Profiling the pharmacology of GPCRs by time-resolved impedance

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1 AtlaZ for sophisticated high-throughput quantitative live-cell analysis

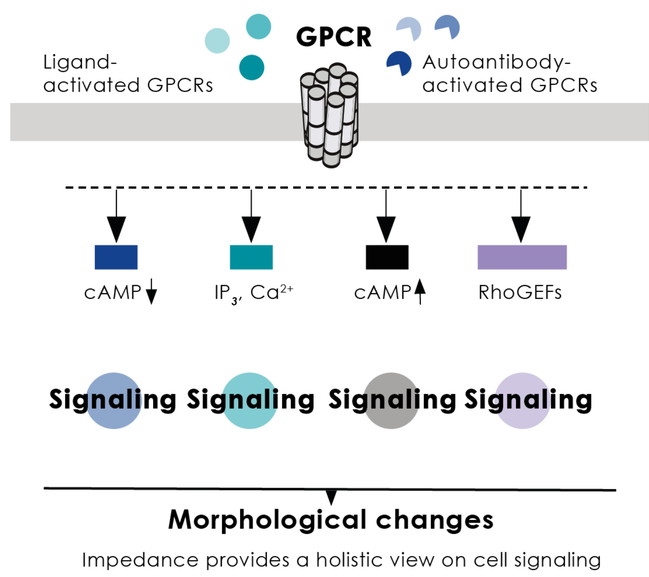
Key benefits:

- **Real-time, label-free, long-term** impedance recordings (Cell Signal)
- **High throughput** with 6 x 96 well plates
- **Electrical impedance spectroscopy**
- **Continuous** cell monitoring with **high time resolution**
- **Automated data analysis** with access to raw data
- 21 CFR Part 11 **compliance in GLP/GMP** laboratories

Application areas:

- Cell proliferation, viability and cytotoxicity
- Cell signaling
- GPCR signalling:
  - Pharmacology: drug MOA (agonist, antagonist, inverse agonist, allosteric modulator)
  - Pathway deconvolution
  - Orphan GPCRs
- Immuno-oncology; Cell and gene therapy
- Virology; Oncolytic viruses
- TEER; Barrier function

2 Why is label-free impedance sensing so attractive?

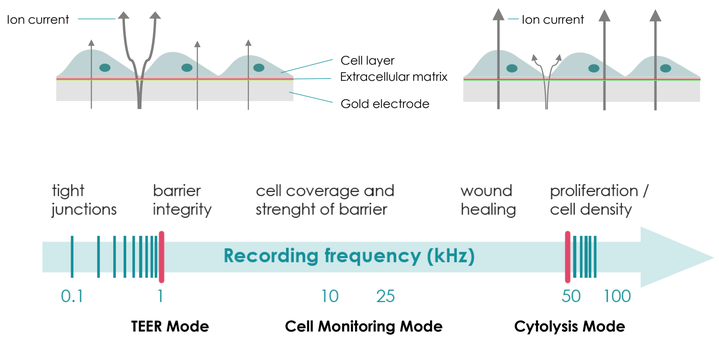


Impedance provides a holistic view on cell signaling

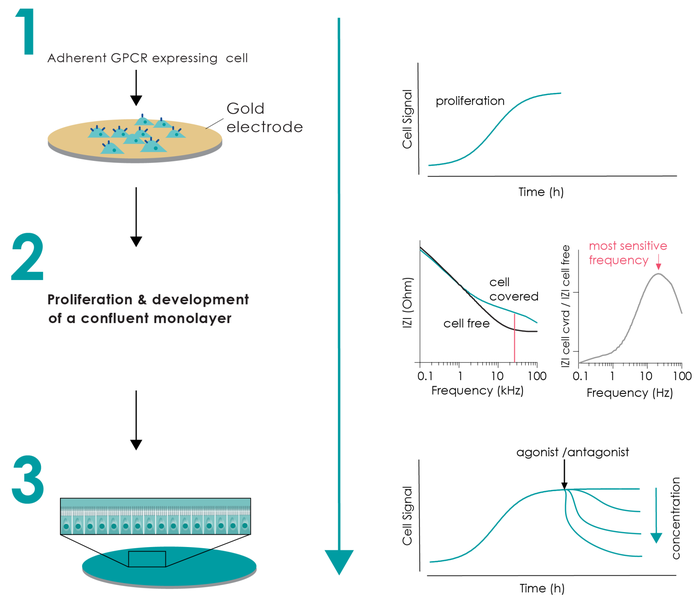
Different GPCR-dependent signaling pathways induce morphological changes of cells. Independent of which pathway being active, these changes can be measured via impedance.

