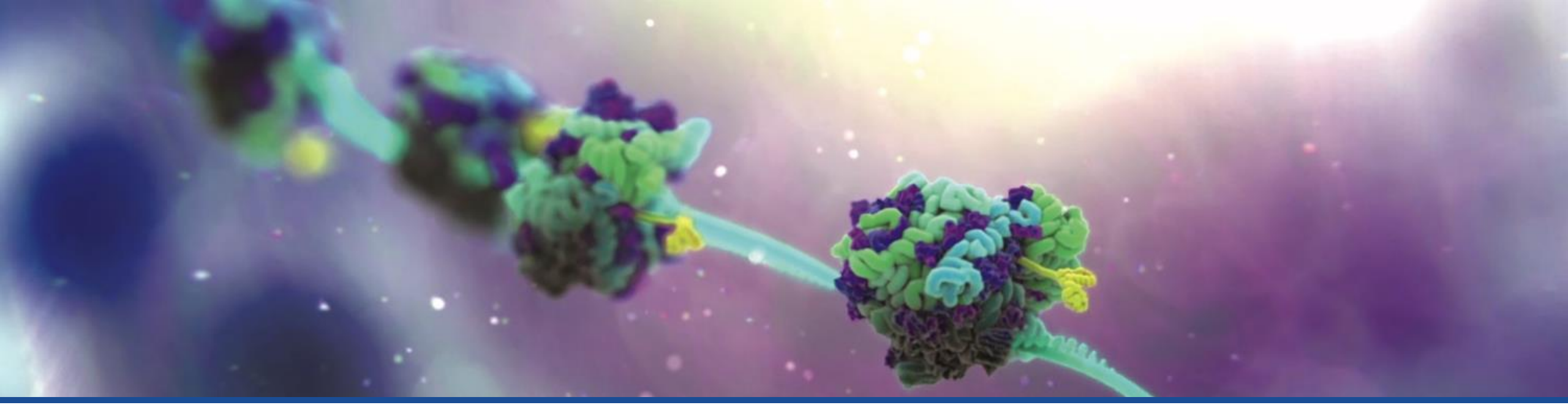

A Novel Serum-Insensitive Fusogenic Polyvalent Cationic Lipid for *in vivo* mRNA Delivery

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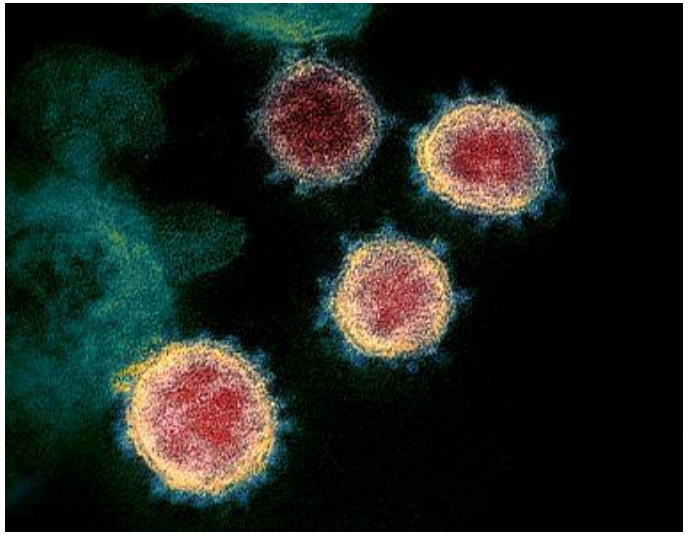
Classes of mRNA therapeutics

Vaccines

- Rapid development and manufacturing
- Non-microbial targets possible



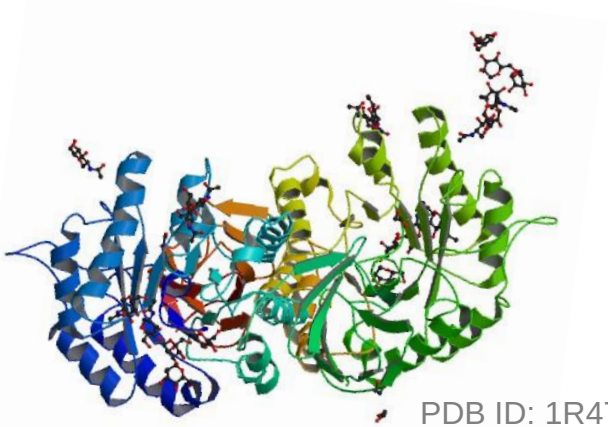
NIAID-RML

E.g., mRNA vaccine clinical trials:
NCT04283461 (SARS-CoV-2)
NCT03164772 (cancer vaccine)

Protein replacement

- Protein purification not required
- Post-translational modification occurs *in vivo*

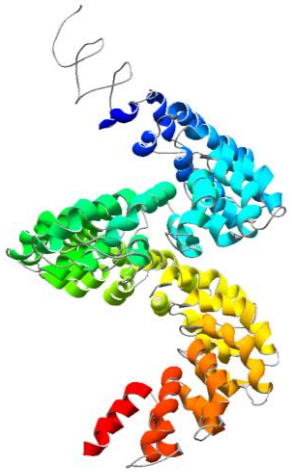
E.g., α-galactosidase A mRNA used to treat Fabry disease



PDB ID: 1R47

DeRosa, F., et al. *Mol Therapy* **2019**, *27* (4), 878–889.

Gene editing

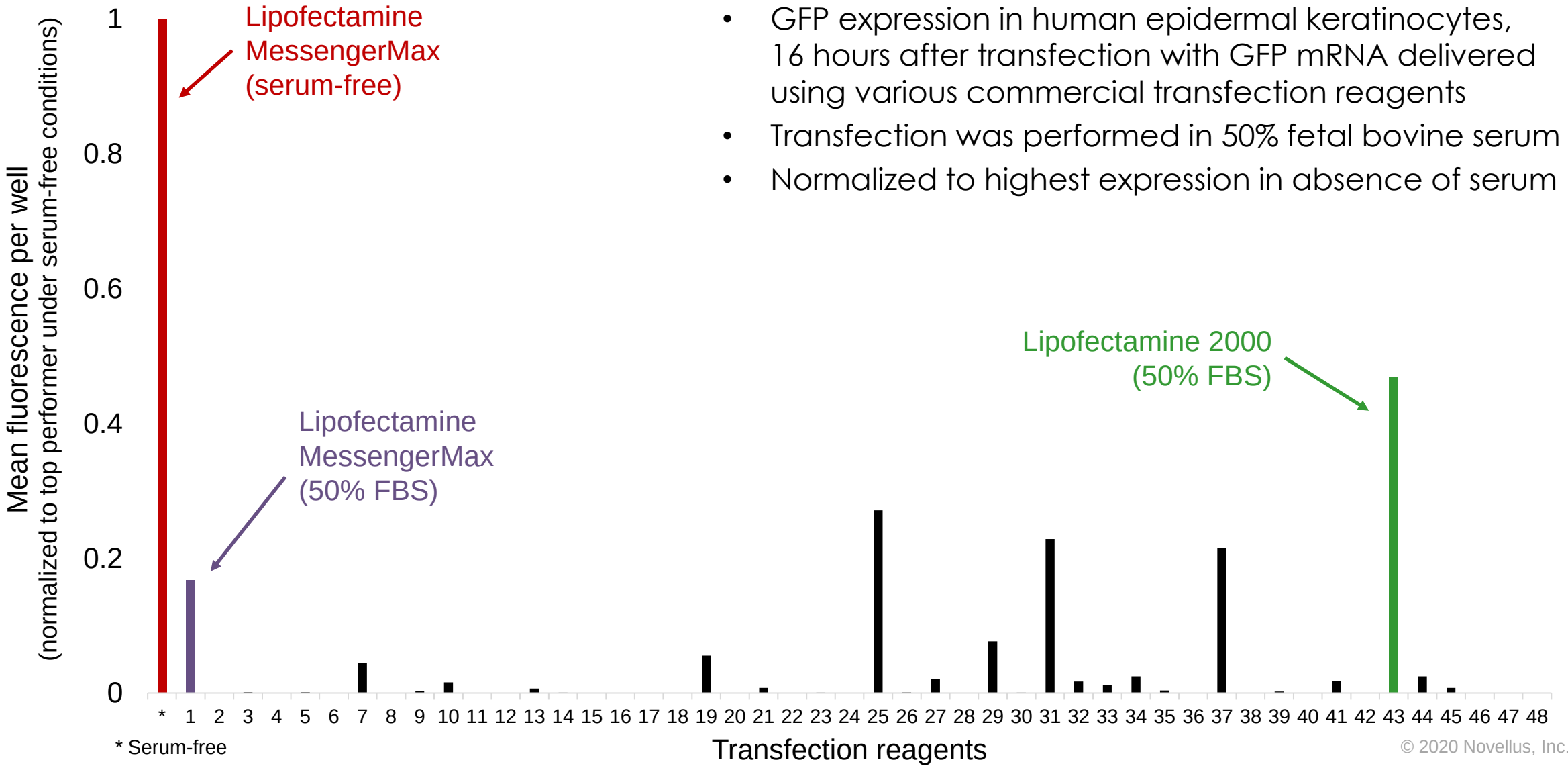


- Transient expression of gene-editing proteins
- No risk of vector incorporation into host genome
- Edits made to host DNA are durable

E.g., T-cell receptor knockout for allogeneic CAR-T therapy

Kopacz, M., et al. *Mol Ther*, Vol 28 No 4S1, **2020.** Poster 642.

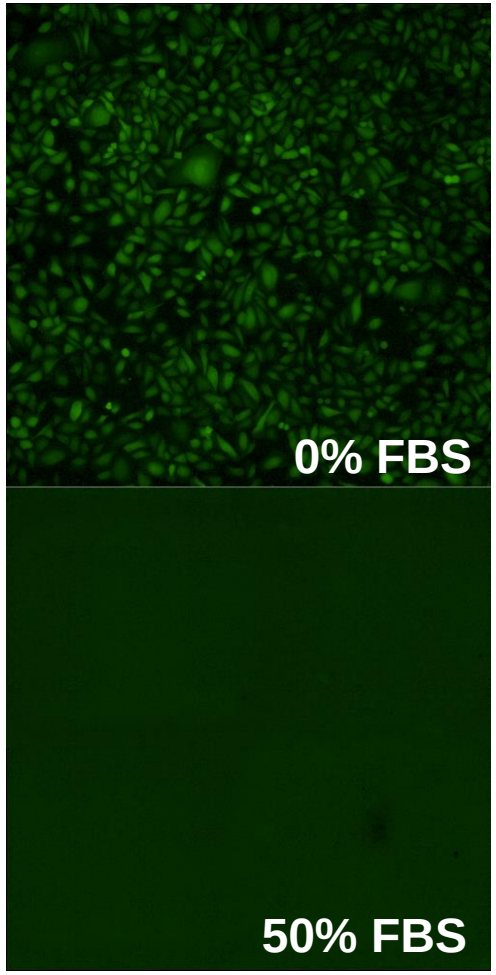
Serum can inhibit nucleic acid delivery



- GFP expression in human epidermal keratinocytes, 16 hours after transfection with GFP mRNA delivered using various commercial transfection reagents
- Transfection was performed in 50% fetal bovine serum
- Normalized to highest expression in absence of serum

