Modelling of Nef Interaction with ABCA1 Revealed Potential Binding Sites For Inhibitor Compounds

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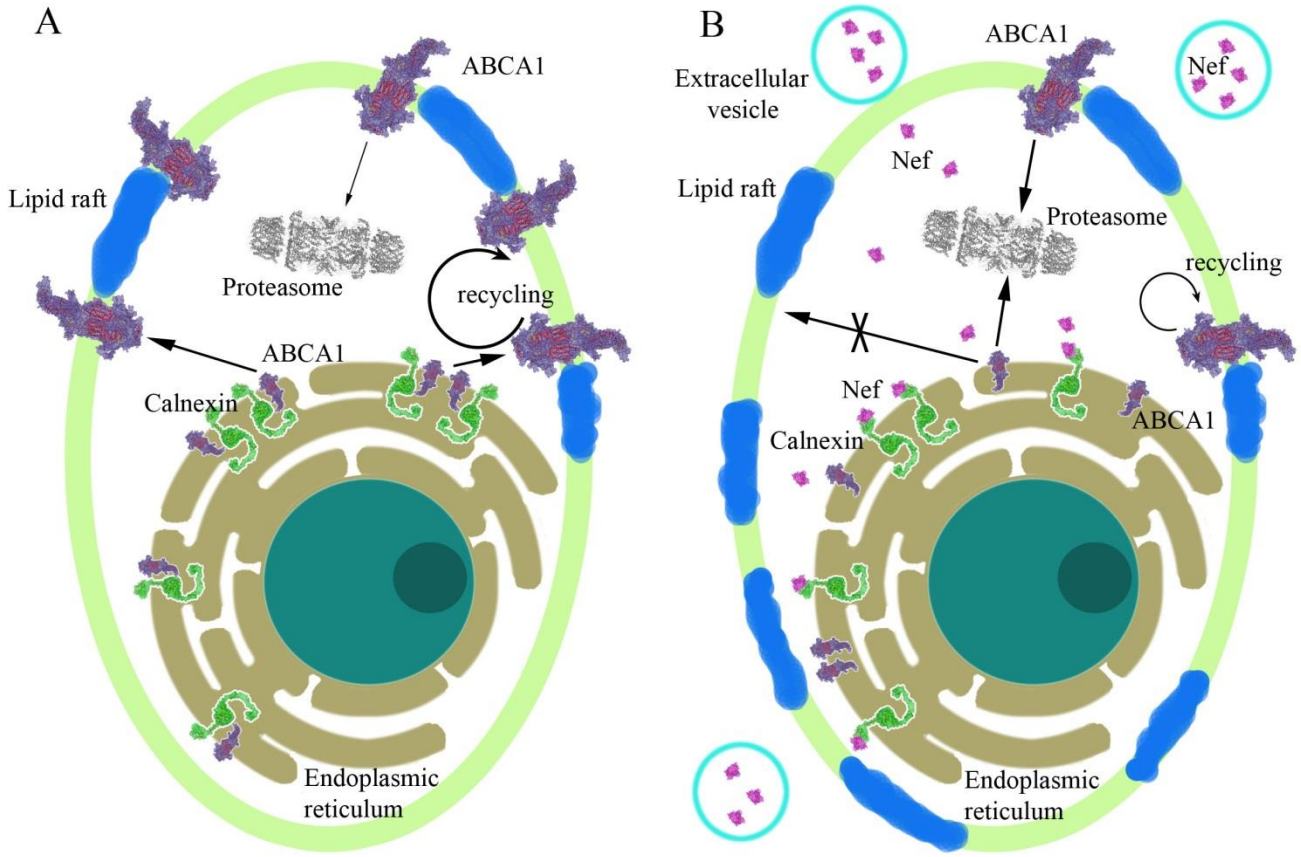
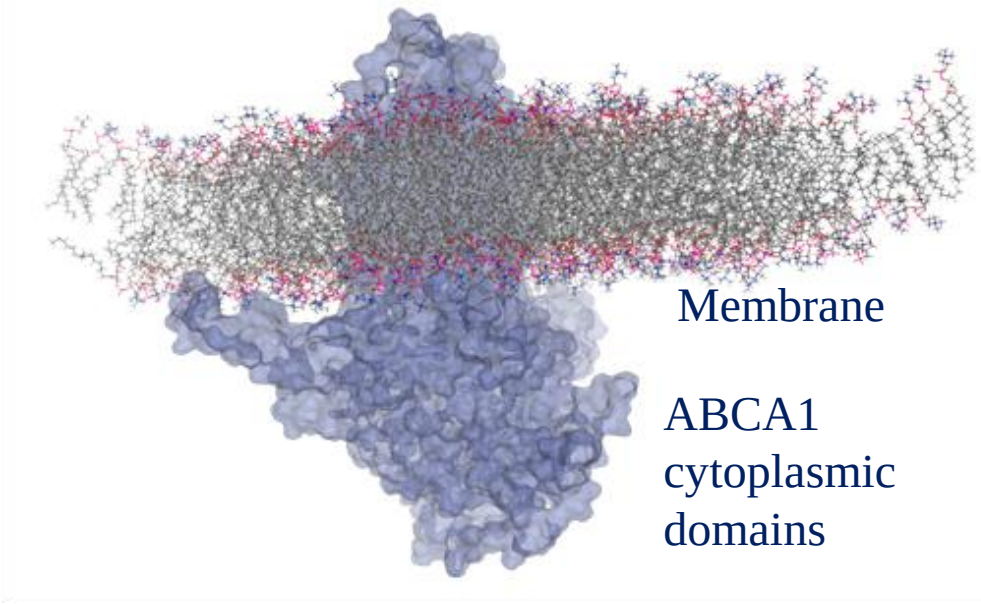
(**A**) Cell without Nef. ABCA1 matures in ER with the help of calnexin and is directed to the cytoplasmic membrane, where it mediates cholesterol efflux. ABCA1 is recycled from the cell membrane and to a smaller extent internalized to the proteasomes and degraded.



