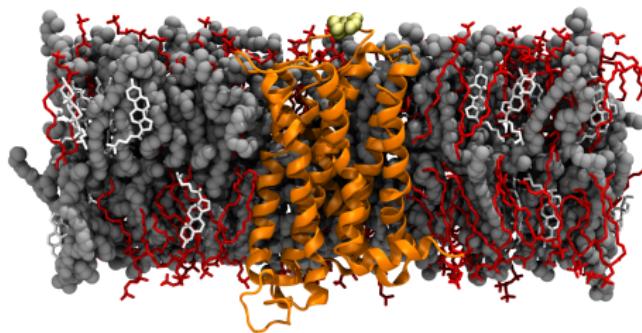


LAB 2

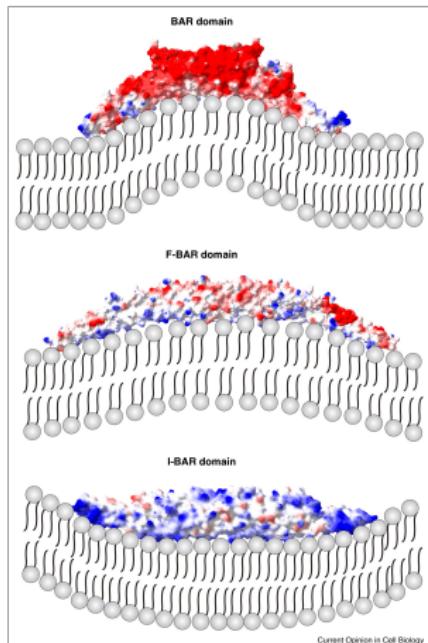
Constructing the Simulation System

Waldemar Kulig Giray Enkavi Matti Javanainen



Scientific Background

- The Bin-amphiphysin-Rvs (BAR) superfamily proteins are membrane deforming proteins.
- The BAR and the F-BAR domain proteins induce positive membrane curvature.
- the inverse BAR (I-BAR) domain proteins (studied here) induce negative curvature to generate plasma membrane protrusions.
- I-BAR domain proteins bind phosphoinositide (PIP)-rich membranes and induce the formation of PI(4,5)P₂ clusters
- They also interact with actin and regulators of actin dynamics.



Types of BAR domains

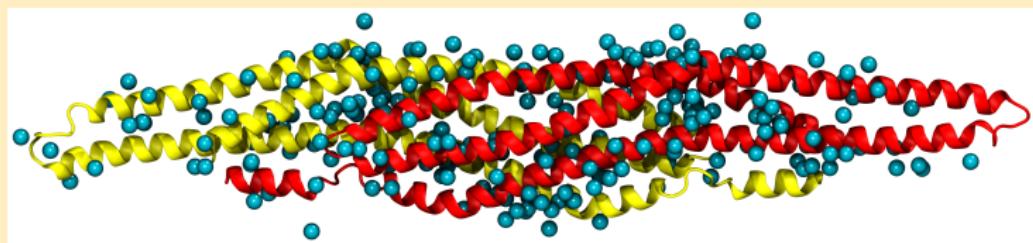
Zhao, H., Pyklinen, A., and Lappalainen, P. Current Opinion in Cell Biology 23, 1421 (2011).

Let's Start!

Please open your Instruction Book. We will start from the section 2.1.2 on page 15.

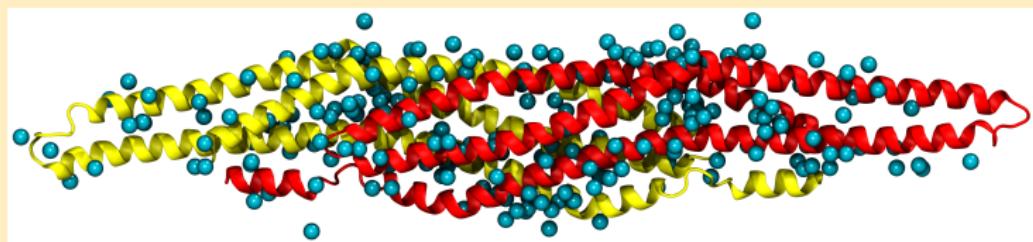
Downloading, Examining, and Fixing the Structure

Question 13. Why do you think there were oxygen atoms scattered around in our protein model?



