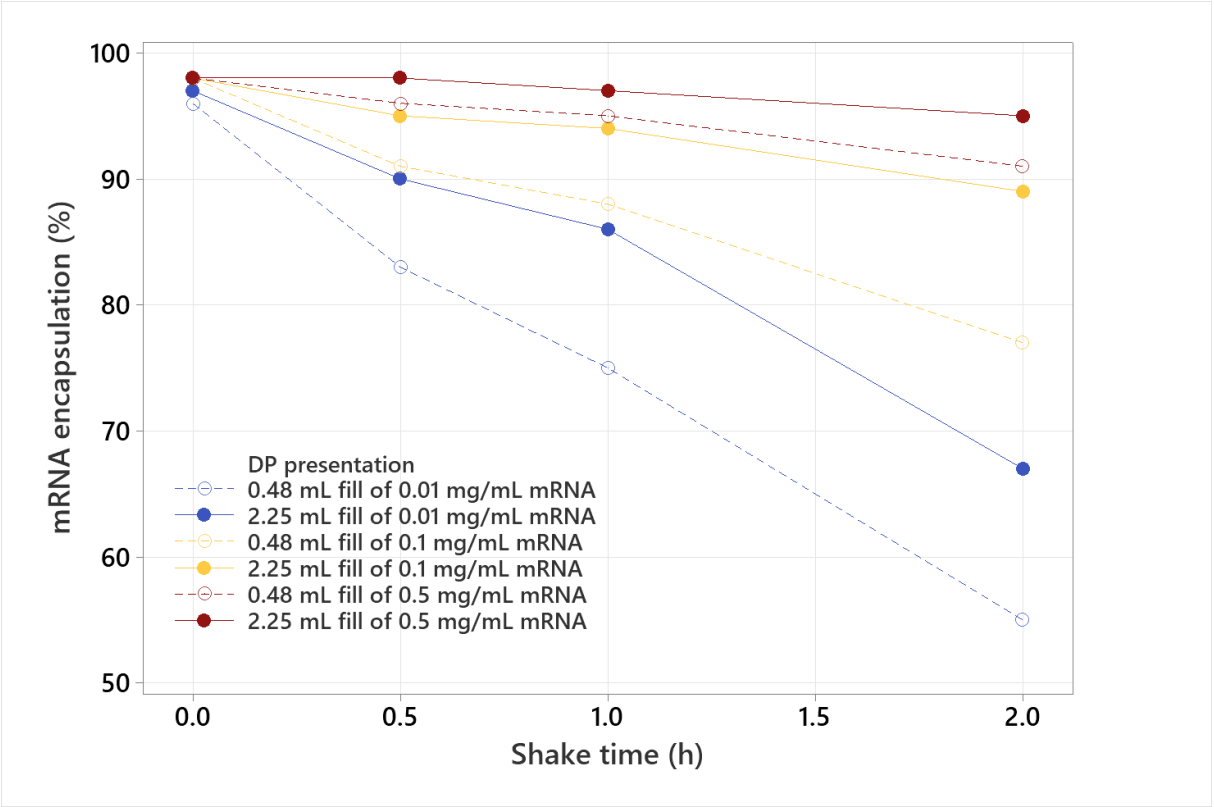

Figure S9: mRNA encapsulation (measured with Ribogreen) as function of shake time. Different concentrations and fill volumes were tested. mRNA strain with double strain length compared to 16-hour shake tests was used.

