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Molecular Dynamics Simulations of Active, Membrane-Engaged Complex

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We use this protocol and it's working

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Abstract

This protocol details molecular dynamics simulations of the active PI3KC3-C1 complex bound to RAB1A on a lipid membrane.

Attachments



[852-2196.pdf](#)

38KB

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: $1000 \text{ kJ mol}^{-1} \text{ nm}^{-2}$) 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 $2 \mu\text{s}$ during further simulations upon removal of any steering force.

Preparation of membrane-engaged complex containing RAB1A

- 20 Superimpose the cryo-EM structure of the active-state complex including RAB1A onto the final structure of the previous MD run.
- 21 Model the two RAB1A geranylgeranyl groups and the VPS15 N-myristate using CHARMM-GUI.
- 22 Repeat above-described SMD protocol to adjust the remaining membrane interactions.
- 23 Allow the complex to relax for $1 \mu\text{s}$ (3 independent replicas with random velocities).