Decoding Lipid Nanoparticle Structure: From Lipid Composition to pH through Multiscale Simulations

CATRIN
Czech Advanced Technology and Research Institute

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Date 6th November, 2026

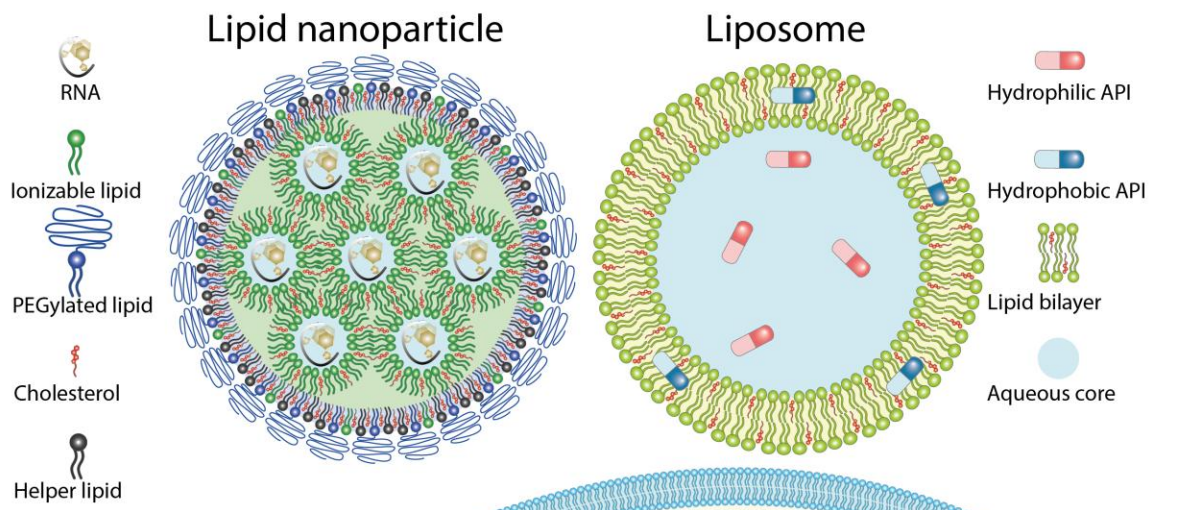
Co-funded by the European Union

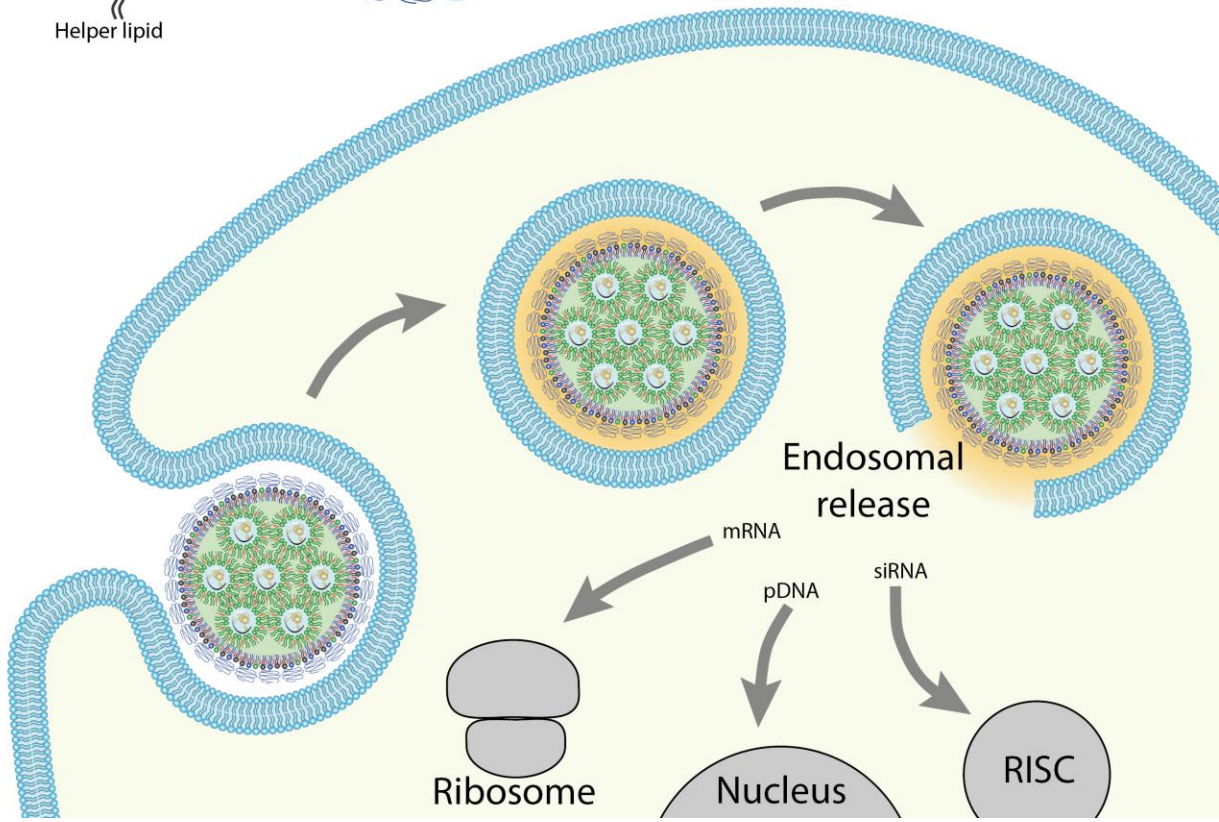
Ministry of Education, Youth and Sports of the Czech Republic

The work was supported from ERDF/ESF
Projekt TECHSCALE No. CZ.02.01.01/00/22_008/0004587

- RNA delivery vehicles

- 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>

- Predict LNP organization and stability with MD
  LNP organization, pH response
- 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.

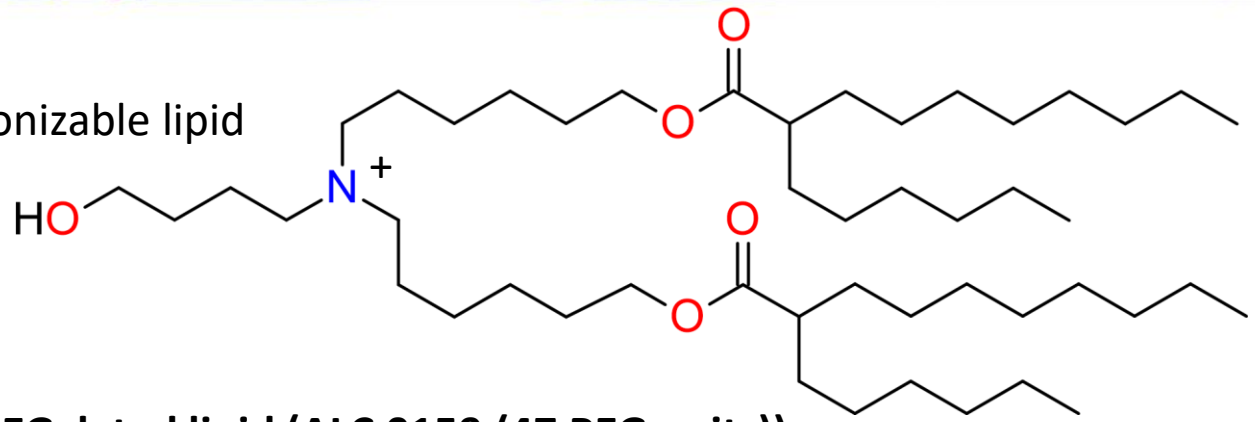
Lipid Nanoparticle Composition

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

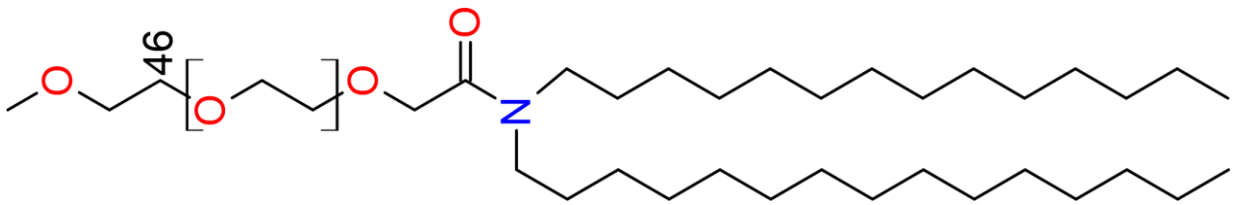
- Phenomenon to study – what is our question?
  - Do we need a model of a discrete particle?
    - Internal organization?
    - Surface layer?
  - Do we need all parts of the system?
    - PEGylation?

- Resolution
- Method

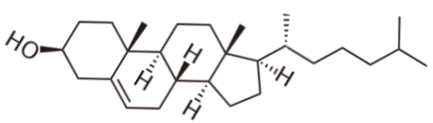
Ionizable lipid



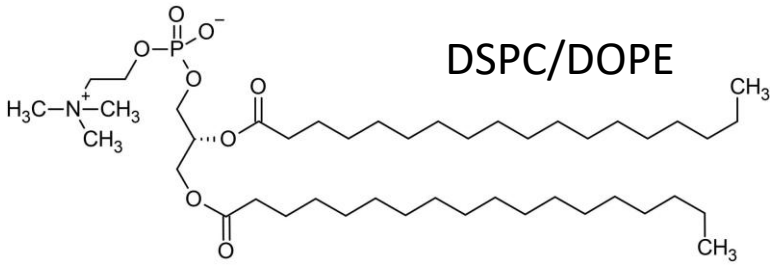
PEGylated lipid (ALC 0159 (47 PEG units))



Cholesterol

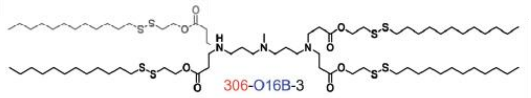


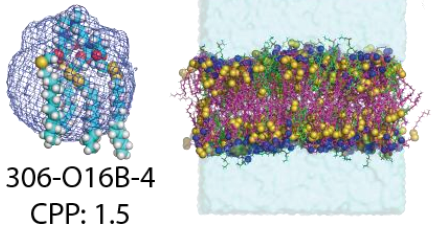
DSPC/DOPE



MD Simulation Approaches to LNP

All-atom lipid bilayer screening



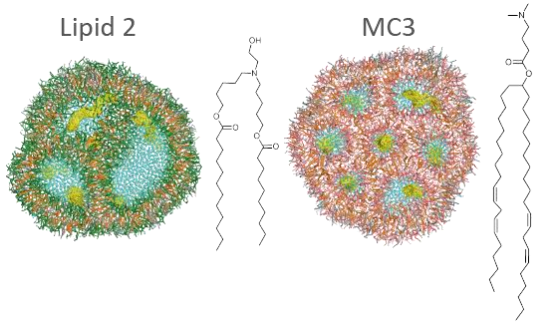


306-O16B-4
CPP: 1.5

306-O16B-3
CPP: 2.2

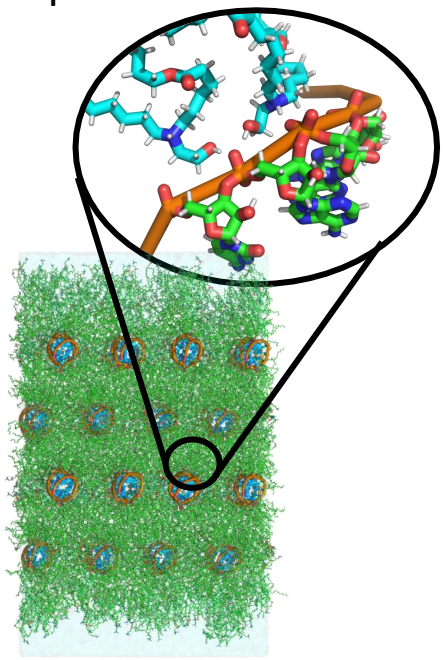
Bilayer stability
Lipid shape

Coarse-grained LNP simulation



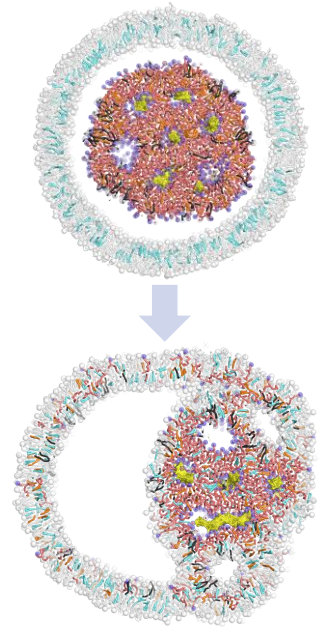
Structure
Curvature
Lipid mobility
pH induced phase change