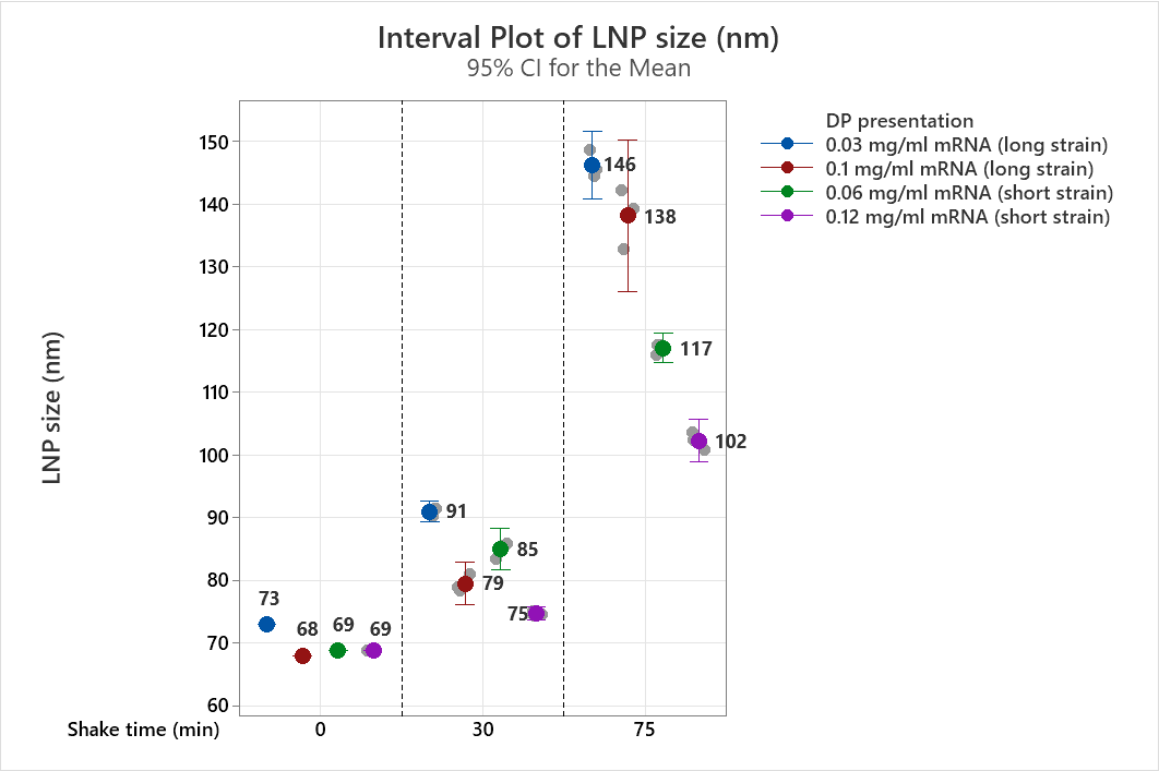

Figure S10: Interval plot of LNP size (measured with Zetasizer) in function of shake time for different formulations with different mRNA content. 3 separate shaking tests were performed for each of the formulations. The 0.03 and 0.1 mg/mL presentations consist of mRNA strain with double strain length compared to the 0.06 and 0.12 mg/mL presentations. All presentations have a 0.48 mL fill volume in a 2 mL glass vial.

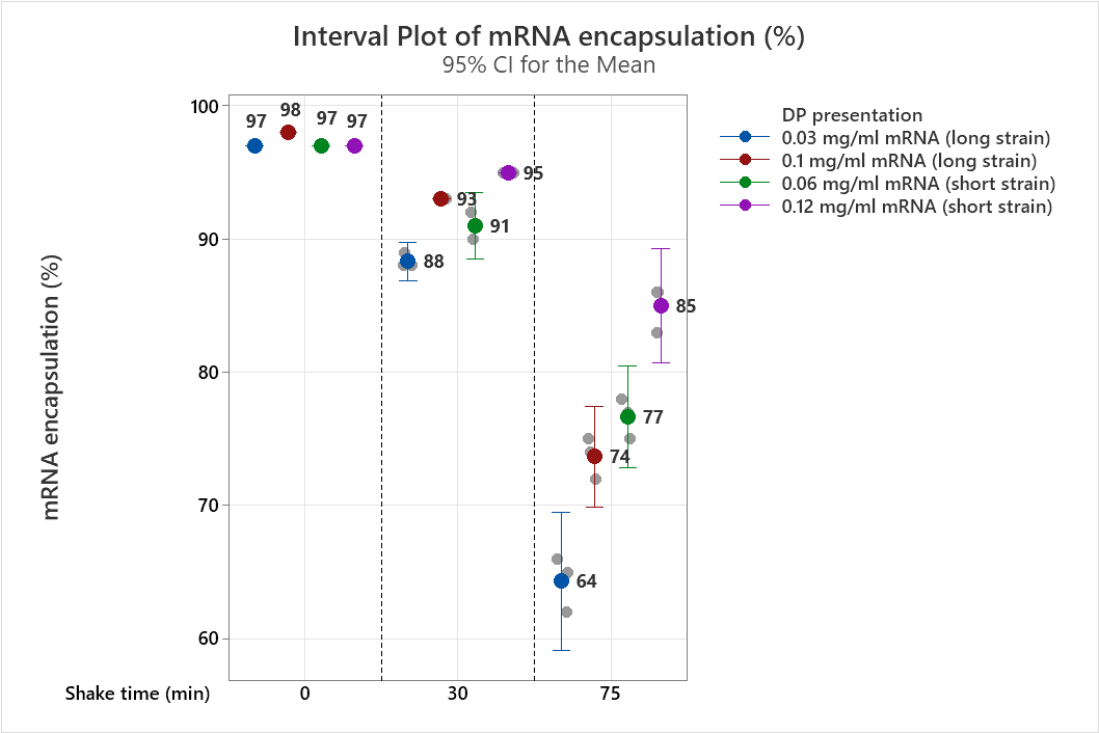

Figure S11: Interval plot of mRNA encapsulation (measured with Ribogreen) in function of shake time for different formulations with different mRNA content. 3 separate shaking tests were performed for each of the formulations. The 0.03 and 0.1 mg/mL presentations consist of mRNA strain with double strain length compared to the 0.06 and 0.12 mg/mL presentations. All presentations have a 0.48 mL fill volume in a 2 mL glass vial.