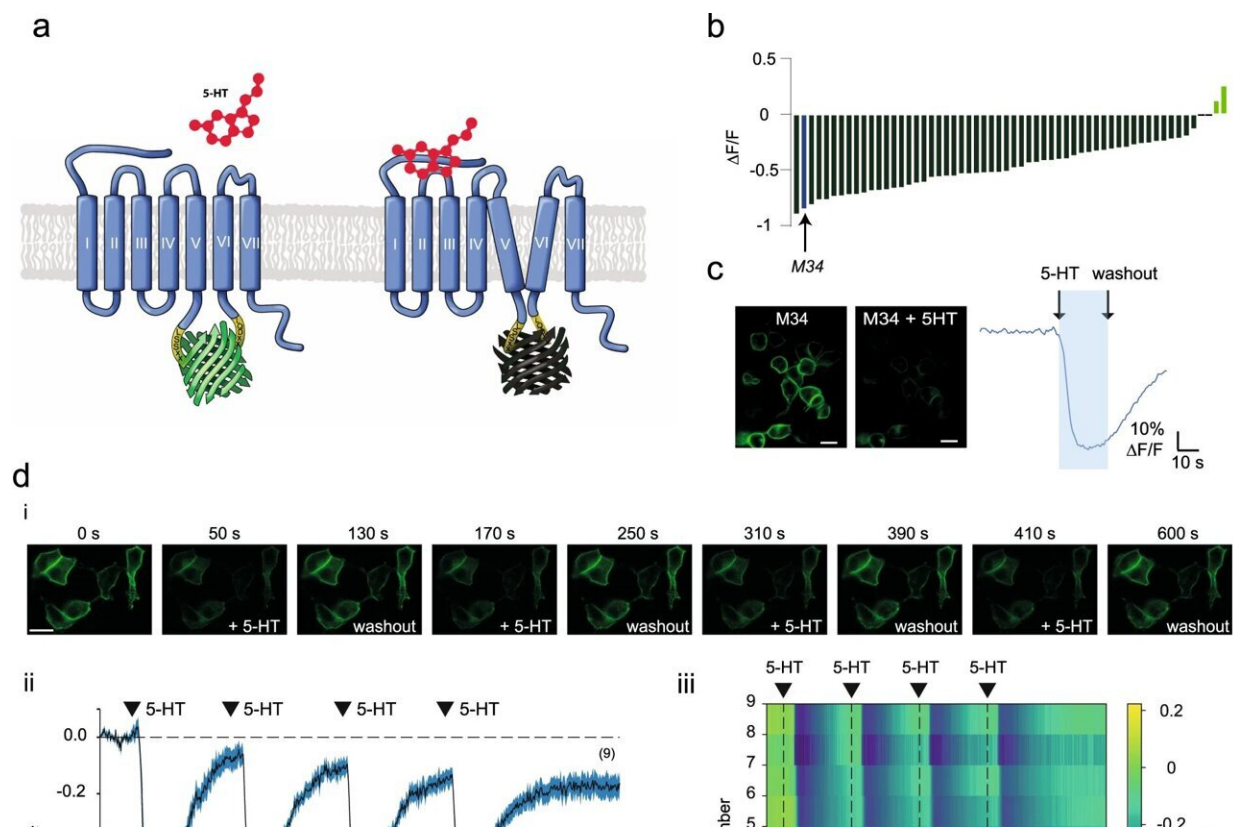


Synthetic biology: Next generation genetically encoded fluorescent sensors for serotonin

December 16 2022, by Sarah Batelka



Characterization in HEK cells. **a** Design principle of *sDarken*. **b** Screening result of linker variants. Bars indicate fluorescence changes upon application of serotonin. Blue bar represents mutant M34. **c** Expression of mutant M34 (*sDarken*) in HEK cells. Scale bar 20 μm . Fluorescence before and after the application of 800 nM 5-HT. Representative fluorescence trace of sensor variant M34 expressing HEK cell during the application of 800 nM 5-HT. Experiments were at least repeated three times. **d** (i) Representative image sequence of repetitive 5-HT application via wash in. (ii) Example fluorescence measurement,

repetitive application of 5 μ M 5-HT in 9 example cells in two dishes. Application of 5-HT leads to a reversible reduction of fluorescence. No baseline corrections were performed. (iii) Heatmap of all analyzed ROIs. Scale bar in the first frame applies to all following frames. Scale bar 20 μ m. **e** Example trace of 5-HT uncaging (405 nm, laser power: 90%, bleaching period: 90 ms, interval: 50 s) evoked *sDarken* responses. Blue circle indicates uncaging area. White line indicates the part of the membrane that was used for analysis. Fluorescence signal of *sDarken*. $\Delta F/F$ values of part of membrane (white line) over time (exemplary trial). Blue bars indicate uncaging (i.e., bleaching) intervals. Repetitive uncaging of 5-HT resulted in decreasing $\Delta F/F$ values of *sDarken*. **f** Fast 5-HT application to an outside-out patch. Representative image from an outside-out patch containing *sDarken* 48 h post transfection (left). Fluorescence intensity changes in the patch. The fluorescence recovery after 5-HT wash out is significantly slower than the activation. No background/baseline corrections were performed. **g** Dose response curve measured in response to different 5-HT concentrations. Group data $n = 20$ from at least 2 replicates, mean and \pm SEM are shown. **h** Fluorescence changes after application of serotonin, related substances or neurotransmitters (if not mentioned differently, concentration 10 μ M ($n = 10$)). Box represents the 25% percentile to the 75% percentile. The line in the middle of the box represents the median. One-Way ANOVA Multiple Comparison, *** p

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