Lipid-RNA interactions



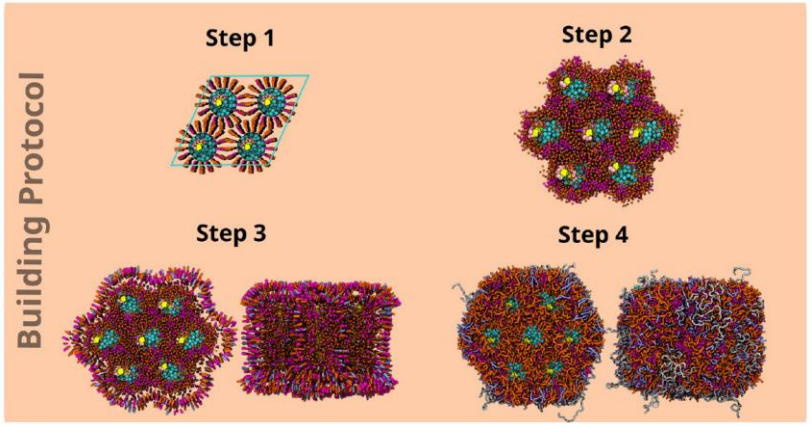
Atomistic interactions
H_{II} phase stability

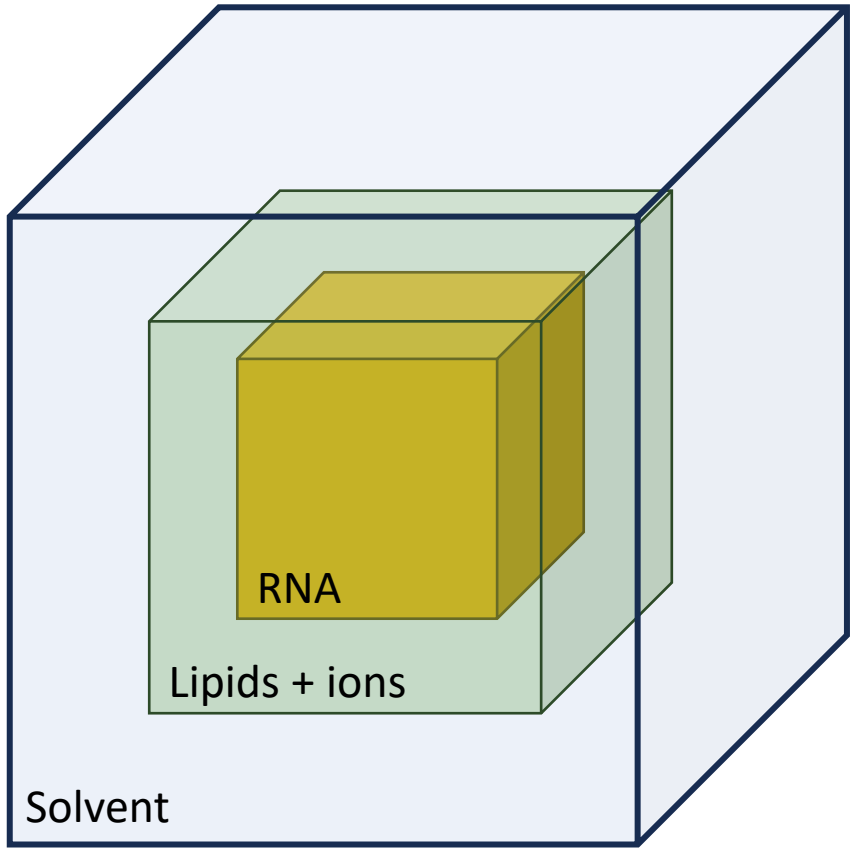
Endosomal escape simulation



LNP-endosome
interaction mechanism

Finite LNP Building





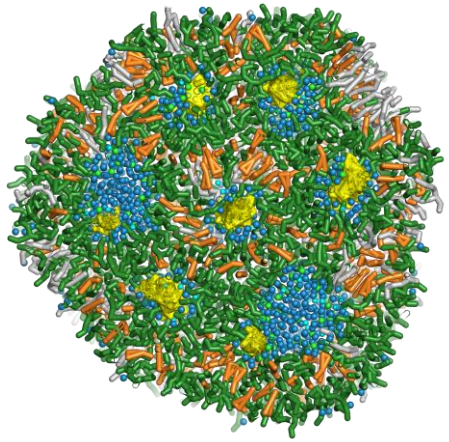
Kjølbye, L. R.; et al. Martini 3 Building Blocks for Lipid Nanoparticle Design. *J. Chem. Theory Comput.* **2025**. https://doi.org/10.1021/acs.jctc.5c01207.
van der Pol, R. W. I.; Brinkmann, B. W.; Sevink, G. J. A. *J. Chem. Theory Comput.* **2024**, *20* (7), 2888–2900. https://doi.org/10.1021/acs.jctc.4c00082.

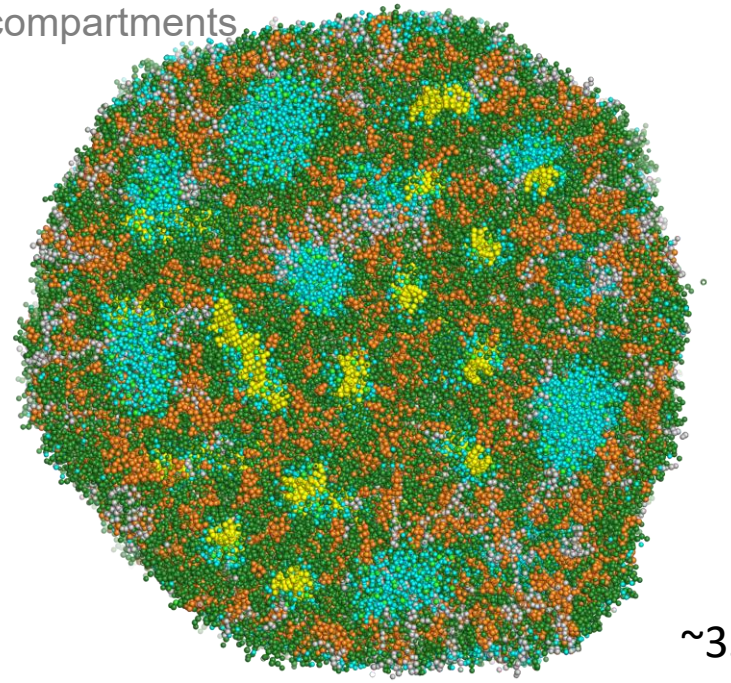
Assembly conditions

- Preparation dependence
  - Water&ions inside/outside
  - Prebuilt/self assembly
  - RNA model