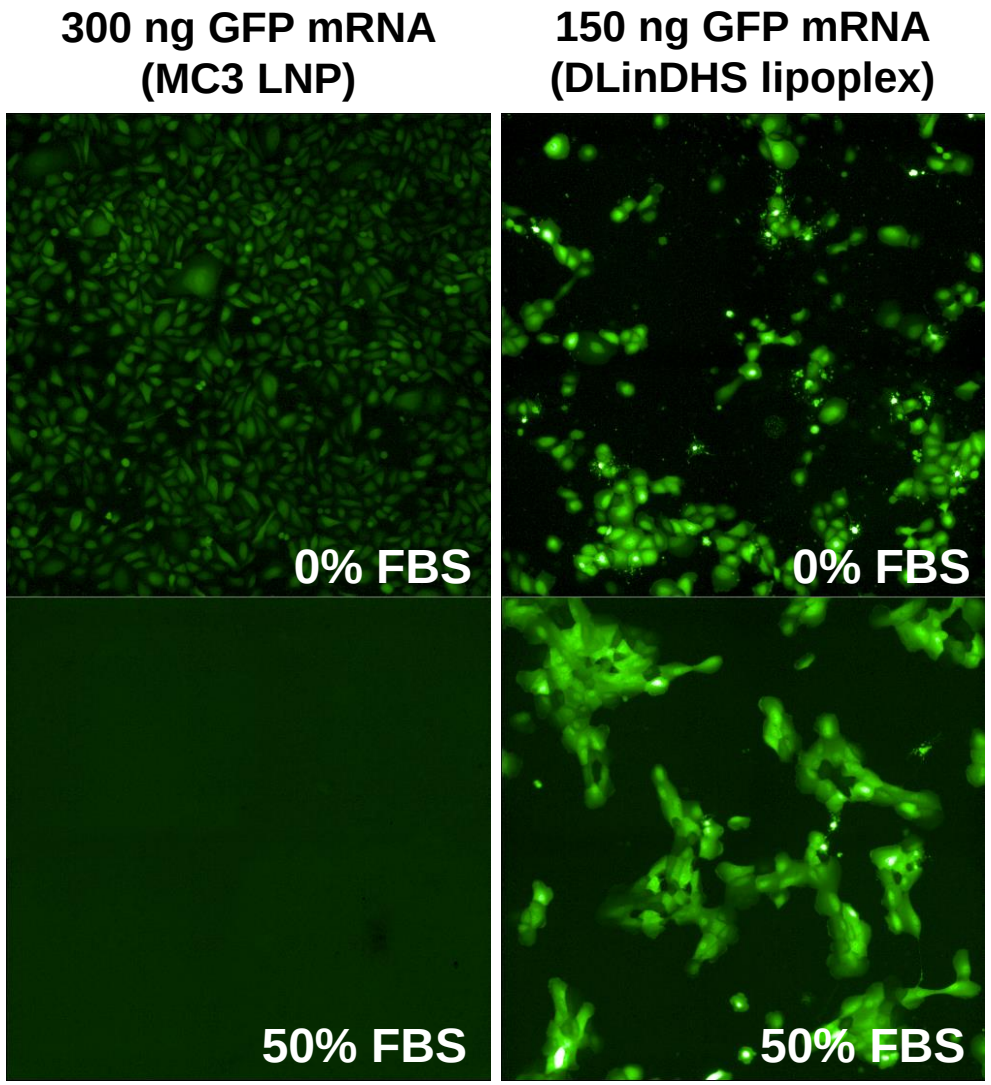
Serum can inhibit nucleic acid delivery using LNPs

300 ng GFP mRNA (MC3 LNP)

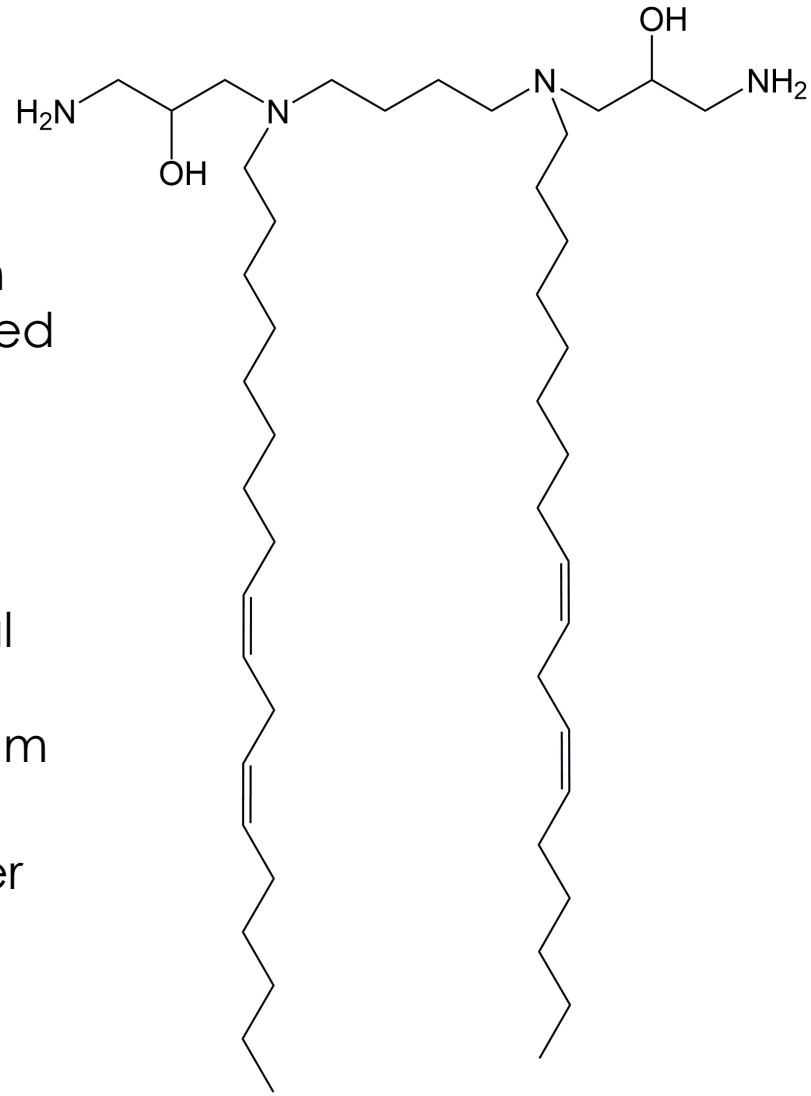


- LNP: DLin-MC3-DMA, DOPE, cholesterol, DMPE-PEG (30:30:38.5:1.5)
- mRNA-LNPs were added to 20,000 human epidermal keratinocytes in serum-free medium or 50% FBS
- Cells imaged after 16 hours

DLinDHS efficiently delivers mRNA in 50% serum

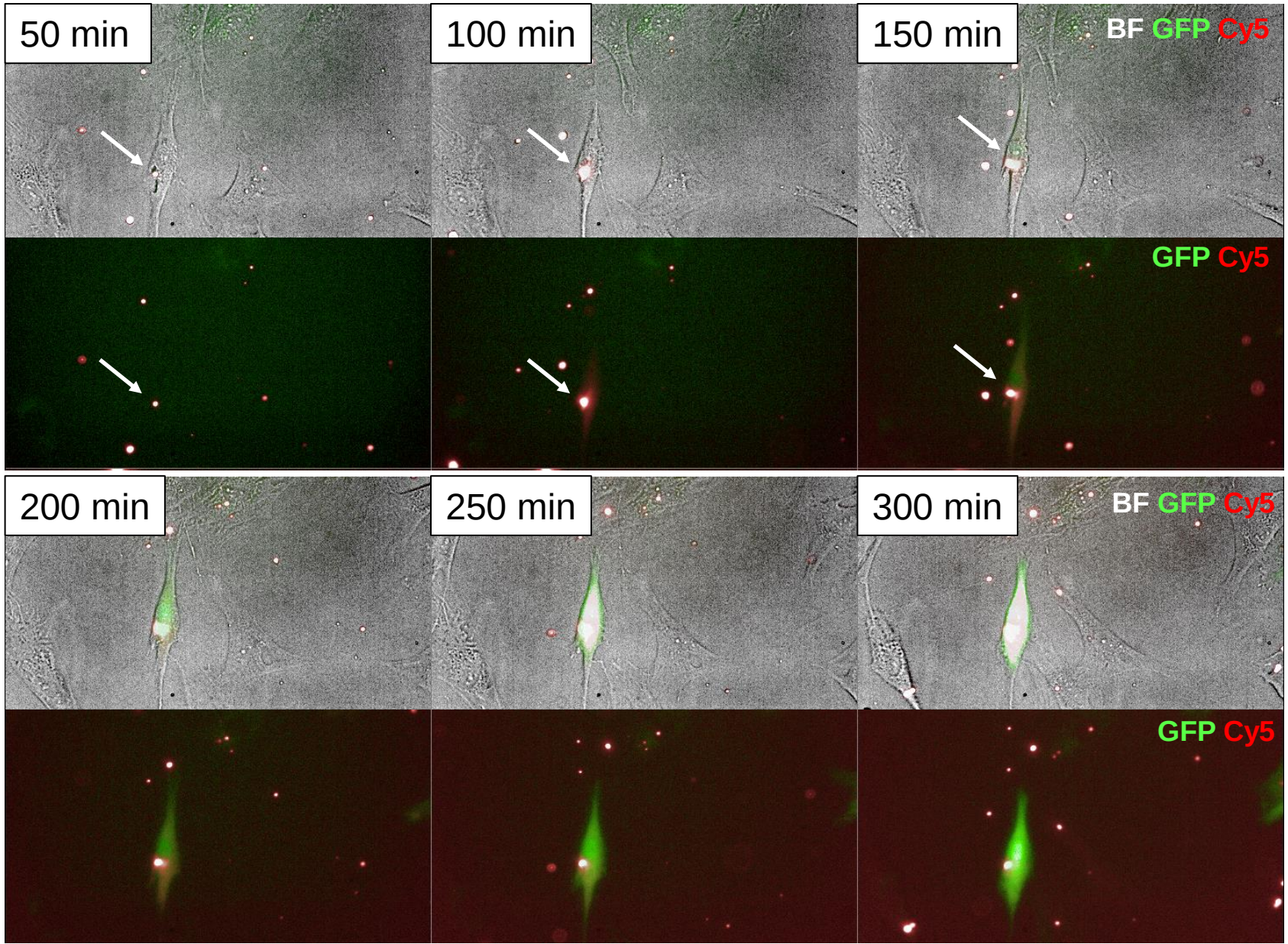


- DLinDHS lipoplex: 375 ng DLinDHS in ethanol was added to 150 ng RNA diluted in DMEM
- Complexes were added to 20,000 human epidermal keratinocytes in serum-free medium or 50% FBS
- Cells imaged after 16 hours



