Case study

Using automated patch clamp to study CFTR mutations and modulators

Tools:
Patchliner

Prof. Frédéric Becq
featured by Nanion Technologies

Frédéric Becq is Professor of Physiology at the University of Poitiers, France. His research focuses on the pharmacology and function of chloride channels, in particular CFTR, and its role in cystic fibrosis. His research group is screening small molecules with the aim to identify new therapeutics for CF patients.

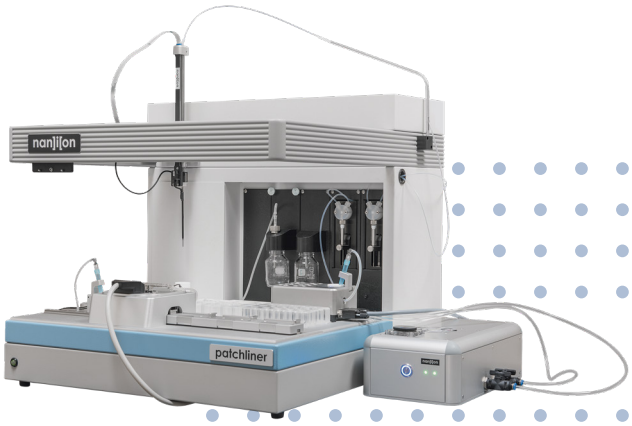
Cystic fibrosis (CF) is a severe genetic disorder affecting approximately 100,000 individuals globally. This autosomal recessive condition is caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene, which encodes an anion channel essential for proper mucosal hydration in the lungs, pancreas and other organs. CFTR protein regulates the movement of chloride and bicarbonate across epithelial cell membranes, and its dysfunction results in the buildup of thick, sticky mucus.

Over 2,000 CFTR mutations have been identified so far, each contributing to the protein's malfunction in a variety of ways, ranging from misfolding and premature degradation to gating abnormalities, thermal instability and trafficking defects. CF patients with different CFTR mutations can exhibit different symptoms with varying degrees of severity. Therefore, treatment plans for CF are often individualized and depend on the specific symptoms and mutation type.

Since the discovery of the CFTR gene in 1989, advances in CF treatment, especially with the introduction of CFTR modulators, have significantly improved the prognosis and life expectancy for many people with CF. The approved CFTR modulator therapies like ORKAMBI™ (ivacaftor/lumacaftor) and TRIKAFTA™ (elexacaftor/tezacaftor/ivacaftor) are small molecule drugs that can correct the functioning of the defective CFTR protein with certain mutations, and have shown improved clinical benefits for many patients harboring theF508del-CFTR mutation. Still, many people with rare CF variants are not eligible for such modulator treatments and are unresponsive or intolerant to these drugs, indicating the need for developing new CFTR modulators.

The diverse and numerous mutations present a significant challenge in CF research, necessitating a robust method to study CFTR ion channel function under various conditions.

Traditional methods, particularly the manual patch clamp technique, while offering high-resolution data, are notoriously low throughput and labor intensive, making them impractical for screening the large array of CFTR mutations or for assessing the myriad small-molecule correctors, potentiators, stabilizers and amplifiers.



Nanion's Patchliner platform
is a fully automated patch clamp system which records from either 4 or 8 cells simultaneously. The HEKA EPC10 amplifiers, full control over the pipette speed, ability to change protocols on-the-fly and flexible add-ons for temperature control and dynamic clamp make this instrument the most versatile APC system on the market. The Patchliner is tried and tested and is appreciated in academia, industry and CROs alike since its introduction in 2006.

"After almost ten years of use we are very satisfied with the Patchliner which was instrumental to analyze the function and pharmacology of CFTR and F508del-CFTR and to characterize the effects of therapeutic modulators."

