Receptomics: Calcium imaging of sensory receptor cell arrays in a microfluidic system and novel applications for food screening

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Background

Reverse-transfected cell arrays in microfluidic systems have strong potential to perform large-scale parallel screening of GPCR libraries. We have successfully combined the multi-variable nature of a receptor cell array with microfluidics allowing for controlled and sequential sample dosing [1]. Our receptomics platform uniquely allows for dosing of small sample quantities against a large receptor library including controls for host cell responses and sample colour. Compared to existing microtiter-plate systems it can be applied more efficiently for the screening of off-target effects and the discovery of bioactivities in complex extracts with strong and variable host cell responses. The sequential injection format allowed the development of powerful spot-based statistical models to discriminate between a host cell response and the superimposed specific GPCR response.

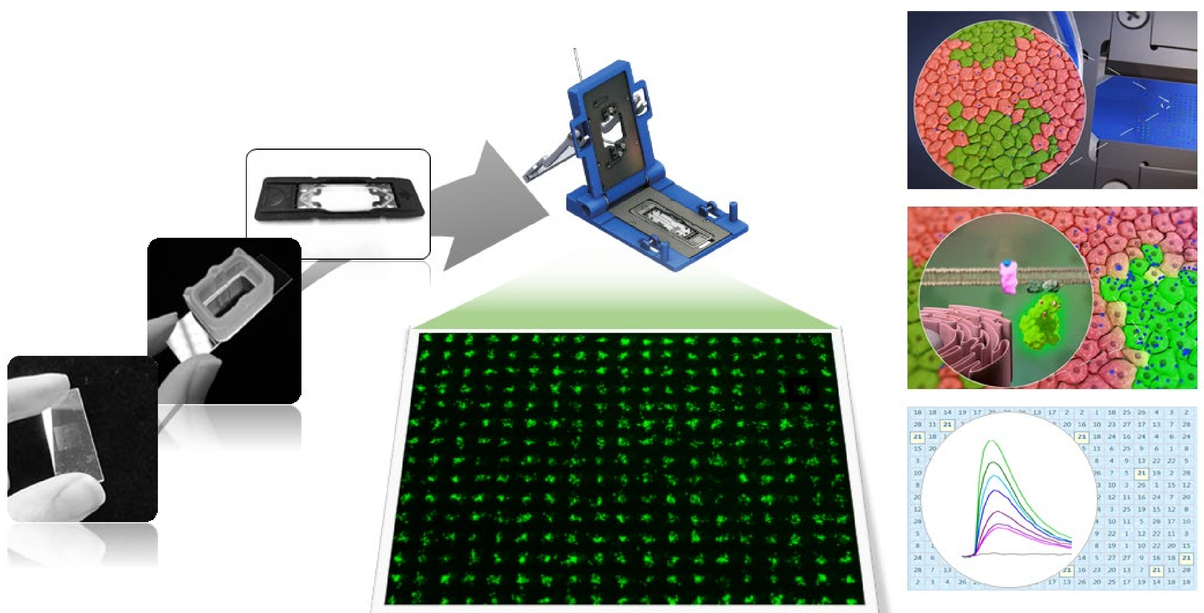
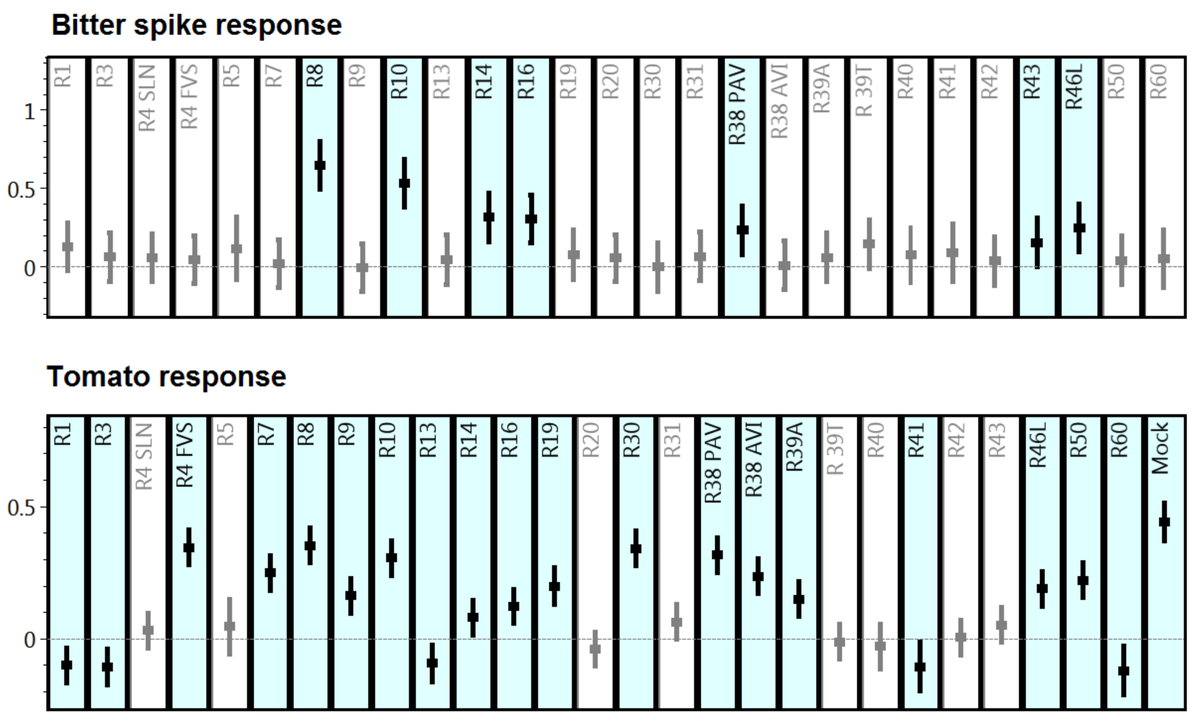


