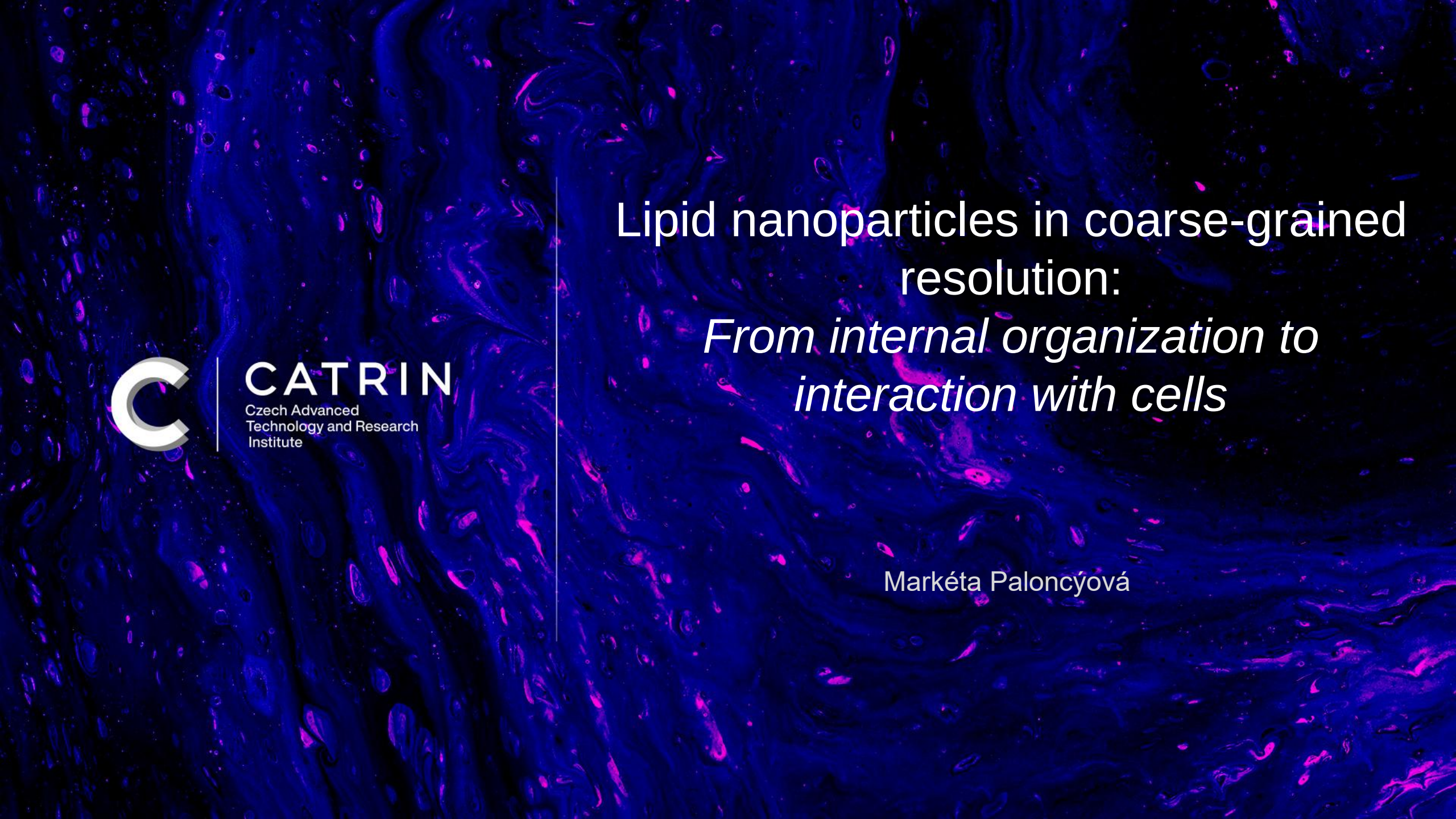

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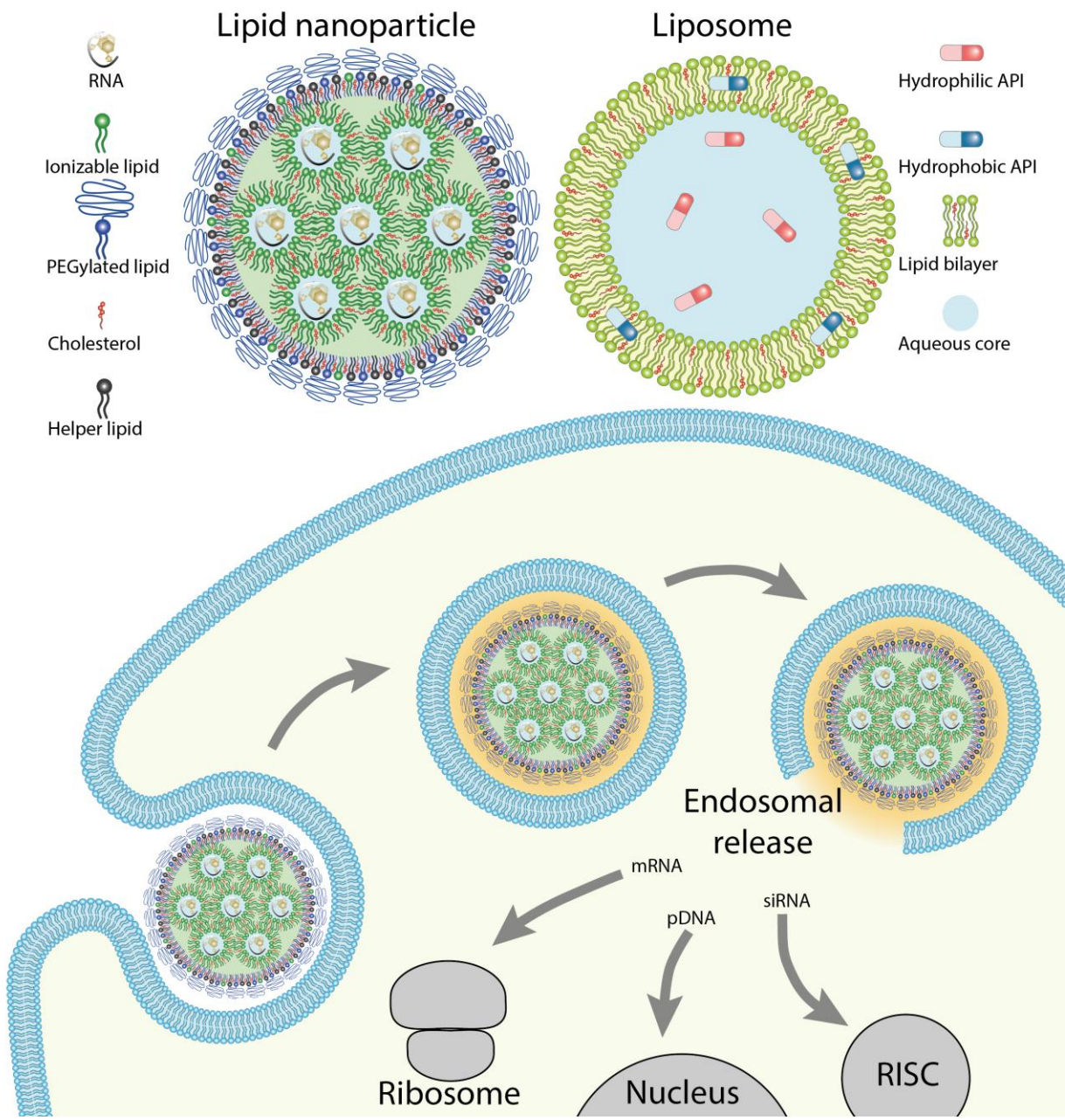
Lipid nanoparticles in coarse-grained resolution:
From internal organization to interaction with cells

Markéta Paloncýová

- Lipid nanoparticles
  - Soft/solid core
  - Lipid inverse hexagonal phase
  - Onion-like/Bleb phase
  - pH responsive
- Liposome
  - Aqueous core
  - APIs in lipid or aqueous phase



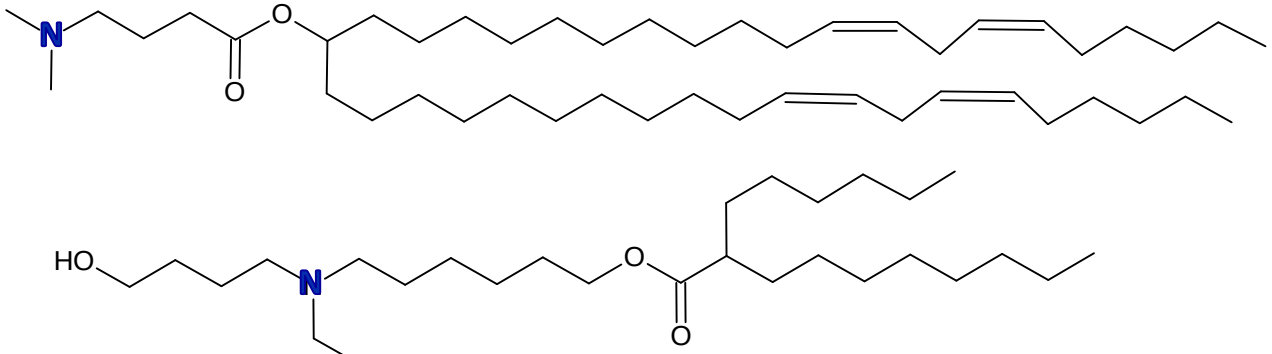
- <u>In-silico driven cargo delivery optimization</u>
  - Can we predict LNP organization and stability with MD?
    - LNP organization, pH response
  - Can we quantify RNA delivery efficiency with MD?
    - LNP-cell membrane interactions

Paloncýová, M. et al. Computational Methods for Modeling Lipid-Mediated Active Pharmaceutical Ingredient Delivery. *Mol. Pharm.* **2025**, *22* (3), 1110–1141. https://doi.org/10.1021/acs.molpharmaceut.4c00744.