  - Fairly regular internal organization
  - Large bilayer-like compartments

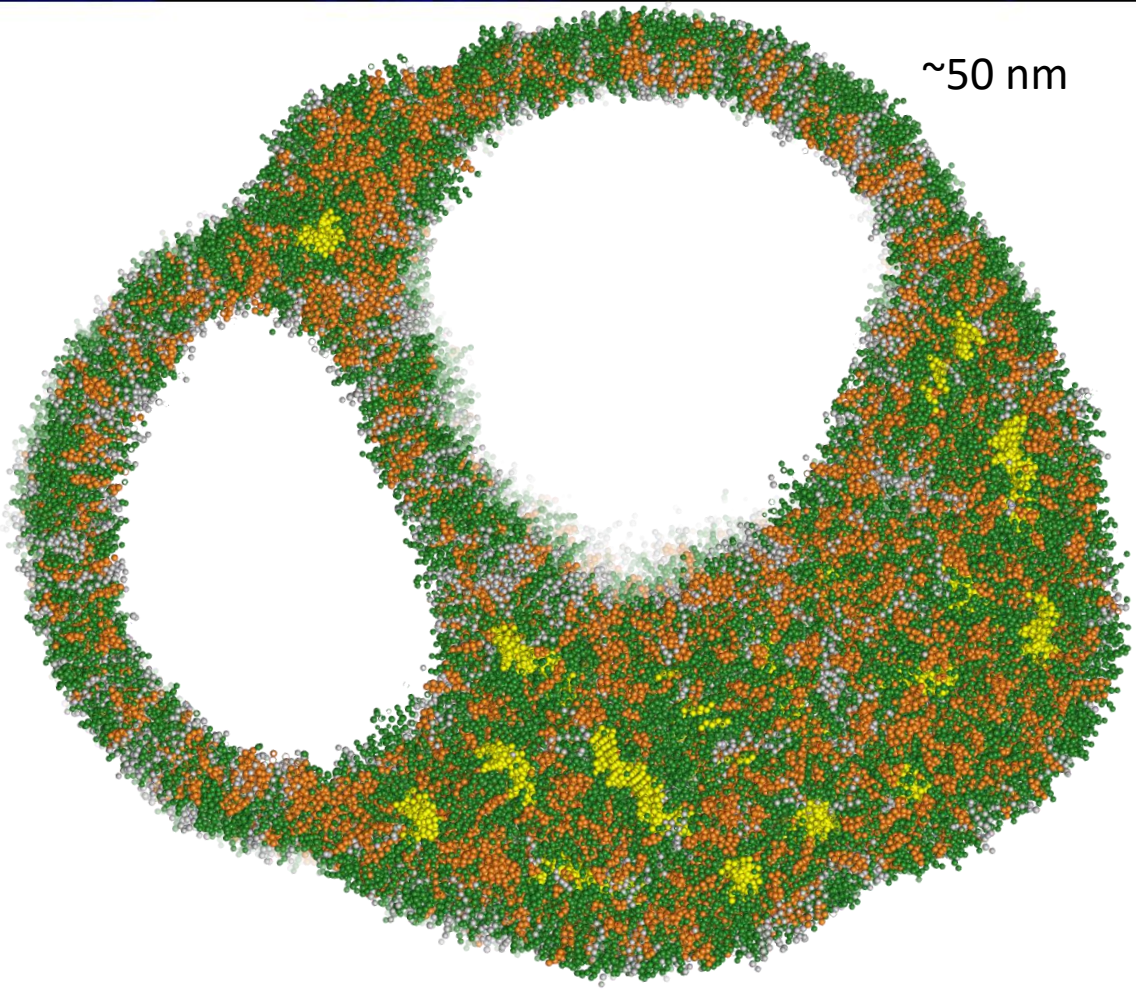
~20 nm





~35 nm

~50 nm

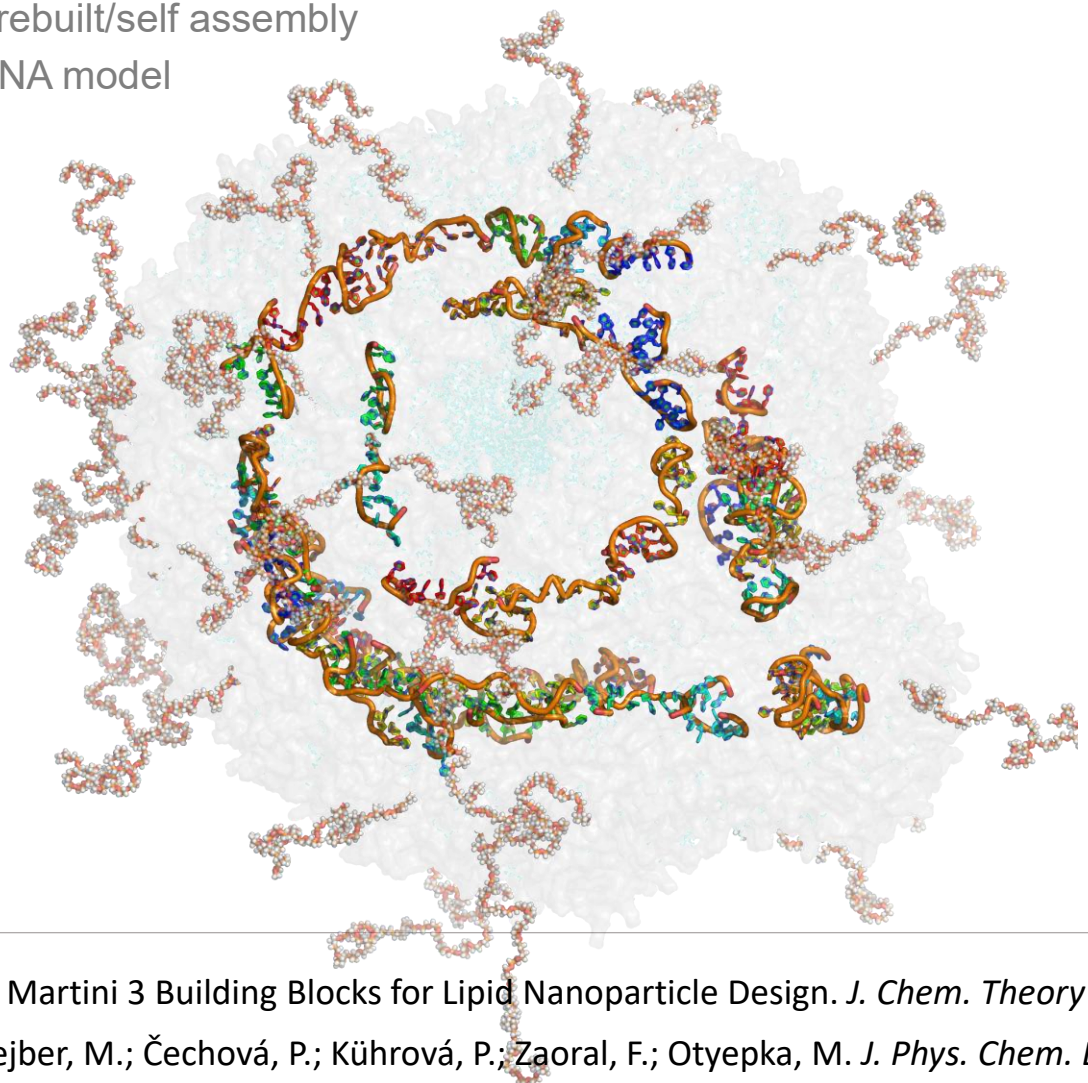


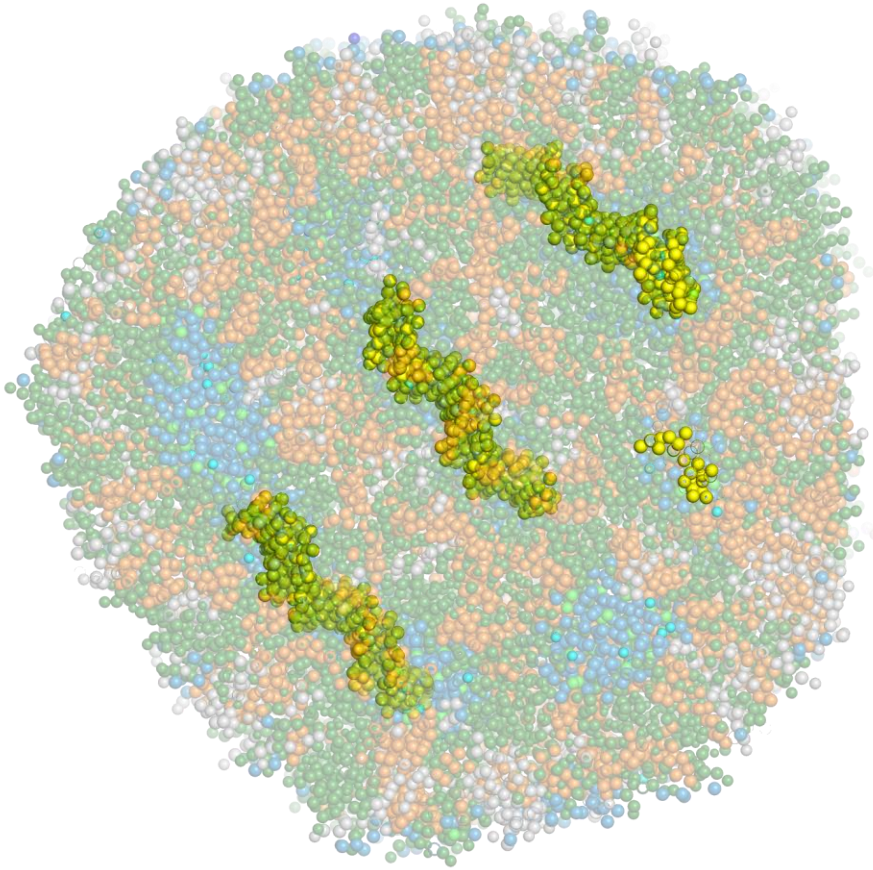
~20,000 lipids, reaction-field, simulated annealing, MARTINI 3, cholesterol, 10 µs

Unpublished data

Assembly conditions

- Preparation dependence
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  - RNA model





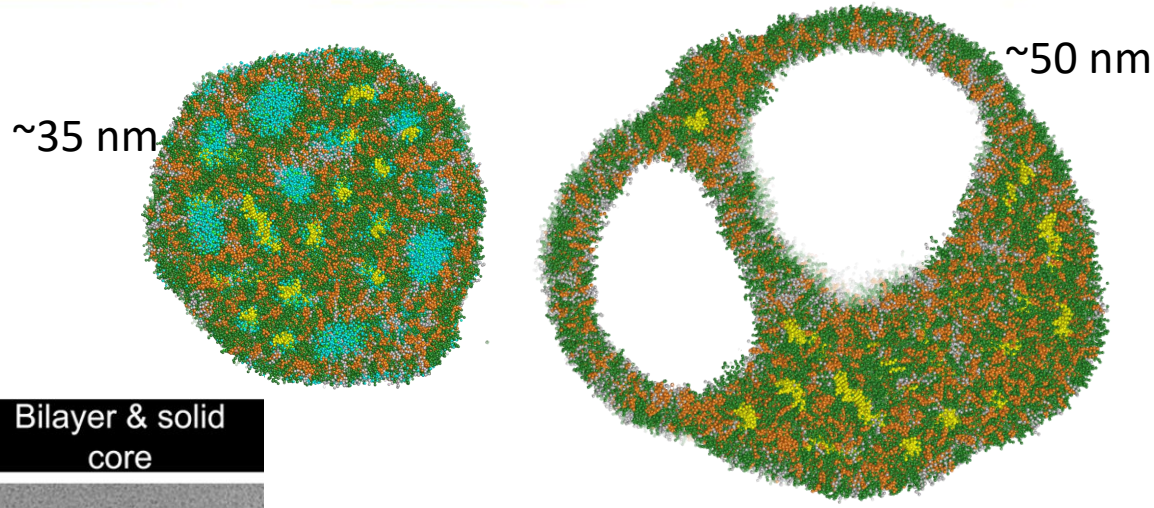
Kjølbye, L. R.; et al. Martini 3 Building Blocks for Lipid Nanoparticle Design. *J. Chem. Theory Comput.* **2025**. https://doi.org/10.1021/acs.jctc.5c01207.

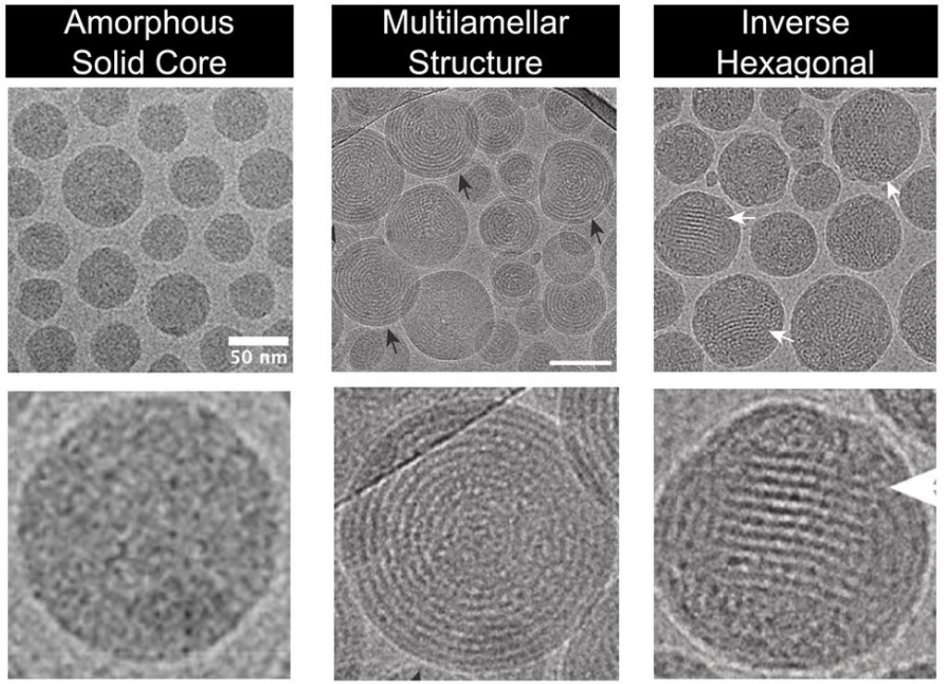
Paloncýová, M.; Šrejber, M.; Čechová, P.; Kührová, P.; Zaoral, F.; Otyepka, M. *J. Phys. Chem. B* **2023**, *127* (5), 1158–1166.