(**B**) Extracellular vesicles (EV) deliver Nef molecules into the cell. Nef interacts with cytoplasmic domain of calnexin disrupting interaction of its intra-ER domains with ABCA1. This impairs ABCA1 maturation, targeting most ABCA1 to proteasomes. Abundance of lipid rafts is increased, ABCA1 recycling is decreased, most ABCA1 is targeted to proteasomes.

- Nef EVs modify cholesterol metabolism of uninfected cells by inhibiting ABCA1 activity and suppressing cholesterol efflux
- Defect in cholesterol efflux impairs cholesterol delivery to oligodendrocytes and affects myelination of axons
- ABCA1 suppression leads to increased abundance of lipid rafts
- Increased lipid rafts promote inflammation by enhancing response to inflammatory stimuli
- Nef EVs induce ‘trained memory’ in myeloid cells promoting pro-inflammatory phenotype
- Compounds blocking the effects of Nef on ABCA1 reverse pathogenic activity of Nef EVs

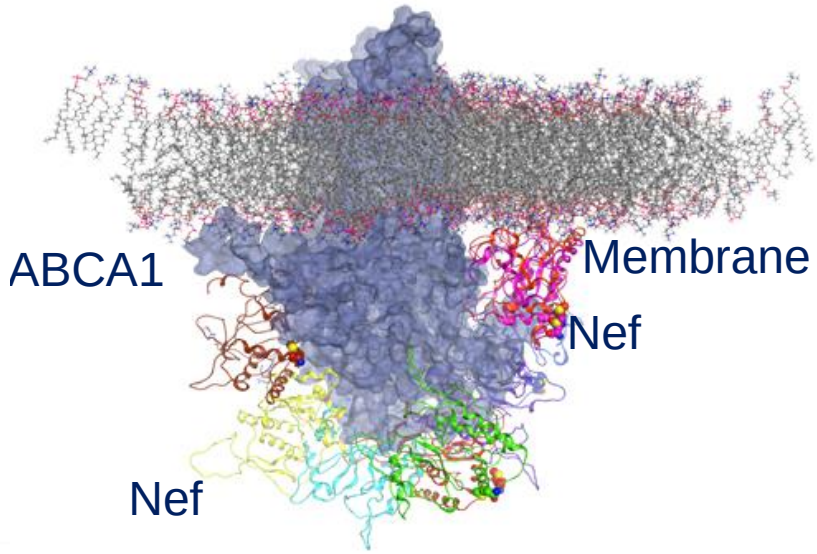
ABCA1 model



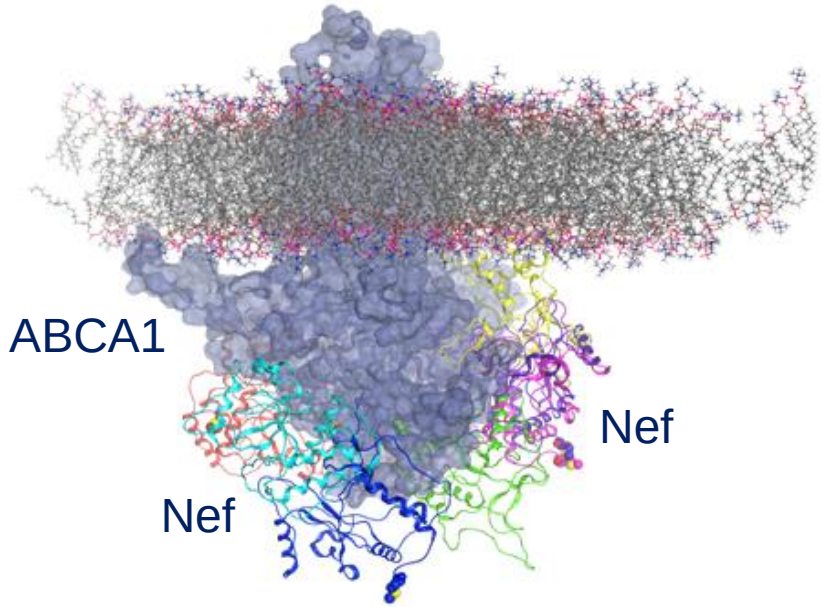
ABCA1 with POPC bilayer

Model structure containing ABCA1 transmembrane and cytoplasmic domains was inserted into bilayer consisting of 312 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) molecules, using charmm-gui Membrane Builder. Extracellular domain residues 49-623 and 1371-1635 were clipped and replaced by sort flexible loops. Resulting system was placed into hexagonal unit cell (125x125x125A), solvated with TIP3P water, ionized with NaCl to 0.15M, minimized using steepest descent minimizer, and then equilibrated using Berendsen temperature and pressure coupling. For the production run of 60 ns length, NPT ensemble was simulated using Nose-Hoover temperature coupling and semi-isotropic Parrinello-Rahman pressure coupling method. Temperature during simulation was 310K. Simulations were performed with full-atom CHARMM36 force field, using GROMACS software.