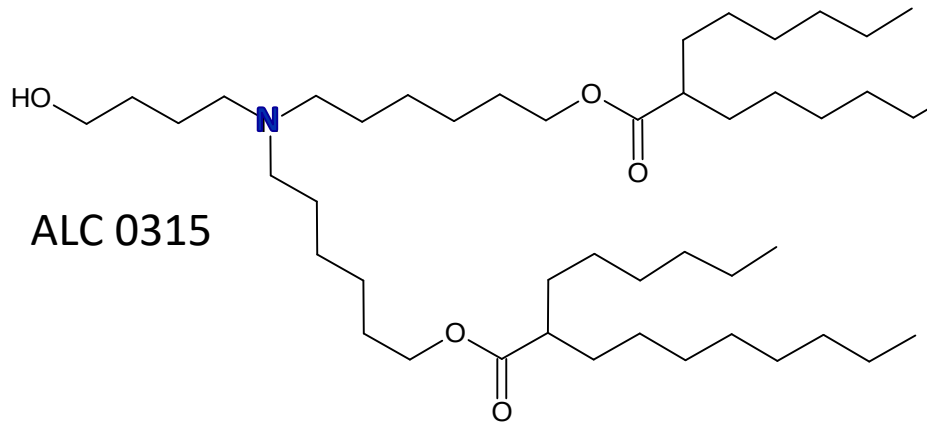
Zoo of Ionizable Lipids in Lipid Nanoparticles

- Protonable amine-based headgroup
  - Apparent pKa 6-7
  - Affected by the overall composition – helper lipids, PEGylated lipids
  - Differing position
- Biodegradable tails
- Branching/unsaturation

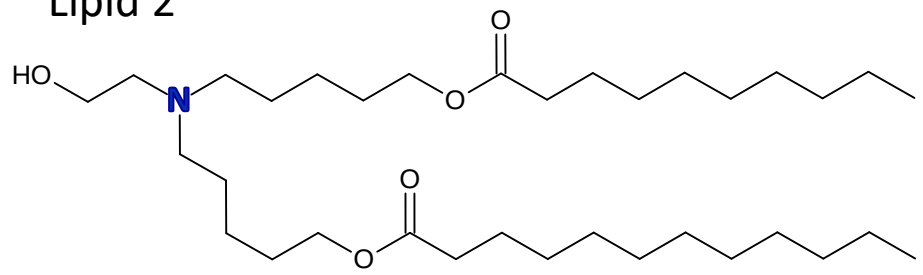
Dlin-MC3-DMA



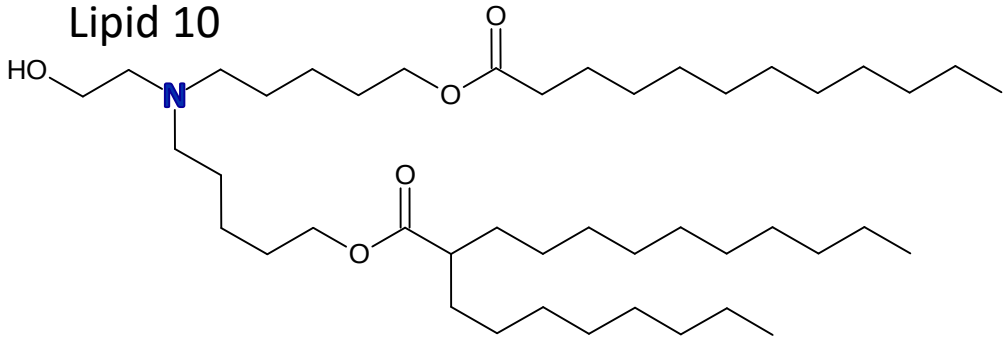
ALC 0315



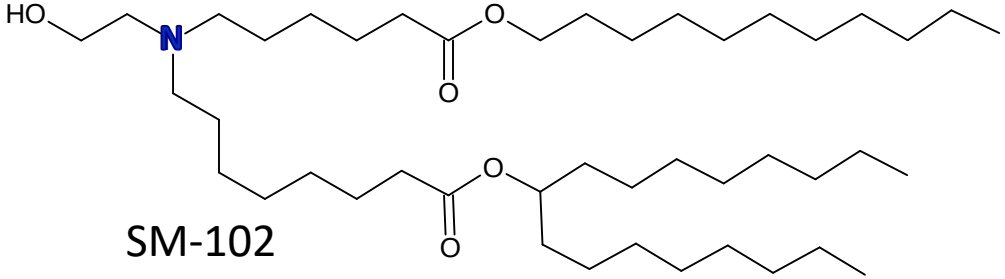
Lipid 2



Lipid 10

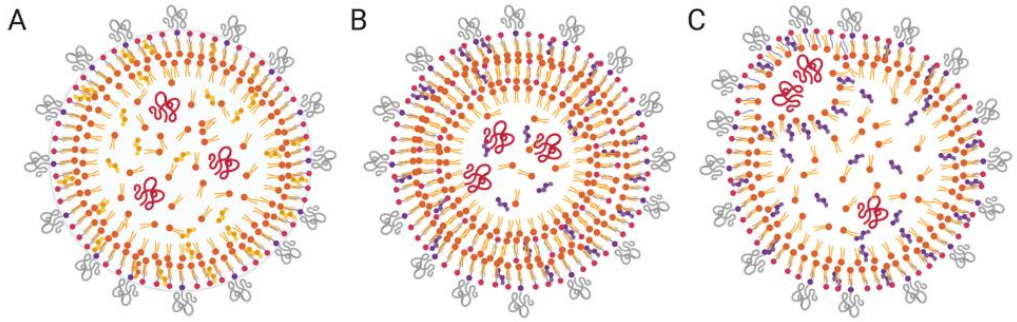


SM-102

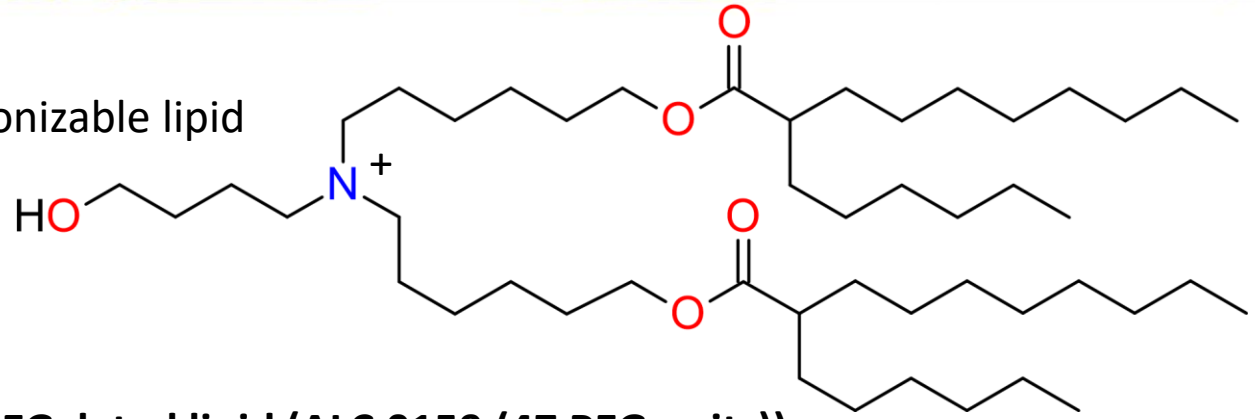


Lipid Nanoparticle Composition

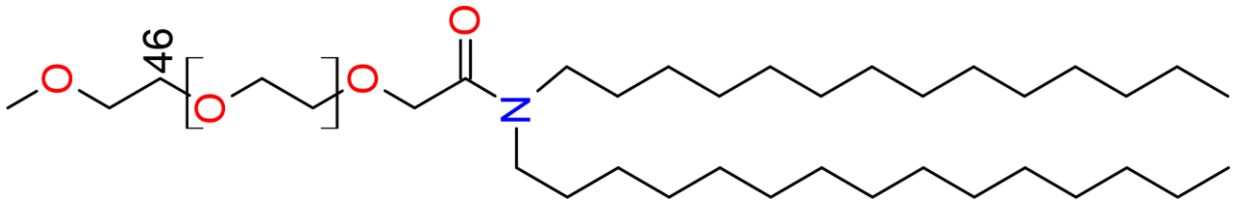
- Internal LNP structure not fully resolved
- Depends on
  - Lipid composition
  - Encapsulated RNA



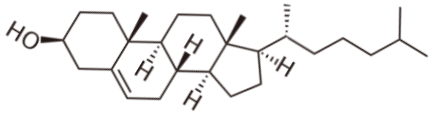
Ionizable lipid



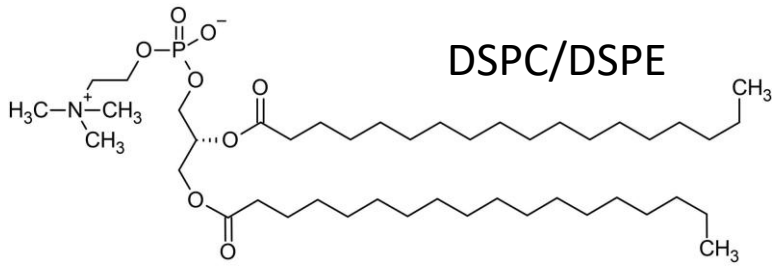
PEGylated lipid (ALC 0159 (47 PEG units))



Cholesterol



DSPC/DSPE

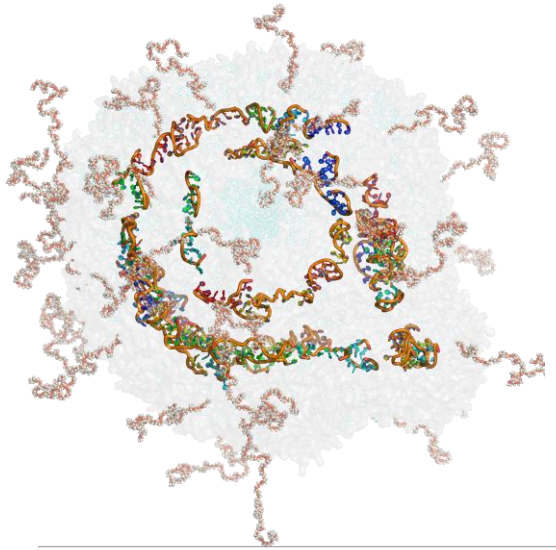


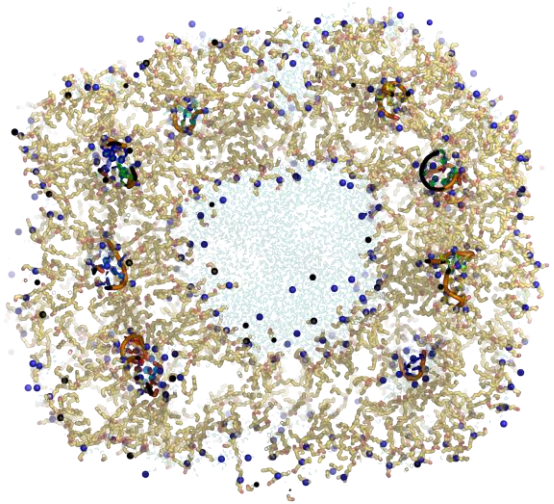
Eygeris, Y.; Patel, S.; Jozic, A.; Sahay, G.; Sahay, G. *Nano Lett.* **2020**, *20* (6), 4543–4549.