DLinDHS

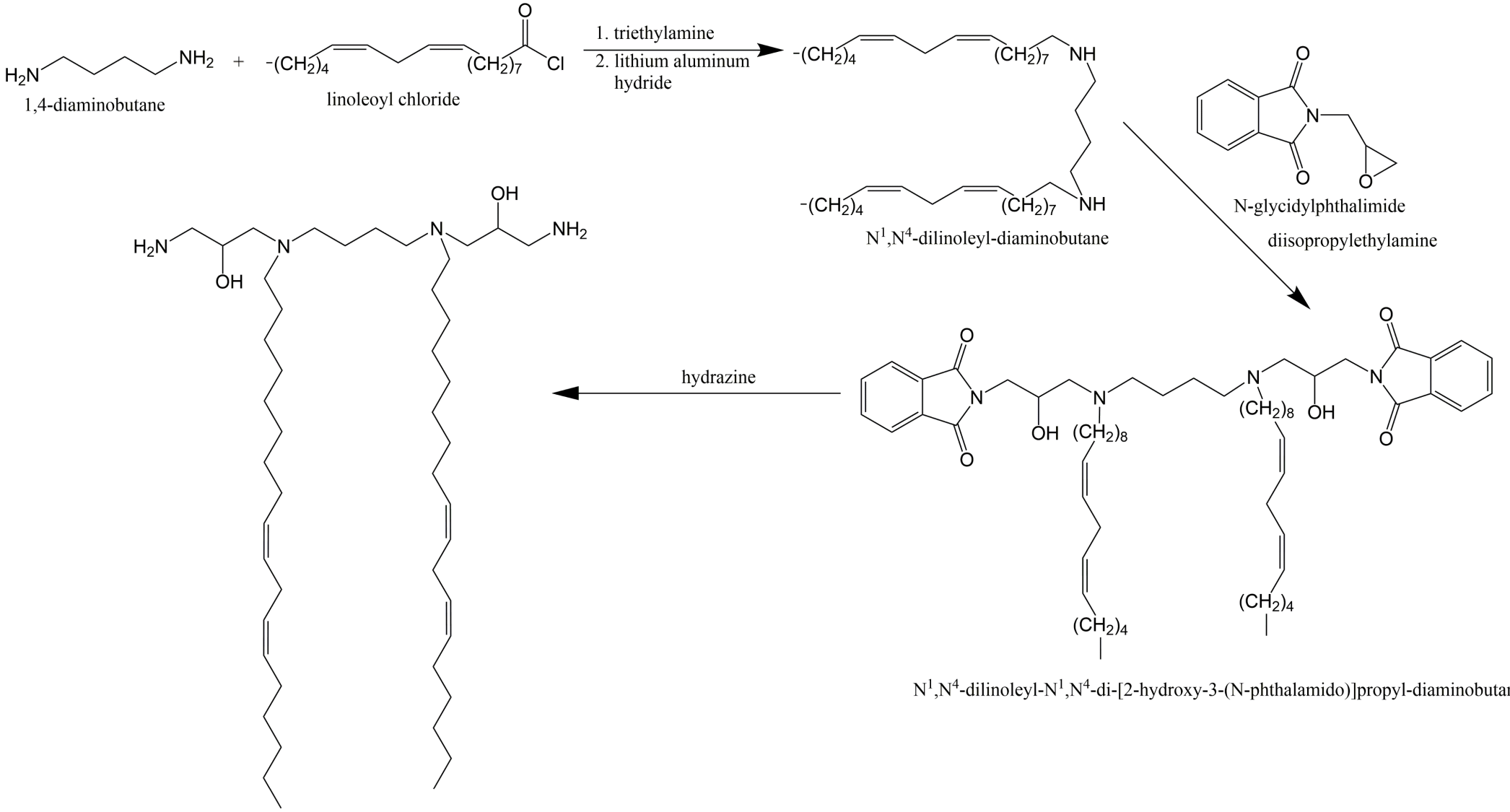
Protein expression begins within 3 hours after transfection



- 100 ng GFP mRNA, 20% of which was labeled with Cy5, was complexed with DLinDHS
- Complexes were added to 20,000 human dermal fibroblasts in 10% FBS
- mRNA was detected in cytoplasm within 50 min of contact with lipoplex

Red: Cy5 (mRNA)
Green: GFP

DLinDHS synthetic scheme

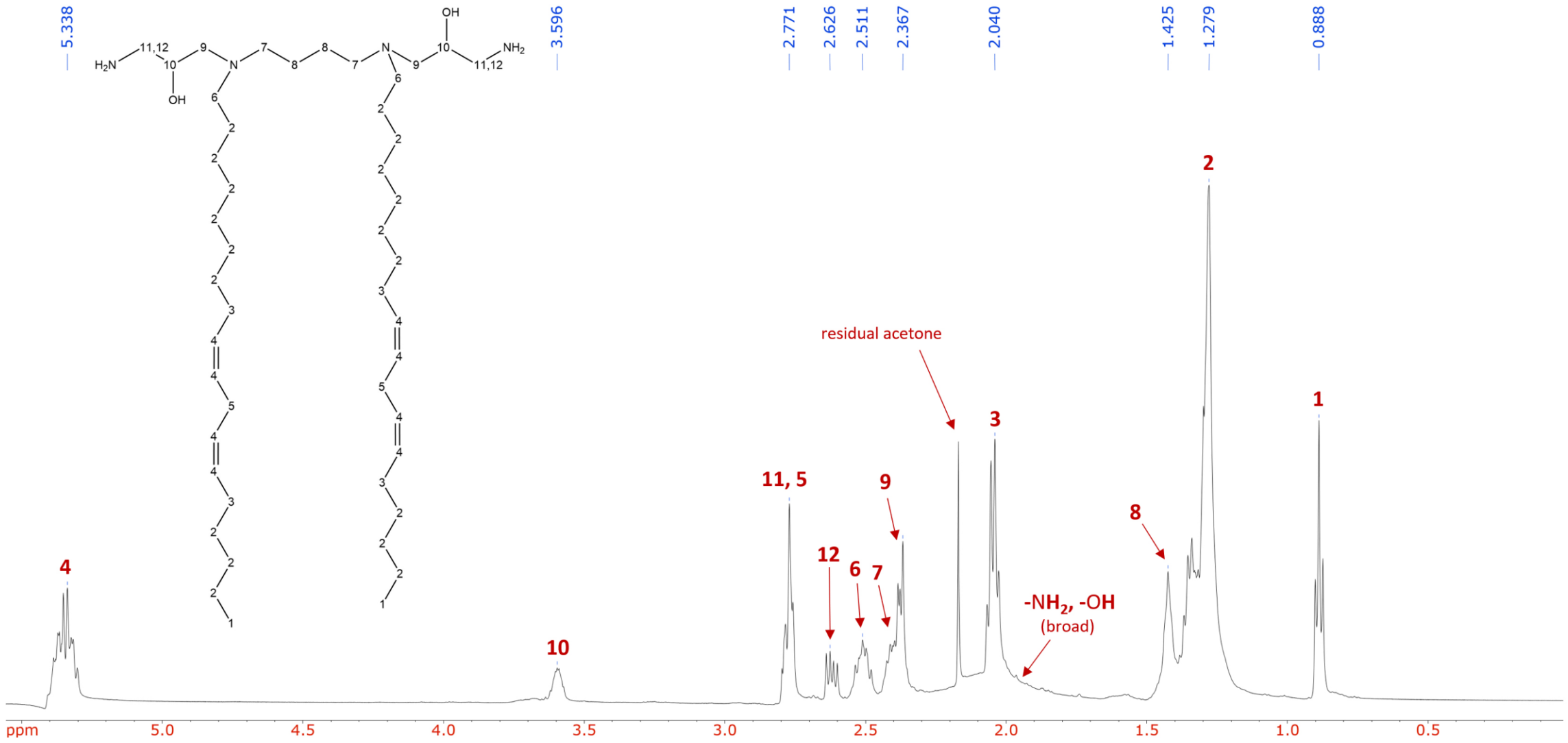


N^1,N^4-dilinoleyl-diaminobutane

N^1,N^4-dilinoleyl-N^1,N^4-di-[2-hydroxy-3-(N-phthalamido)]propyl-diaminobutane

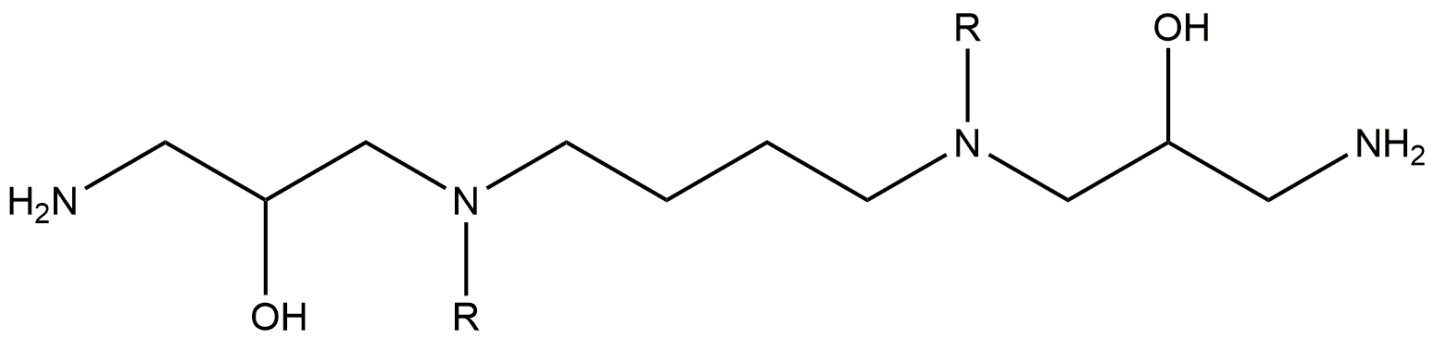
N^1,N^4-dilinoleyl-N^1,N^4-di-(2-hydroxy-3-aminopropyl)-diaminobutane
(DLinDHS)