Downloading, Examining, and Fixing the Structure

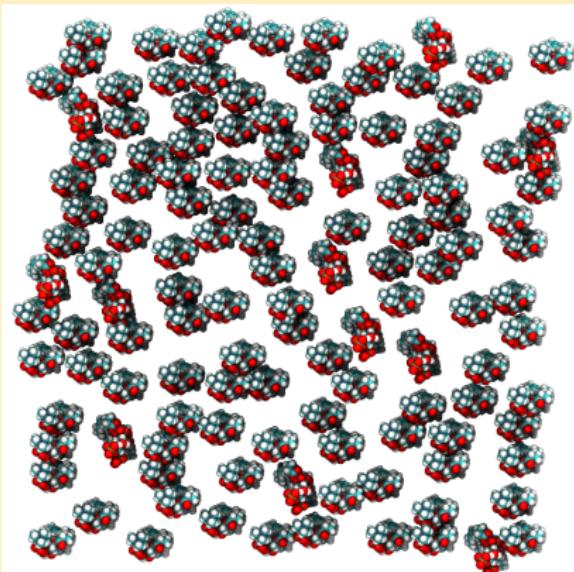
Question 13. Why do you think there were oxygen atoms scattered around in our protein model?



These oxygen atoms represent crystallographic water molecules, which were resolved together with the protein structure in the X-ray experiment.

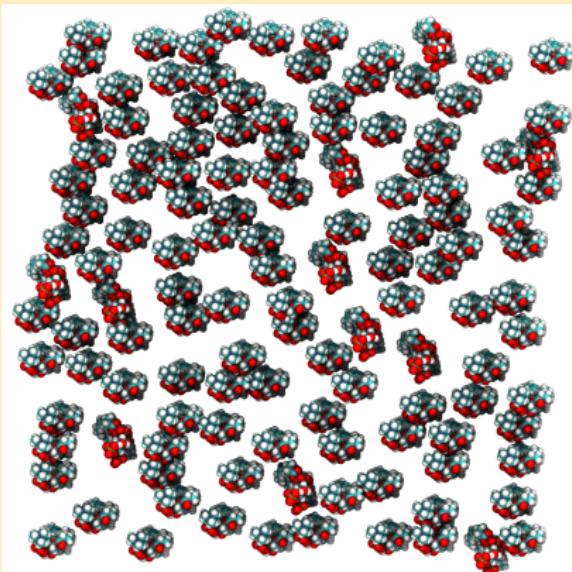
Bilayer Construction

Question 14. Does the structure you have attained seem reasonable?



Bilayer Construction

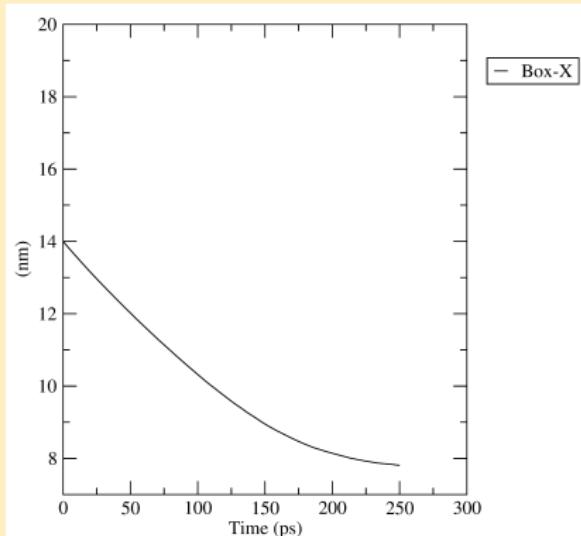
Question 14. Does the structure you have attained seem reasonable?



It seems that there is way too much space in between lipids. This is not a realistic starting point for the MD simulation.