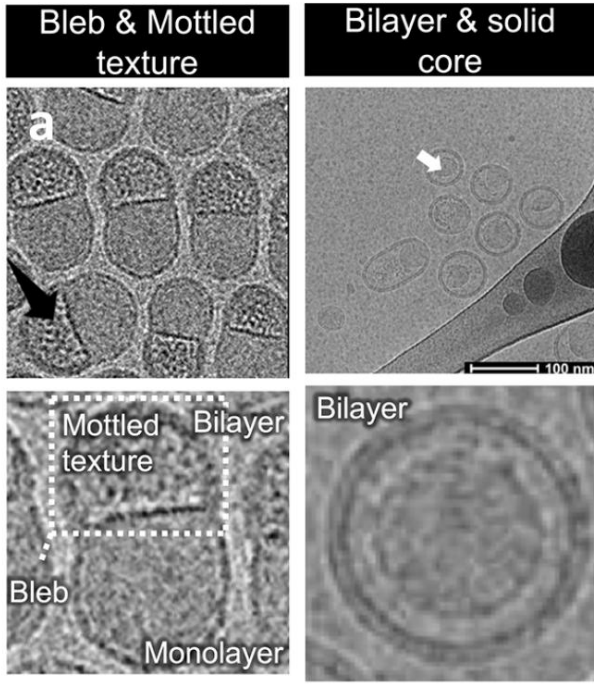
Assembly conditions

- Preparation dependence
  - Water&ions inside

  - Fairly regular internal organization
  - Large bilayer-like compartments







- Kulkarni, J. A.; On the Formation and Morphology of Lipid Nanoparticles Containing Ionizable Cationic Lipids and siRNA. ACS Nano 2018, 12 (5), 4787−4795.
- Pattipeiluhu, R.; Liquid Crystalline Inverted Lipid Phases Encapsulating siRNA Enhance Lipid Nanoparticle Mediated Transfection. Nat. Commun. 2024, 15 (1), No. 1303.
- Brader, M. L.; Encapsulation State of Messenger RNA inside Lipid Nanoparticles. Biophys. J. 2021, 120 (14), 2766−2770.
- Cheng, M. H. Y. Liposomal Lipid Nanoparticles for Extrahepatic Delivery of mRNA. Nat. Commun. 2025, 16 (1), No. 4135.

Mo, Y.; Zheng, G. From Morphology to Mechanism: Cryo-Electron Microscopy Insights into Lipid Nanoparticles for RNA Delivery. *ACS Nano* **2026**, *20* (28), 19906–19929. https://doi.org/10.1021/acsnano.6c09354.

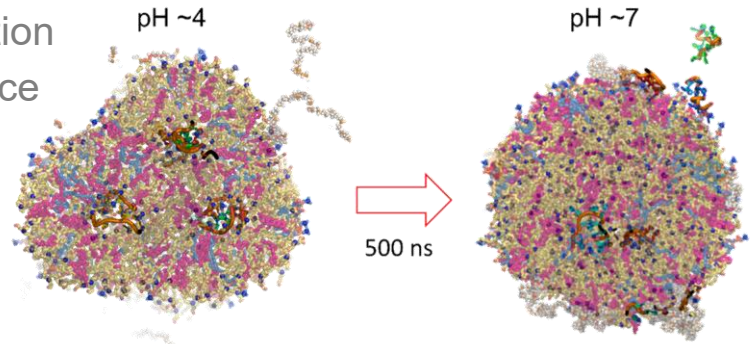
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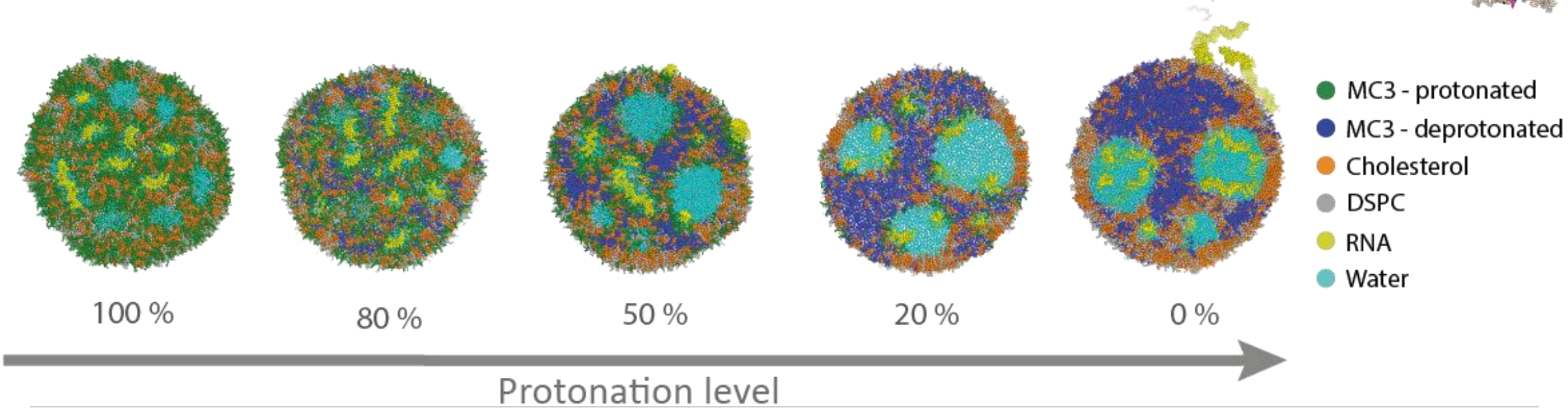
pH Dependent Behavior

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

- Dehydration, phase separation
- RNA „bleb“ phase

- RNA
  - Interaction with IL(+1) - accumulation
  - Interaction with water + LNP surface
  - mRNA structure would be highly valuable





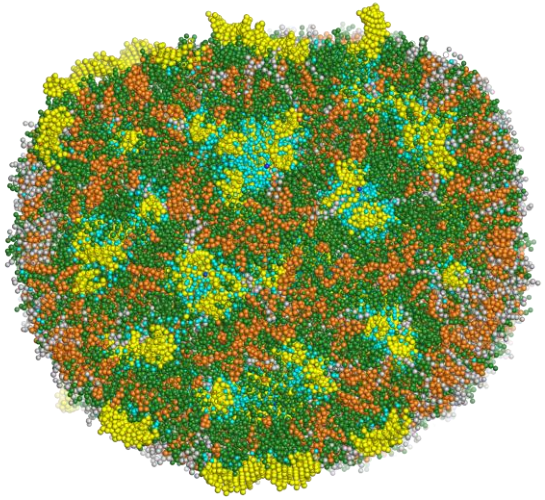
~4000 lipids, MC3, reaction-field, simulated annealing, MARTINI 3, cholesterol, 1.5 µs

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

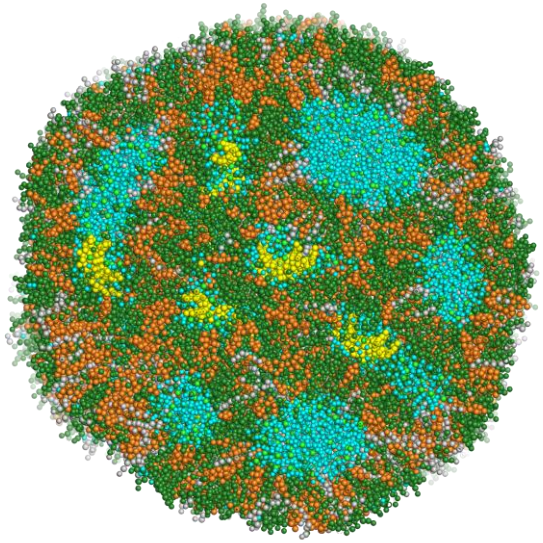
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CG self assembly in acidic pH

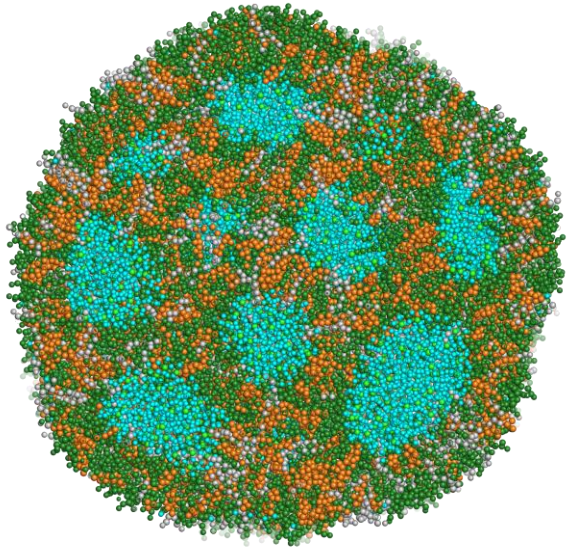
- RNA loading alters the structure



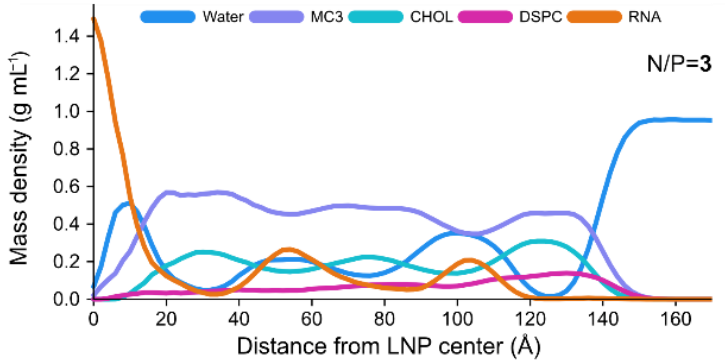
NP 1

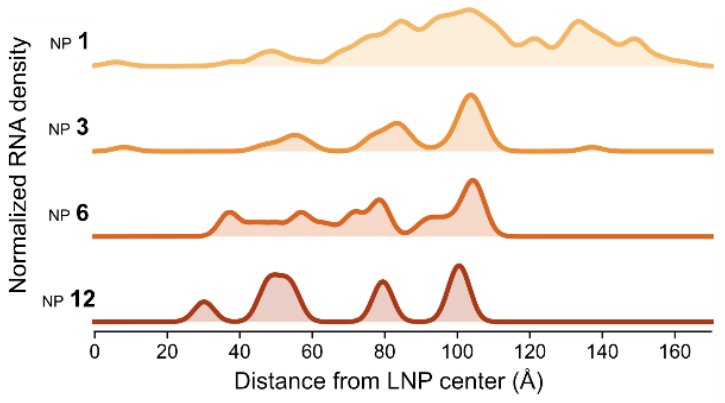


NP 6



RNA free





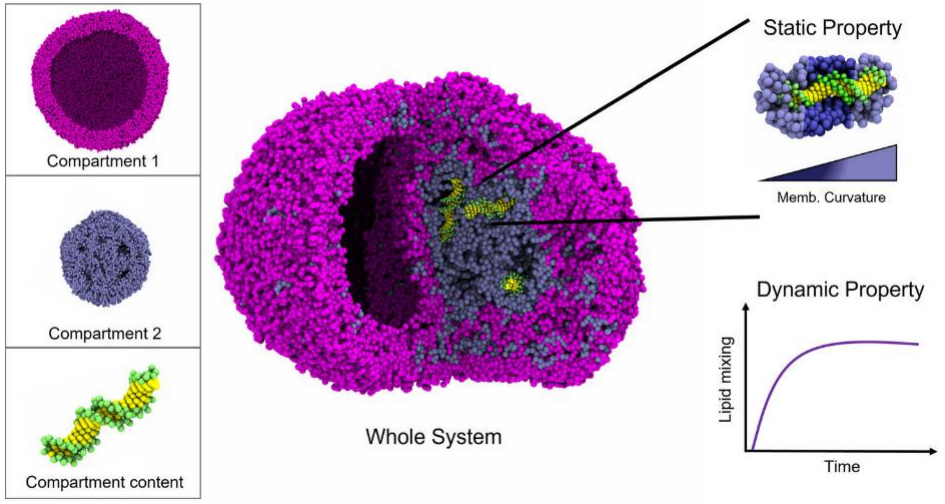
MC3, 10000 lipids, rigid siRNA, simulated annealing, MARTINI 3, cholesterol, 10 µs