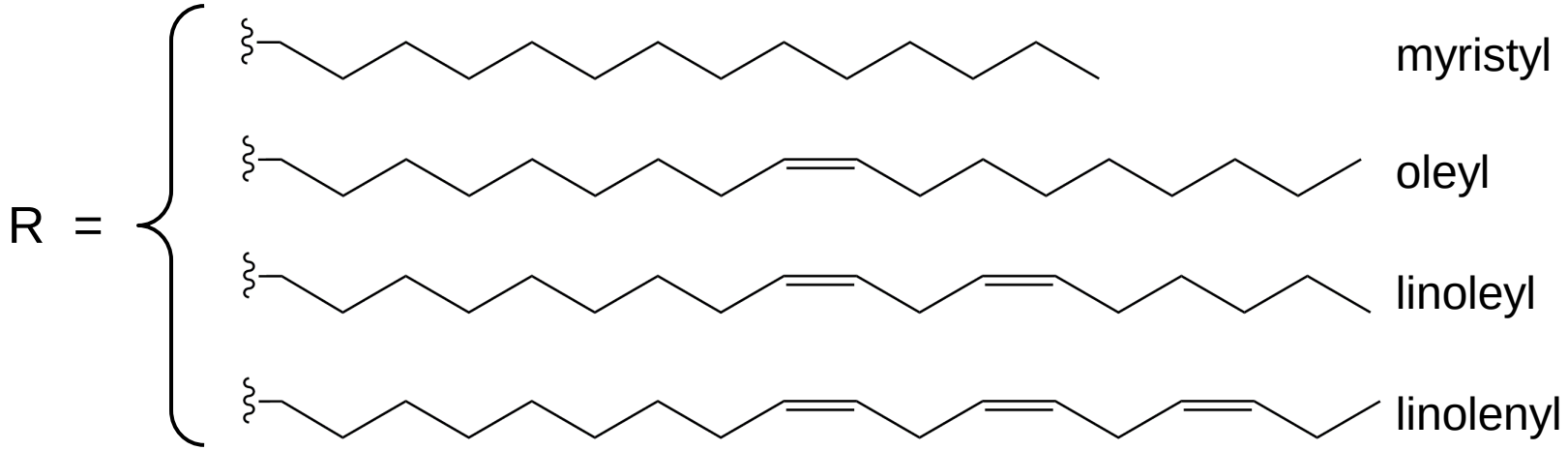
DLinDHS 500 MHz NMR spectrum



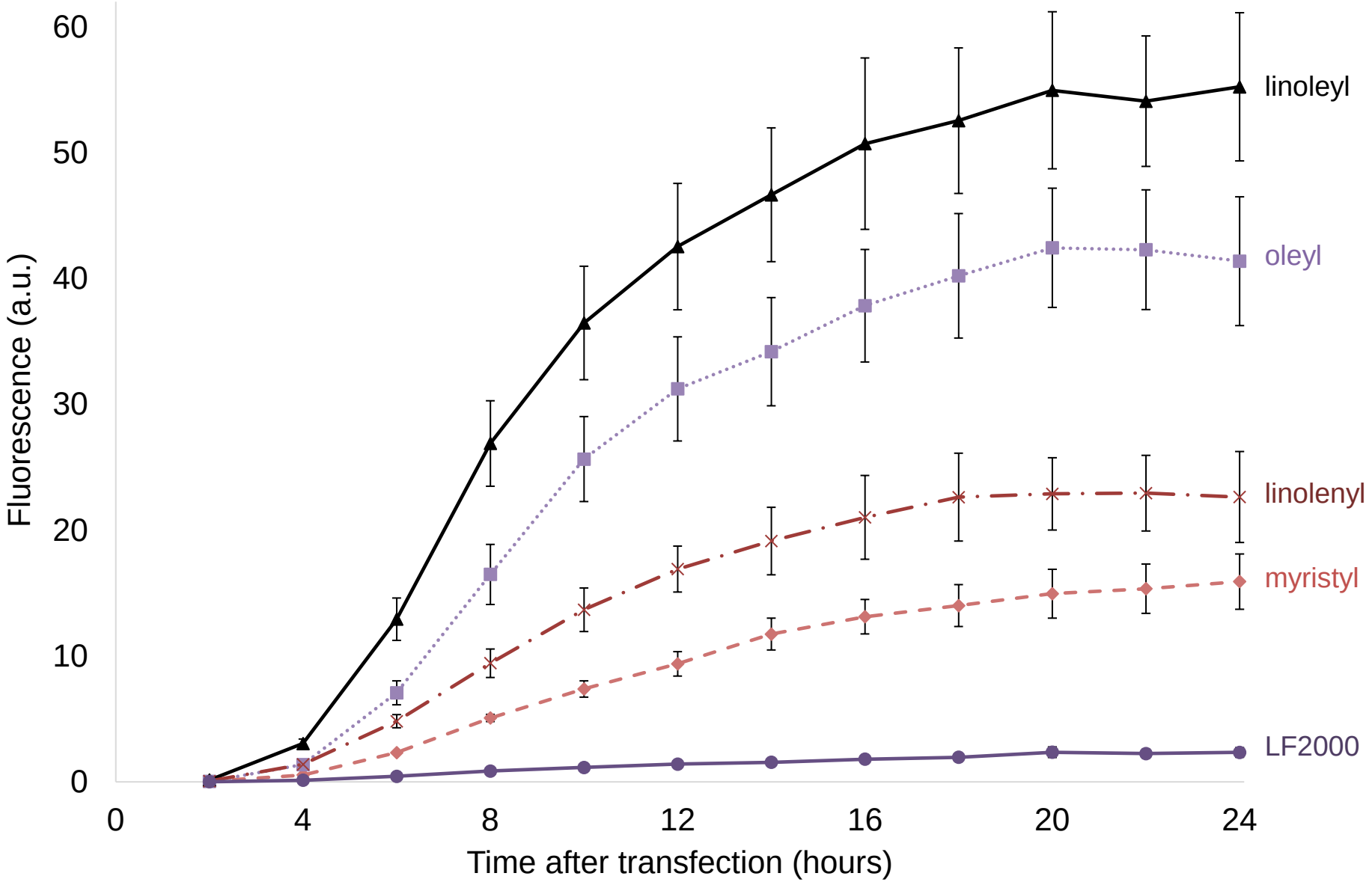
Synthesized compounds – tail unsaturation

Dihydroxyspermine (DHS) headgroup

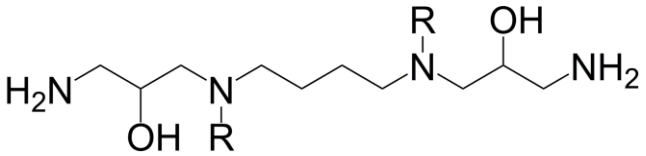


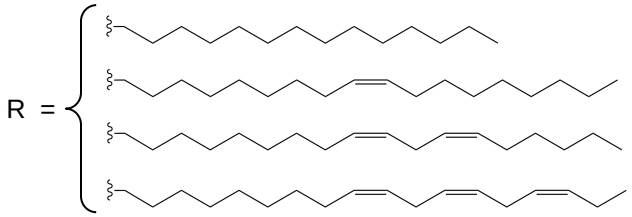


Synthesized compounds – effect of tail unsaturation



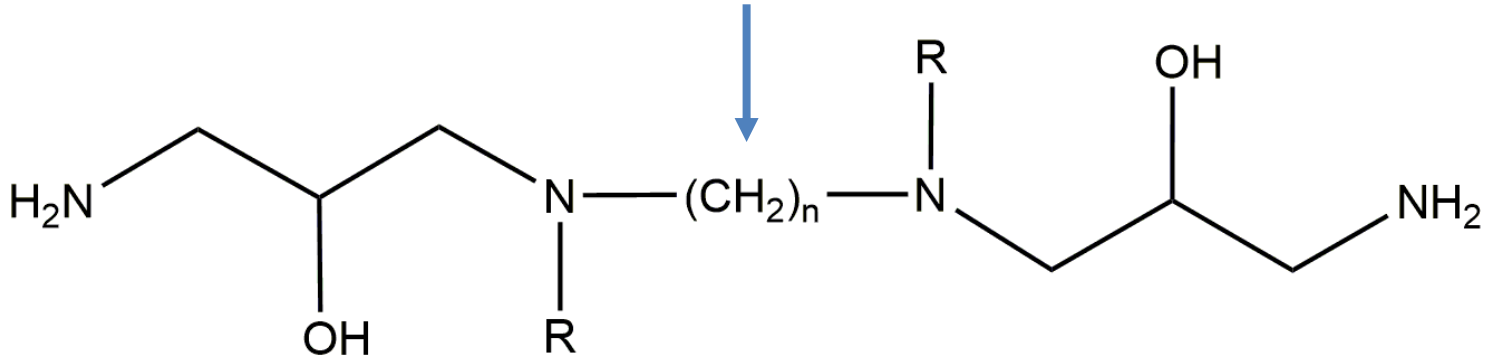
- GFP mRNA lipoplexes
- 100 ng mRNA added to 20,000 keratinocytes
- 50% FBS





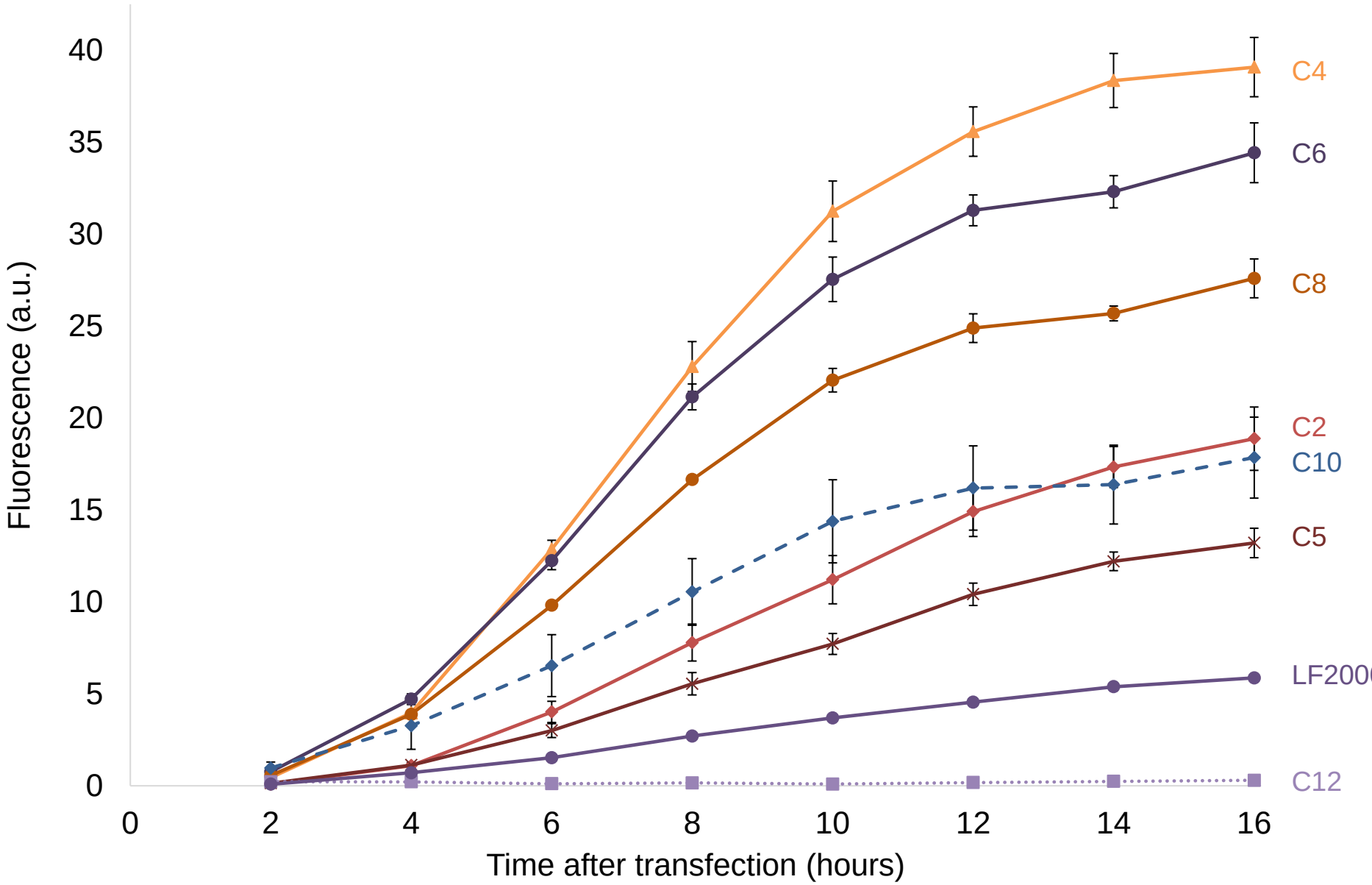
Synthesized compounds – inter-amino spacing