3 Accelerating your research
… workflow of a GPCR assay

Impedance assays are used in cellular biology to measure various biological processes and drug effects. To measure impedance, small electrical currents are delivered to electrodes embedded in the cell culture substrate. When no cells are present, the electrical current flows easily, resulting in low impedance. Upon introducing cells onto the substrate, the flow of electrical currents Is obstructed, leading to an increase in impedance. Impedance changes induced by various treatments can then be monitored.



AtlaZ utilizes Electric Cell-substrate Impedance Sensing (ECIS) assay technology. Advanced information content is obtained by using multi-frequency impedance readouts (0.1 kHz – 100 kHz). High frequency impedance is sensitive to differences in cell-confluency, making it useful for measuring proliferation or cytotoxicity. Low frequency impedance data reveal barrier integrity, allowing to study tight junctions in health and disease.



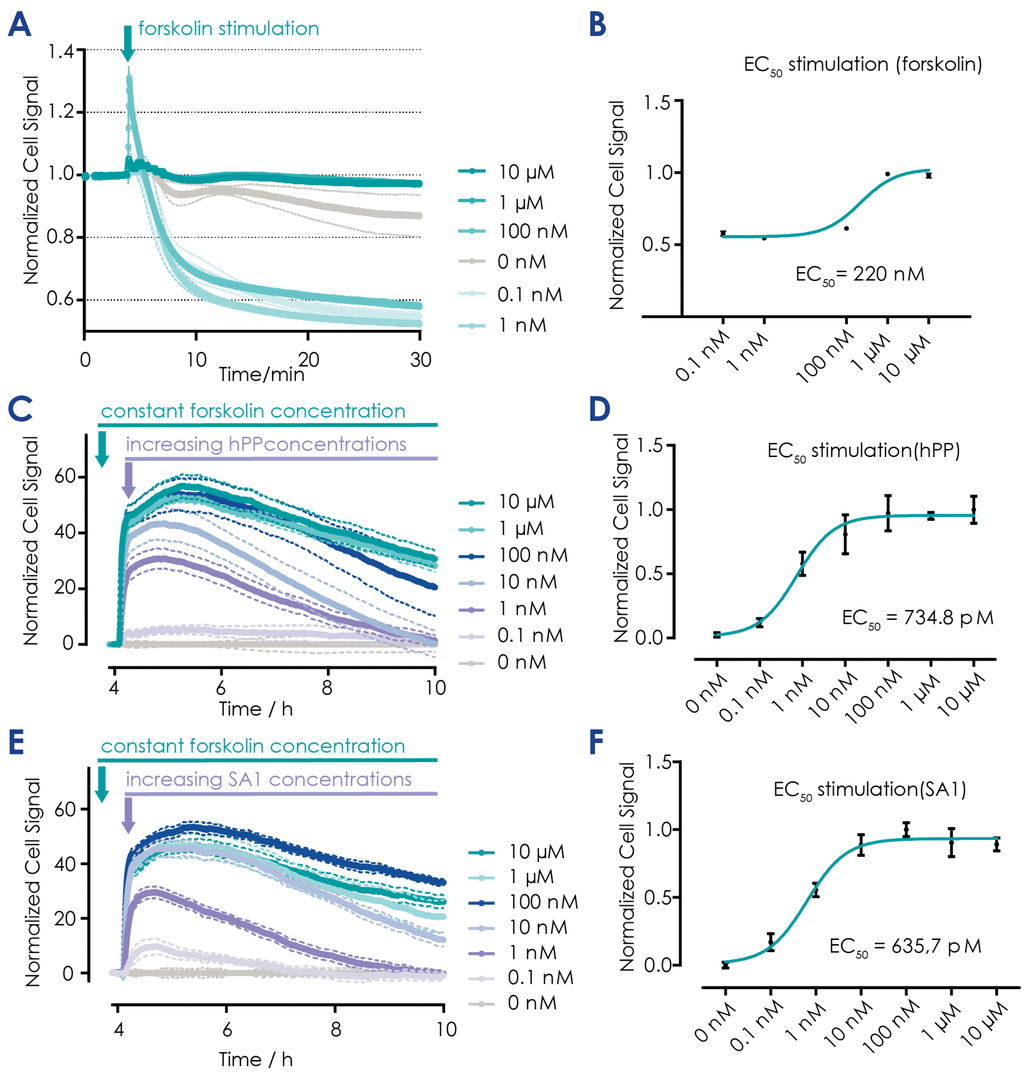
Workflow of a GPCR assay:

Target cells adhere and proliferate (<= 48 h) (**1**).

Upon reaching a plateau phase, spectra for cell-free and cell-covered electrodes reveal the most sensitive frequency (**2**) by plotting the ratio of the impedance magnitude |Z| of electrodes with cells and the impedance |Z| of electrodes without.

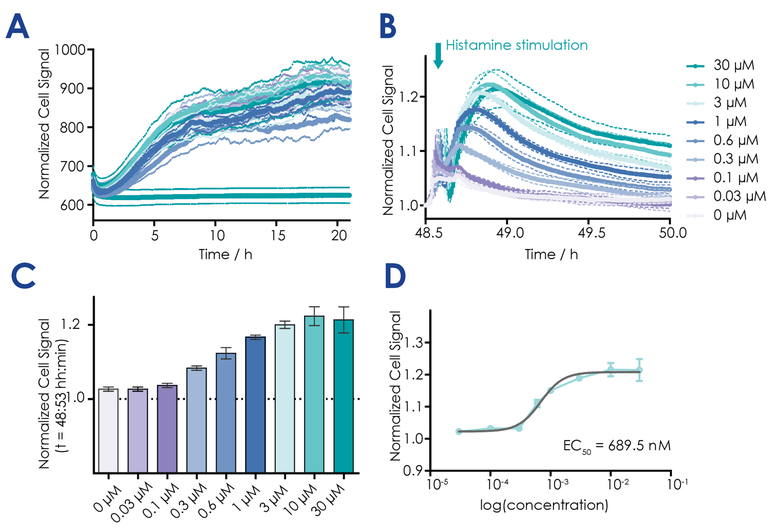
Next, the treatment can be executed (**3**). The Cell Signal is monitored continuously over time, revealing the kinetics of stimulus-induced effects.

4 Time-resolved impedance profiles of Y4R stimulation in CHO cells



Time-resolved impedance profiles of Y4R stimulation in CHO cells, **A** stimulation via increasing forskolin conc., **B** dose-response relationship yielding an EC_{50} of 220 nM. **C** Stimulation of Y4R with the endogenous ligand hPP, **D** dose-response relationship yielding an EC_{50} of 735 pM. **E** Stimulation of Y4R with increasing conc. of SA1, **F** dose-response relationship yielding an EC_{50} of 636 pM. Data are shown +/- SD, n = 6 each.

5 Time-resolved impedance profiles of human glioblastoma cells (U373)



Time-resolved impedance profiles of human glioblastoma cells (U373), **A** raw traces for adhesion and growth (seeding at time zero, n = 14 wells), **B** normalized traces for stimulation of U373 cells with Histamine at 0.03 µM - 30 µM (normalized to cell signal in L-15 medium before Histamine addition), **C** bar graph for normalized cell signal at t = 48:53 hh:mm. **D** EC_{50} estimated to be 690 nM. Data are shown +/- SD, n = 6 each.

6 Conclusions

- AtlaZ is a quantitative label-free and real-time live-cell analysis system.
- Our results demonstrate the capability of AtlaZ to characterize the pharmacology of GPCRs and monitor major G-protein-dependent pathways.
- The strength of AtlaZ-based assays for GPCRs as drug discovery targets lays in its (i) time resolution, (ii) throughput, (iii) label-free and (iv) independent of genetic engineering.
- Recordings can be performed for up to 6 x 96 = 576 samples either simultaneously or independently.