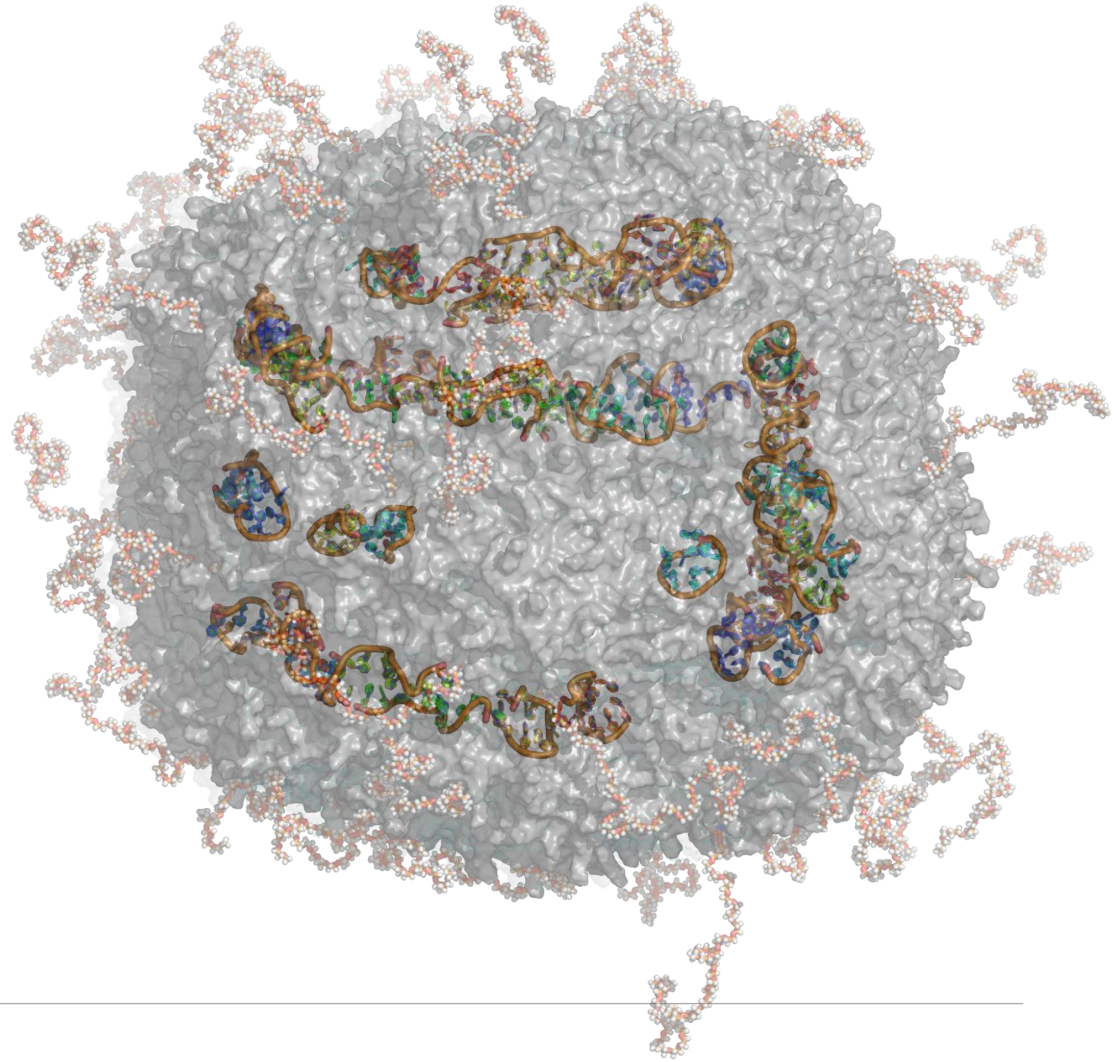
pH ~ 4

CG self assembly in acidic pH

- Rapid assembly
- RNA encapsulated inside LNP
- Water droplets inside LNP
- Rough shape





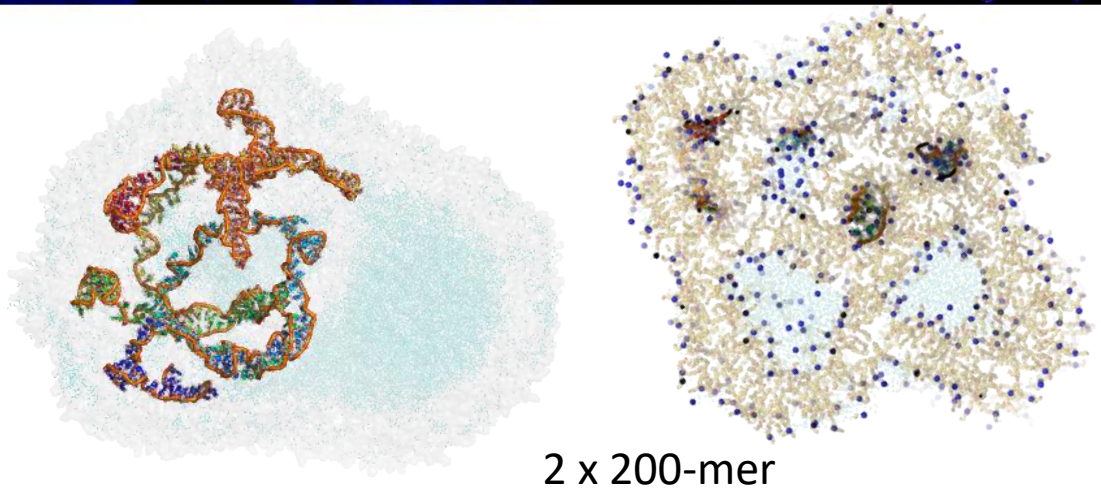


4000 lipids, PME, simulated annealing, MARTINI 3, cholesterol beta, 10 µs

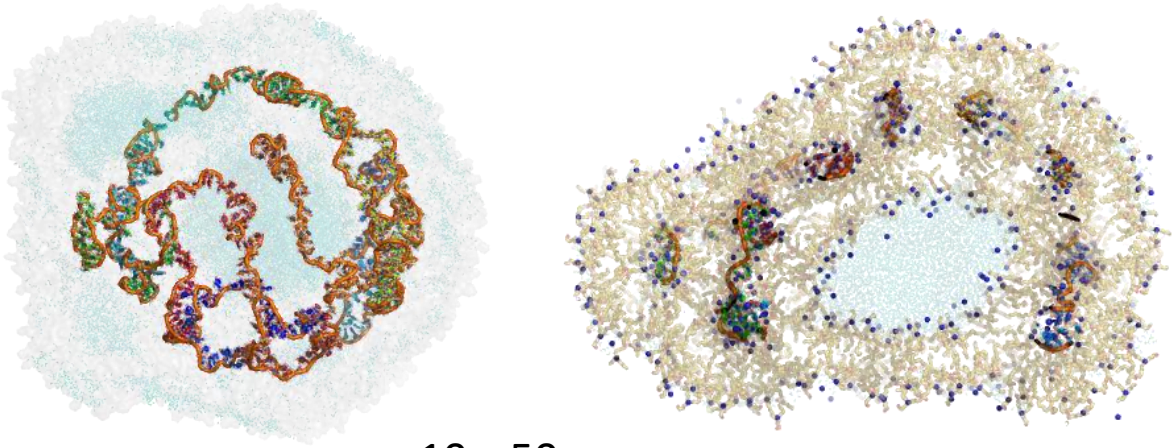
Paloncýová, M.; Šrejber, M.; Čechová, P.; Kührová, P.; Zaoral, F.; Otyepka, M. *J. Phys. Chem. B* **2023**, *127* (5), 1158–1166.

Longer RNA chains

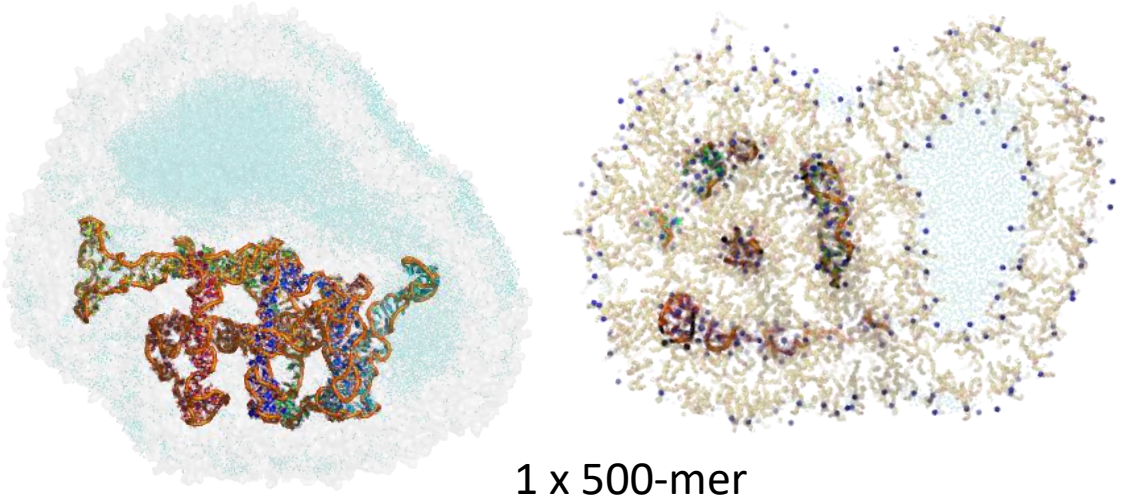
- RNA-rich region – unequally distributed
- Large water droplet
- Lipid hexagonal phase



2 x 200-mer



10 x 50-mer



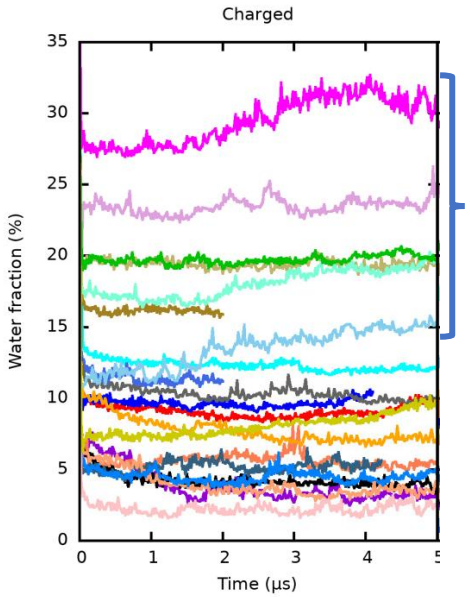
1 x 500-mer

Paloncýová, M.; Šrejber, M.; Čechová, P.; Kührová, P.; Zaoral, F.; Otyepka, M. *J. Phys. Chem. B* **2023**, *127* (5), 1158–1166.