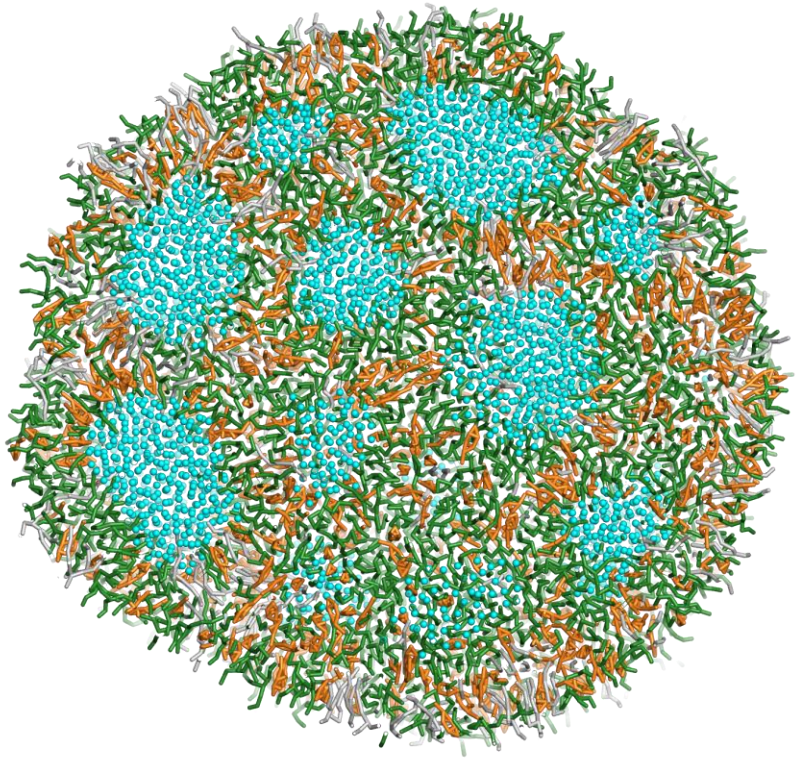
Unpublished data, to be submitted soon

LNP compartmentalization

CG self assembly in acidic pH

- Does the inner structure differ?
- Do the lipids exchange between compartments?
- How to define a compartment?





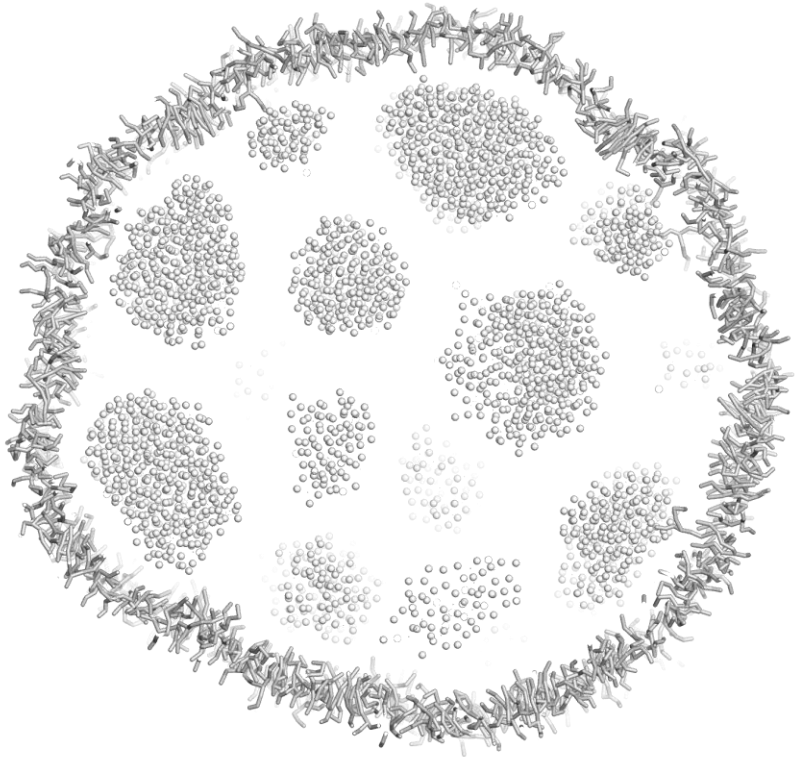
Čechová, P.; Kührová, P.; Šrejber, M.; Valério, M.; Borbuliak, M.; Souza, P. C. T.; Otyepka, M.; Paloncýová, M. *Small Struct.* **2026**, *7* (3). https://doi.org/10.1002/sstr.202500697.

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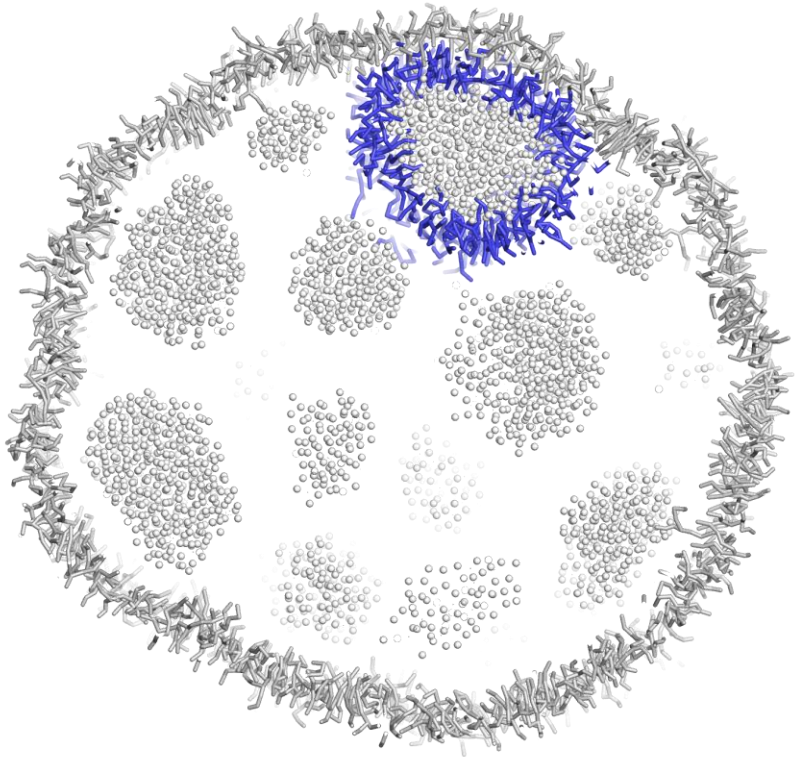


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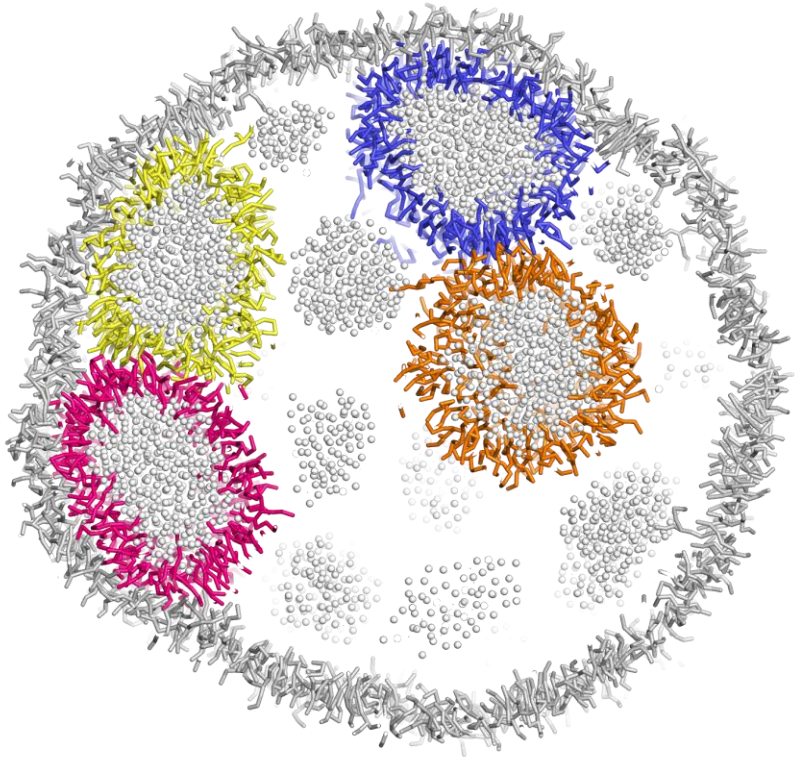


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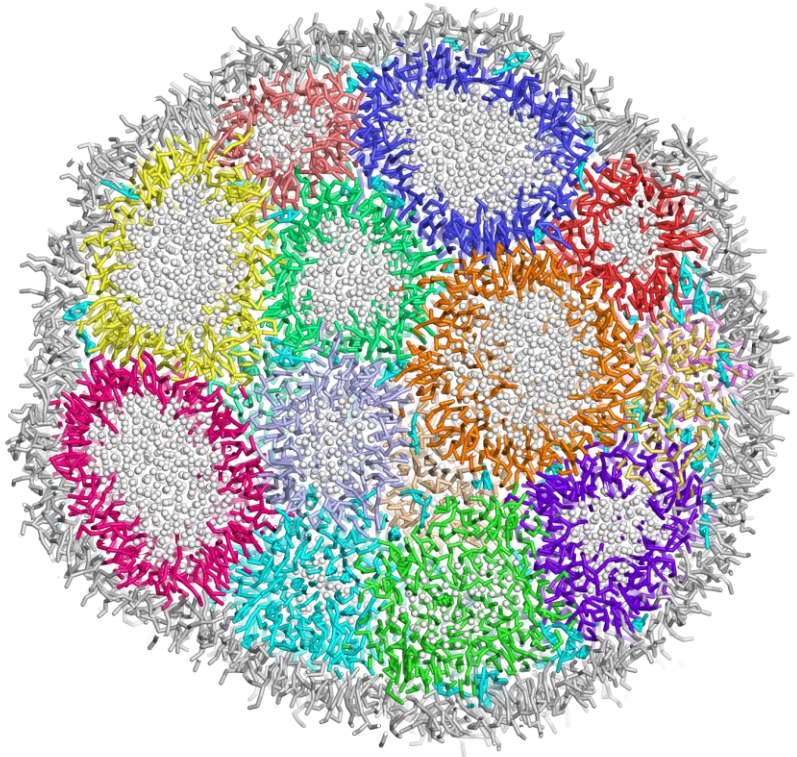