Variable tetra-amino headgroup

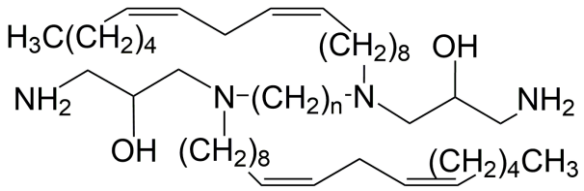


R = linoleyl

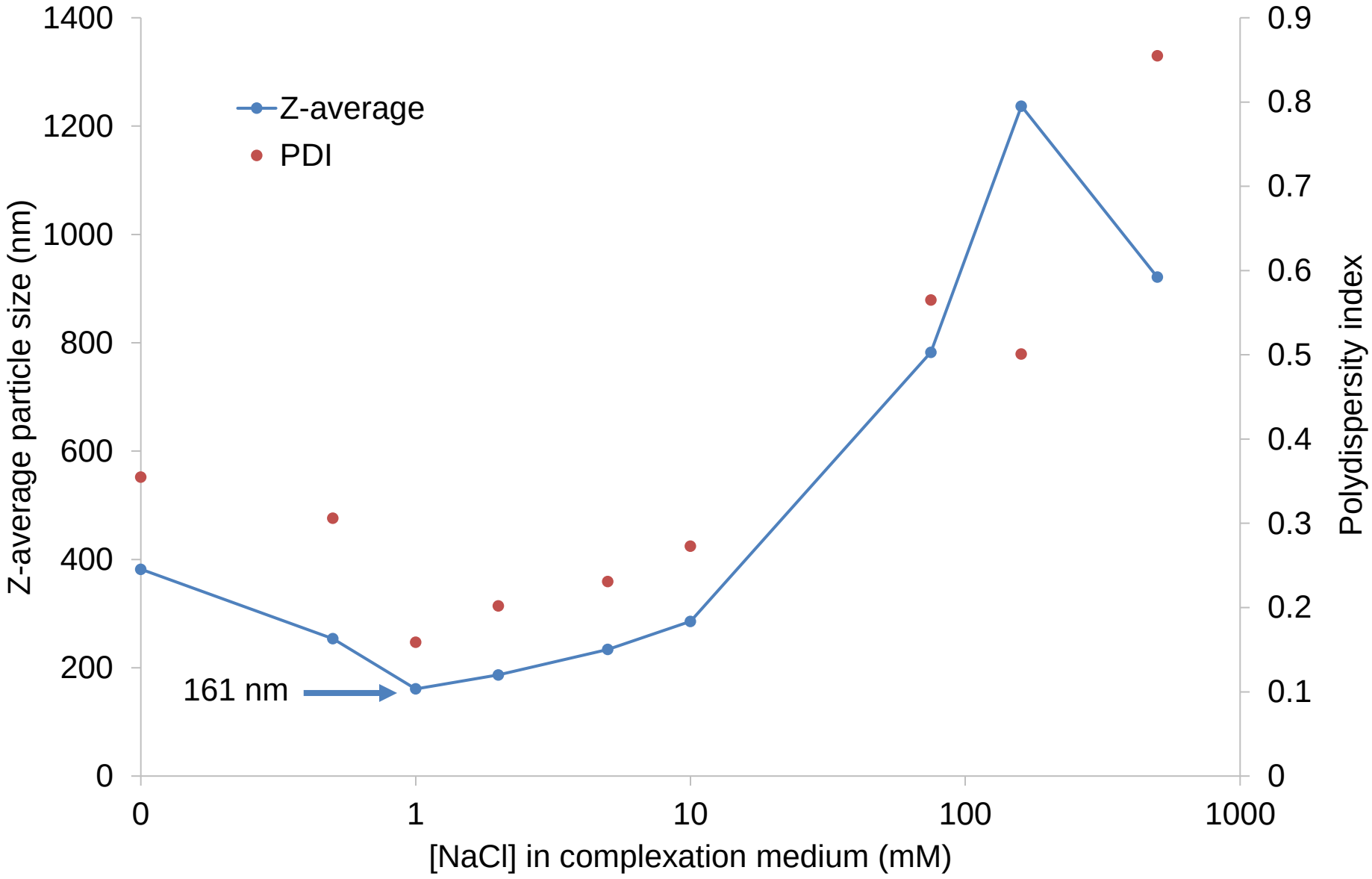
Synthesized compounds – effect of inter-amino spacing



- GFP mRNA lipoplexes
- 100 ng mRNA added to 20,000 keratinocytes
- 50% FBS

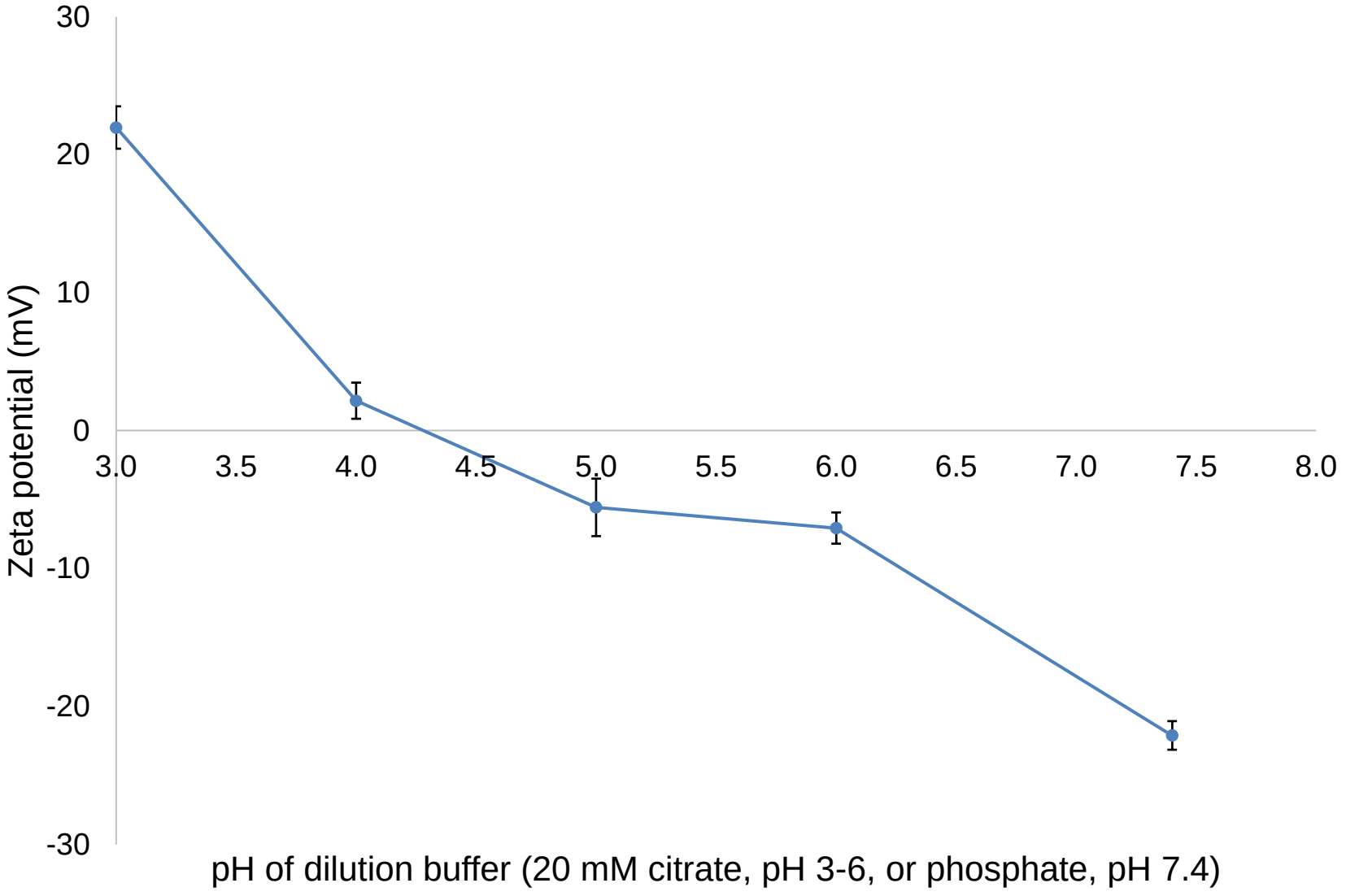


DLinDHS lipoplex particle size is ionic-strength dependent



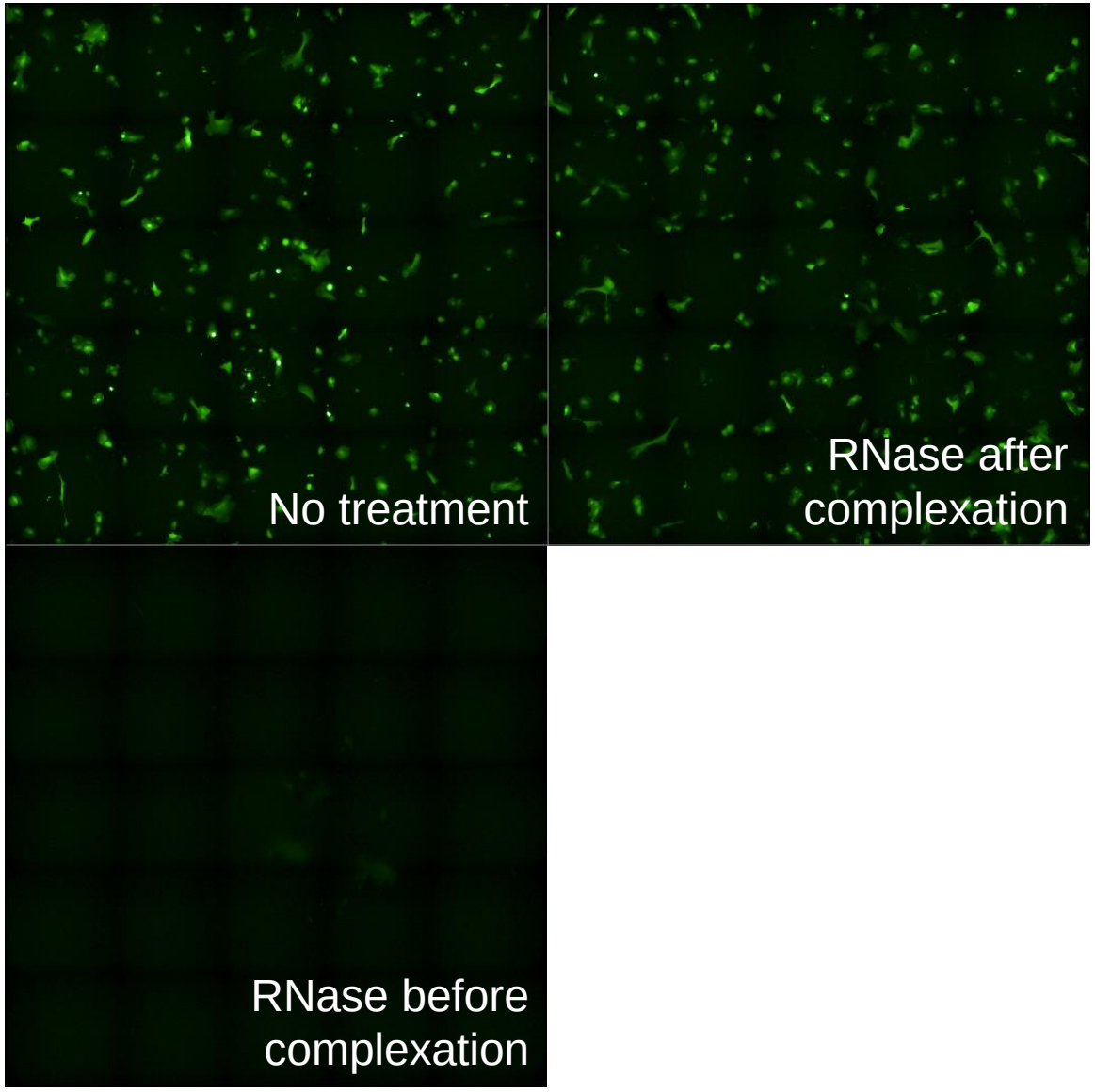
- DLinDHS/mRNA complexes formed by dilution of lipid from ethanol stock into aqueous mRNA solution
- Particle size measured by dynamic light scattering

DLinDHS lipoplexes have pH-dependent zeta potential

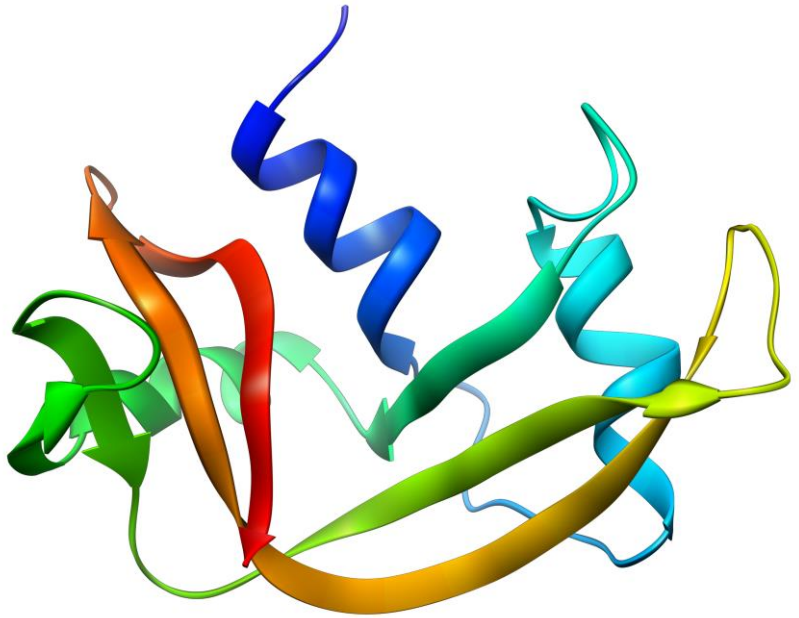


- DLinDHS/mRNA complexes formed by dilution of lipid from ethanol stock into aqueous mRNA solution (160 mM NaCl)
- -22.1 mV zeta potential at pH 7.4 implies stable dispersion