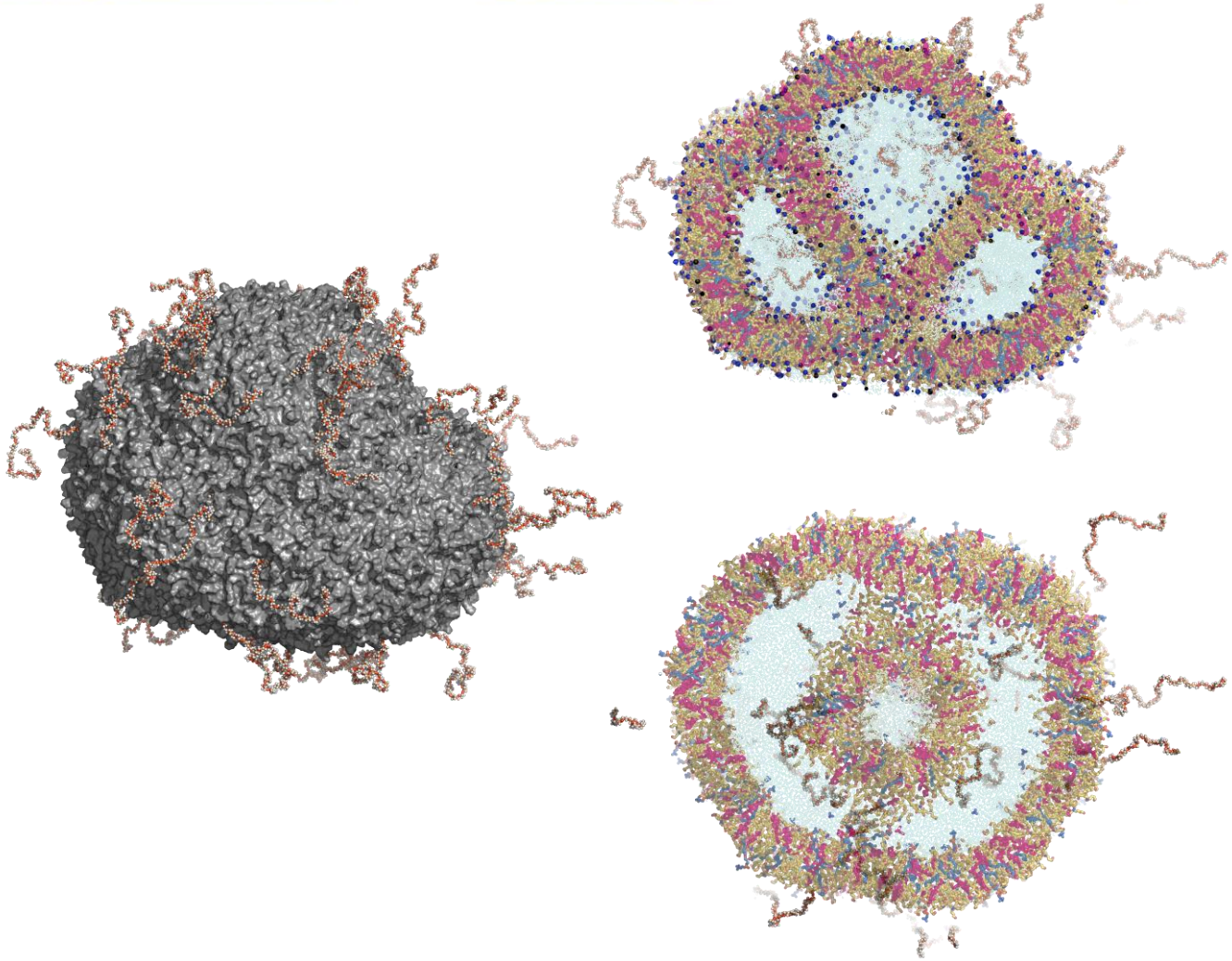
pH ~ 4

RNA-free LNPs

- Higher hydration level
- Several water droplets inside
- Separated by lipid bilayers

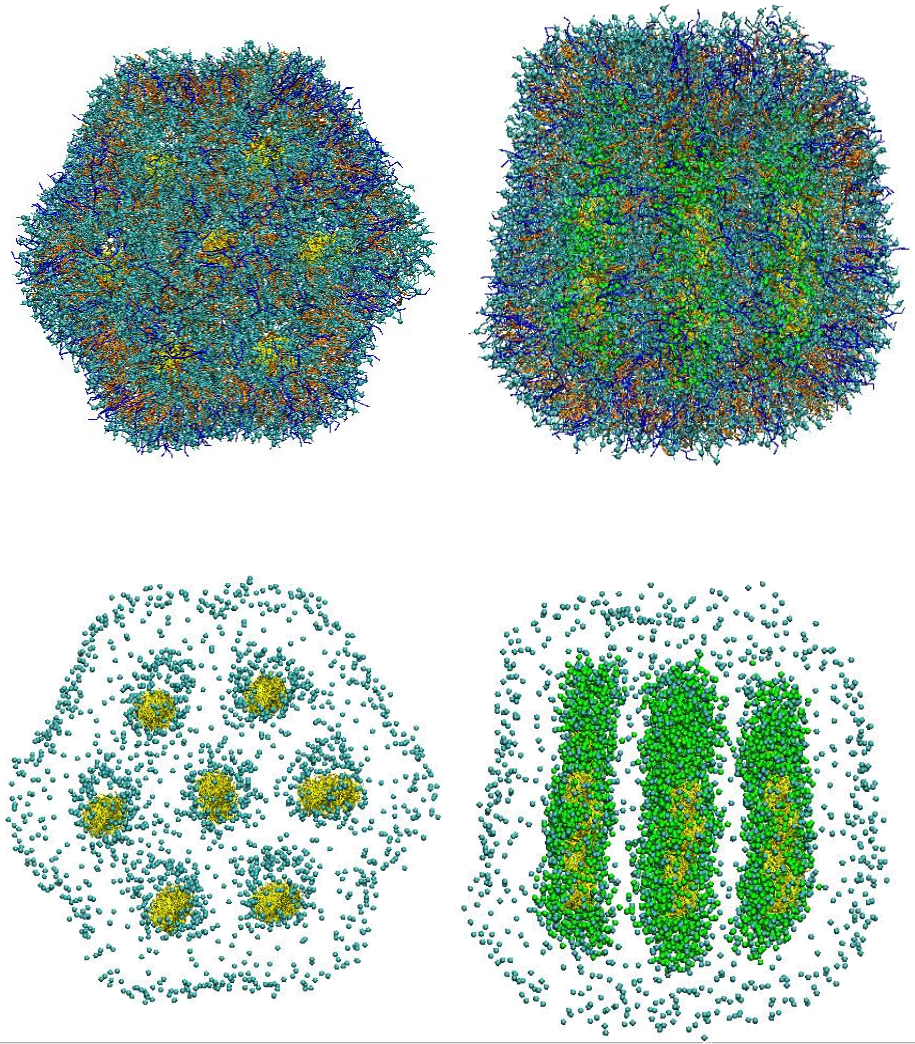


noRNA



Paloncýová, M.; Šrejber, M.; Čechová, P.; Kührová, P.; Zaoral, F.; Otyepka, M. *J. Phys. Chem. B* **2023**, *127* (5), 1158–1166.

LNP Building



- polyAU siRNA
- 20 nm in diameter
- Inverted hexagonal phase – IL, cholesterol
  - Hydration, neutralization
  - TS2CG
- Covered by mixture of IL, cholesterol, DSPC

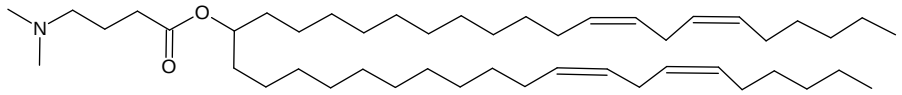
Lisbeth R. Kjølbye

Kjølbye, L. R. et al. Martini 3 Building Blocks for Lipid Nanoparticle Design. *ChemRxiv* **2025**, 02 January 2025. https://doi.org/10.26434/chemrxiv-2024-bf4n8-v2.
van der Pol, R. W. I.; Brinkmann, B. W.; Sevink, G. J. A. *J. Chem. Theory Comput.* **2024**, *20* (7), 2888–2900. https://doi.org/10.1021/acs.jctc.4c00082.

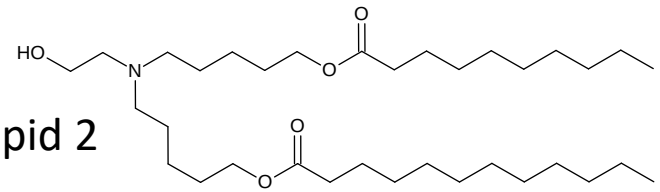
Lipid phase preference

- Lipid 10, MC3 – preserve inverse hexagonal phase
- Lipid 2 – multicompartmental vesicle

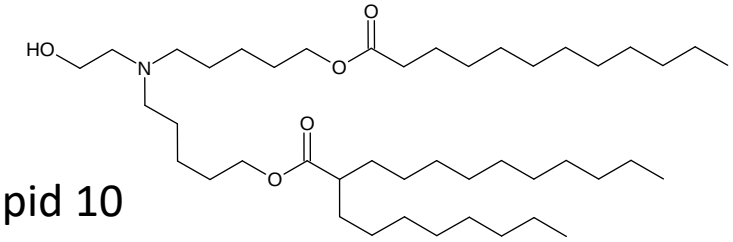
Dlin-MC3-DMA

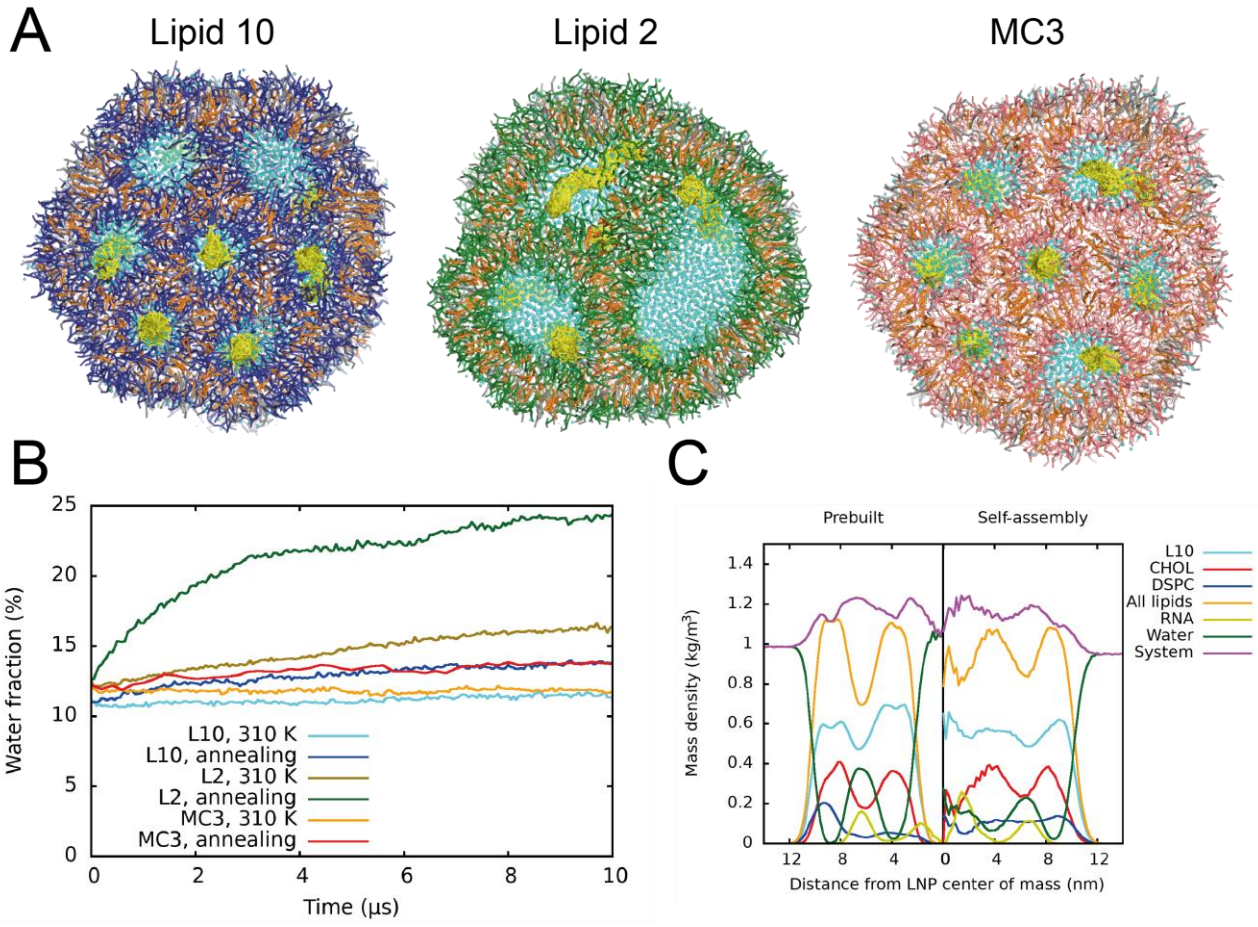


Lipid 2



Lipid 10





~4000 lipids, reaction-field, simulated annealing, MARTINI 3, cholesterol, 10 μs

Kjølbye, L. R. et al. Martini 3 Building Blocks for Lipid Nanoparticle Design. *ChemRxiv* **2025**, 02 January 2025. https://doi.org/10.26434/chemrxiv-2024-bf4n8-v2.

