AFM imaging of lipidic nanosomes containing olfactory receptors

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Olfactory receptors (OR) constitute the key elements in the fantastic performance of the animal olfactory system. These receptors are located in the plasmic membrane of the ciliae of olfactory neurons, which are bathed by a layer of aqueous mucus that protects the olfactory epithelium [1]. Olfactory receptors can be expressed *in vitro* in various expression systems, as in yeast cells for instance [2]. These heterologously expressed olfactory receptors could be used in the development of artificial olfactory biosensors [3]. In order to transfer and immobilize the olfactory receptors onto the biosensors, one possibility is to prepare small lipidic nanosomes from the membrane fraction of the cells. In the present communication we report on the AFM characterisation of these nanosomes in the case of the rat I7 OR.

The rat I7 olfactory receptor has been expressed in yeast (*S. ceverisae*) [2]. Localization of I7 olfactory receptor expressed in yeast has been performed by immunocytochemistry and confocal microscopy, revealing the presence of the receptor at the plasma membrane. A high-level of functional expression has been demonstrated through a luciferase reporter. In addition, immunogold labeling of the I7 OR expressed in *S. cerevisae* followed by electron microscopy revealed its ultrastructural functional localization in the yeast membrane and in its trafficking pathway. Immunoblot analysis and glycosylation studies has shown that the I7 receptor is present as a mannose-glycosylated monomer. The level of expression of the I7 receptor has been quantified to be around 1.5×10^5 receptors/yeast cell, which is quite high for this type of receptors.Yeast cells were then mechanically disrupted and the plasma membrane fractions were separated from unbroken cells and cell walls by serial centrifugation steps. Inspection by transmission electron microscopy (TEM) of negatively stained membrane fraction adsorbed onto formvar-coated grids reveals that it is composed of circularized nanosomes or unclosed fragments with sizes ranging from 500nm to 40nm. Their size can be homogenized to 40-60nm by additional sonication. TEM images however, do not provide a three dimensional representation of the adsorbed nanosomes. In order to overcome this limitation, atomic force microscopy images were performed.

To this end, the membrane fraction was simply deposited and adsorbed onto a mica susbtrate and imaged in tapping mode in air with a commercial Atomic Force Microscope. Probes with constant force k=0.9 N/m and resonant frequency 40 kHz were used in order to minimize the damaging of the nanosomes.Nanosomes with diameters ranging from 200 nm down to 40 nm, and heights ranging from 30 nm down to 10 nm, respectively, have been identified in the images (see Fig 1). The 3 dimensional structure of the adsorbed nanosomes (Figure 2) is compatible with the spherical structure of the nanosomes in solution although due to the adsortion process the aspect ratio is far from unity (as would be the case for a sphere). The important point to be noted is that the nanosomes do not break upon adsorption, since the height is much larger than twice the membrane thickness (10 nm), therefore indicating that the nanosomes still contain some aqueous solution inside. This may depend on the nanosome-surface interaction and be different upon deposition on a functionalized surface. To our knowledge, this is the first time this type of nanosomes incorporating olfactory receptors has been imaged upon adsorption onto a substrate. Note that the number of receptors in the smallest nanosomes is expected to be between one and ten.

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