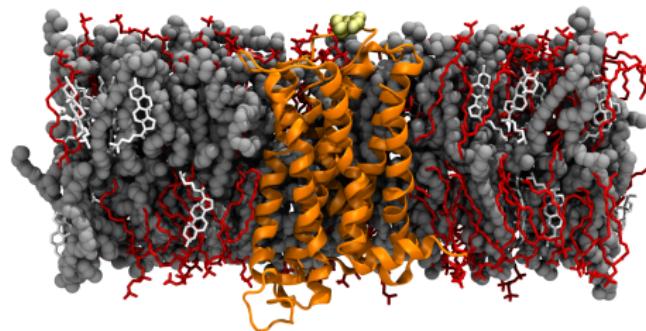


ProLipids Midsummer Symposium

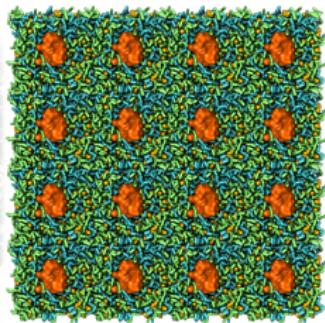
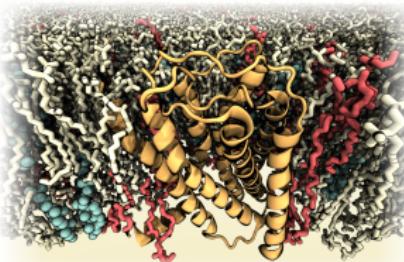
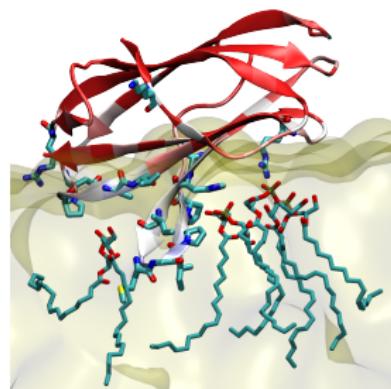
Module A: Introduction to Biomolecular Simulations: From Theory to System Preparation

Waldemar Kulig Giray Enkavi Matti Javanainen



Content of this Module

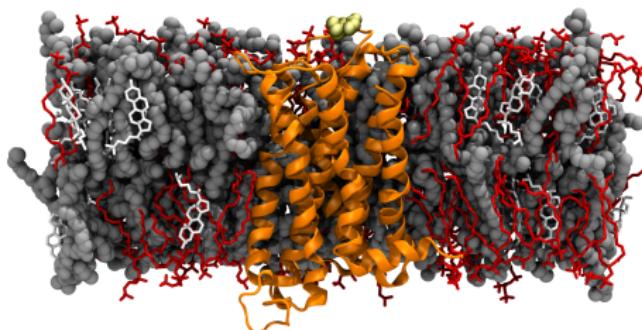
- Absorption of Peripheral Proteins to Membrane
- Constructing the Simulation System
- Intramembrane Protein–Lipid Interactions and Their Effect on Protein Conformation



LAB 1

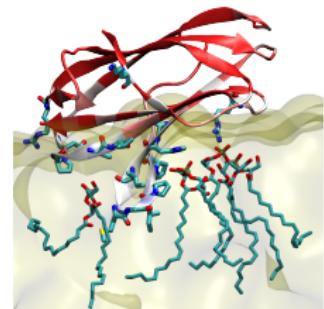
Adsorption of Peripheral Proteins to Membrane

Amina Djurabekova Juho Liekkinen Mia Creuz Santeri Paajanen Hanna
Korolainen Mykhailo Girych Waldemar Kulig Giray Enkavi



Scientific Background

- Peripheral membrane proteins are amphipathic proteins;
- They adsorb onto the membrane surface reversibly;
- They are functionally diverse, working as carriers of hydrophobic molecules such as lipids, as electron carriers in the electron transport chain, and as hormones;
- Niemann-Pick C2 protein is a cholesterol carrier found in the late endosomes and lysosomes.
- Responsible for shuttling cholesterol between the intra-organellar membranes;
- Mutations result in Niemann-Pick C disease, a genetic neurological disease.



NPC2 adsorbed
onto the
membrane

Interesting Research Questions

- specific protein–lipid interactions that stabilize binding;
- the protein conformational changes upon membrane binding;
- the local perturbation on the membrane structure;
- and in the case of carriers, how the above listed are coupled to uptake and release of the ligand.