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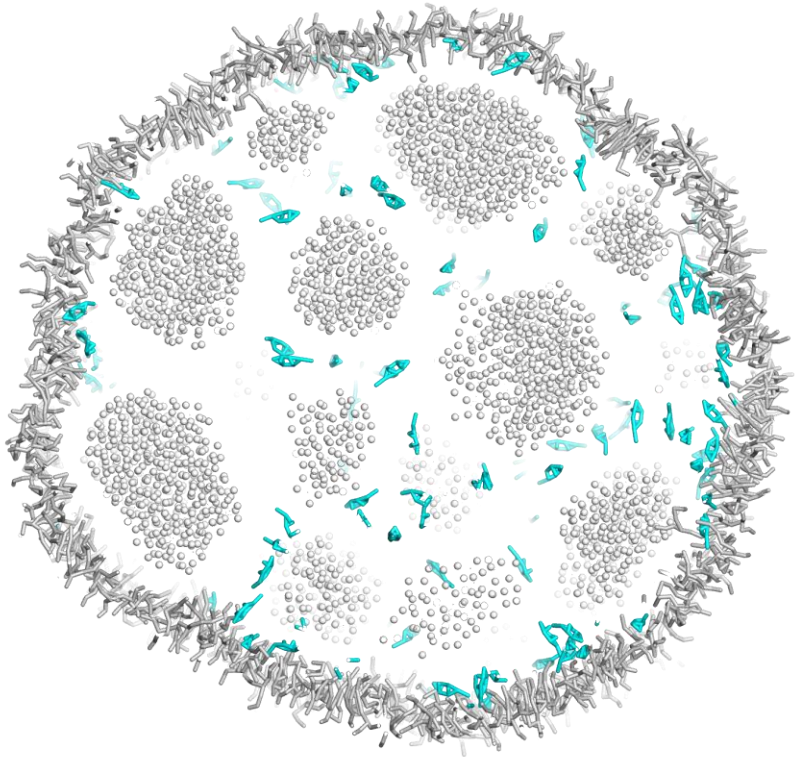


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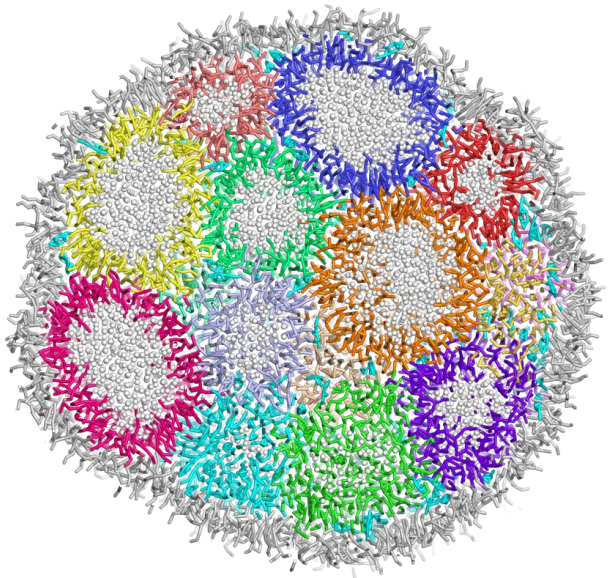


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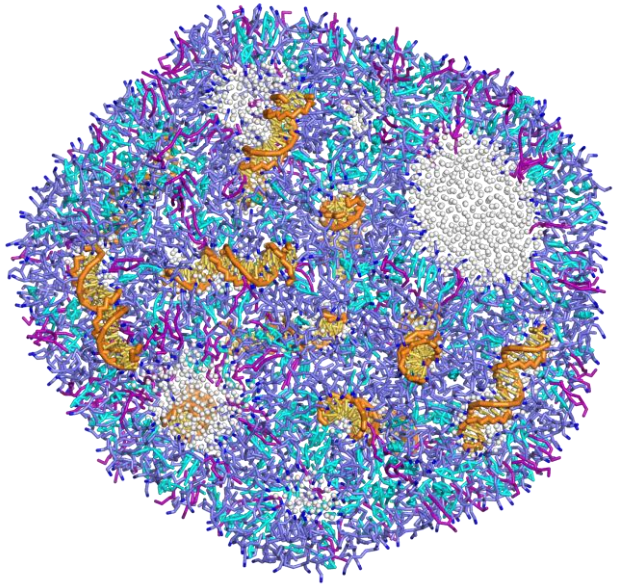
- Each snapshot – compartments defined by water/RNA clusters (0.7 nm gap)
- Final frame compartments – lipid composition
- Going backwards – most similar composition

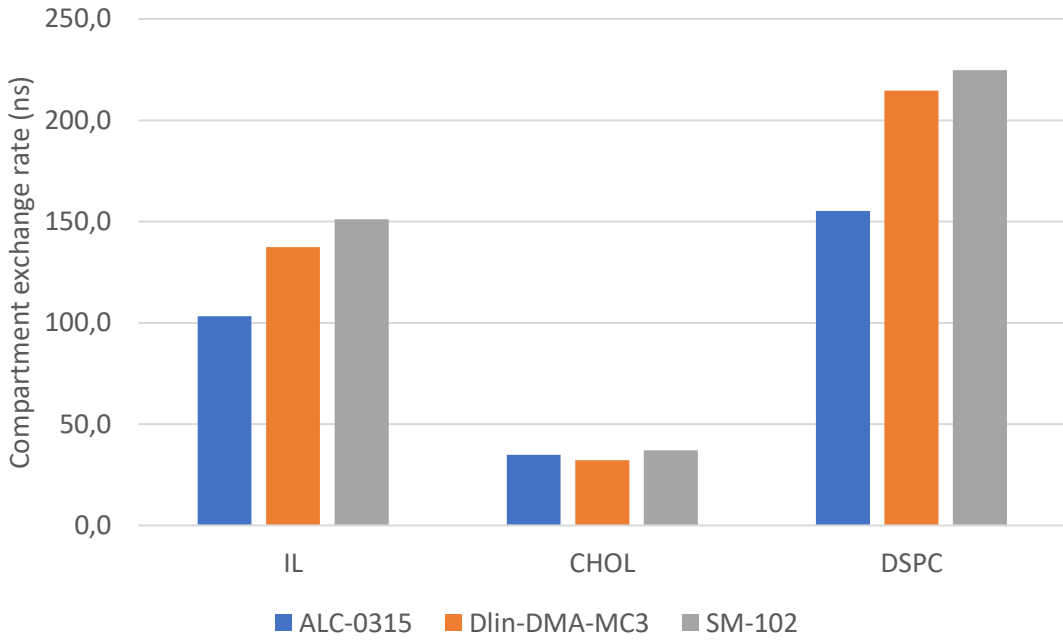
- Aqueous bubble size
- Composition of individual compartments
  - surface
  - unassigned
- Lipids mobility