DLinDHS protects mRNA from RNase A degradation



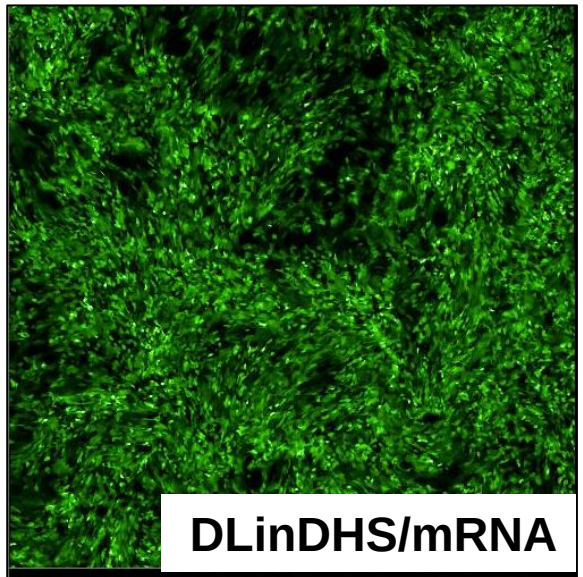
- GFP mRNA was incubated with RNase A either before or after complexation with DLinDHS
- Complexes were added to 20,000 keratinocytes in serum-free medium

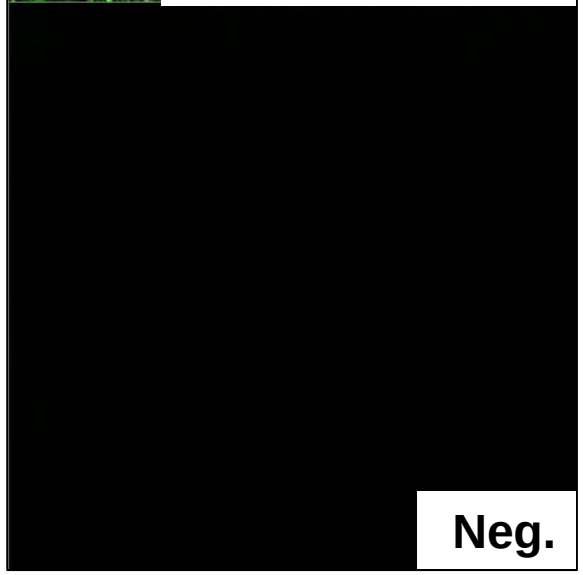


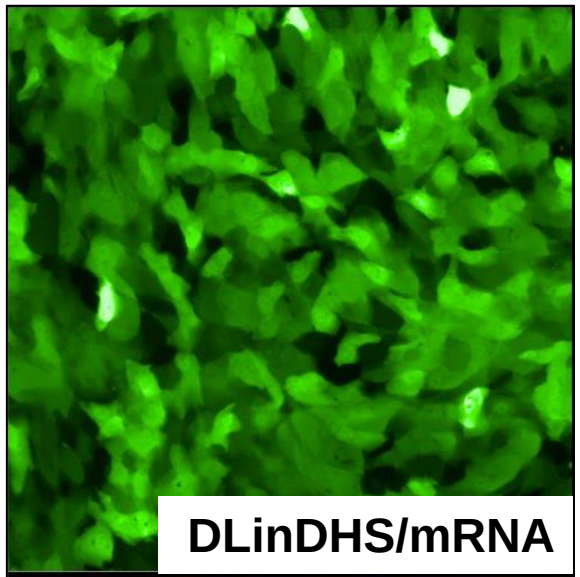
PDB ID: 2AAS

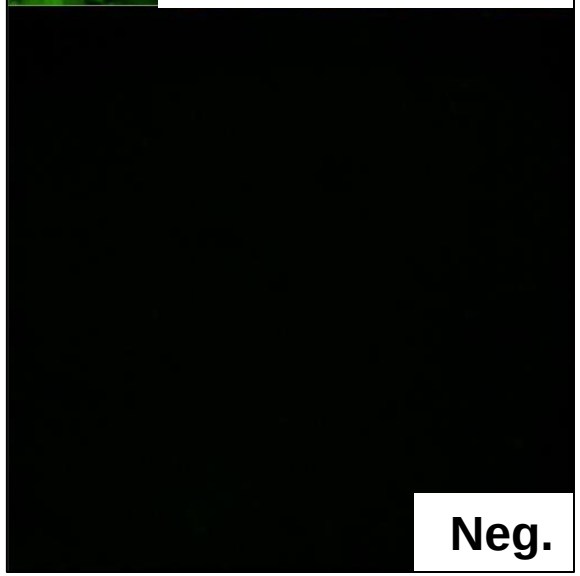
RNase A

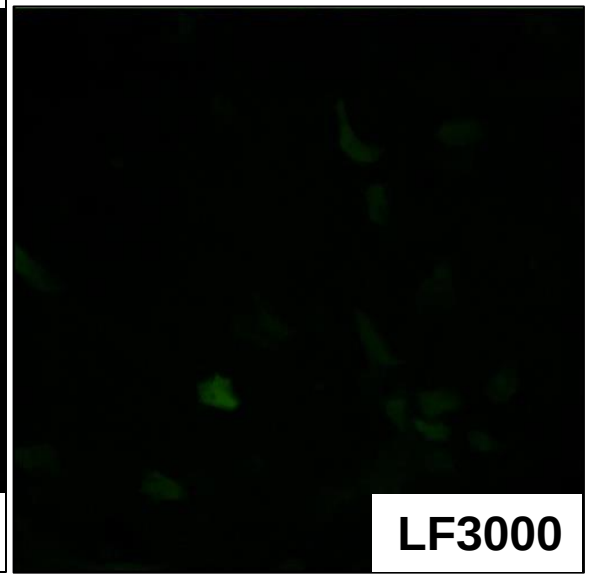
DLinDHS delivers mRNA to confluent keratinocytes in 100% serum





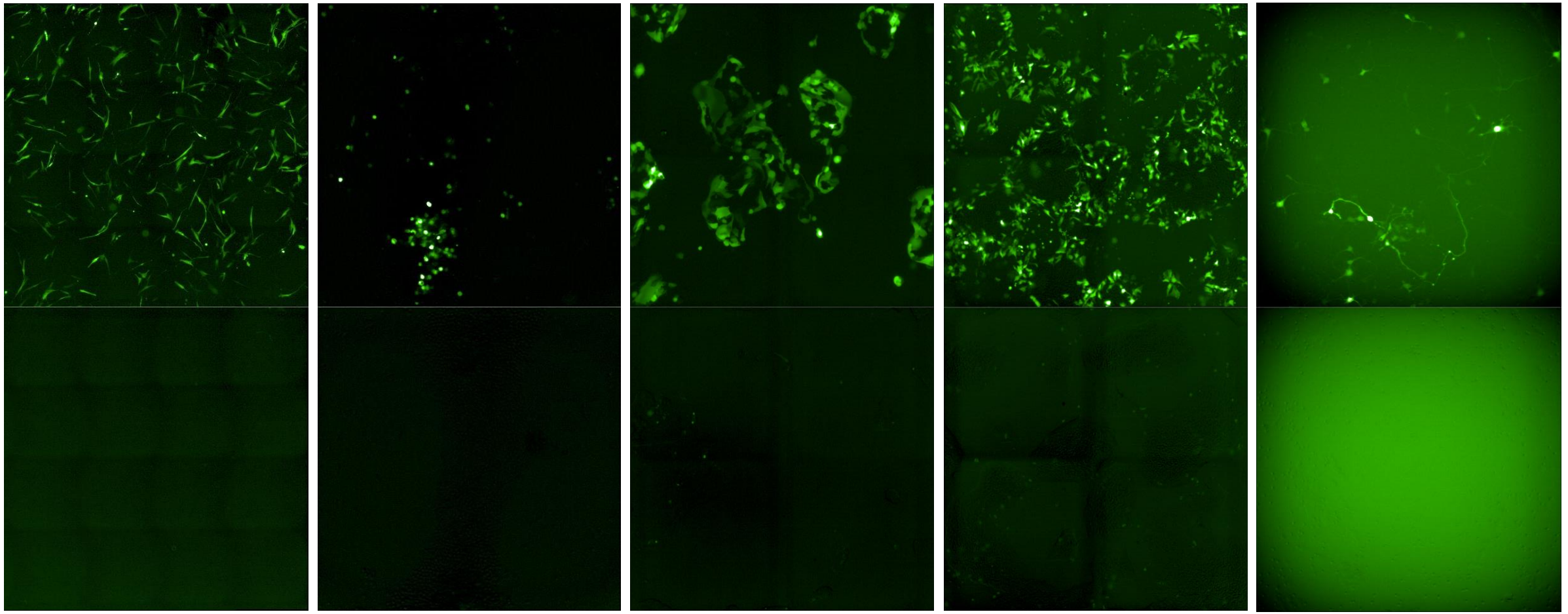




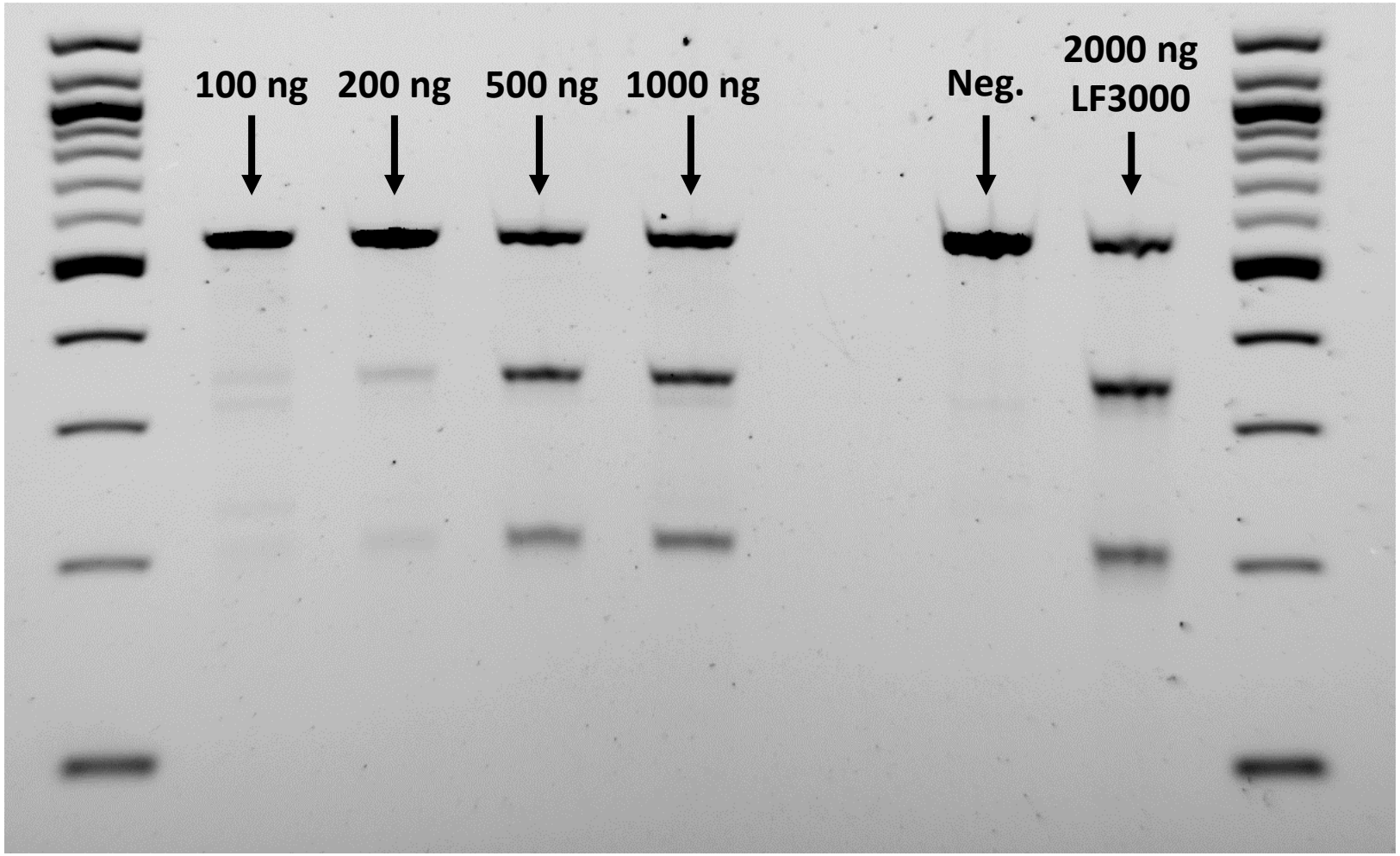


- GFP mRNA was complexed with DLinDHS or Lipofectamine 3000
- Complexes were added to confluent keratinocytes in 100% FBS

