

pKa tuning of ionisable lipids and cytosolic delivery of siRNA

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Background

Ionisable lipid nanoparticles deliver siRNA to cells, but most cargo stays trapped in endosomes. The apparent pKa of the ionisable lipid controls when particles become cationic during endosomal acidification.

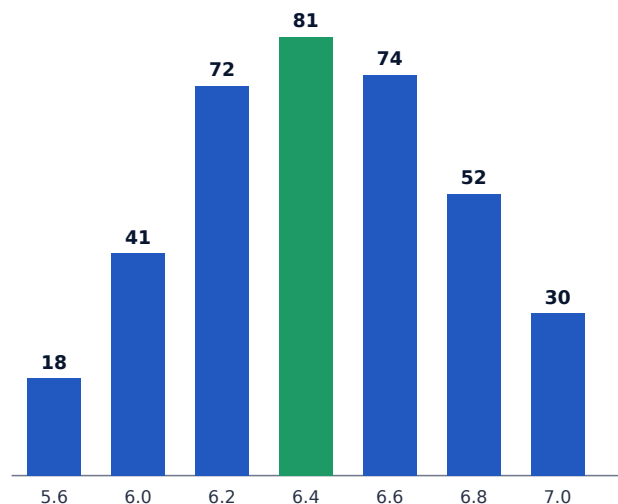
Aim

To relate apparent pKa to gene silencing in a hepatocyte model and a tumour cell model, using a matched lipid series.

Methods

- Seven ionisable lipids, apparent pKa 5.6 to 7.0 (TNS assay).
- LNPs by microfluidic mixing; 70 to 85 nm, PDI below 0.12.
- Reporter siRNA at 10 nM in hepatocyte and tumour cells; knockdown at 48 h.

Silencing vs apparent pKa



Reporter knockdown (%) in hepatocytes by apparent pKa.
Representative data.

Results

- Silencing peaked for lipids with apparent pKa 6.2 to 6.6 in both cell models.
- Lipids below pKa 6.0 were taken up but gave weak silencing, consistent with poor endosomal escape.
- Above pKa 6.8, particles were cationic at neutral pH and viability fell.

Conclusions

- An apparent pKa window of about 6.2 to 6.6 is a practical first screen.
- Uptake and escape should be measured separately when ranking formulations.