Let's start!

Please open your Instruction Book. We will start from the section 1.2 on page 3.

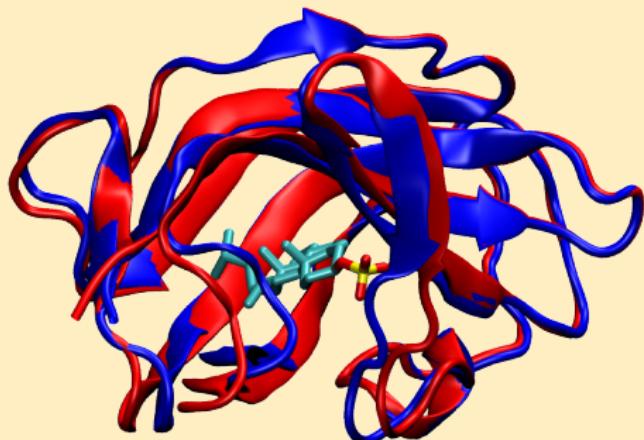
Visualizing the crystal structures

Question 1. What are the structural effects of cholesterol binding to NPC2?

Visualizing the crystal structures

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Chains B and C in 2hka (cholesterol-bound) structure contain a cholesterol analog. Close examination and comparison of the crystal structures do not show any major structural difference with respect to the 1nep (*apo* structure). However, chains B and C have slightly higher RMSD in comparison to chain A, which is also present in the *apo* form. The difference is mostly due to the loop at the opening of the cholesterol binding site.



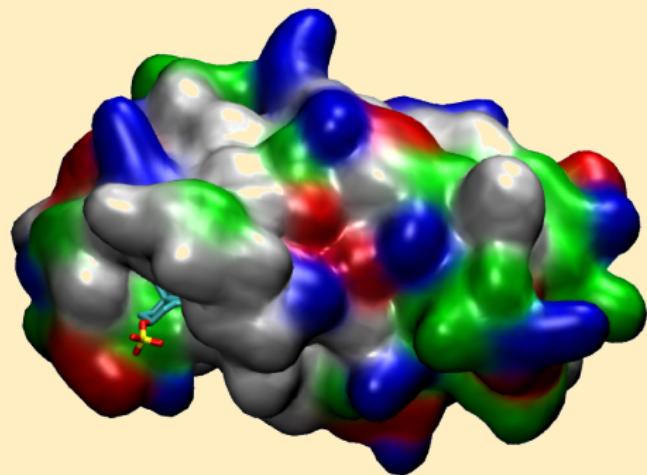
Visualizing the crystal structures

Question 2. Based on the structures, can you come up with any hypotheses on membrane binding mode or residues of NPC2?

Visualizing the crystal structures

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The outer surface of the protein is made up of polar and charged residues. A short hydrophobic loop is also visible. One could hypothesise that the hydrophobic loop may insert into the membrane. Additionally, one could assume that cholesterol binding pocket should face the membrane.



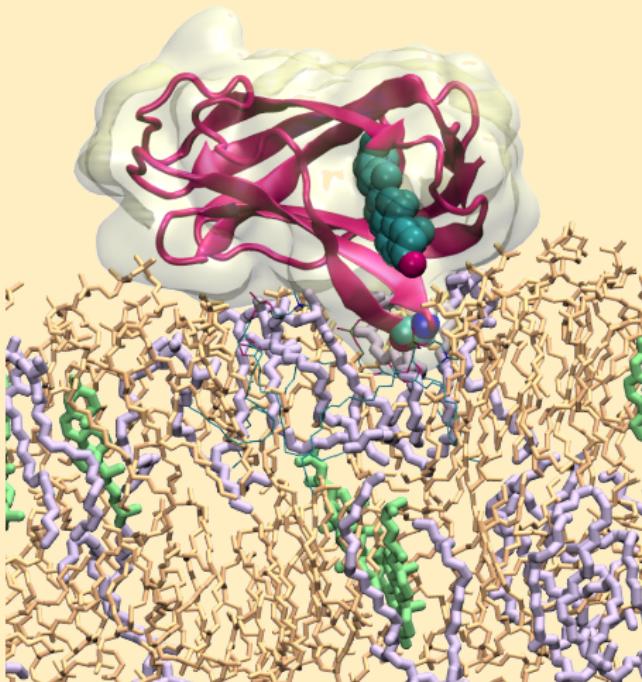
Visualizing the crystal structures

Question 3. Can you hypothesize a mechanism for cholesterol uptake and release?

