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Model Building of PI3KC3-C1/RAB1A

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Croll, T. I. ISOLDE: a physically realistic environment for model building into low-resolution electron-density maps. *Acta Crystallogr D Struct Biol* **74**, 519-530 (2018). **https://doi.org:10.1107/S2059798318002425**
Pavel V Afonine, et al. Real-space refinement in *PHENIX* for cryo-EM and crystallography, ,**Acta Crystallogr D Struct Biol.** 2018 Jun 1; 74(Pt 6): 531–544.

Protocol status: Working
We use this protocol and it's working

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Abstract

Details model building of the PI3KC3-C1/RAB!A complex using chimeraX1.5-1.6 and ISOLDE. Refinement was done using Phenix real space refinement.

Generate Starting model

1 Use Alphafold2 to generate a starting model containing the PI3KC3-C1 components (VPS34, VPS15, ATG14, BECN1) and RAB1A respectively.

Docking and model building/refinement

2 Dock each starting model into the experimental map

3 Use ISOLDE to relax the entire PI3KC3-C1 complex and RAB1A into the experimental data in ChimeraX, selectively using restraints and ignoring clashes until the model simulates correctly. Trim obvious disordered regions by deleting them in Chimera.

4 Build ligands into the map, use the ignore command to ignore clashes selectively until the clashes have been eliminated.

5 Iteratively refine the model by building sidechains into density features, and improving the Ramachandran angles with selective restraints.

6 Assess the model to map fit using the built in Q-score function and Ramachandran plot

7 When model fit to density and Ramachandran angles start to look good, export the model and refine using Phenix real space refinement.

8 Repeat steps 5-7 until you are satisfied with the final model and the metrics cease improvement.