Palmitoylation of CB_1 receptor finely tunes its interaction with G proteins

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Keywords: CB_1, palmitoylation, membrane and G proteins interactions.

We previously demonstrated that CB_1 receptor is palmitoylated at cysteine 415, and that such a post-translational modification affects many aspects of its biological activity, including association with the plasma membrane, segregation within lipid rafts, signal transduction and coupling to specific G proteins [1].
In this study, we combined computational and experimental approach in order to address the structural reasons and the molecular mechanisms at the basis of these features of CB_1 receptor. We built the three-dimensional model of CB_1 receptor based on the sequence alignment with the A_{2A} adenosine receptor in the activated state (PDB code: 3QAK), and embedded it within a POPC/cholesterol membrane bilayer. In parallel we conducted experiments of co-immunoprecipitation, and assessed the physical association of the wild-type and the mutated (*i.e.*, non-palmitoylable) receptors with distinct G proteins. All experiments were run in the presence or absence of CP55940, a synthetic agonist of CB_1 receptor. Our data show that after 120 ns of MD simulation the non-palmitoylated active form of CB_1 receptor is unstable, and is converted into the inactive form. Instead, the palmitoylated receptor maintains its conformation in an active-like state. Experimental data demonstrate that the non-palmitoylable CB_1 receptor, although retaining its ability to bind to $G_{\alpha i2}$ protein, was no longer able to activate it upon stimulation with CP55940. Taken together, our results suggest that palmitoylation of CB_1 seems to anchor the H8 in a position which stabilizes the receptor active form, finely tunes its interaction with G proteins, and might serve as a signal for its subcellular targeting.

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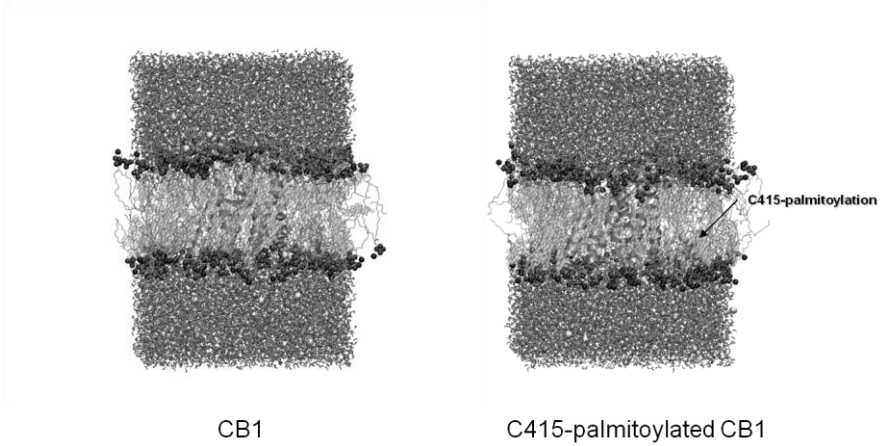

Figure 1. Three-dimensional model of CB_1 receptor (ribbon) based on the sequence alignment with the A_{2A} adenosine receptor (A_{2A}AR) in the activated state [2] (PDB code: 3QAK), and embedded within a POPC/cholesterol membrane bilayer. The initial three-dimensional model of CB_1 receptors was built using the MODELLER software [3], and refined by 40 ns molecular dynamics simulation according to an earlier described protocol [4] with the ACEMD software [5]. Afterwards, the refined CB_1 model was subject to 100 ns production run.

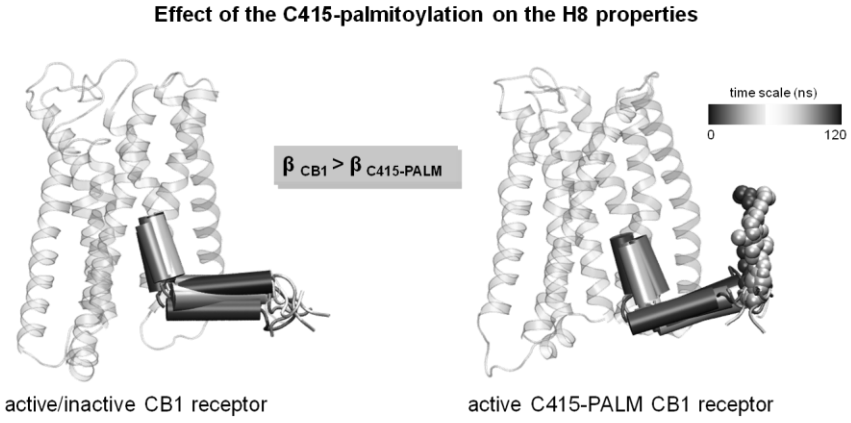

Figure 2: The image shows the dynamic properties of H8 during the 120 ns as superimposition of H8 over time whereas red 0 ns and blue 120 ns. Angle between TM7 and 8 (here called β) differs between CB_1 and C415-PALM CB_1. C415-palmitoylation seems to anchor the H8 in a position which stabilizes the complete receptor in its active form (e.g. ionic lock and TM5-7).