Visualizing the crystal structures

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Cholesterol hydroxyl group points out of the binding pocket. When the protein is adsorbed onto the membrane so that the cholesterol binding pocket faces the membrane, the cholesterol has to rotate during uptake and release.



Visualizing the crystal structures

Question 4. Speculate how cholesterol binding is related to the structural deviation between the two structures?

Visualizing the crystal structures

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There does not seem to be any major difference in terms of RMSD between the *apo* and cholesterol-bound crystal structures. Most of the structural deviation is due to the loops at the opening of the cholesterol binding pocket.

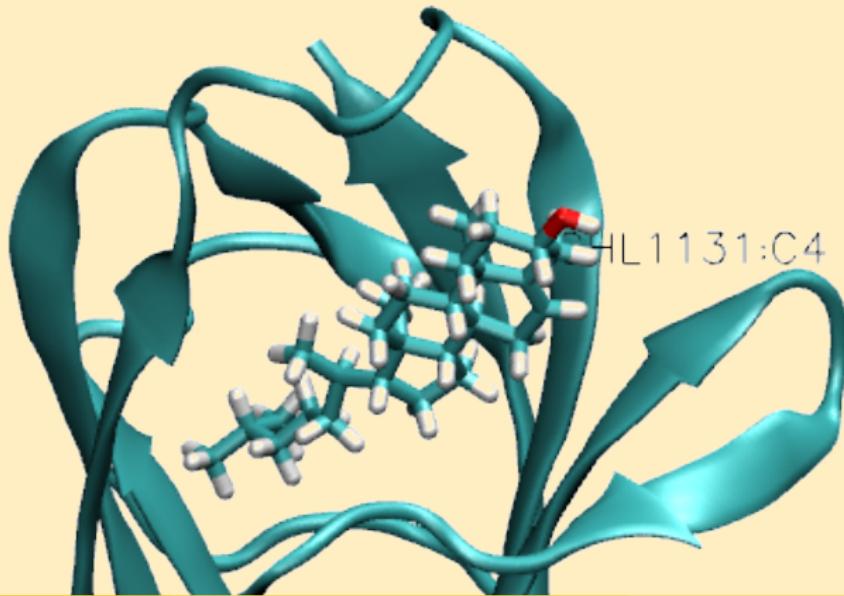
Visualizing a complete system

Questions 5 and 6. What is the bound state of the protein? If it is in *holo* state, what is the residue id of the ligand?

Visualizing a complete system

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system_1.pdb is in cholesterol-bound state and system_2.pdb is in *apo* state. The resid of bound cholesterol in system_1.pdb is 131



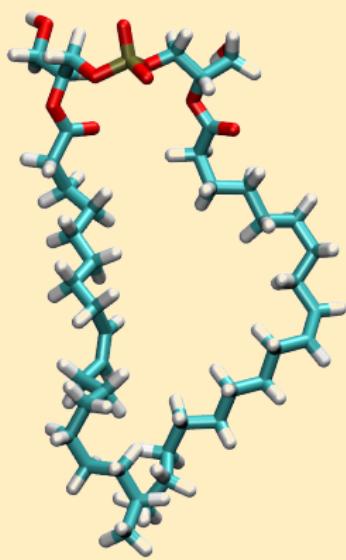
Visualizing a complete system

Question 7. How is structure of LBPA different from POPC?

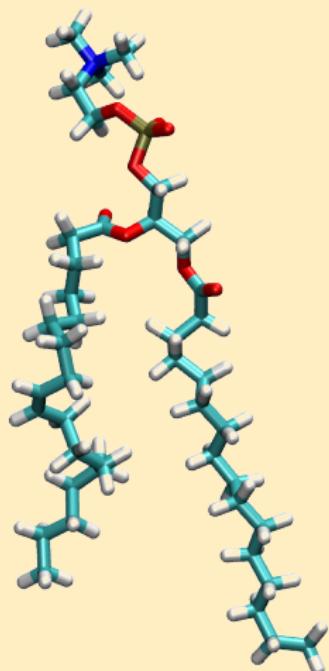
Visualizing a complete system

Question 7. How is structure of LBPA different from POPC?

LBPA does not have choline group attached to the phosphorus atom. Besides, it is an anionic lipid, while POPC is neutral.