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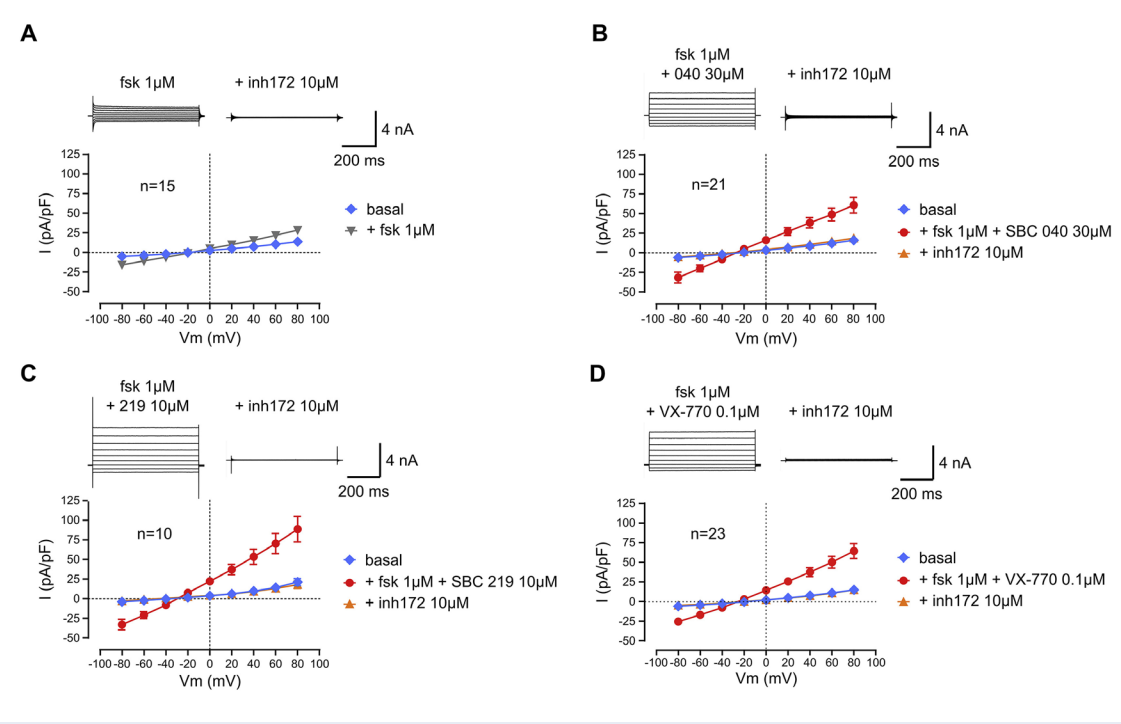
The automated patch clamp (APC) approach has emerged as a pivotal advancement in this context. It offers a high-throughput alternative, capable of generating precise electrophysiological characterizations of CFTR function, thus accelerating the pace of discovery and evaluation of potential therapeutic compounds. Modern APC systems are able to measure ion channel currents from 1 to 384 cells simultaneously. These systems are designed for ease of use and frequently offer automated data analysis, saving researchers a significant amount of time. Therefore, APC systems are increasingly utilized, not only for drug discovery in Pharma and Biotech companies, but also in academia.

Professor Frédéric Becq leads one of those academic groups that leverage APC technology to enhance our understanding of CFTR function and drug interactions. His work, as detailed in several publications below, underscores the efficacy of the Patchliner APC system in accelerating the discovery and characterization of CFTR modulators.

Interestingly, before purchasing an APC system, Frédéric Becq and colleagues performed a comparative study to assess the benefits of automated over manual patch clamp (MPC) for studying CFTR. This study provides a comprehensive validation of the Patchliner APC system against traditional MPC, demonstrating its effectiveness and efficiency in recording CFTR chloride currents[1].

Here is how Frédéric Becq concludes about their findings: „We have shown that whole-cell parallel planar APC is suitable for recording CFTR activity and gives robust results similar to those obtained by MPC but with higher throughput and standardized protocols. Screening with APC is an attractive option for pharmacological investigations including hit



Potentiator effect of the Small Binders of CFTR (SBCs) on F508del-CFTR. *Each panel shows the representative traces of test and CFTR(inh)-172 conditions and grouped data of I/V curves obtained in the whole cell patch-clamp configuration (automated patch-clamp) on F508del-CFTR HeLa cells with fsk (1 µM) alone (A) or in addition to SBC040 (30 µM) (B), SBC219 (10 µM) (C) or VX-770 (0.1 µM) (D). Mean current values are normalized to cell capacitance and expressed in pA/pF.*

validation, trafficking correction, potentiation, thermal stability restoration, and the functional characterization of mutant CFTR."