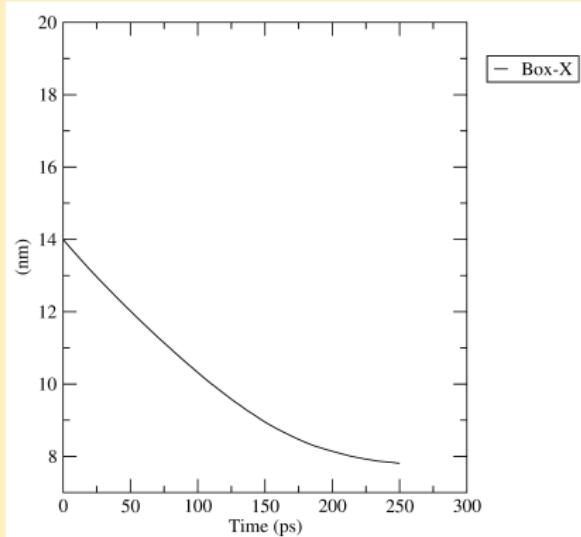
Bilayer Construction

Question 15. Has the system size converged to a specific value?



Bilayer Construction

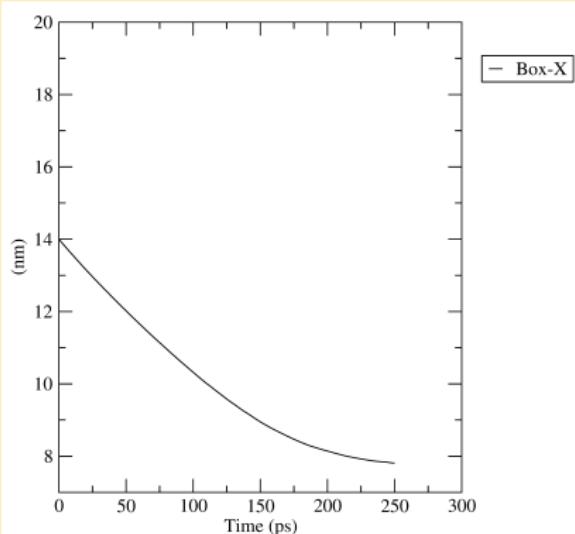
Question 15. Has the system size converged to a specific value?



Not yet, but it started plateauing.
If we run this simulation a little
bit longer it will converge to some
specific number.

Bilayer Construction

Question 16. What is the average area per lipid (APL) at the end?



The final size of the box was 7.81 nm.

Using the equation given in the Instruction Book and remembering that the monolayer contains 128 lipids (12 PIP2 and 116 POPC), the APL is equal to 47.65 \AA^2 .

Bilayer Construction

Questions 17 and 18. Is the concentration of ions physiologically relevant ($\sim 0.2 \text{ mol/L}$)? In case the concentration is not physiological, would you add or remove water molecules to the system to reach a suitable concentration?

Bilayer Construction

Questions 17 and 18. Is the concentration of ions physiologically relevant ($\sim 0.2 \text{ mol/L}$)? In case the concentration is not physiological, would you add or remove water molecules to the system to reach a suitable concentration?

ion conc. (mM)	sea water	<i>E. coli</i>	<i>S. cerevisiae</i>	mammalian cell (heart or RBC)	blood plasma
K ⁺	≈ 10	30-300	300	100	4
Na ⁺	≈ 500	10	30	10	100-200
Mg ²⁺	≈ 50	30-100 (bound); 0.01-1 (free)	50	10 (bound) 0.5 (free)	1
Ca ²⁺	≈ 10	3 (bound); 100 nM (free)	2 (bound)	10-100 nM (free)	2
Cl ⁻	≈ 500	10-200 media dependent		5-100	100