LBPA



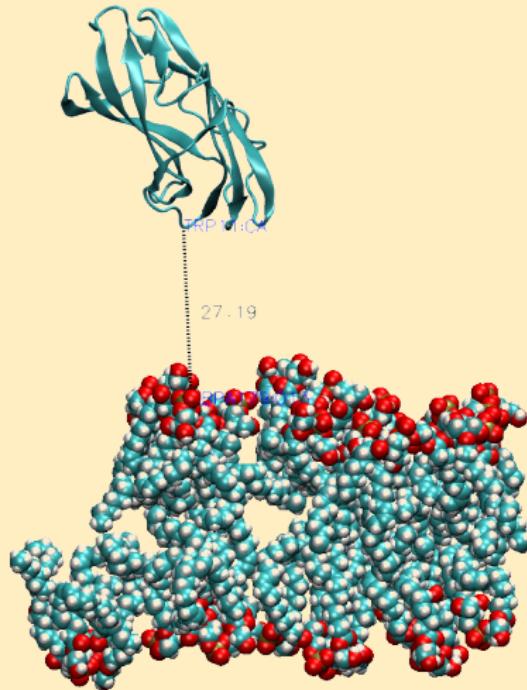
POPC

Visualizing a complete system

Question 8. Measure the distance between roughly the closest amino acid of the protein and the head group of a LBPA molecule.

Visualizing a complete system

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Qualitative Evaluation of Membrane Binding

Question 9. Does the membrane contain LBPA?

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Only the second trajectory contains LBPA.

Qualitative Evaluation of Membrane Binding

Question 10. Can you conclude that the protein bind to the membrane in this simulation?

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Only in the second trajectory the protein binds to membrane. Note, however, that multiple simulations and advanced methods may be needed to reach scientifically sound conclusions in these systems. After all, the nature of these interactions is statistical.

Qualitative Evaluation of Membrane Binding

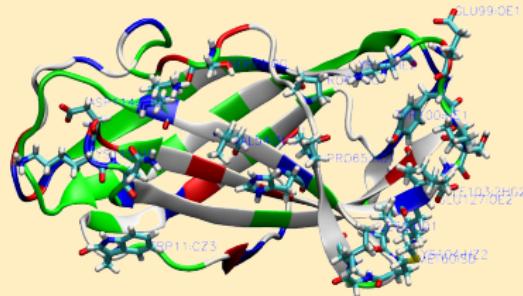
Question 11. If protein binds to the membrane, which residues are involved in binding?

Qualitative Evaluation of Membrane Binding

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You can have a quick look at the residues involved in binding by choosing an appropriate frame. Use the following selection

protein and same residue as within 3
of resname POPC LBPA CHL1

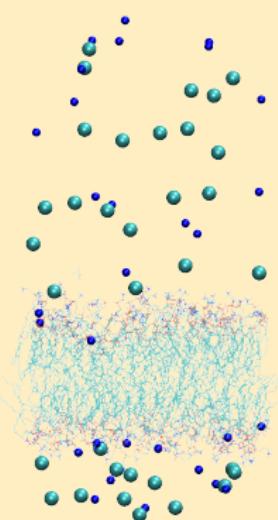


Qualitative Evaluation of Membrane Binding

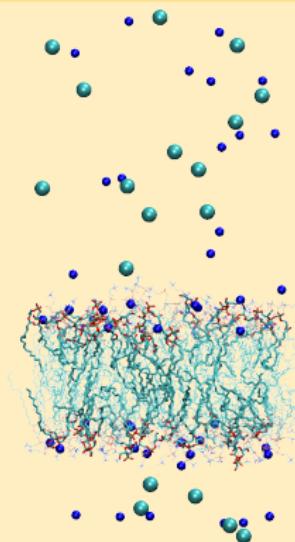
Question 12. Pay attention to the ions. Do you see anything interesting in ion distribution? Are they evenly distributed within the box? Are they interacting with protein or lipids?

Qualitative Evaluation of Membrane Binding

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Trajectory 1: Ions are evenly distributed in the aqueous phase.



Trajectory 2: NA ions are adsorbed to the membrane due to the presence of anionic LBPA.

Conclusions

We performed atomistic molecular dynamics simulations to elucidate the roles of the physiologically relevant key lipids associated with NPC2-membrane binding. We showed in atomistic detail the mechanism and energetics of the binding process. Our research show that LBPA is required for membrane association and proper protein orientation on the membrane surface (Enkavi *et al.* PLOS Computational Biology 13, e1005831 (2017)).

