Title: Demonstration of NPC1 as a lipid binding protein *in Vitro*

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Lay summary

This present study aims at testing the possibility that NPC1 may directly bind cholesterol and/or other lipid. *In vitro (cell free)* photo-crosslinking study with the photoactivatable cholesterol analog (azocholestanol: AC) demonstrated that drosophila NPC1 can bind to cholesterol just like mouse NPC1 that has been using for my photo-crosslinking experiments so far. In order to identify a binding site within the SSD of NPC1 protein directly binding to AC, I tried to establish the efficient isolation system to separate the AC-labeled fragments with the biotinylated theta-toxin (BC-theta), which has been known to be the cholesterol-binding toxin. But unfortunately, it did not worked for the isolation purposes, although it did work for the detection of the intracellular cholesterol still as previously reported. Finally, *intact cell* photo-crosslinking experiment show that the association between ^{3}H-AC and NPC2 in cells with or without added NPC1 is approximately equal, suggesting that binding between AC and NPC2 does not require NPC1.