Technical Note

Electrical Impedance Spectroscopy in GPCR Research

Tools:
AtlaZ

Key aspects

- *Discovering GPCR ligands is essential for advancing new drug development.*
- *The AtlaZ´s high sensitivity is primarily driven by the use of Electrical Impedance Spectroscopy.*
- *AtlaZ excels in studying orphan receptors with its unbiased readout, independent of specific signaling cascades but sensitive to all.*
- *User-friendly software with ready-to-use recording modes, automated analysis of the most sensitive frequency, and the ability to record 576 samples simultaneously or independently enables efficient research and screening.*

Introduction

Investigation of cellular responses to GPCR activation provide valuable data for drug discovery and development. Distal effects of GPCR stimulation in cells are morphological changes of the cells under investigation. Such changes in cell morphology can be characterized by size, shape, and structural alterations. These physical rearrangements in cells result in corresponding alterations in their electrical properties as the cell acts as both a resistor and a capacitor. Thus, electrical impedance spectroscopy (EIS) for the quantification of such electrical properties[1,2] is a perfectly suited methodology to investigate GPCR pathways in an unbiased way. Whether occurring independently or concerted, ligand induced alterations of cell adherence, shape, volume or cell-cell interactions impact the extracellular and transcellular current flow that is being measured (Figure 1 A), consequently influencing the magnitude and characteristics of the recorded impedance.

Impedance-based cell monitoring provides a *holistic approach* as the impedance integrates over the entire cell body without any molecular specificity as, for instance, provided by most label-based assays. This means, it provides an unbiased perspective on the cell under study. The lack of molecular specificity may require some careful assay procedures and controls to avoid false-positive results. But it unfolds its full strength when orphan receptors are being studied. The search for a yet unknown endogenous agonist typically goes along with no information on the coupling pathways of the receptor. Thus, an unbiased assay readout that is independent of the downstream signaling cascade but sensitive to all of them is the most efficient readout in de-orphanizing campaigns. Within the assay development it is therefore extremely valuable to be able to identify the cell´s most sensitive frequency (Figure 1) ad hoc by running a full spectrum, thus making use of the full potential of the technology to quantify even small or fast effects. The high sensitivity of the technology allows it to be applicable to primary cultures or finite cell lines with endogenous receptor density.

Another advantage over standard assays that rely on labels for optical or radiometric detection, is the continuity of cell monitoring. Endpoint assays using dyes or labels just report on the status of the cells at a single time point but neglect

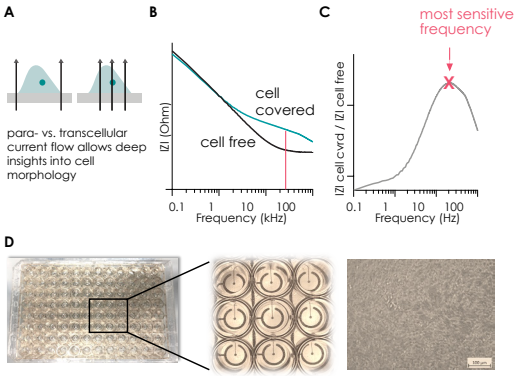


Figure 1: **A** *Schematic side view of a cell-covered electrode with current flow at low (left) and high (right) AC frequencies.* **B** *Frequency dependent behavior of a cell-free & cell-covered electrode reflecting the impedance increase due to the cell layer between 0.1 – 100 kHz.* **C** *Ratio of the normalized impedance - calculated by dividing |Z| for a cell-covered electrode by that of the same cell-free electrode is used to estimate the monitoring frequency with maximum sensitivity.* **D** *NSP-Z 96-well sensor plate, each well has a central recording electrode and a reference electrode (see magnification); the plate is transparent to facilitate observation of the cell layers and multiplexing. Right: confluent cell layer of CHO cells.*

the kinetics of the cell response. Thus, the EIS method has found significant applications in cancer research and the study of cell surface receptors such G-protein-coupled receptors (GPCRs) or Receptor Tyrosine Kinases (RTKs), which are critical in various physiological processes and disease mechanisms.

