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## **Localization of olfactory receptors heterologously expressed in *S. cerevisiae* cells**

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Our group previously showed that *S. cerevisiae* yeast cells constitute an efficient means to express heterologous olfactory receptors upon a galactose induction carried out at the low temperature of 15°C. In the present work, we study the kinetics of a human olfactory receptor, hOR17-40, and its localization and functional response in sucrose gradient density subfractions, or in subfractions of a detergent gradient (Optiprep), starting from a membrane fraction preparation. The abundance of olfactory receptors co-localizing with various protein markers, such as the yeast endoplasmic reticulum (Dpm1p), Golgi (Vps10p), or lipidic rafts of the plasmic membrane (Pma1), evolves with time post-induction, as shown on immunoblots. Olfactory receptors were also localized and their amount comparatively evaluated by immunogold transmission electron microscopy using a gold-labeled secondary antibody, in various sucrose subfractions. The presence of co-expressed Golf (the G $\alpha$  subunit of G proteins specific of olfactory sensory neurons) was also examined in the various fractions.

Lipids analysis revealed various compositions for the sucrose subfractions. In addition, Pma1 marker located in the high sucrose subfraction (plasmic membrane) proved to be attached to lipidic rafts, since it was found in the detergent-resistant fraction of a subsequent Optiprep separation. On the contrary, olfactory receptors from any sucrose subfraction, including the plasmic membrane subfraction, proved to be present only in detergent-sensitive domains, i.e. not attached to rafts. Thus, a given sucrose subfraction may include both rafts and detergent-sensitive microdomains.

The functional response of olfactory receptors present in the various sucrose or Optiprep subfractions was monitored by Surface Plasmon Resonance as already reported. Whatever the fraction considered, i.e. independently of the cellular localization as indicated by the various protein markers (Pma1, Dpm1p, and Vps10p), olfactory receptors exhibited a functional response, and the level of functional response is not directly correlated to the total amount of olfactory receptors and Golf revealed on the immunoblots. Finally, this functional response is obtained regardless of lipidic rafts presence, and especially in the detergent-sensitive fraction of the Optiprep gradient.

Altogether, our results thus demonstrate that localization of olfactory receptors within rafts lipidic microdomains is not an absolute requirement for their efficient functional response.