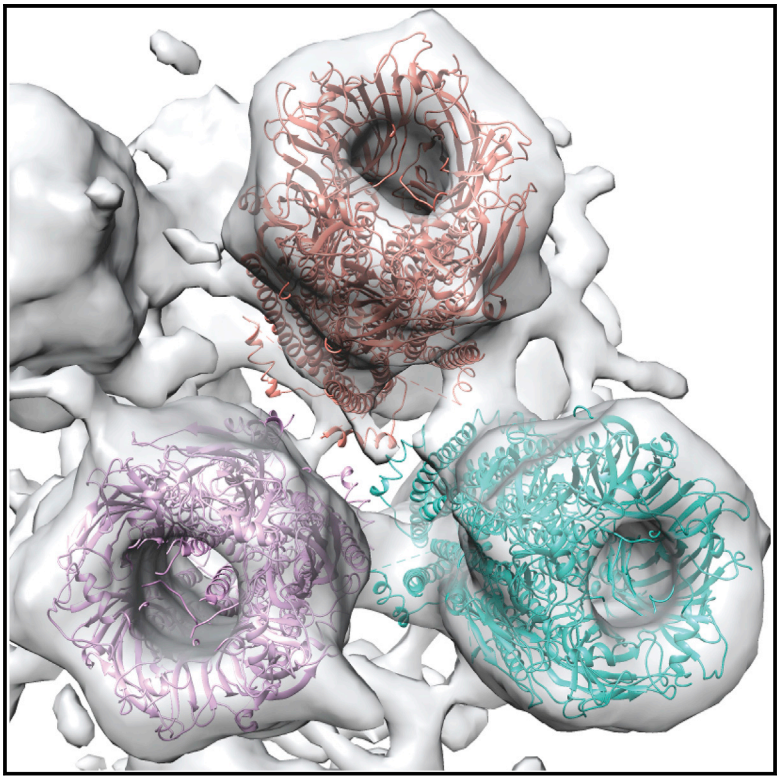
Short Article

Structure

The Structure of the Mouse Serotonin 5-HT_3 Receptor in Lipid Vesicles

Graphical Abstract



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In Brief

Membrane proteins may change structure in the absence of lipids. A 12-Å resolution structure of a ligand-gated ion channel in a lipid bilayer was obtained by cryo electron tomography. This approach is generally applicable to resolve 3D structures of non-crystalline proteins in membranes.

Highlights

- 5-HT_3 receptors are imaged in lipid bilayers by cryo electron tomography
- Subtomogram averaging resulted in a 3D structure at 12 Å resolution
- No major differences were found between EM and X-ray structures
- Inter-receptor interactions in the membrane are mediated by short horizontal helices

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The Structure of the Mouse Serotonin 5-HT_3 Receptor in Lipid Vesicles

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SUMMARY

The function of membrane proteins is best understood if their structure in the lipid membrane is known. Here, we determined the structure of the mouse serotonin 5-HT_3 receptor inserted in lipid bilayers to a resolution of 12 Å without stabilizing antibodies by cryo electron tomography and subtomogram averaging. The reconstruction reveals protein secondary structure elements in the transmembrane region, the extracellular pore, and the transmembrane channel pathway, showing an overall similarity to the available X-ray model of the truncated 5-HT_3 receptor determined in the presence of a stabilizing nanobody. Structural analysis of the 5-HT_3 receptor embedded in a lipid bilayer allowed the position of the membrane to be determined. Interactions between the densely packed receptors in lipids were visualized, revealing that the interactions were maintained by the short horizontal helices. In combination with methodological improvements, our approach enables the structural analysis of membrane proteins in response to voltage and ligand gating.

INTRODUCTION

Membrane proteins perform a variety of vital functions in living organisms, and knowledge of their structure is a prerequisite to understanding their function (Bill et al., 2011; Vinothkumar and Henderson, 2010). Recent developments in direct electron detection and software for single-particle cryo electron microscopy (cryo-EM) allow atomic resolution structures of non-crystalline proteins and protein complexes to be solved (Cheng, 2015). Reports indicate that lipids and detergents affect the conformations of membrane proteins and their mutual interactions (Li et al., 2014; Sun et al., 2015).

High-resolution structures of membrane proteins in lipid bilayers can be obtained by cryo electron crystallography (Abeyrathne et al., 2010). This, however, requires the production of ordered two-dimensional (2D) crystals. An alternative approach is to image non-crystalline membrane proteins in lipid vesicles by cryo electron tomography (cryo-ET) and process the volumes by subtomogram averaging (STA) (Eibauer et al., 2012). Several reconstructions have been obtained at subnanometer resolution (8–9 Å) using this method, namely, for the large stable chaperonin GroEL (Bartesaghi et al., 2012), a large icosahedral virus (Bharat et al., 2015), the structural protein of HIV (Schur et al., 2013), and native virions (Schur et al., 2015). The resolution obtained by cryo-ET and STA is still a critical issue; however, the advantages of direct electron detectors (DEDs) for tomography have so far been exploited to a lesser degree than for single-particle cryo-EM.

Here we concentrate on the serotonin-gated 5-HT_3 receptor (Maricq et al., 1991), which is a pentameric ligand-gated ion channel belonging to the superfamily of Cys-loop receptors (Thompson et al., 2010). The 5-HT_3 receptor is expressed in the CNS in the regions responsible for vomiting, pain, the reward system, cognition, and anxiety control; rodent models suggest that 5-HT_3 receptors are involved in pain perception, emotions, memory, and psychiatric and gastrointestinal disorders (Walstab et al., 2010).

The 3D structure of the detergent-solubilized 5-HT_3 receptor in complex with a high-affinity nanobody, VHH15, has been solved at a resolution of 3.5 Å by X-ray crystallography (Hassaine et al., 2014), which revealed the architecture of the homopentameric receptor, including extracellular, transmembrane, and intracellular domains. The bound nanobody stabilizes a non-conducting channel conformation. Consequently, imaging of the receptor reconstituted in a native-like lipid bilayer in the absence of a restricting nanobody is useful in understanding the physiologically important conformation and the gating mechanism of the receptor.

Here, our aim was to determine the structure of the native, non-crystalline 5-HT_3 receptor in a lipid bilayer. For this we reconstituted the purified, detergent-solubilized receptor in lipid vesicles and performed cryo-ET using direct electron detection