applied nano

Supplementary materials

1. Size distribution of CPP-liposomes

The size distribution by intensity of CPP-liposomes is shown in Figure S1.

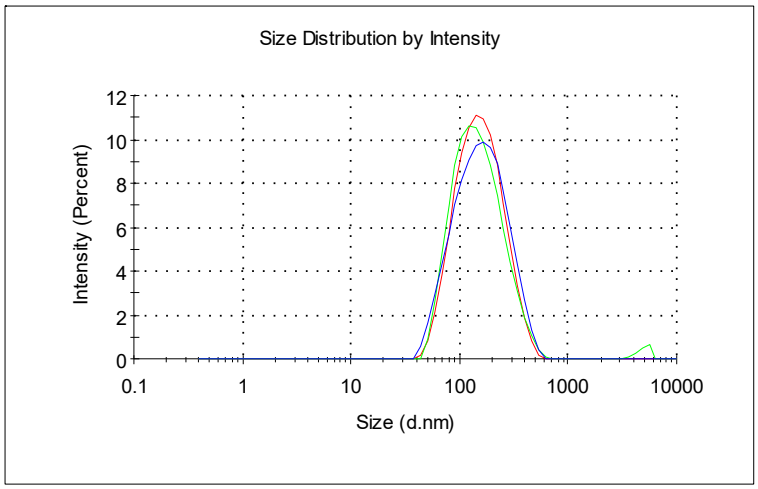

Figure S1. Size distribution by intensity of CPP-liposomes (n=3).

2. Influence of mRNA loading on liposomal characteristics

Liposomal characteristics depending on mRNA loading are shown in Figure S2.

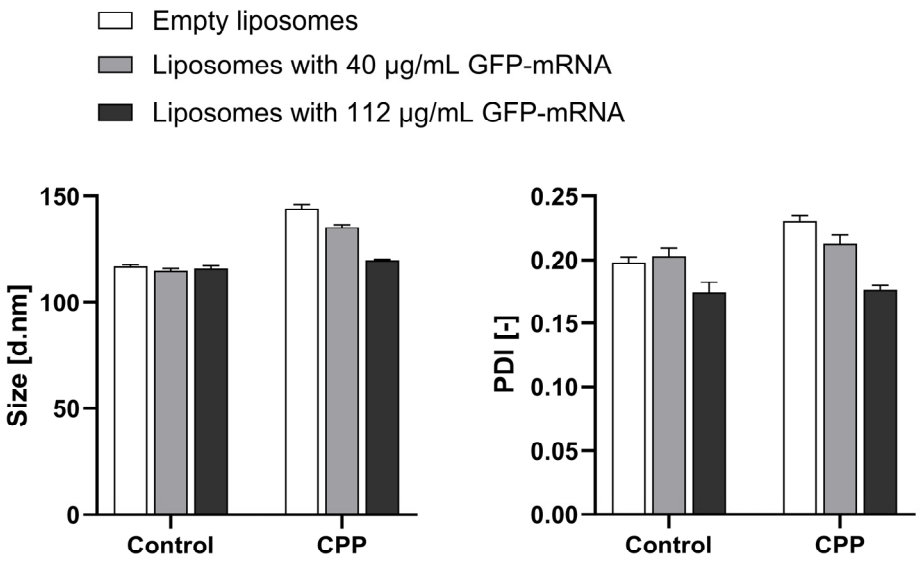

Figure S2. Influence of mRNA loading on liposomal characteristics.

3. Stability and release of mRNA out of liposomes in simulated gastric and intestinal fluid

Methods:

The stability and release of mRNA out of liposomes was tested in simulated gastrointestinal fluids. For this, control liposomes and CPP-liposomes loaded each with 40 µL/mL GFP-mRNA were prepared as described previously. Subsequently, liposomes were mixed with either fasted state simulated gastric fluid (FaSSGF), or fasted state

simulated intestinal fluid (FaSSIF) in a 1:1 ratio and incubated at 37 °C on a shaker at 400 rpm for 1 h. Samples were withdrawn before starting the study, and during the study at the following time points: 0, 15, 30 and 60 min. To assess stability and release, the concentration of free mRNA (mRNA not encapsulated in the liposomes) was determined by using Quant-iT RiboGreen RNA Assay Kit (Invitrogen). The fluids were prepared according to manufacturer's instructions (FaSSGF: 1.839 g buffer, 48.095 mL deionized water, 3 mg powder; FaSSIF: 2.082 g buffer, 48.06 mL deionized water, 112 mg powder; Biorelevant, London, UK).

The stability and release of mRNA out of liposomes in FaSSGF and FaSSIF is shown in Figure S3.