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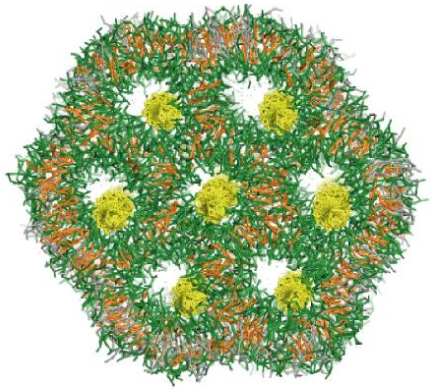
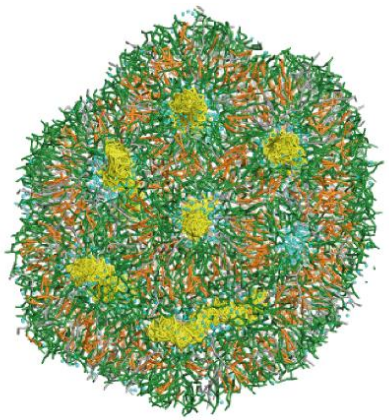
LNP Hydration/ion content

- Water equilibrates during the simulation
- Ions depend on initial structure



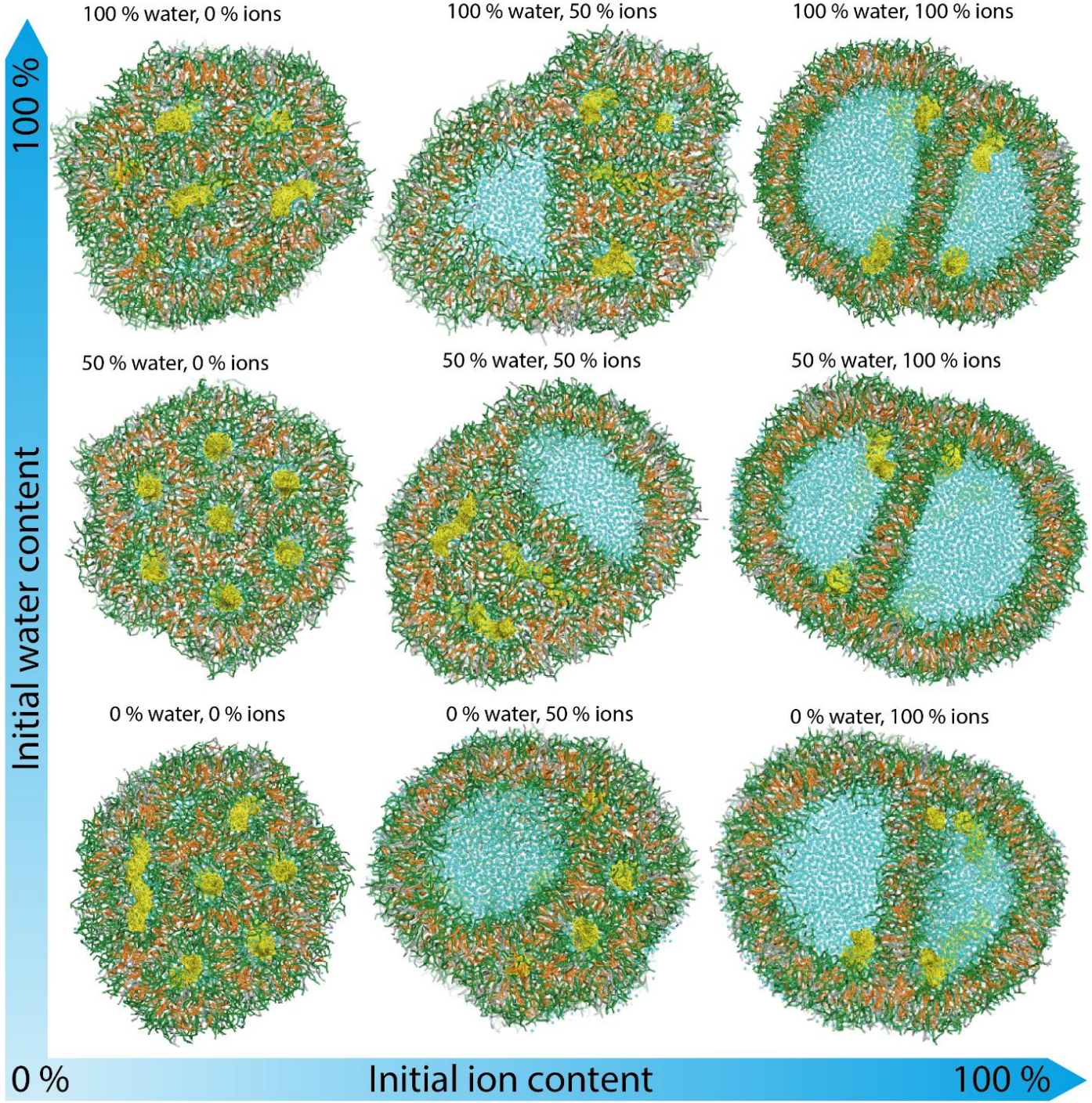
Initial LNP lipid organisation



Self assembly structure

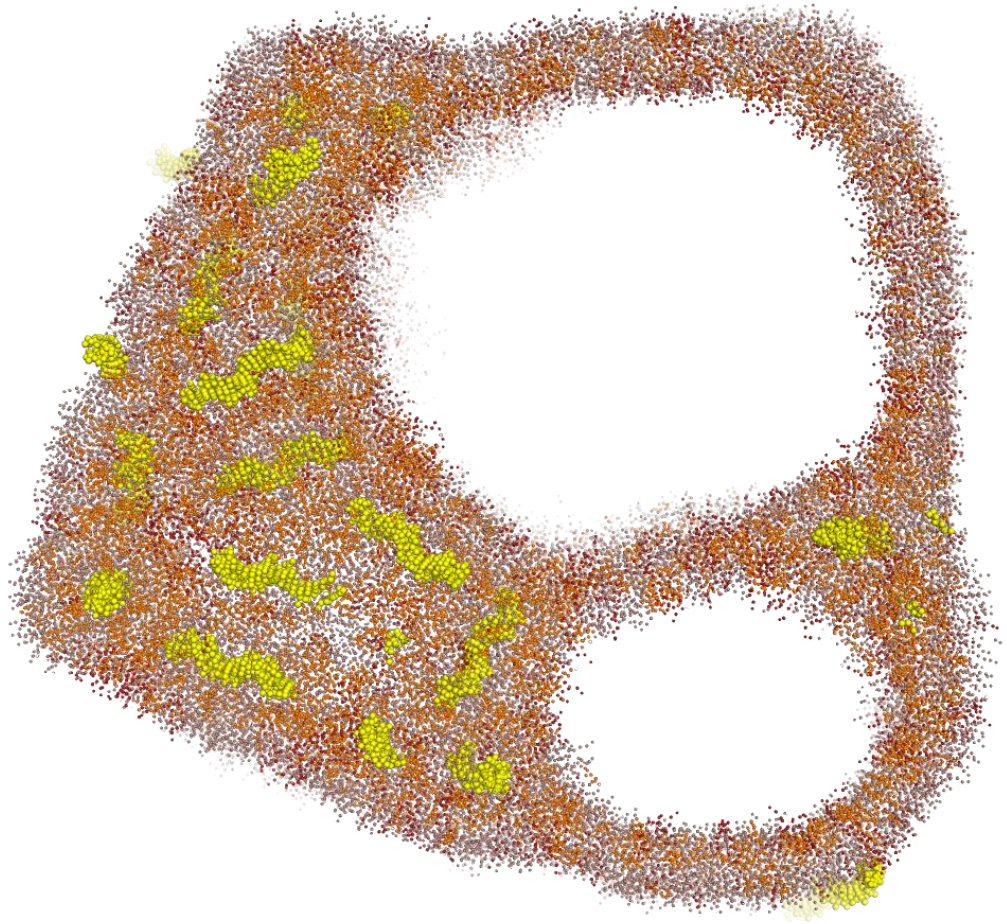
~4000 lipids, reaction-field, simulated annealing, MARTINI 3, cholesterol, 10 µs

Kjølbye, L. R.; et al. Martini 3 building blocks for Lipid Nanoparticle design, ChemRxiv 2024. 10.26434/chemrxiv-2024-bf4n8



Real-scale LNP

- MC3-based LNP
- Self-assembly simulation
- ~50 nm in diameter
- More bleb-phase organization
  - RNA-rich phase
  - Water-rich phase
  - Non-spherical organization



~40,000 lipids, reaction-field, simulated annealing, MARTINI 3, cholesterol, 6 µs

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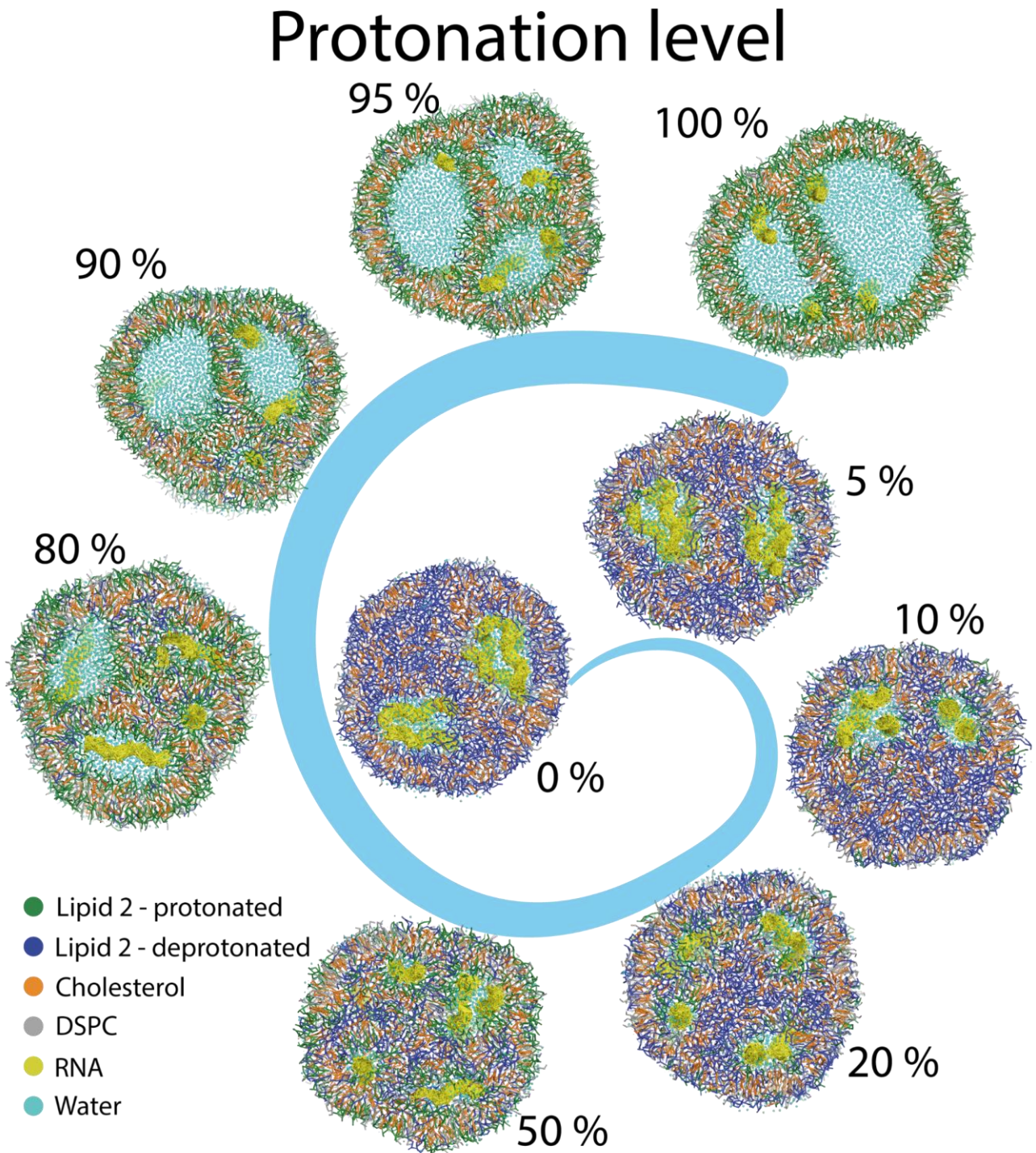
LNP Deprotonation