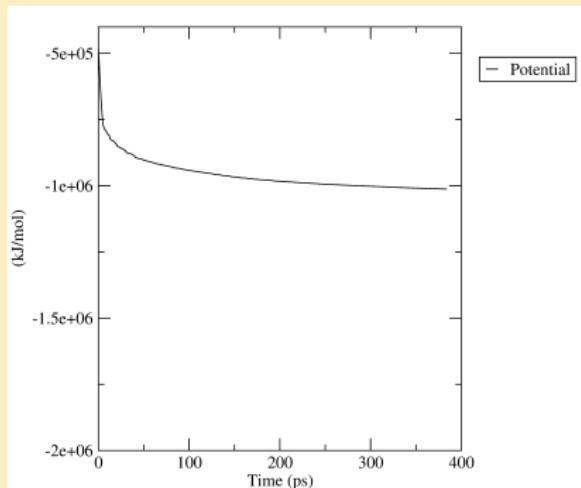
The physiological concentration of ions differs with the cell type. Assuming that we want to simulate the human plasma conditions, the concentration of sodium ions should be somewhere in the range of 0.1–0.2 mol/L. In this case, we could add more water molecules (or, alternatively, remove some ions) to the system to be closer to the middle of this range.

Bilayer Construction

Question 19. Does the potential energy converge to some value?

Bilayer Construction

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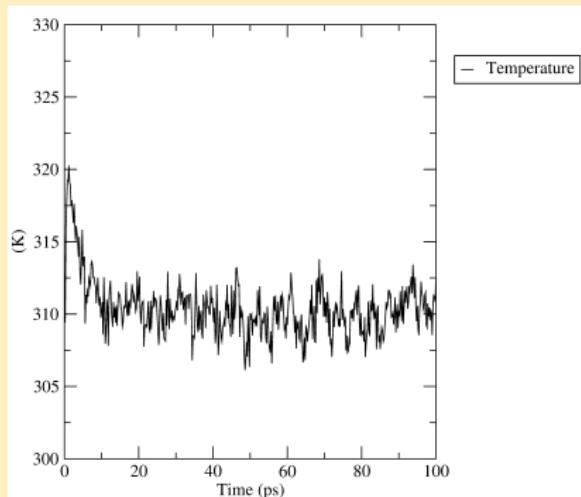
It seems that the potential energy started converging nicely. One could run this equilibration a little bit longer to make sure that it reaches the plateau.

Bilayer Construction

Question 20. Could you have used a shorter NVT equilibration simulation to stabilize the temperature?

Bilayer Construction

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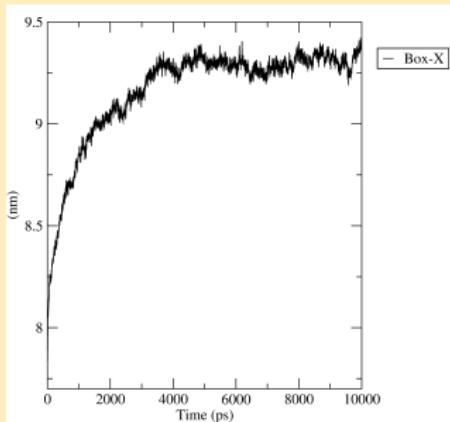
Yes, in this case the temperature stabilizes around 310 K after 30–40 ps, so 50 ps of the equilibration would be enough to stabilize the temperature.

Bilayer Construction

Questions 21, 22, and 23. Has the system size converged to a specific value? How many nanoseconds does it take for the bilayer to reach stable APL? What is the average APL of the bilayer at the end of the equilibration? How does this correlate with the approximate experimental APL values of 67.4 and 68.3 Å² for PIP2 and POPC, respectively?

Bilayer Construction

Questions 21, 22, and 23. Has the system size converged to a specific value? How many nanoseconds does it take for the bilayer to reach stable APL? What is the average APL of the bilayer at the end of the equilibration? How does this correlate with the approximate experimental APL values of 67.4 and 68.3 Å² for PIP2 and POPC, respectively?



Yes, the system reached the equilibrium. It took 4–5 ns to reach the stable system size. This box size corresponds to the area per lipid equals to 66.85 Å².

Constructing the Protein–Membrane Systems

Question 24. How do you think we should check if our equilibration simulations have been sufficient?

There are many different ways to check the equilibration of the systems simulated in MD. You have already seen some of them in this tutorial, namely area per lipid, temporal behavior of temperature, pressure, and so on.

Conclusions

In this Lab, we prepared the model of biological system composed of protein and lipid bilayer. We subsequently energy minimized it and performed the equilibration of our model. Finally, we performed short production run and briefly analysed the data. More advanced analysis of the protein–lipid system will be given in the **Lab 3**.