Unpublished data, to be submitted soon

Lipid mobility

- Cholesterol the most mobile
- ILs/lipid mixtures differ in mobility

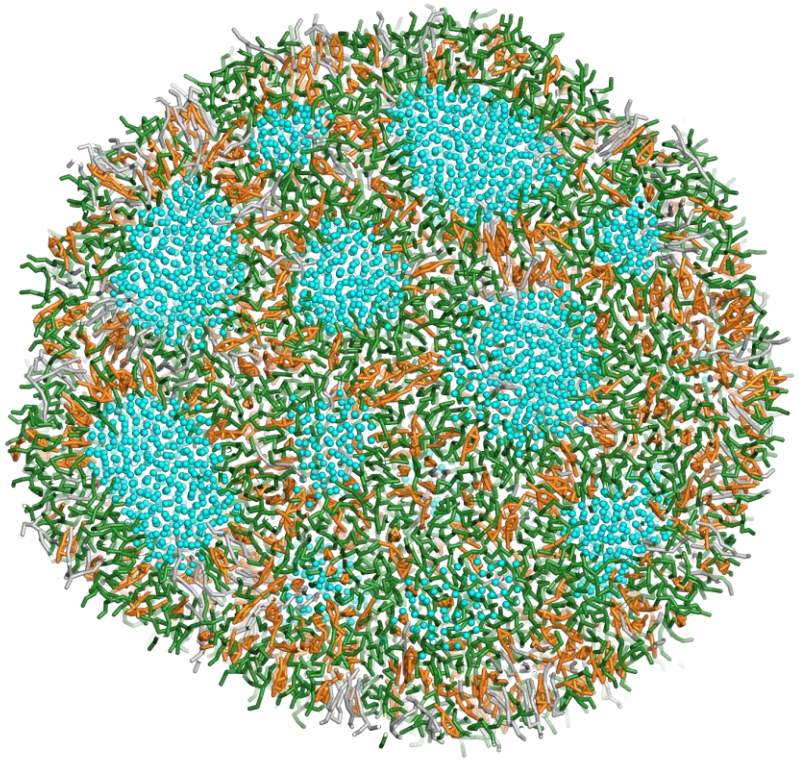


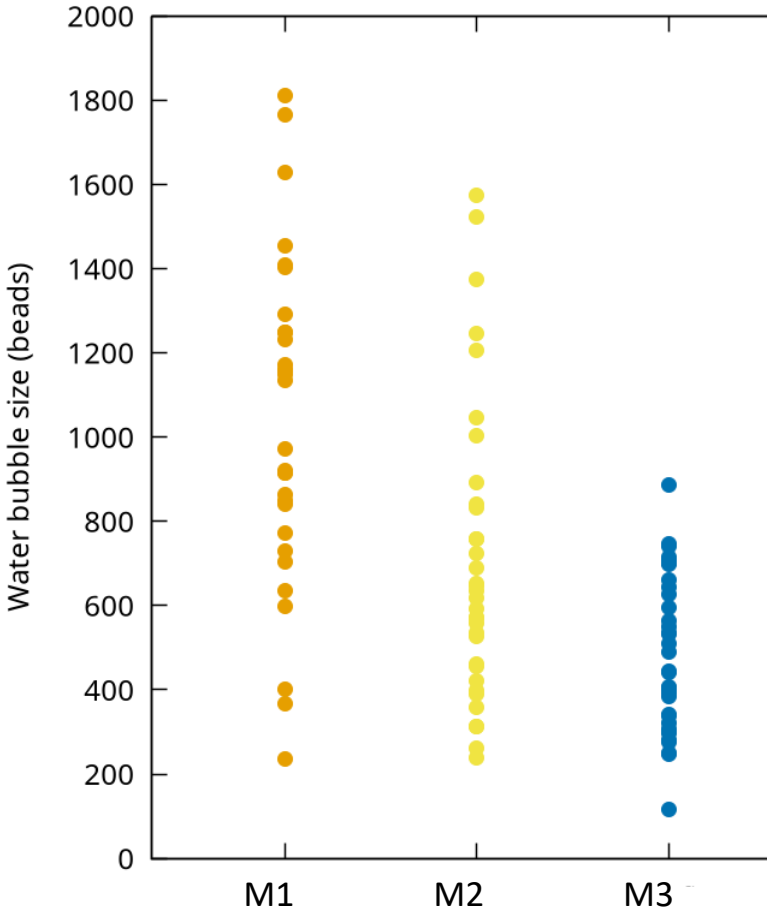


Unpublished data, to be submitted soon

Acqueous compartments

- Helper lipid/cholesterol composition

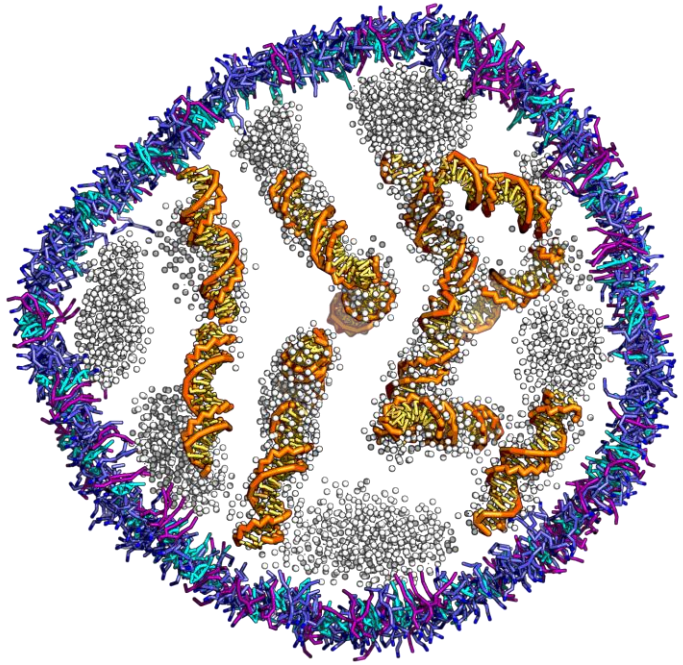


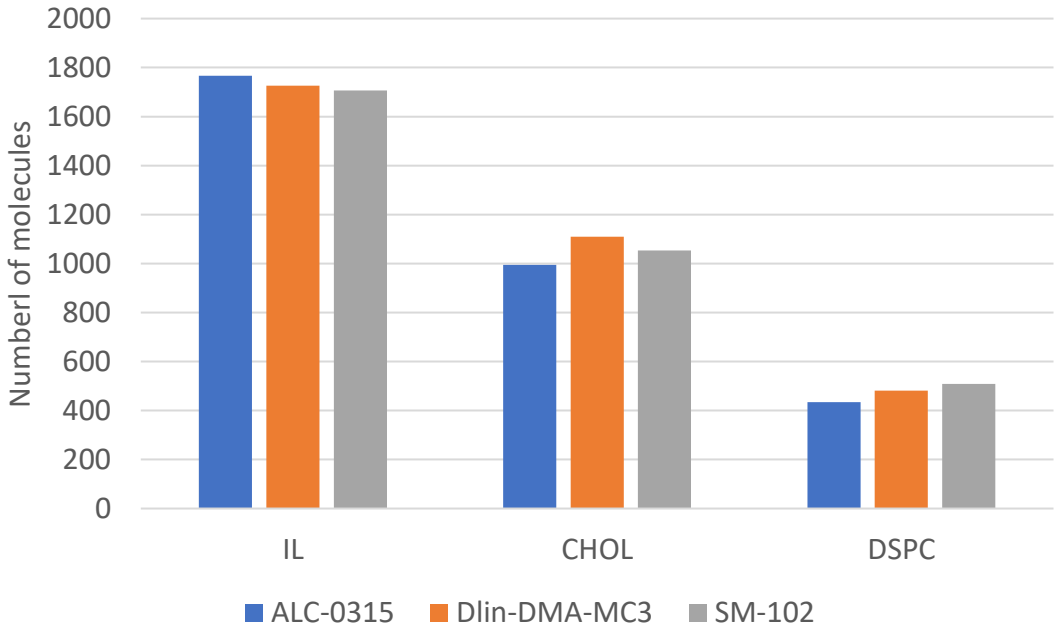


Unpublished data

Surface composition

- Surplus of DSPC
- Majority of DSPC not exchanging from the surface layer

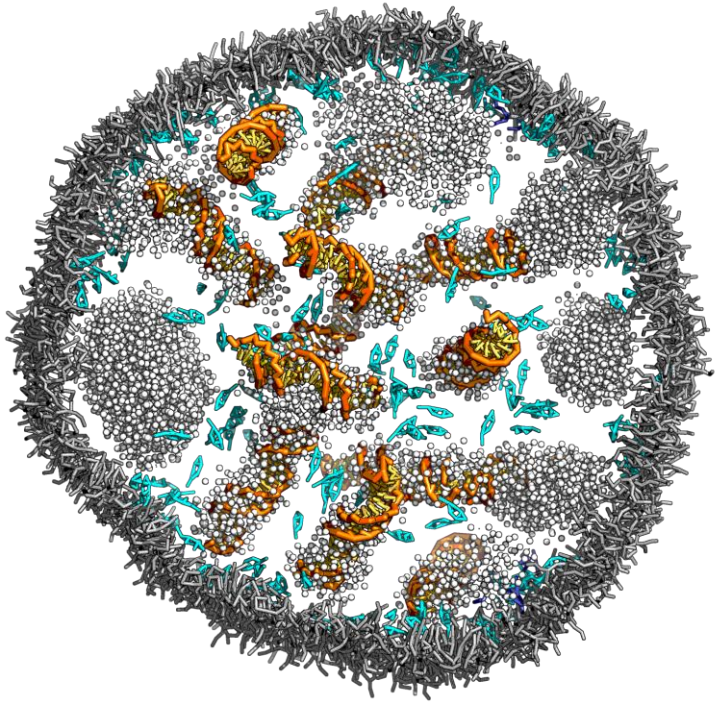




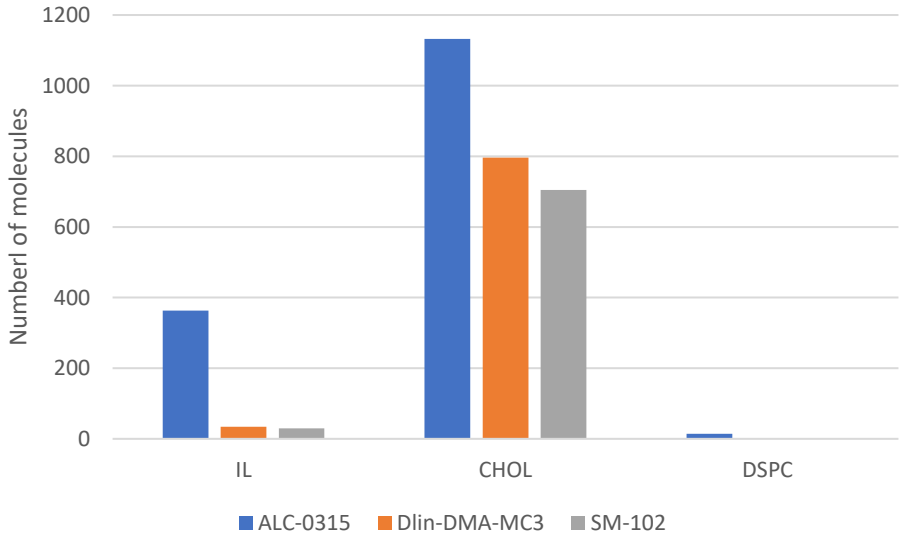
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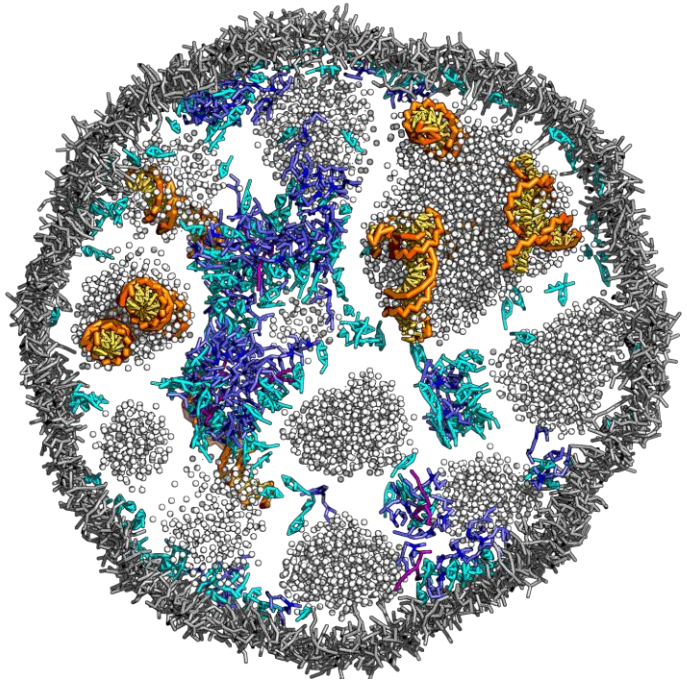
Unassigned lipids

- Majority made by cholesterol
- ALC-0315 forms tiny clusters around few molecules of water



MC3



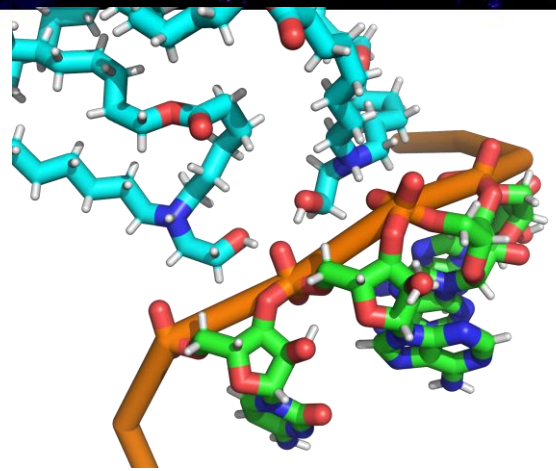


ALC-0315

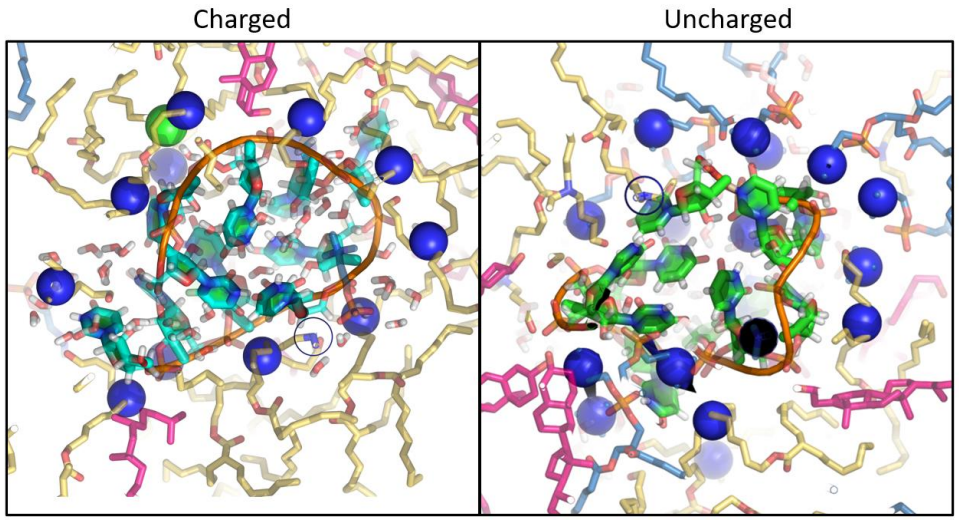
Unpublished data, to be submitted soon

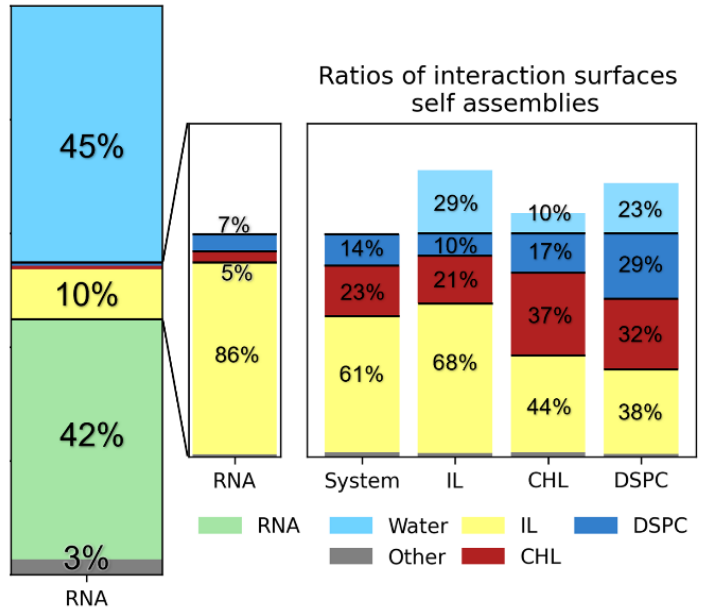
Atomistic Insight into RNA-lipid Interactions