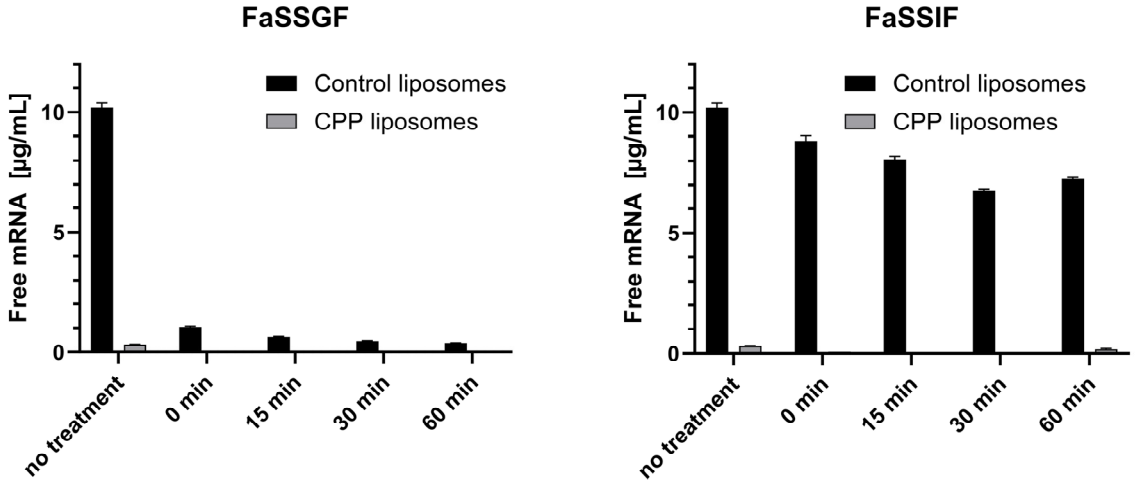

Figure S3. Stability and release of mRNA out of liposomes in FaSSGF and FaSSIF.

4. Liposomal stability in simulated gastric and intestinal fluid

Methods:

For stability testing of liposomes in simulated gastrointestinal fluids, liposomes were mixed with either fasted state simulated gastric fluid (FaSSGF), or fasted state simulated intestinal fluid (FaSSIF) in a 1:1 ratio and incubated at 37 °C on a shaker at 400 rpm for 1 h. Samples were withdrawn before incubation and after 15, 30 and 60 min. To assess the stability, the size and PDI of liposomes were determined. The fluids were prepared according to manufacturer's instructions (FaSSGF: 1.839 g buffer, 48.095 mL deionized water, 3 mg powder; FaSSIF: 2.082 g buffer, 48.06 mL deionized water, 112 mg powder; Biorelevant, London, UK).

The stability of the different liposomal formulations in FaSSGF and FaSSIF is shown in Figure S4.

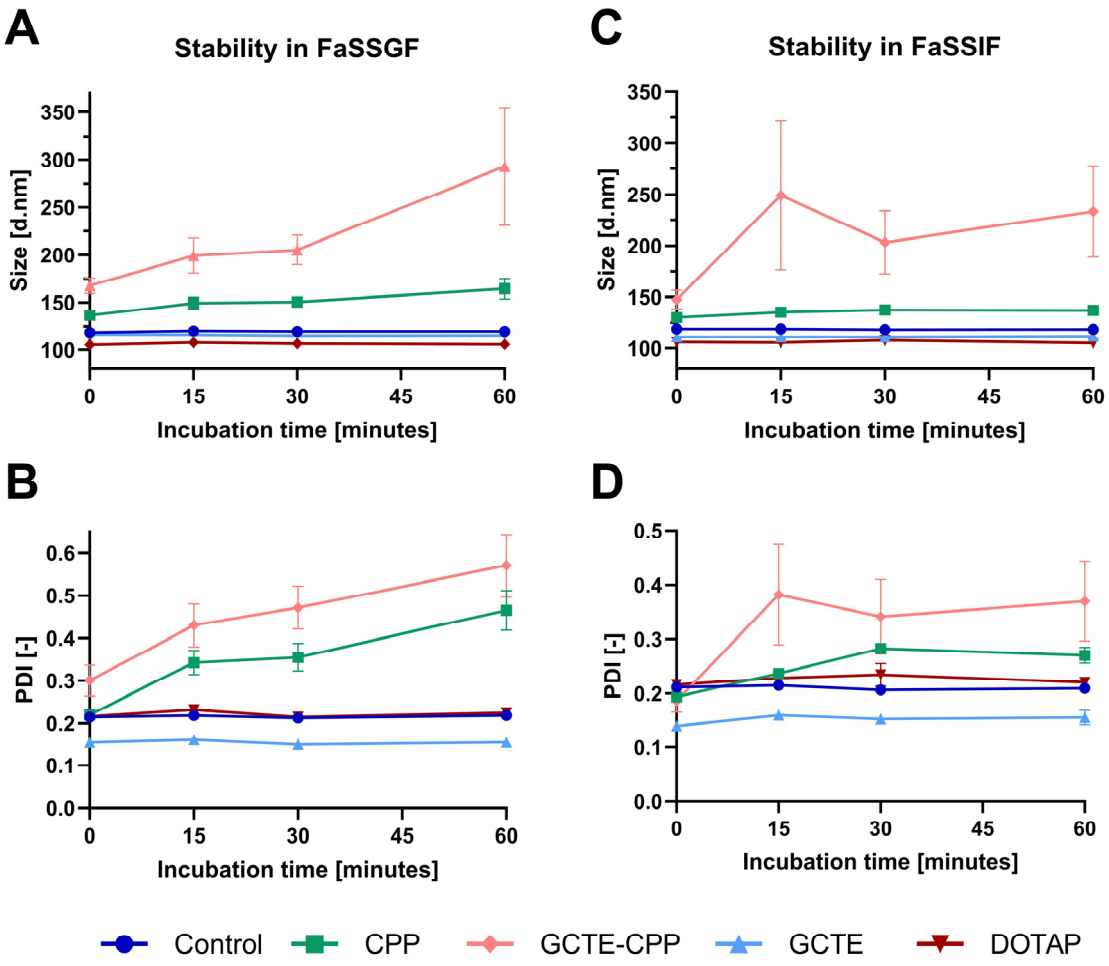

Figure S4. Stability of liposomal formulations in FaSSGF and FaSSIF.