Technical solution

EIS derived measurements offer advantages such as real-time kinetic measurements and GPCR activation (agonist/antagonist mode) in target cells. Specifically, the strength of the AtlaZ assay approach with regards to GPCR as a target is its **(i)** time resolution, **(ii)** throughput, **(iii)** the fact that it is label-free and **(iv)** independent of genetic engineering. And, since the methodology allows to measure effects at a spectrum of different recording frequencies, another strength of the technology is to, **(v)**, easily identify the most sensitive frequency of the cells being investigated. The rationale behind here is that different cellular responses to GPCR activation can manifest at different frequencies. Identifying the most sensitive frequency ensures the highest signal-to-noise ratio for detecting specific cellular changes.

The optimal frequency is cell-type specific and is determined empirically, mostly it ranges between 1 kHz and 50 kHz[3]. Depending on the data acquisition frequency (0.1 - 100 kHz), the system detects predominantly the resistive part of cell-cell and cell-matrix contacts at lower frequencies (Figure 1 A left) or predominantly capacitive currents across the cell membranes at higher frequencies (Figure 1 A right) as an indicator for electrode coverage. The latter is the basis for cell adhesion or migration assays. Thus, impedance data provide insights into various cell phenotypes, such as cell morphology changes as a result of signaling, proliferation, lateral migration or cytotoxicity in real-time and over prolonged periods of time. Specifically, AtlaZ Control allows detection of impedance signals at 100 different frequencies, ranging from 0.1 kHz to 100 kHz. For example, cell signals representing barrier integrity can be recorded in parallel to cell attachment strength when analyzing the data output at 1 kHz or 50 kHz, respectively.

As a general guideline, the most sensitive frequency for detection of changes in cell layer properties can be determined by dividing the impedance magnitude |Z| of a cell-covered electrode by the impedance of a cell-free electrode along the frequency spectrum (Figure 1 B). When these ratios are plotted for each individual frequency, one obtains a bell-shaped curve from which the maximum ratio reflects the frequency with the broadest range of relative change in impedance (Figure 1 C), i.e. the most sensitive frequency.

Different experimental setups (e.g. various cell types within particular research areas) own a specific "sweet spot" (most sensitive frequency) and the available frequency spectrum (0.1 – 100 kHz) allows to exploit a multitude of experimental conditions.

Methods

It is important to maintain healthy cell cultures to generate optimal and consistent results. The efficacy and reliability of cellular assays are dependent on the overall viability and health of the experimental cell population. This critical factor is predominantly influenced by the accuracy and consistency of the cell plating process. To enhance data quality, it is essential to determine the optimal cell density for plating and employ surface coatings to enhance the adherence of less firmly attached cells.

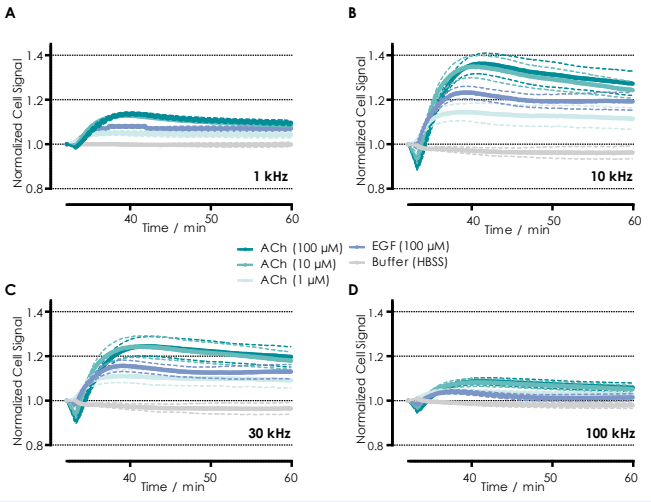
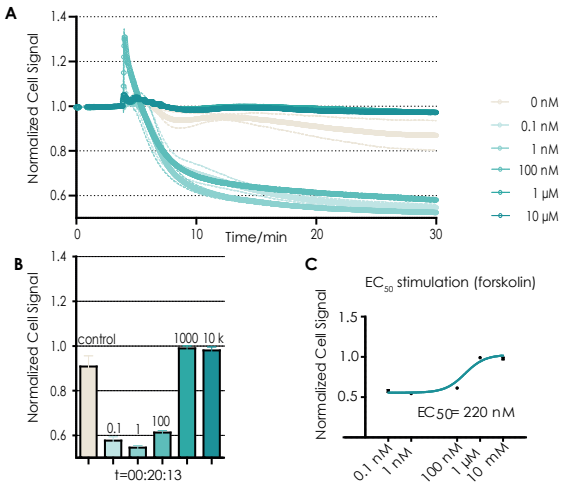


