Homework

## mRNA-LNP Delivery System:

1. Afterwards, various studies have been done to properly deliver mRNA across the cell membrane into cytosol, because mRNA directs the synthesis of proteins in the cytoplasm using various nanoparticles such as polymeric particles and liposomes [2, 16, 17].
2. . Loading mRNAs into LNPs is stable, leading to a successful delivery into cells [21].
   1. <https://pubmed.ncbi.nlm.nih.gov/26264835/>
   2. <https://www.researchgate.net/publication/280997102_Expression_kinetics_of_nucleoside-modified_mRNA_delivered_in_lipid_nanoparticles_to_mice_by_various_routes>
   3. Paper uploaded in folder.
   4. Subcutaneous, intramuscular and intradermal injection of the LNP-encapsulated mRNA translated locally at the site of injection for up to 10days.
3. As membrane fusion occurs, the entrapped nucleic acids in the LNPs escape and are released into the cytosol [29].
   1. Necessary for therapeutic effects to come into action.
   2. Paper uploaded in folder.
   3. <https://pubmed.ncbi.nlm.nih.gov/33786376/>
   4. <https://www.researchgate.net/publication/349018716_Cytosolic_Delivery_of_Nucleic_Acids_The_case_of_Ionizable_Lipid_Nanoparticles>
4. However, it has been reported that less than 2-3% of nucleic acids escape from the endosome and are released into the cytosol [30, 31].
   1. <https://www.nature.com/articles/nbt.2612>
   2. <https://www.researchgate.net/publication/280242406_Visualizing_lipid-formulated_siRNA_release_from_endosomes_and_target_gene_knockdown>
      1. siRNA release occurred invariably from maturing endosomes within ∼5-15 min of endocytosis
      2. Both papers above deal with siRNA only
5. In general, these helper lipids...enhance the delivery efficacy by promoting intracellular uptake and cytosolic entry [32, 33]
6. ...it can lead to disruption of the lipid bilayer, which promotes endosomal escape [34-38]

**On composition of LNPs and endosome release:**

One of the commonly used phospholipids is a phosphatidylcholine (PC), such as 1,2-distearyol-sn-glycero-3-phosphocholine (DSPC) and hydrogenated soybean PC (HSPC). DSPC is clinically applied such as siRNA therapeutics (Patisiran) and mRNA vaccines  
against SARS-CoV-2 (mRNA-1273 and BNT126b2) [5, 33]. **DSPC contains a saturated acyl chains, containing one or more double bonds, in the tail of the lipid and a relatively larger head group, which forms a cylinder-shaped geometry. Furthermore, the high melting temperatures (Tm ) value of this lipid provides highly stable LNP structure [39-41]. Due to its high stability, it inhibits membrane fusion with the endosomal membrane, which inhibits endosomal escape.** On the other hand, DOPE is considered as a fusogenic lipids. It contains two unsaturated acyl chains, containing hydrocarbon chain with single bond only, in the tail and relatively smaller head group, which forms cone-shape geometry [39, 42]. These unsaturated acyl chain lipids have low melting  
temperature (T m ) values and stabilize the non-bilayer hexagonal II (HII) phase. In the physiological temperature, DOPE forms a non-lamellar lipid phase due to the inverted hexagonal (H II) phase. This enables membrane fusion, bilayer disruption, that  
leads to endosomal escape [36, 43, 44]. **Additionally, DOPE is reported to enhance** the transfection efficacy when present in cationic lipid formulations by facilitating membrane fusion [44-46]

1. PEG immuno-genicity and anti-PEG antibodies need to be considered in developing LNP-based vaccines and therapeutics. According to recent studies, both SARS-CoV-2 mRNA LNP-based vaccines, mRNA-1273 (Moderna) and BNT126b2 (Pfizer/BioNTech), induced the amount of anti-PEG IgM and anti-PEG IgG in human samples [64, 65].
   1. Both studies show a common detection of anti-PEG antibodies before the vaccination due to previous exposure to PEG from cosmetics and PEG-containing medicines. However, both mRNA-1273 and BNT126b2 vaccines significantly induced anti-PEG IgM and anti-PEG IgG after the administration.
   2. Increase in anti-PEG antibodies depend on type of terminal chain and shedding rate

**Skipping part on mRNA-LNP production methods**

1. As various gene therapies enter clinical trials, LNPs have been considered as the most optimized and applicable delivery system for nucleic acids such as siRNA and mRNA because negatively charged nucleic acids interfere with the delivery to cell membranes and are degraded by endogenous nucleases in the body, making efficient delivery difficult without the aid of LNPs [88, 108].