- Neutral pH
- 80 – 20% - large structural changes

- Dehydration
- RNA „bleb“ phase
- Protonated IL surround RNA

- RNA
  - Interaction with IL(+1)
  - Interaction with water + LNP surface

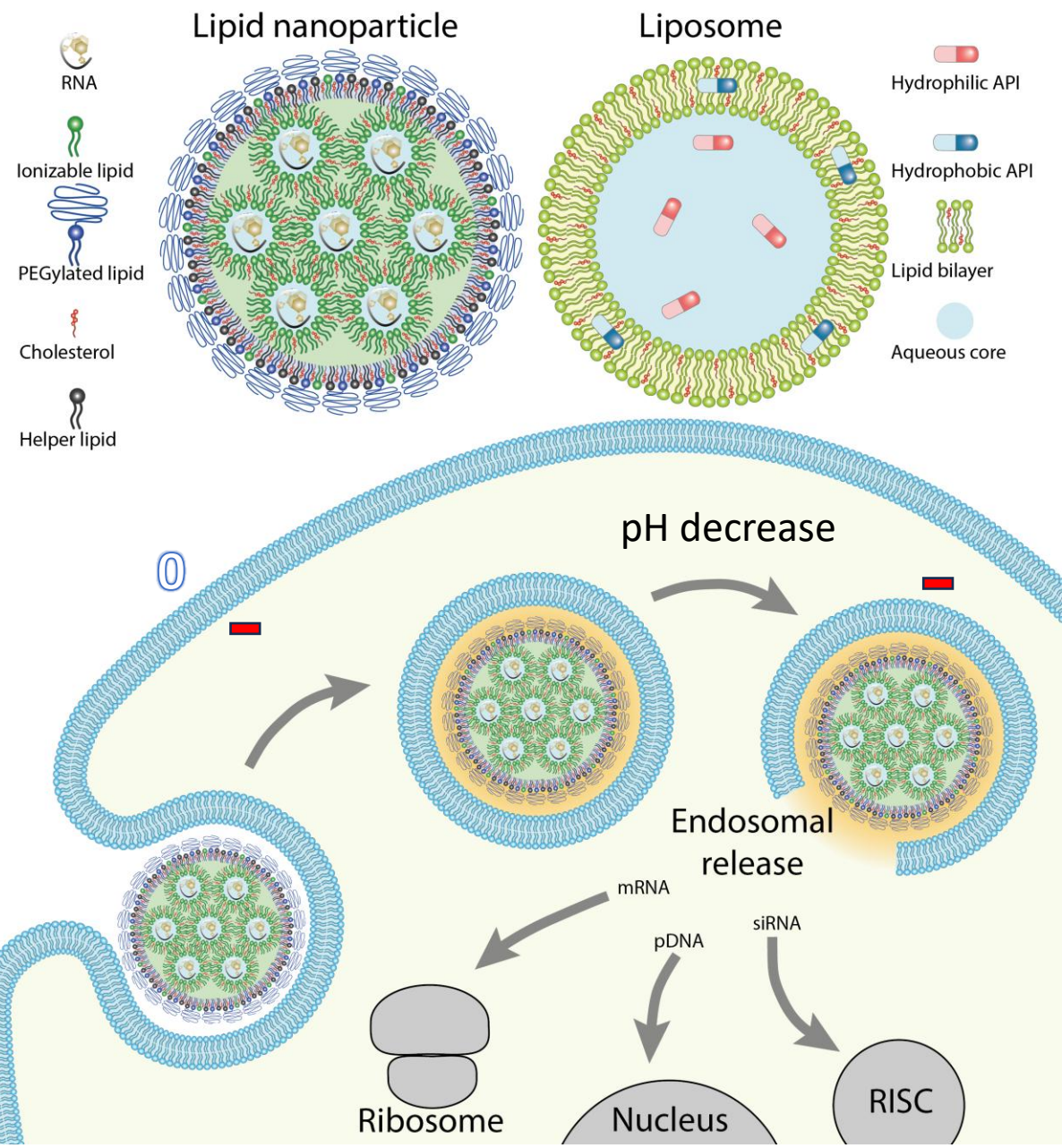


~4000 lipids, reaction-field, simulated annealing, MARTINI 3, cholesterol, 10 µs

Kjølbye, L. R.; et al. Martini 3 building blocks for Lipid Nanoparticle design, ChemRxiv 2024. 10.26434/chemrxiv-2024-bf4n8

Interaction with membranes

- Plasma membrane
  - Outer - neutral
  - Inner - negative
- Endosomal membrane - negative
- Lysosomal membrane – negative



Paloncýová, M. et al. Computational Methods for Modelling Lipid-Mediated Active Pharmaceutical Ingredient Delivery, Submitted to Molecular Pharmaceutics

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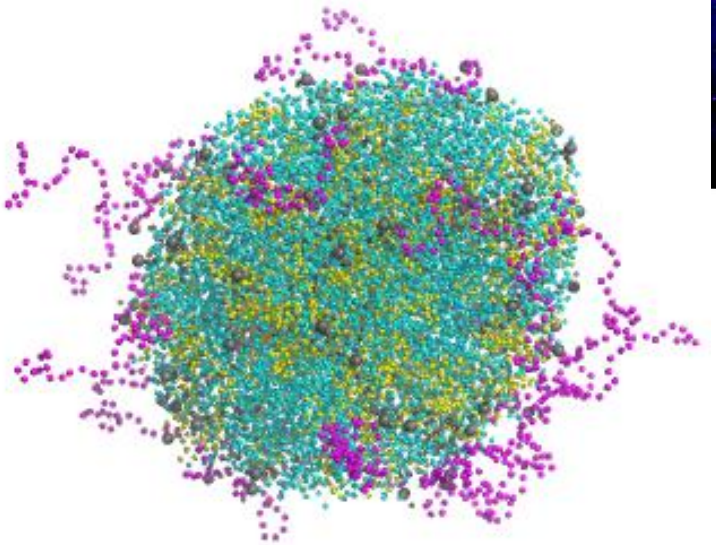
Uncharged LNP with Cell Membranes

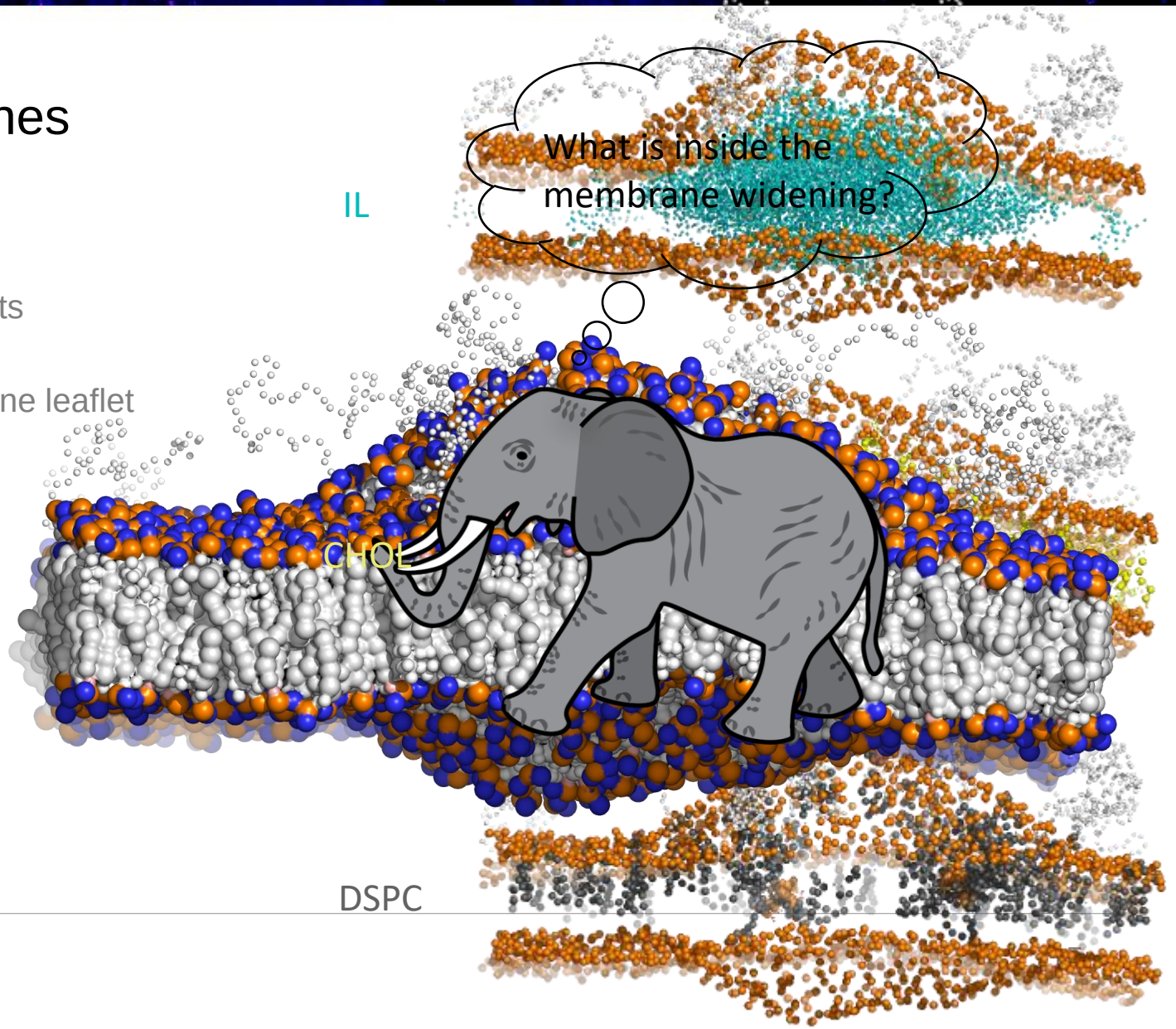
- Rapid merging of LNP with cell membrane