Figure 2: *HEK cells endogenously expressing Gq-coupled muscarinic acetylcholine M3 receptors, and cell responses to ACh concentrations of 1 µM, 10 µM and 100 µM, furthermore to 100 µM EGF. Note the difference of the size of the negative peak at various measurement frequencies,* ***A-D****, 1 kHz, 10 kHz, 30 kHz and 100 kHz. The largest effect size is seen at 10 kHz, which is the most sensitive frequency here.*

Figure 3: *Time-resolved impedance profile of Y4R stimulation in CHO cells.* ***A*** *Impedance raw data showing stimulation via increasing forskolin concentrations,* ***B****, normalized signal amplitude shown in a bar graph at t = 20 min.* ***C*** *Dose-response relationship yielding an* EC_{50} *of 220 nM.*



Cell preparation

Many cell types are compatible and have been used on the AtlaZ, including cultured cells, primary cells or patient-derived cells. In general, cells should be thawed as specified by the cell supplier. Before seeding the cells on the AtlaZ NSP-Z sensor plate, it is important to count the viable cells. A Trypan Blue staining is very well suited to perform viability check. Previously cultured cells can be transferred directly onto the NSP-Z.

Seeding the cells

Target cells are seeded on NSP-Z plates and they adhere on the surface with embedded gold electrodes (Figure 1 D). Each well of the 96-well plate contains one center gold electrode with 0.6 mm of diameter and one reference electrode. Cells should be seeded according to their specific optimal conditions. It is possible for seeding to occur as early as several days in advance until the day before the assay. However, for most adherent cell types, seed the cells into the NSP-Z plate on the day prior to the experiment, and incubate and monitor in the AtlaZ system at 37°C with 5% CO_2 overnight. In the case of loosely adherent cells, the plates can be treated with surface coatings such as collagen, fibronectin, poly-D-lysine or similar reagents prior to cell plating to improve cell adherence. Cells should be confluent when executing a GPCR experiment (Figure 1 D). Note that assay conditions like cell density and compound concentrations may need to be optimized for each receptor[4].

Experimental planning

Once the cells are confluent, the most sensitive frequency has been identified and the compounds are set, the experiment can begin. Before compound addition, and at the latest 1 h prior to running the assay, replace cell culture media with the final volume of assay buffer or control medium. After having recorded the impedance in control buffer solution, the measurement is being paused, the plate is being removed from the recording unit and the compounds are added. Replacing the measurement plate back into the recording unit and resuming the experiment continues the monitoring and the compound effects are recorded. The user does have the ability to modify treatment layouts on the fly if needed.

Data analysis

The AtlaZ Control user interface allows both data acquisition and data analysis. Data analysis can proceed online during experiments or offline after experiments have concluded. The software allows easy loading of recorded files, furthermore, you have the option to choose the frequency you want to view (e.g. 1 kHz, 10 kHz, or 50 kHz). The software analyzes the impedance curves to quantify compound-induced GPCR-mediated responses. Various parameters can be assessed, including response amplitude, kinetics, and EC_{50}/IC_{50} values.