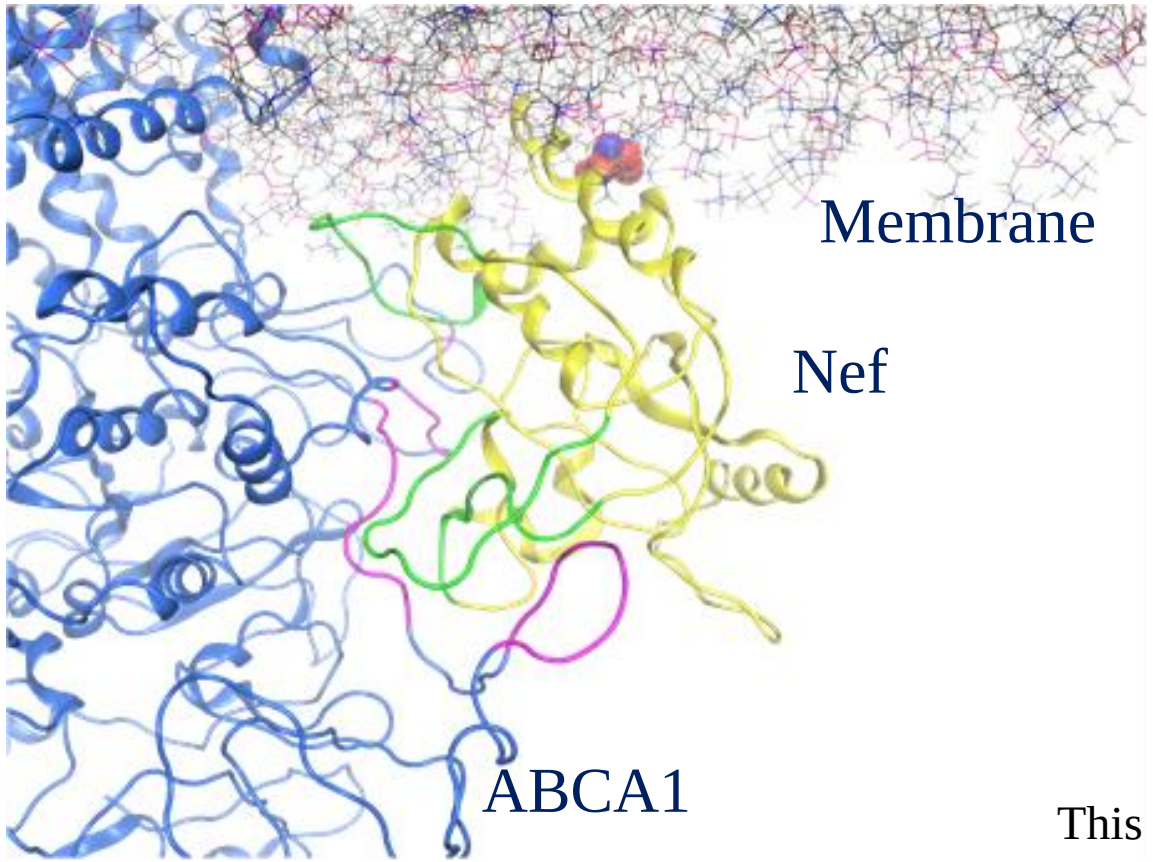
Modelling interaction between Nef and ABCA1 – docking results



- Nef molecules are positioned at the alternative binding locations predicted by docking
- From all models, one model has been selected where Nef is linked to the membrane (current virtual screening run)
- Other models will be also analysed to identify consensus binding sites



Modelling interaction between Nef and ABCA1 – docking results and virtual screening site



Considering that the N-terminus of Nef protein is lipidated at the Gly2 residue with the myristic acid residue, which serves to anchor Nef in the membrane, there remains only one ABCA1-Nef docking model, satisfying such spatial criteria. Using the QASDOM server [2], we determined the ABCA1 and Nef protein regions that form the interaction interface. The binding sites are centered on residues for which the largest number of intermolecular atomic interactions is shown. For ABCA1, these are Ser1023, Leu1026, Leu1972, Thr1261, Pro1267, Leu1282, Pro1284, Thr1305. For Nef: Asp32, Asp40, Asn51, Cys59, Trp61, Tyr85, Asp127. These putative binding sites will be used for structurally oriented virtual screening.

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- Currently we have selected the docking model where Nef is linked to the membrane at Gly 2 via myristoyl group
  - We have previously shown that ABCA1–Nef interaction requires membrane localization of Nef (PLoS Biol. 2006)

Anashkina AA. et al. Meta-server for automatic analysis, scoring and ranking of docking models. Bioinformatics 2018. Vol. 34. P. 297.

Hunegnaw R. et al. Interaction Between HIV-1 Nef and CalnexinHighlights // Arteriosclerosis, thrombosis, and vascular biology 2016. Vol. 36. P. 1758.