- Lipid – RNA interactions
  - Atomistic resolution and the role of individual groups
  - ILs accumulate around RNA
- Ion behavior
- ALC-0315



Amber 16
500 ns isotropic
200 ns anisotropic
OPC water
298 K
Lipids:
AMBER Lipid 17 + GAFF
RNA:
ff99SB16, OL3, parmbsc0, gHBfix19

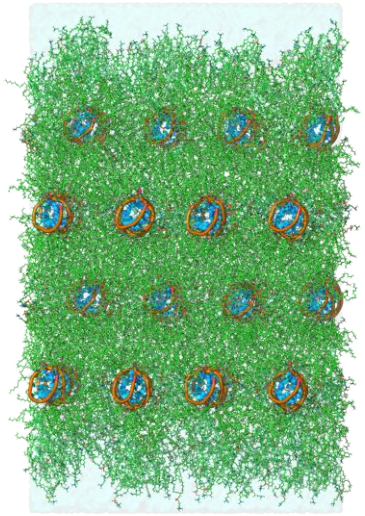


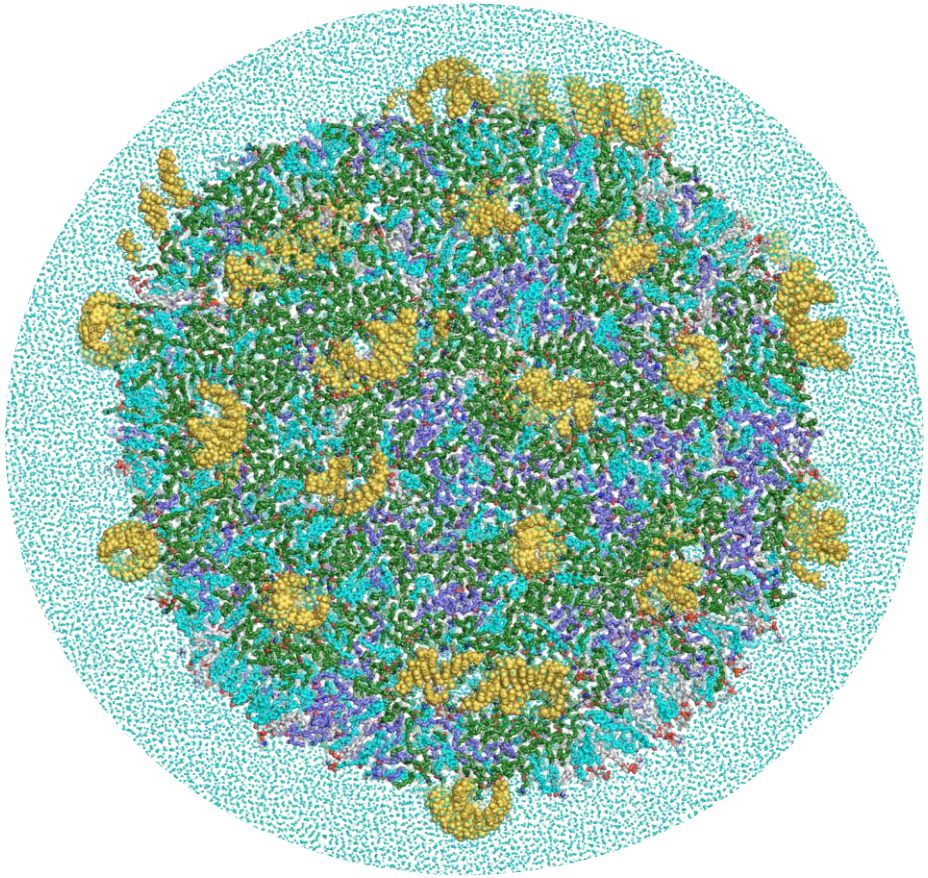


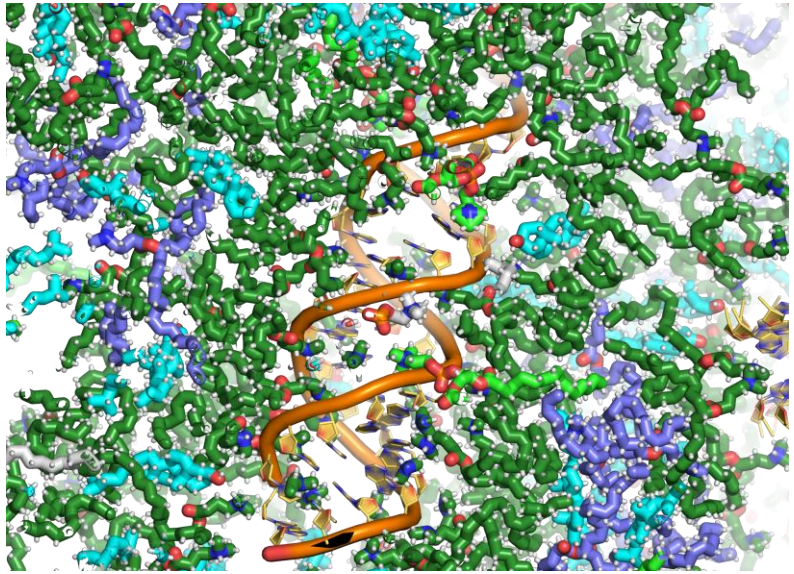
Paloncýová, M. et. al. *J. Phys. Chem. Lett.* **2021**, *12* (45), 11199–11205. https://doi.org/10.1021/acs.jpclett.1c03109.

All atom structure of H_{II} phase

- ~4.5 M atoms
- Atomistic resolution
  - RNA-IL interaction
- CG validation



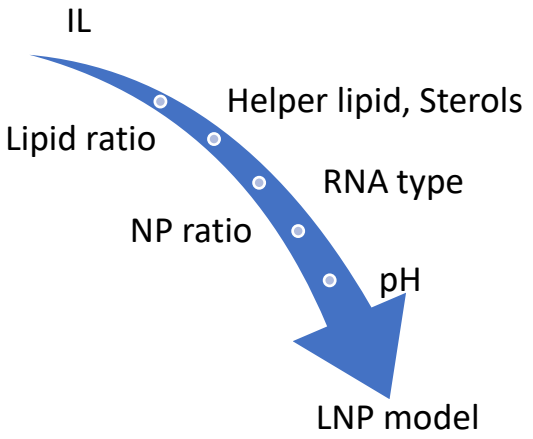


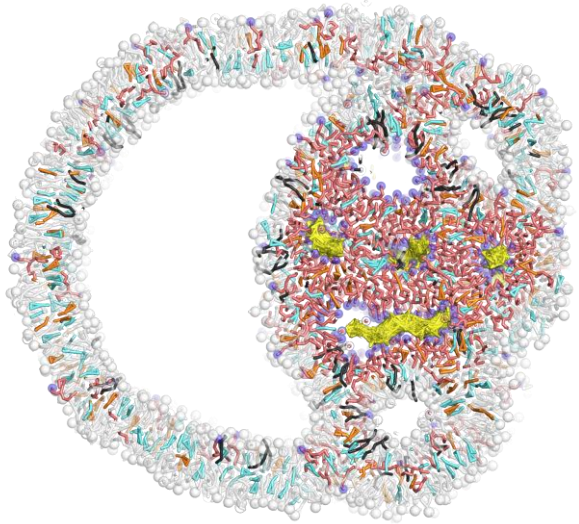


Unpublished data

Conclusion

- Insight into LNP organization and stability
  - Organization -> CG simulations
    - H_{II} phase mostly, mixture of phases
    - Lipid mixture governs phase and curvature
    - pH induced change
    - mRNA structure issue
    - Lipid-RNA interactions - AA
  - Stability -> AA and CG simulations
    - pH-induced phase changes
    - Lipid mobility, curvature





Computational Demand

- OPEN-36-23, OPEN-34-46

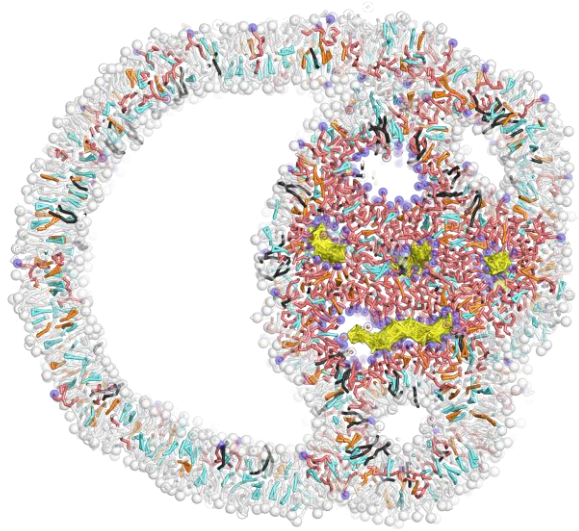
- CG simulations
  - CPU
  - GROMACS
  - 1-4 nodes/job
    - 10k lipids: 1.3 μs/day/node Karolina
    - 20k lipids: 0.9 μs/day/node LUMI

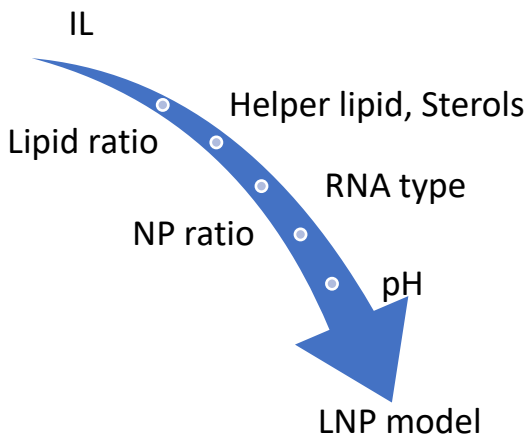
- Analyses
  - Initial – Jupyter notebook
  - Batch – interactive job
    - Cut the trajectory
    - Run analyses separately in parallel

- AA simulations
  - GPU
  - GROMACS
    - 4.5 mil atoms: ~2 ns/day/GPU

Thank you for your attention

- Insight into LNP organization and stability
  - Organization -> CG simulations
    - H_{II} phase mostly, mixture of phases
    - Lipid mixture governs phase and curvature
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    - Lipid-RNA interactions - AA
  - Stability -> AA and CG simulations
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