Case study: endogenous receptor expression

The AtlaZ EIS method is sensitive enough to detect responses from endogenously expressed GPCRs. Thus, researchers can work with primary cells or patient-derived samples expressing native receptor levels. Furthermore, potential artifacts from receptor overexpression systems can be avoided. In this example here, response profiles of HEK293 cells endogenously expressing GPCRs (muscarinic M3 receptors) have been investigated. We could show that the initial transient drop in impedance that is characteristic of Gq-coupled GPCRs is most prominent at 10 kHz (Figure 2). In contrast to 10 kHz, the most sensitive frequency, signals were less prominent when measuring the ACh (Acetylcholine) or EGF (epidermal growth factor) responses at 1 kHz or 30 kHz or 100 kHz. Here, wells have been coated with fibronectin in order to allow the HEK293 cells to tightly attach to the surface of NSP-Z plates.

Case study: Setting up a new assay

The search for a yet unknown endogenous agonist typically goes along with no information on the coupling scheme of the receptor. Thus, an unbiased assay readout that is independent of the downstream signaling cascade but sensitive to all of them is the most efficient readout in de- orphanizing campaigns. In such a case, a time-resolved impedance profile of the cells & compounds under investigation is being performed. To identify even fast kinetics of an effect, a high repetition interval between sequential impedance readings can be chosen. In the example shown here, the aim was to identify a forskolin concentration response curve. Forskolin is a well-established, receptor-independent inducer of adenylate cyclase, and here CHO cells overexpressing the Neuropeptide-Y Receptor 4 (Y4R) were exposed to 0.1 nM, 1 nM, 10 nM, 100 nM, 1 µM and 10 µM forskolin (Figure 3 A, B), and a high repetition interval of 1 s was chosen. We estimated an EC_{50} of 220 nM (Figure 3 B), and the optimal forskolin concentration identified was 400 nM, inducing a maximum effect. A similar experimental setup, i.e. high repetition interval and multiple concentrations of a compound could be recapitulated in de-orphanizing studies: When attempting to de-orphanize GPCRs, typically a diverse library of potential ligands is prepared, including known GPCR agonists, tissue extracts, and novel compounds. These compounds then are applied to the cells in a systematic manner, often using high-throughput screening approaches, which could be realized in an AtlaZ platform by using 6 x 96 well plates (576 wells) in parallel. As a next step, compounds are identified that produce reproducible and dose-dependent responses, and hits are confirmed using secondary assays (e.g., calcium mobilization, cAMP production).

This methodology leverages the high sensitivity of impedance-based assays to detect subtle alterations in cellular morphology and adhesion patterns resulting from GPCR activation. The frequency with the highest ratio of cell-covered to cell-free impedance provides the broadest range for detecting changes, allowing for more sensitive measurements of GPCR-induced cellular responses. Such precision enables the identification of novel ligands for orphan receptors, thereby playing a pivotal role in expanding our understanding of GPCR signaling networks.

References

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2. *Lukic, S.; Wegener, J. (2015) Impedimetric monitoring of cell-based assays. Encyclopedia of Life Sciences, Wiley & Co. https://doi.org/10.1002/9780470015902.a0025710*
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4. *Doijen, J. et al. (2019) Advantages and shortcomings of cell-based electrical impedance measurements as a GPCR drug discovery tool. Biosensors and Bioelectronics,137: 33-44, https://doi.org/10.1016/j.bios.2019.04.041*

Find out more

- *Application note:*
  Profiling the pharmacology of GPCRs by time-resolved impedance measurements
- *Webinar Joachim Wegener (Professor, University of Regensburg & Fraunhofer EMFT):*
  Profiling the pharmacology of GPCRs using time-resolved impedance
- *Website info:*
  How AtlaZ works
- *Application note:*
  TEER – "Monitoring cell – attachment and tight junctions in the same assay"

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