- Fusion into the cell membrane
  - Composition of outer plasma membrane



Uncharged LNP with Cell Membranes

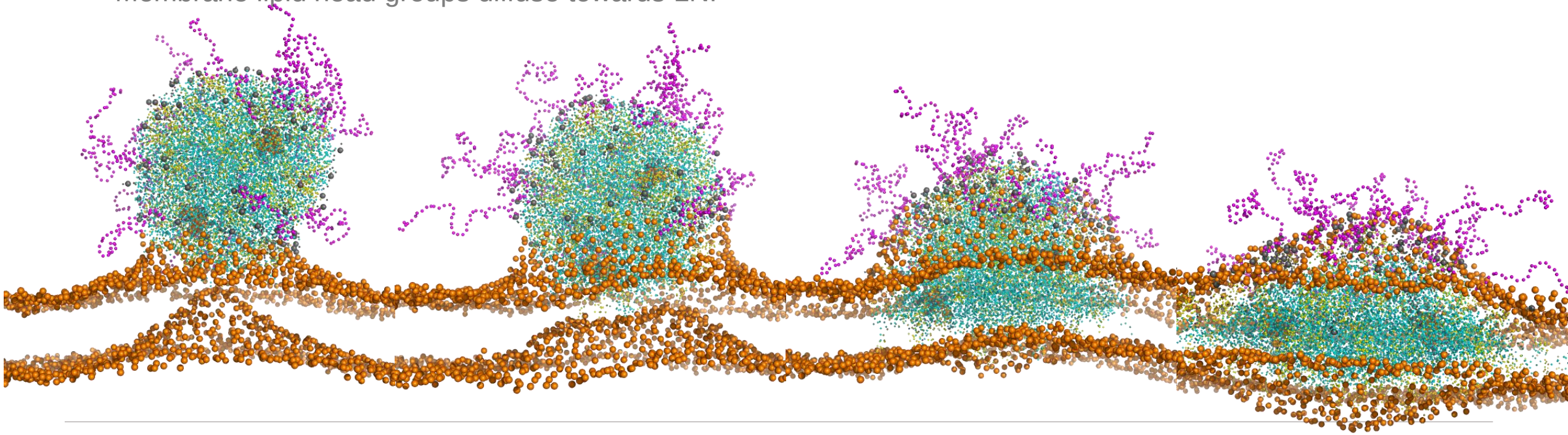
- Rapid merging with cell membrane
  - IL – stays in compact droplet
  - CHOL – dissolves into both membrane leaflets
  - DSPC – dissolves into one membrane leaflet
  - PEGylated lipid – dissolves into one membrane leaflet
    - PEG stays in water



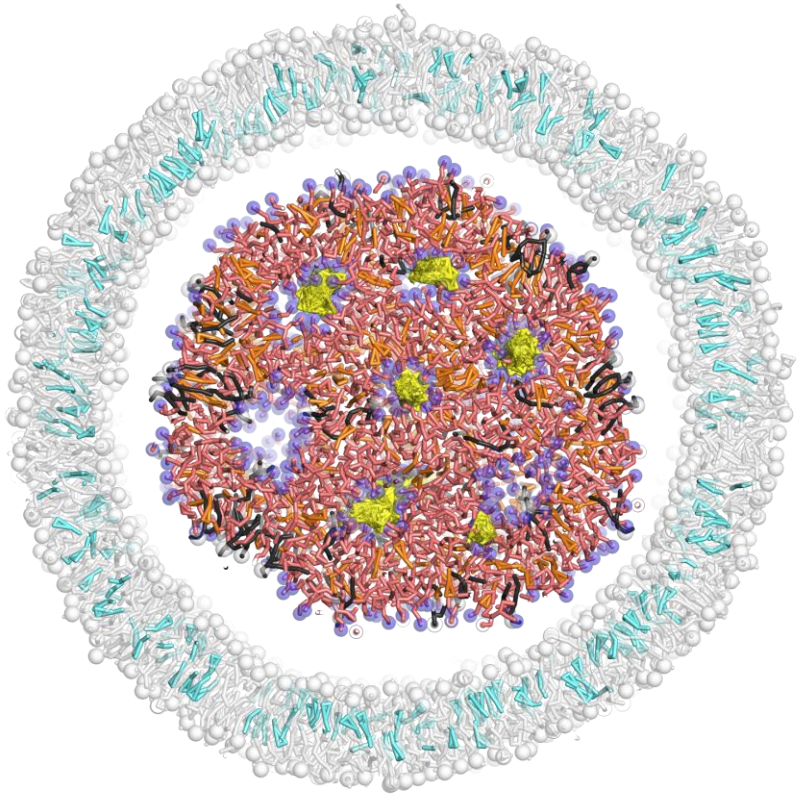
Mechanism of LNP merging with the cell membrane

- Non-PEGylated surface

- Membrane lipid head groups diffuse towards LNP

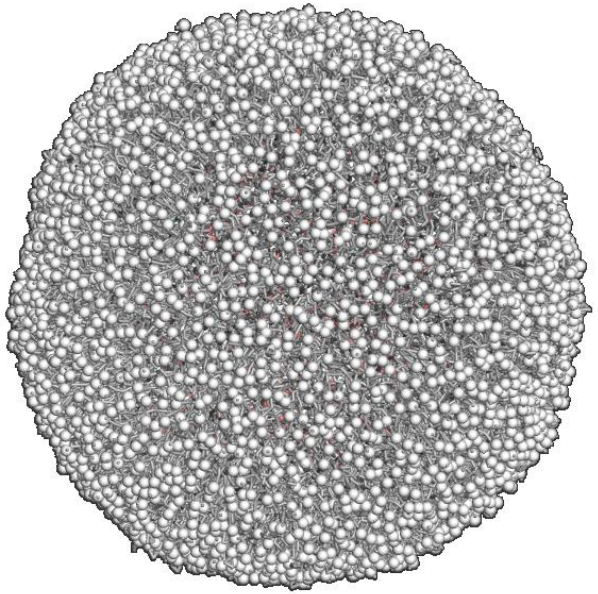


Fusion with Endosome



Kjølbye, L. R.; et al. Martini 3 building blocks for Lipid Nanoparticle design, ChemRxiv 2024. 10.26434/chemrxiv-2024-bf4n8

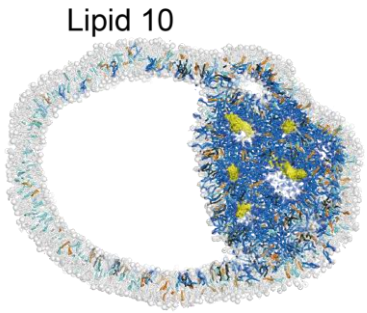
Fusion with Endosome

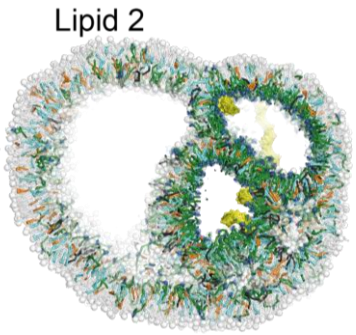


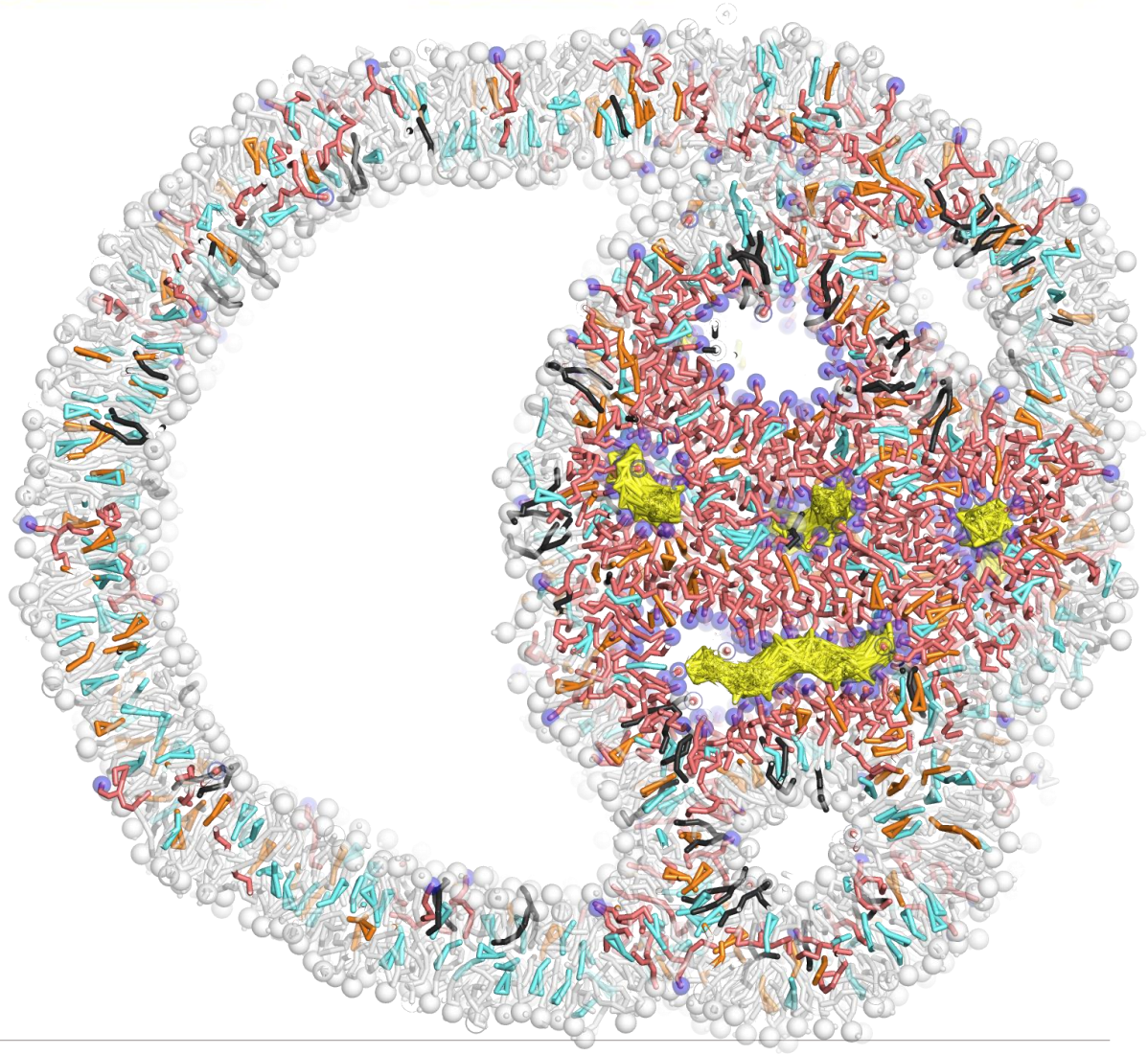
Kjølbye, L. R.; et al. Martini 3 building blocks for Lipid Nanopa
ChemRxiv 2024. 10.26434/chemrxiv-2024-bf4n8