DLinDHS delivers mRNA to other cell types



DLinDHS facilitates efficient gene editing in primary cells

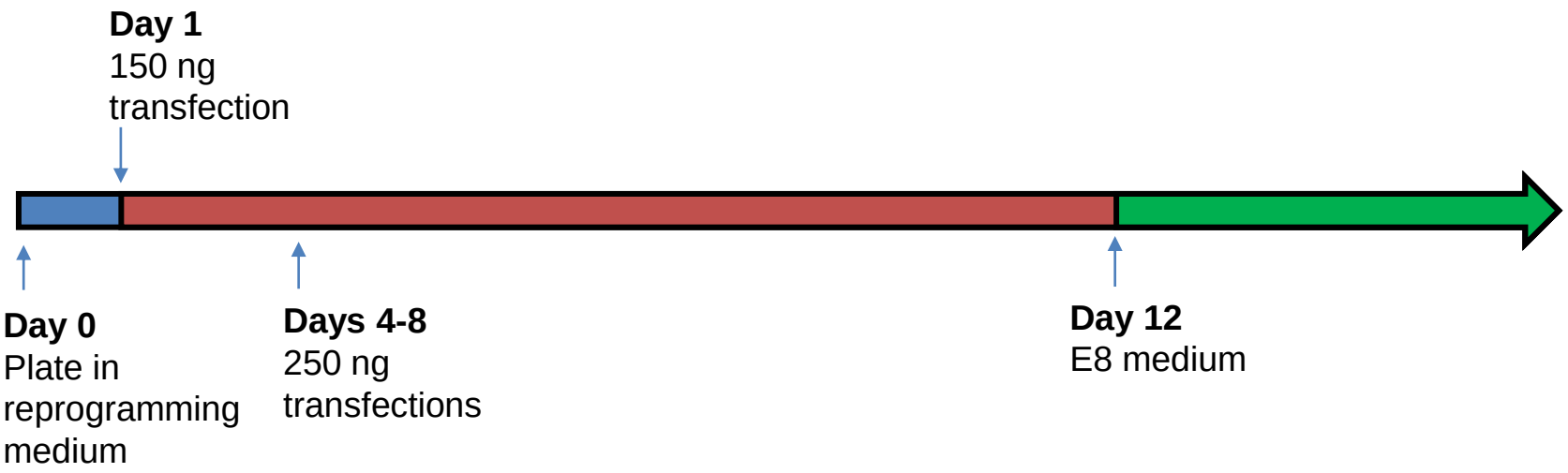


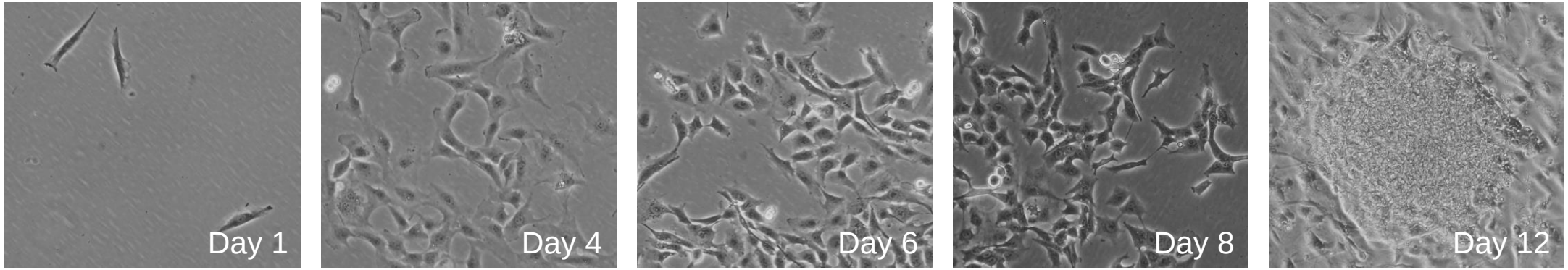
- TALEN mRNA was complexed with DLinDHS or Lipofectamine 3000
- Complexes were added to 50,000 keratinocytes
- After 48 hours, genomic DNA was extracted and assayed using the T7E1 assay
- Target site: COL7A1 exon 73 splice acceptor

Mealmaker, C., et al. *Mol Ther*, Vol 28 No 4S1, **2020.** Poster 198.

DLinDHS enables mRNA reprogramming to create iPS cells

- Primary human dermal fibroblasts were transfected 6 times with mRNAs encoding Oct4, Sox2, Klf4, c-Myc, and Lin28

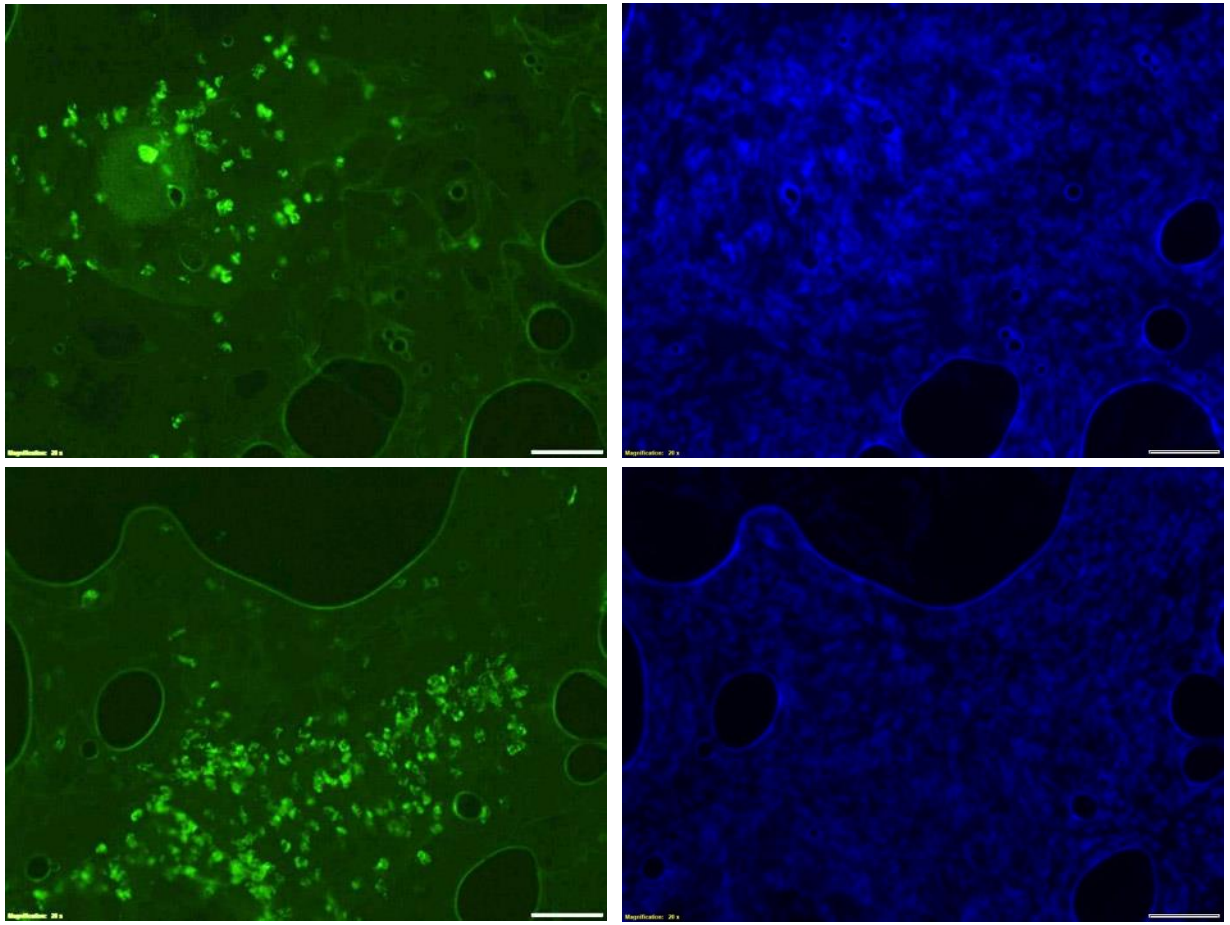




Brightfield images showing fibroblast reprogramming into iPS cells

Harris, J., et al. *Mol Ther*, Vol 28 No 4S1, **2020.** Poster 830.