Figure 1, **Schematic overview of array preparation and measurement in the microfluidic system.**



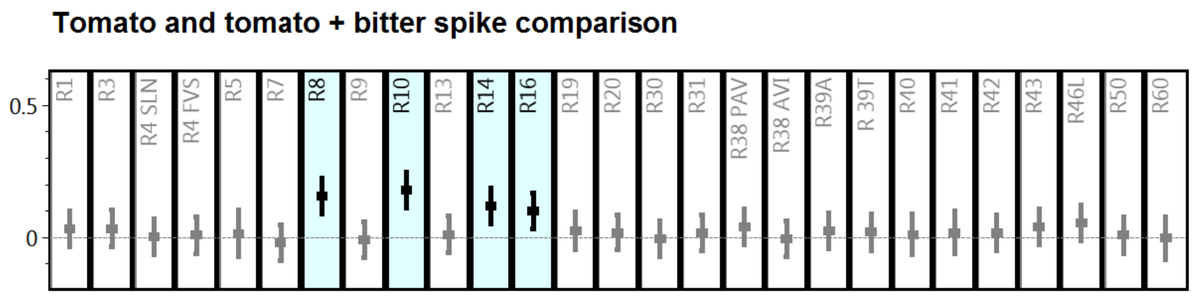


Figure 3, **Bitter receptor array response results from serial injections.** (top) Response to the bitter compounds mix. (middle) Response to the tomato sample. (bottom) Response difference between spiked and non-spiked tomato juice. Bitter taste receptors R8, R10, R14 and R16 show a significant increase in response in addition to the host cell response. Receptors R38PAV, R43 and R46L also show an increased response difference, but not significantly.

Method

The receptomics method involves reverse transfection of HEK293 cells, imaging by stereo-fluorescence microscopy in a flowcell format, real-time monitoring of cytosolic Ca^{2+} fluctuations, and automated statistical analysis of GPCR responses to sequential sample exposures.

Results

As proof of principle, cleared tomato juice was diluted 5x and spiked with 6 bitter compounds*. A bitter receptor array was exposed to alternating injections of spiked and non-spiked tomato juice. Despite the matrix interference and host cell response of the tomato juice, the bitter spikes were detected after a spot-based comparison. Figure 2 and 3 (top) show the 7 receptors that respond to the exposure of the 6 bitter compounds. When the bitter compounds were combined with tomato juice, 4 out of 7 receptors responded to the bitter spikes. The strong effects of tomato juice on the intracellular calcium prevented the other three receptors to give a significant signal.

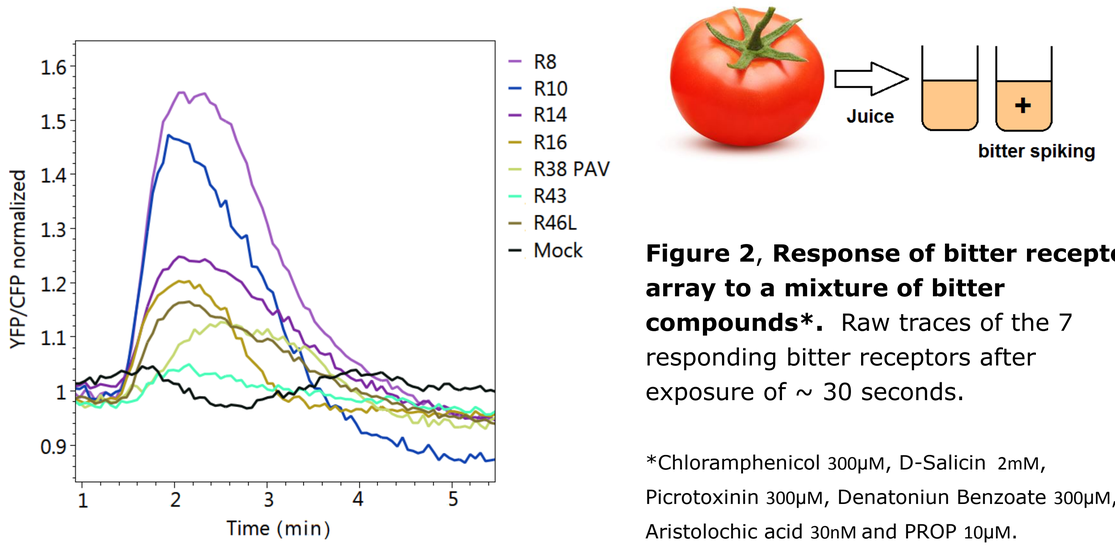


Figure 2, **Response of bitter receptor array to a mixture of bitter compounds*.** Raw traces of the 7 responding bitter receptors after exposure of ~ 30 seconds.

*Chloramphenicol 300µM, D-Salicin 2mM, Picrotoxinin 300µM, Denatoniun Benzoate 300µM, Aristolochic acid 30nM and PROP 10µM.

Conclusions

These findings provide a confident outlook on introducing this system as a novel high throughput GPCR screening platform, applicable for the screening of food products and with important applications in the area of sensory research.

For more information www.receptomics.com

1. Roelse et al., Calcium Imaging of GPCR Activation Using Arrays of Reverse Transfected HEK293 Cells in a Microfluidic System. Sensors Feb 2018

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