Fused Charged LNP with lipid vesicle

- IL – close to RNA
- Chol – diffuse into the vesicle
  - Flip
- DSPC – diffuse into the vesicle, do not flip
- No RNA release

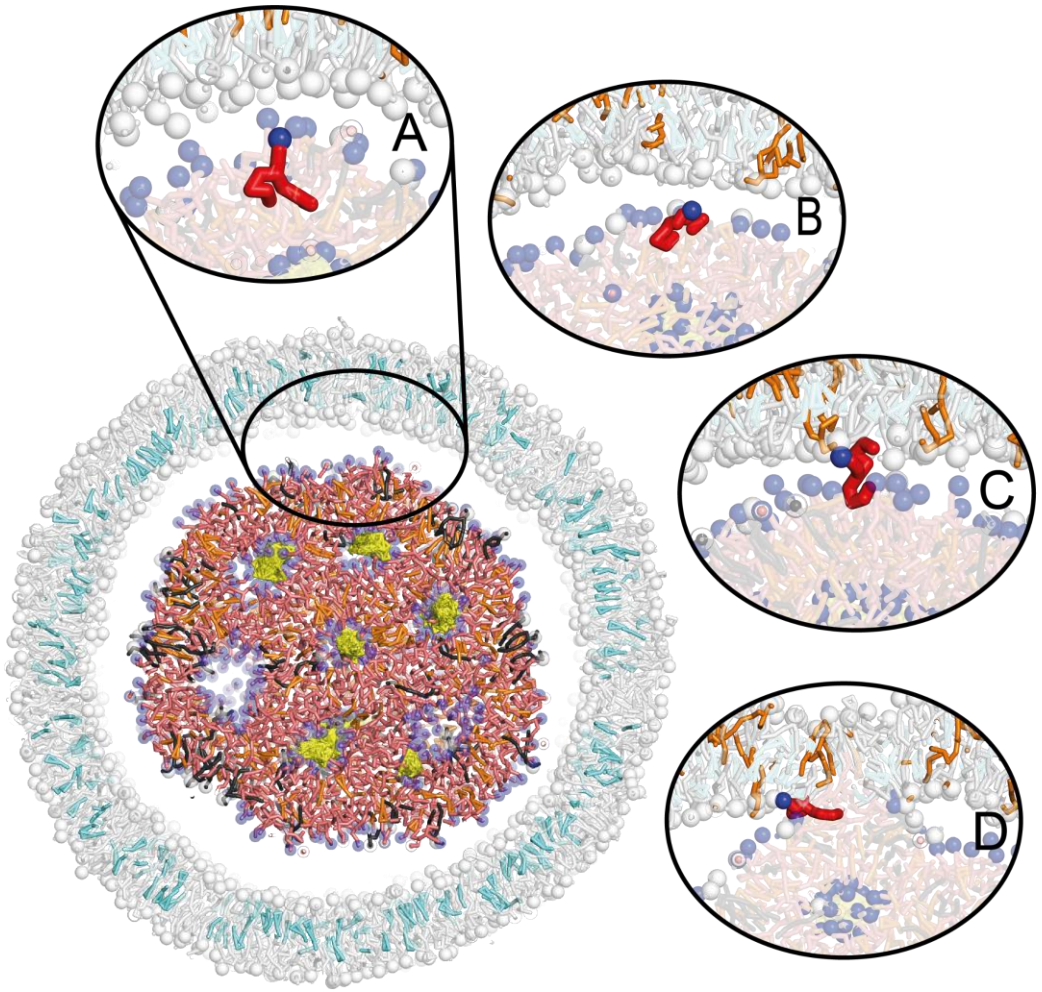






Kjølbye, L. R.; et al. Martini 3 building blocks for Lipid Nanoparticle design, ChemRxiv 2024. 10.26434/chemrxiv-2024-bf4n8

Fusion process

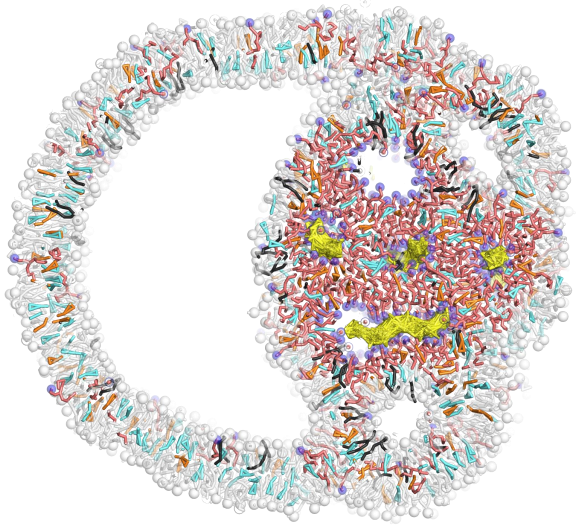


- Initiation
  - Lipid handshake
  - IL in contact with PS
- Stalk formation
- Rapid fusion

Kjølbye, L. R.; et al. Martini 3 building blocks for Lipid Nanoparticle design, ChemRxiv 2024. 10.26434/chemrxiv-2024-bf4n8

Conclusion

- Can we predict LNP organization and stability with MD?
  - Organization – H_{II} phase mostly, bias with scale
    - Initial structure
      - Water permeates through lipid matrix
      - Ions stay trapped in initial position
      - Lipids can change their adopted phase
    - Electrostatics treatment plays a role
  - Stability – partially, pH dependence



- Can we quantify RNA delivery efficiency with MD?
  - Not yet
  - Merging of LNP in uncharged state with plasma membrane – proteins in reality
  - Fusion of LNP with endosome
  - So far no RNA release

Project Details

Mostly OPEN-32-24

- Karolina – multiple topics were calculated
  - 12 500 CPU node hours – used fully
  - 1 440 GPU node hours – used fully
- Lumi – mistake in project planning, not all resources used
- Students involved

- Open-On-Demand – Desktop, Jupyter Notebook
  - Env containing Python+Gromacs
- TigerVNC

Simulation setup

- System building
  - Interactive jobs
    - Assembly
    - Minimization + equilibration
      - GROMACS
  - AA – GPU
  - CG – CPU

- Analyses
  - GROMACS tools
  - AMBER tools (cpptraj)
  - MDAnalysis

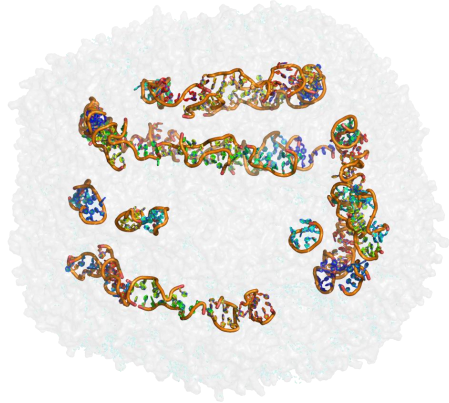
Molecular descriptions of complex biosystems

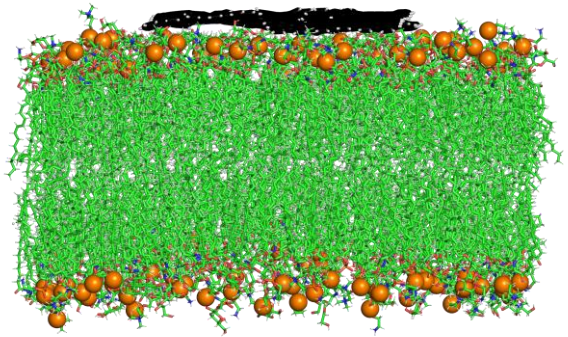
- In-silico description of complex biosystems, focused on MD simulations of systems combining multiple types of biomolecules with nanomaterials

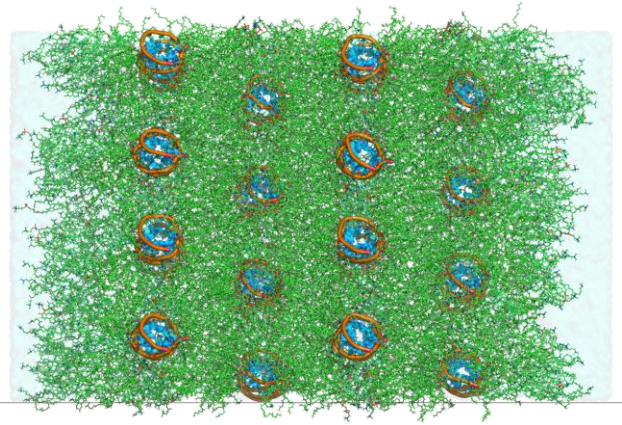
- Date: 8th – 9th September, 2025
- Venue: IT4Innovations, Ostrava, Czech Republic

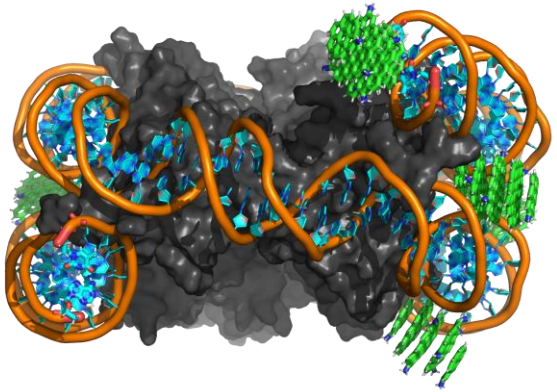
- Lipids, Nucleic acids, Carbon nanomaterials
- Theory of individual systems, FF compatibility
- Practical workshops

https://molecular-descriptions-complex-biosystems.cost-cosy.eu/



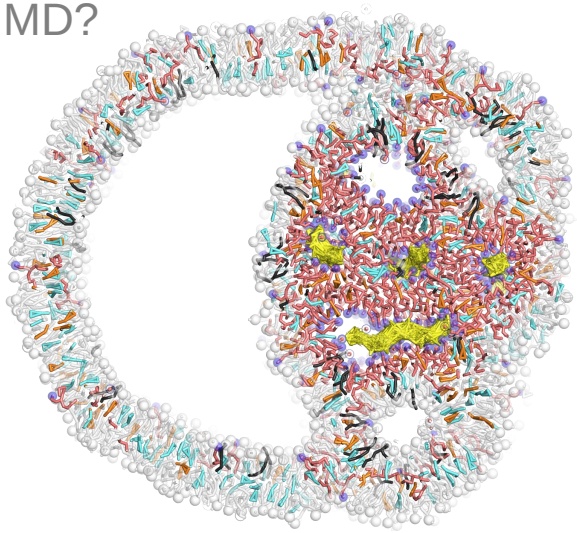






Thank you for your attention

- Can we predict LNP organization and stability with MD?
  - Organization – H_{II} phase mostly, bias with scale
    - Initial structure
      - Water permeates through lipid matrix
      - Ions stay trapped in initial position
      - Lipids can change their adopted phase
    - Electrostatics treatment plays a role
  - Stability – partially, pH dependence



- Can we quantify RNA delivery efficiency with MD?
  - Not yet
  - Merging of LNP in uncharged state with plasma membrane – proteins in reality
  - Fusion of LNP with endosome
  - So far no RNA release

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Martin Šrejber
Petra Kührová
Petra Čechová
Adéla Chadalíková
Pavel Banáš
Michal Otyepka

Lisbeth R. Kjølbye
Mariana Valério
Paulo C. T. Souza

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