DLinDHS delivers mRNA *in vivo* – rat lung



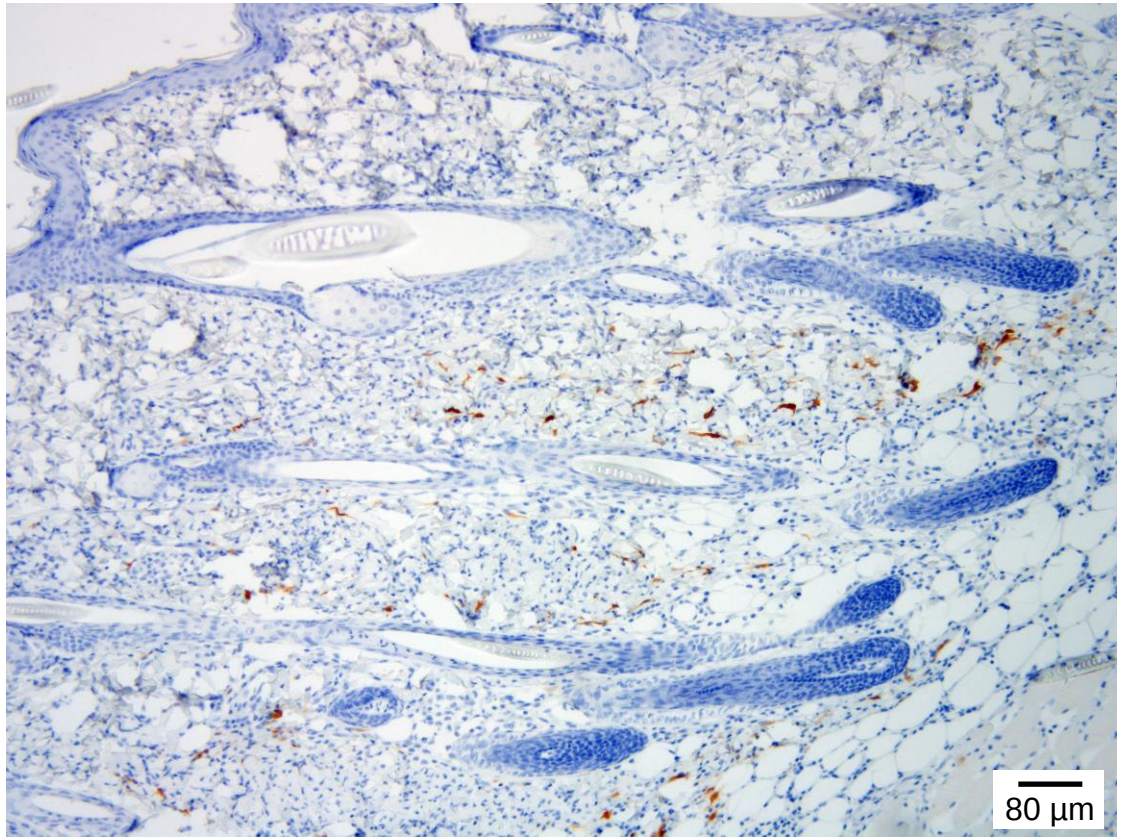
20x magnification

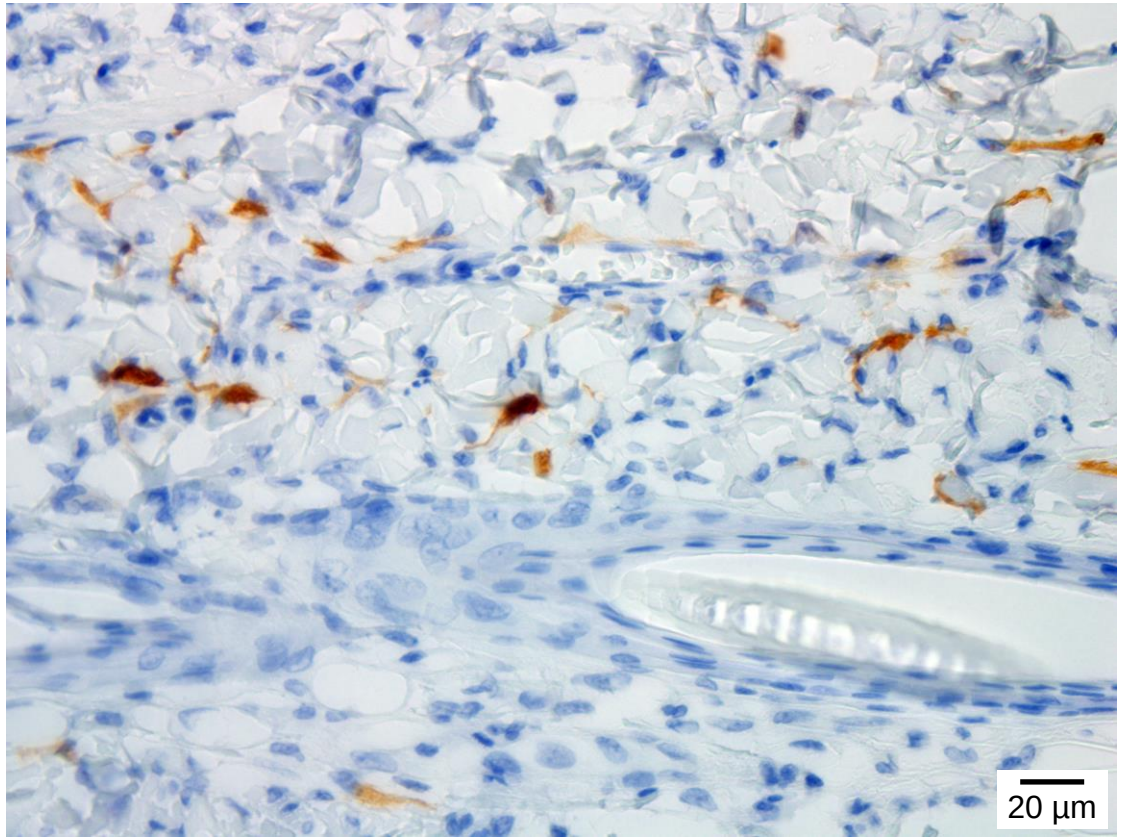
- 40 µg GFP mRNA was complexed with DLinDHS and nebulized using a vibrating-mesh nebulizer (Aerogen Pro) and administered to rats (SCIREQ flexiVent)
- Tissue was cryosectioned (10 µm) and imaged after 48 hours

Aerogen Solo

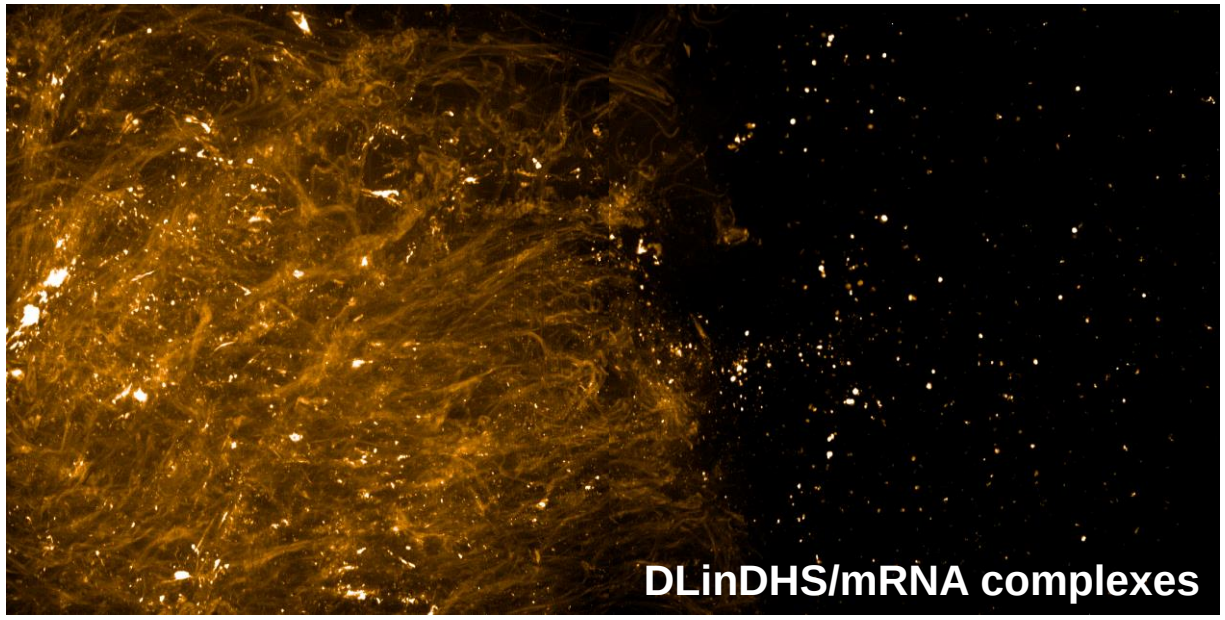
DLinDHS delivers mRNA *in vivo* – rat intradermal injection

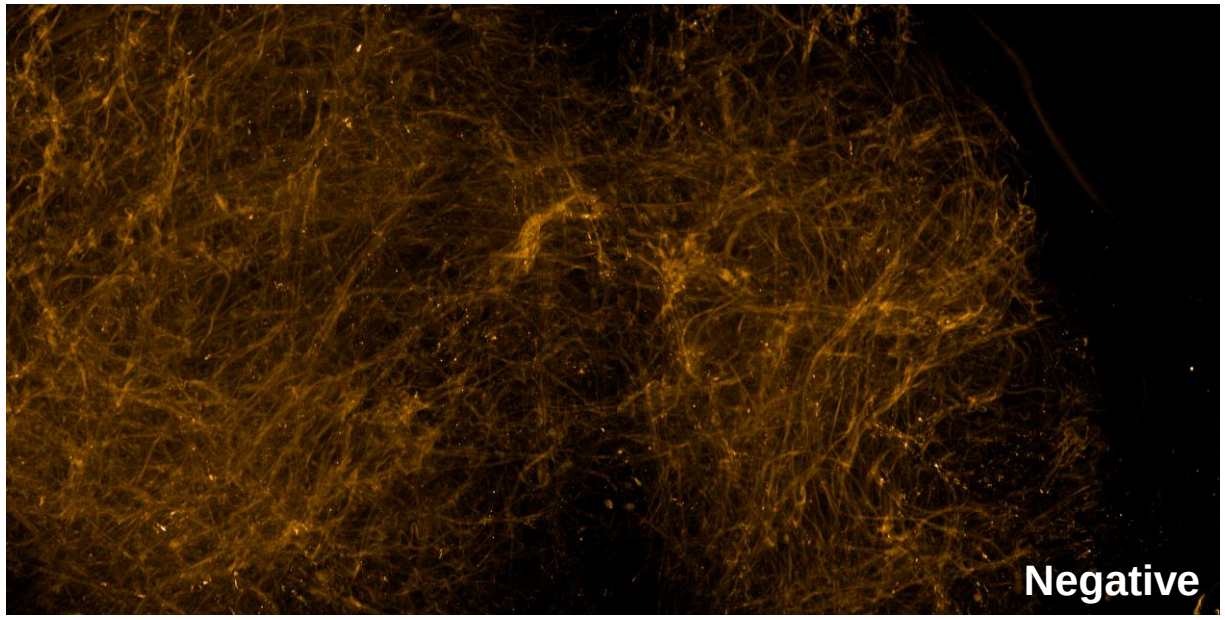
- 2000 ng DLinDHS-complexed GFP mRNA was administered to rats by intradermal injection
- After 48 hours, the injection site was fixed and stained (rabbit anti-GFP, brown)



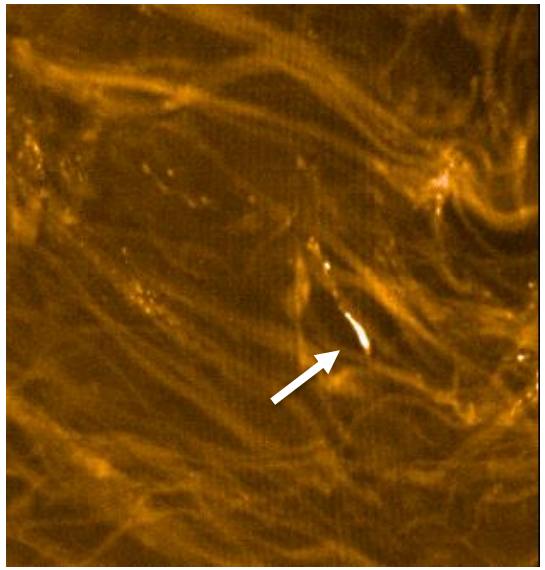


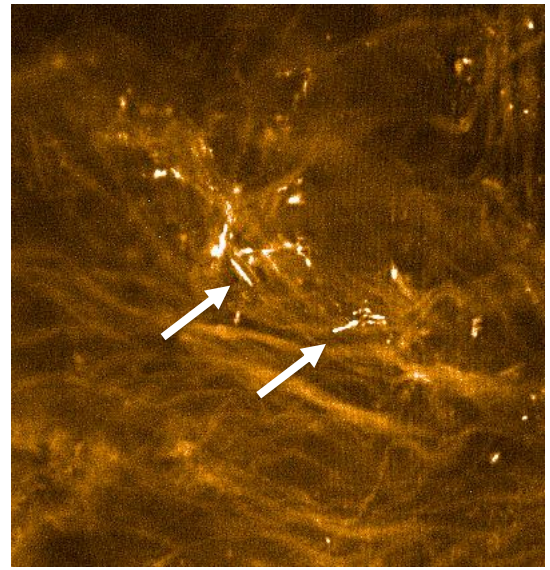
DLinDHS delivers mRNA *in vivo* – human intradermal injection





- Human male, ventral forearm
- 400 ng DLinDHS-complexed RFP mRNA was administered via intradermal injection
- Skin was biopsied and imaged by confocal fluorescence microscopy





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Science Foundation Ireland sfi For what's next

Northeastern University
NMR Core Facility

Author disclosures

F.K., M.K., and J.H. are employees of Novellus, Inc.

C.R. and M.A. are employees of Factor Bioscience Inc.

F.K., C.R., and M.A. are inventors of U.S. Patent Nos. 10,501,404, 10,556,855, and 10,611,722, which are assigned to Factor Bioscience, and licensed to Novellus.

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