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We use this protocol and it's working

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Molecular Dynamics Simulations

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ABSTRACT

This protocol details molecular dynamics simulations of PI3KC3-C1 on a lipid membrane.

ATTACHMENTS

852-2196.pdf

MATERIALS

Material

aqueous NaCl

Model Preparation

1 Prepare all-atom structural models of PI3KC3-C1 based on the active conformation with missing regions predicted by AlphaFold-Multimer 2.2.

2 Assign protonation states to amino acid side chains based on pKa predictions by PROPKA.

Parameterize ATP

3 Parameterize a molecule of ATP in the VPS34 active site using CHARMM-GUI.

Membrane Construction

4 Construct lipid membranes consisting of DOPC (60%), DOPE (20%), DOPS (5%), and POPI (15%) using the insane method.

5 Randomly distribute coarse-grained lipids in the membrane.

Solvation and Equilibration

6 Solvate each membrane patch with [M] 150 millimolar (mM) aqueous NaCl.

7 Perform an initial equilibration of 200 ns.

Conversion to Atomistic Representation

8 Convert the equilibrated membrane patch into an atomistic representation using the CG2AT2 tool.

Protein-Membrane System Setup

9 Place atomistic PI3KC3-C1 above the resulting membranes with a minimum distance of ~2 nm between protein and lipid atoms.

Further Equilibration

10 Subject the protein-membrane systems to an additional 10 ns of equilibration.

11 Apply harmonic positional restraints (force constant: 1000 kJ mol^{-1} nm^{-2}) to non-hydrogen protein atoms during this equilibration phase.

Production Runs

12 Perform 2 µs production runs for each of the six independent replicates of the simulation system.

13 Maintain system pressure at 1 Bar and temperature at 310 °K using the Parrinello-Rahman barostat and velocity-rescaling thermostat.

Electrostatic Interactions

14 Treat long-range electrostatic interactions using the smooth particle mesh Ewald method with charge interpolation through fourth-order B-splines.

Integration Time Step

15 Use a 2 fs integration time step for the simulations.

Bond Constraints

16 Apply the LINCS algorithm to constrain covalent bonds involving hydrogen atoms.

Steered Molecular Dynamics (SMD)

17 For SMD simulations, apply harmonic restraints (force constant: 100 kJ mol^{-1}) to reduce the center-of-mass z-distance between the protein group of interest and the membrane lipids underneath.

18 Apply a negative rate of -0.5 nm ns^{-1} until the protein group reaches the membrane surface.

Relaxation

19 Allow the complex to relax over periods of 2 µs during further simulations upon removal of any steering force.