Now, it should be mentioned that recording CFTR function with APC approach has some peculiarities compared to many other ion channels. It is well known that fluoride is often used in the internal solution in APC experiments to improve the seal resistance and promote longer lasting recordings. While the use of fluoride in the internal solution is no problem for many experiments, there are situations where it is highly desirable to use fluoride-free internal solutions. CFTR recordings is one of such situations.

In fact, it has been shown that fluoride stimulates CFTR activity via activating adenylate cyclase, increased production of cAMP and phosphorylation by protein kinase A. This means that under standard conditions in APC experiments, CFTR will be preactivated by fluoride, which may not present a large problem if you study CFTR inhibitors, but is something to avoid when studying CFTR correctors, activators or potentiators.

To overcome this, one approach is to substitute internal fluoride with other internal ions e.g. phosphates or sulphates and this is the approach that Frédéric Becq's group have used to perform their CFTR studies with the Patchliner.

In one study, the researchers were focused on identifying and characterizing small molecules that could synergistically enhance the activity of wild-type CFTR as well as several CFTR mutants (F508del, G551D, G970R and G1349D) when used in conjunction with known potentiators. The Patchliner system was instrumental in testing the efficacy of two novel cAMP-independent potentiators SBC040 and SBC219. These compounds were found to potentiate CFTR activity at different binding sites, providing a combined effect with the standard potentiators, Forskolin and Ivacaftor. This synergy suggests a new avenue for combinatorial drug therapy in CF, underscoring the necessity for high-throughput screening capabilities provided by APC technologies[2].

In another study, researchers employed the Patchliner to assess the correction efficacy of Trikafta, a triple combination

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"The Patchliner system is also used for training purposes in our lab. Training students in both MPC and APC techniques offers a unique and valuable learning experience. This dual training approach not only broadens their technical knowledge but also enhances their ability to innovate and address complex research questions in the field of pharmacology and beyond."

Prof. Frédéric Becq, Professor of Physiology, University of Poitiers

therapy of two correctors (elexacaftor and tezacaftor) and one potentiator (ivacaftor). This study revealed that while Trikafta markedly improved the maturation, membrane localization, and function of F508del-CFTR in human airway epithelial cells, the presence of ivacaftor paradoxically reduced these corrective effects. Elexacaftor and tezacaftor synergistically improved CFTR function, but ivacaftor's presence destabilized the rescued protein and reduced overall efficacy. Alternative potentiators like genistein and Cact-A1 can enhance CFTR function without this negative effect, suggesting that Trikafta's efficacy might be underestimated and could be improved with different potentiators[3].

"After almost ten years of use we are very satisfied with the Patchliner which was instrumental to analyze the function and pharmacology of CFTR and F508del-CFTR and to characterize the effects of therapeutic modulators. In the future, we will also analyse the function of rare CFTR variants. Likewise, the Patchliner should help to analyze the properties of other chloride channels such as TMEM16A or LRRC8," says Prof. Becq.

"The Patchliner system is also used for training purposes in our lab," continues Frédéric Becq. Each year, master's students are trained in groups of 3-4 through workshops designed to introduce them to high-throughput analysis methods and concepts. These workshops include both theoretical and practical components, utilizing a simple protocol that allows for the recording of CFTR activation and inhibition. Through this training, students can appreciate the advantages of the Patchliner compared to conventional approache (MPC). They learn about its operation modes (such as whole cell mode), the importance of cell membrane quality, and the system's features like speed, temperature control, ease of use, and maintenance. Furthermore, they recognize the importance of having a theoretical background in electrophysiology to develop appropriate protocols and analyze ionic currents (I/V curves, conductance, current density, kinetics for activation and inhibition, current stability, temperature effects, pharmacology), while also learning to identify false positives.

Since these students also receive training in MPC, they can compare both techniques in terms of achieving high-resolution results and addressing the same research questions.

"Training students in both Manual Patch Clamp (MPC) and Automated Patch Clamp (APC) techniques offers a unique and valuable learning experience," concludes Frédéric Becq. "By mastering these complementary methods, students gain a deeper understanding of electrophysiological principles and practical skills, equipping them with the expertise needed to contribute effectively to academic drug discovery research teams. This dual training approach not only broadens their technical knowledge but also enhances their ability to innovate and address complex research questions in the field of pharmacology and beyond."

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