Structural mechanism of taste receptors type 1 activation – Supplementary information

We have been able to successfully express TAS1R1/TAS1R3 and TAS1R2/TAS1R3 heterodimers in Sf9 insect cells. These receptors tended to be toxic for the host cells when heterologously expressed and therefore had a very poor cell surface expression. We were able to solve this problem by implementing bac-to-bac baculovirus expression system under optimized conditions (Fig. 1).
We have utilized three separate constructs with cleavable N- and C-terminal tags for the expression of full length TAS1R family members. The constructs were designed to keep the overall structure of taste receptors and to provide the ability of pulling down only the heterodimeric species. Sample purity and quality were assessed by using SDS-PAGE and SEC (Fig. 2). We are actively working on enhancing the quality of the final samples and we believe that at this point screening samples on a TEM can provide a valuable feedback for further improvements of sample quality in preparation for final data collection and structure determination.

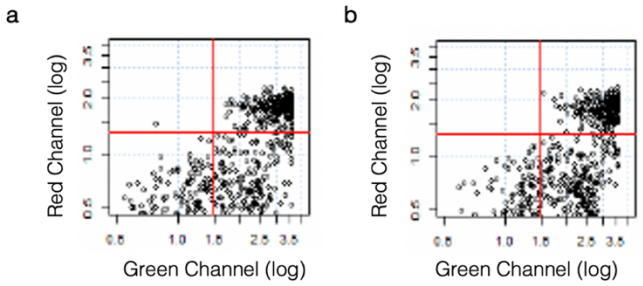

Figure 1 – Expression of TAS1 receptors in insect cells. Flow cytometry data for surface expression of TAS1R1/TAS1R3 (**a**) and TAS1R2/TAS1R3 (**b**) on representative infected sf9 cells. TAS1R1 and TAS1R2 bare a 3xFLAG epitope that is recognized by an anti-FLAG-FITC antibody. TAS1R3 carries an eGFP tag on its C-terminal. Green channel was adjusted to measure the fluorescence signal from FITC or eGFP indicating the expression level of the taste receptors. The red channel was used to indicate the live/dead cells.

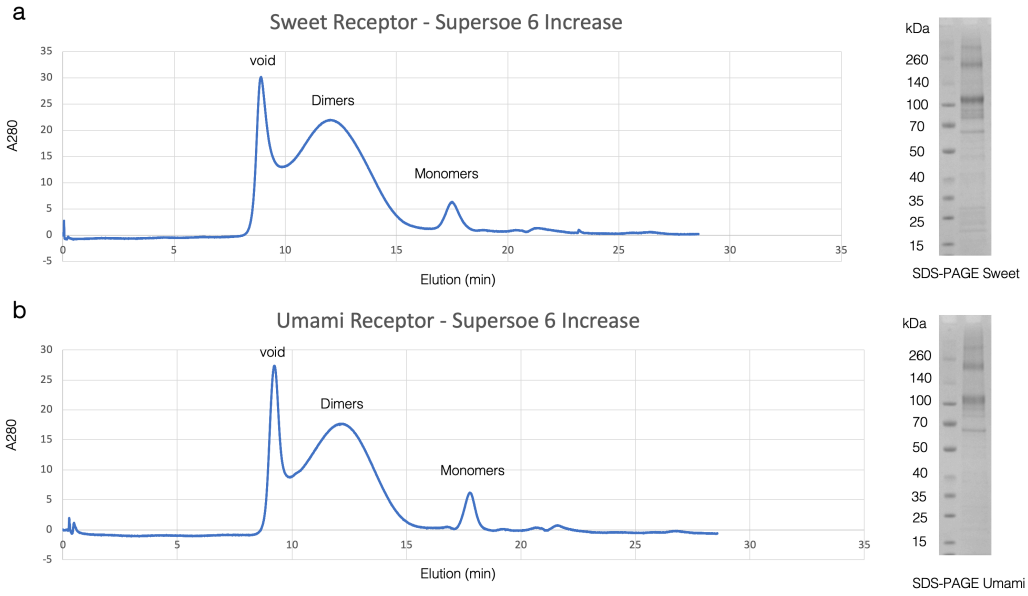

Figure 2– Representative size exclusion chromatography (SEC) and SDS-PAGE of the purified sweet (**a**) and umami (**b**) receptor heterodimers.