“Optical platform for ion channel drug screening”

Background and Description of Invention. We propose an optical platform based on a proprietary method, that is aimed to screen candidate compounds acting on ion channels. The proposed method allows to derive experimentally *a dose-response curve concerning specific agonist-receptor interactions by a fully automated procedure that fits the requirements of high throughput screening and offers a competitive alternative to traditional electrophysiological techniques.*
Briefly, fast-VSD-loaded cells are exposed to electric field stimulation in order to promote an amplification of the ionic currents flowing through the ion channels/transporters in a conductance dependent fashion. Since conductance values are measured under control conditions and after exposure to specific treatments, it is possible to estimate the effect of pharmacological treatments on membrane permeability. A dedicated platform has been designed to exploit the proprietary method, and makes it now possible to obtain the pharmacological profile of molecules acting on ion channels/transporters in a fully automated way.

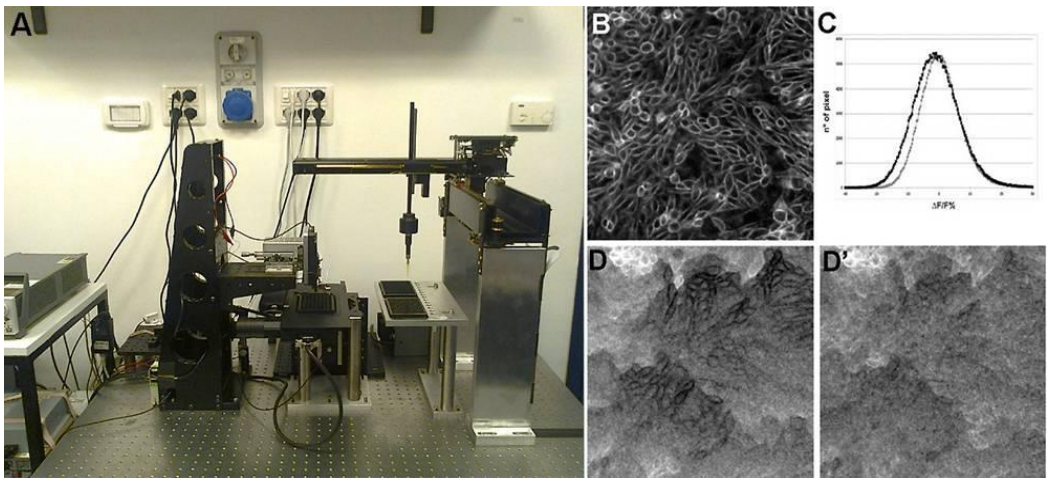

Figure 1. (A) the instrumentation and (B) an example of the measurement of a single data point. VSD-stained cells were exposed to increasing concentrations of capsaicin, an agonist of h-TRPV1. Examples of the analysis of the fluorescence values, before and after capsaicin, are shown as distributions (C, black and grey traces, respectively) as well as black and white maps of positive and negative variations (D and D’).

The platform has been applied to derive experimentally the concentration-response curve of Capsaicin on the h-TRPV1 receptor and that for GABA on ANS1 differentiated gabaergic neurons. Typically, eight concentration/response curves (9 point dilution series, each point calculated from several thousand cells) can be obtained from a 96-well multiwell plate in ~30 min.

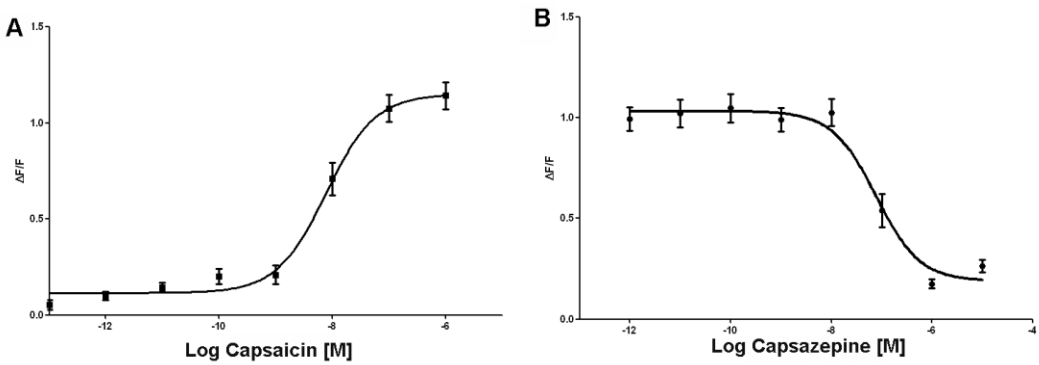

Figure 2. (A) Example of capsaicin concentration response curve (-LogEC_{50} [M]= 8.060; R=0.905). (BEC_{75} concentration of capsaicin is competitively blocked by capsazepine, an antagonist of h-TRPV1 (-LogIC_{50} [M]= 7.112; R=0.825).

Patent information. The international patent application was published as WO2011009825. Patent granted in Europe (EP2457088) and US (US9274100).

Stage of Development. The whole process is fully automated and does not require subjective operator intervention/interpretation. Cell membrane potentials are measured by fast-VSD dyes, upon application of appropriate current stimuli to the cells, before and after drug administration. Membrane conductance is then extrapolated by local measurement of the changes in membrane potential.

Potential Applications and Competitive Advantages. The method can be applied to evaluate:

- direct or indirect activation, modulation or blockade of ion channels or transporters expressed on the plasma membrane, with the possibility to derive experimentally a concentration-response relationship;
- the direct permeabilizing effect of molecules with channel or transport characteristics (i.e. antibiotics and antimycotics);
- changes in the basal conductance of the plasma membrane as a marker of the different physiological states of a cell;
- the cellular and sub-cellular localization of permeability events in identified domains (even in complex and heterogeneous cellular models).

Several advantages can be envisaged:

- Time resolution: changes in membrane conductance (typically caused by drug acting on ion channels) can be measured in the subsecond time scale, i.e. better than in conventional optical drug screening;
- Spatial resolution: it works at the single cell level, thereby allowing the study of conductance changes in identified cells within heterogeneous populations as well as in subcompartments of cells with complex morphology;
- Sensitivity: it does not require overexpression of channels/transporters to have a proper readout of their activity;
- Specificity: it provides a highly specific readout, with no restrictions on the nature of the molecular mechanism responsible for the change in conductance;
- Scalability: it makes cell-based high-content-screening compatible with the requirements of high throughput screening.

We seek a commercial partner with a strong pipeline in high-throughput screening of candidate compounds action on ion channels/transporters.

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