SOPHION BIOSCIENCE

Application Report

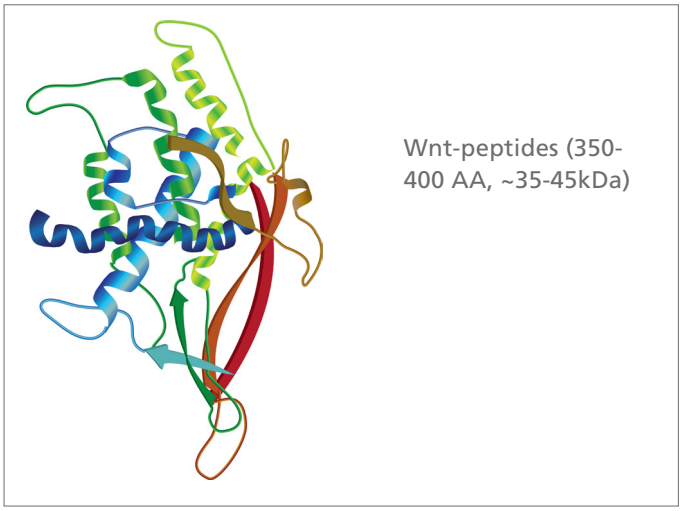
Large Molecules: Wnt signal activation

Optimization of a large molecule assay

Summary

- It was possible to obtain both manual and automated patch clamp (APC) recordings of the labile and scarce Wnt proteins (350-400 AA, 35-45 kDa) after thorough optimisation of com-pound handling.
- Here we highlight aspects of handling of Wnt proteins optimised for APC testing, which could also be applied to other large molecules (e.g. toxins, peptides, antibodies).

Introduction



Wnt-peptides (350-400 AA, ~35-45kDa)

Fig. 1: Wnt comprises a diverse family of secreted signaling glycoproteins that are 350–400 amino acids in length.

Wnt proteins control membrane potential

Wingless-related integration site (Wnt) ligands are conserved, cysteine-rich secreted proteins that act as close-range signalling molecules. Wnt signal activation initiates a complex downstream signal cascade in eukaryotic cells and is critical in development and many diseases, including cancer[1,2], Wnt-ligands activate K^+ currents by elevating intracellular Ca^{2+} and trigger Ca^{2+} release from intracellular stores. Wnt-ligands have significant implications for gene transcription, and open novel avenues to modulate this critical pathway[3, 7].

Large molecule handling

Wnt proteins (Fig.1) are in the class of large molecules (>1 kDa, also known as biologics), a molecule class that has gained attention due to its mode of action, often achieving greater target specificity and potency than small molecule drugs. This, however, comes at a cost. Often, these molecules are expensive and scarce and can have unwanted polyreactivity ("stickiness"). Additionally, they are more sensitive to their environment, as their three-dimensional structure is key to their function and is based on multiple weak bonding interactions.

Here, we show the importance of optimising not only the APC assay but also the compound handling and demonstrate how the QPatch (and Qube) accommodates the testing of labile and scarce large molecules like Wnt proteins.

Results and discussion

Wnt 9B on QPatch 48

The first evaluation in both manual patch clamp (MPC) and APC using Wnt 9B addition to the recording environment resulted in variable and small responses in PC3 cells (Fig. 2, left). This was, however, later shown to be due to the molecule instability: after intense optimisation of the compound handling workflow, it was easily possible to observe a prominent and significant Wnt9B dependent increase in K^+ conductance (Fig. 2, right and Fig. 3).

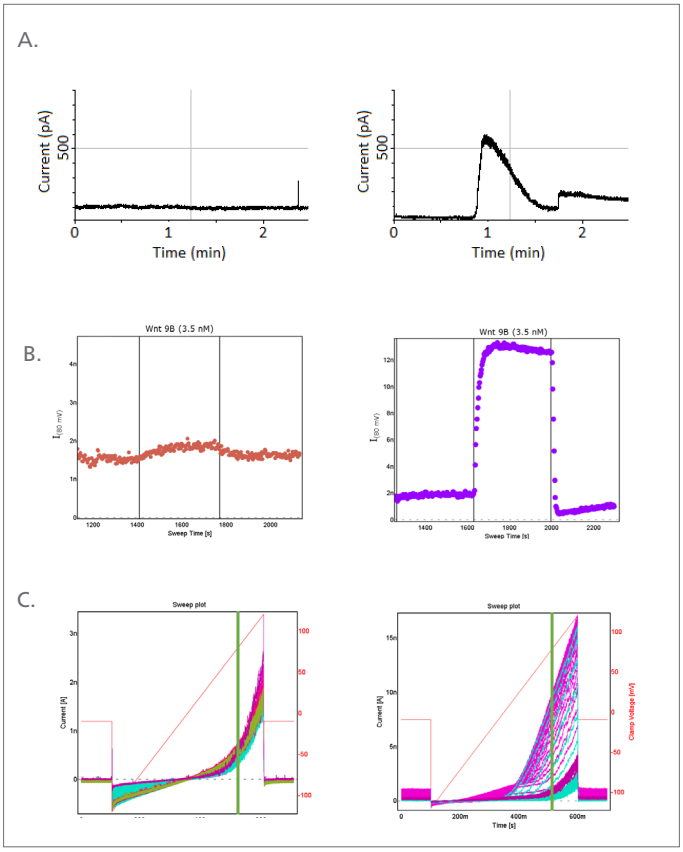


Fig. 2: Optimization of compound handling enables evaluation of the Wnt signaling pathway in both manual patch (top) and automated patch clamp (middle/bottom). Left (A-C): recordings before optimization of compound handling. Right (A-C): recordings after optimization of compound handling. (A) Manual patch clamp data: Wnt 9B induced current elicited by Wnt addition. B1+B2) QPatch data: Current measured at +80 mV plotted against time. (C) Raw current traces elicited by a voltage ramp with and without Wnt 9B.

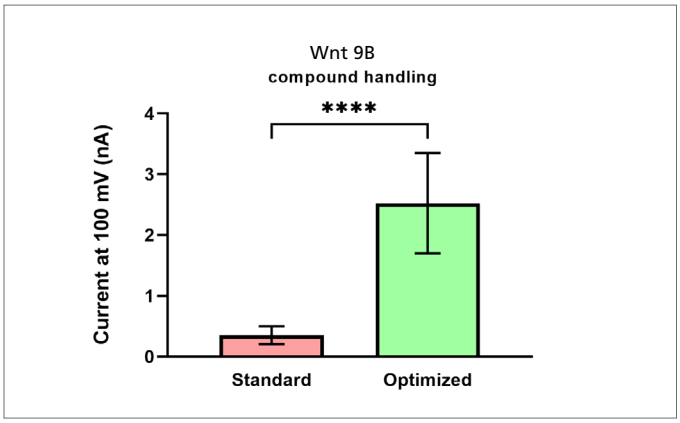


Fig. 3: The current measured at 100 mV upon stimulation with Wnt 9B increased several folds by optimizing the compound handling (From 0.3 nA +/- 0.15 nA to 2.5 nA +/- 0.8 nA, n = 21 and 18, respectively. p <0.0001).

Storage, handling and surface of consumables

Several factors need to be considered when handling large, complex molecules.

1. QPlate: Minimised compound adsorption

The microfluidic channels connecting the extracellular liquid inlet to the patch site are covered with a layer of pure, hydrophilic glass, which minimises compound adsorption. Additionally, the channel design allows solution exchange using volumes as low as 3 µL, thereby limiting compound usage[4].

2. Compound handling

The three-dimensional structure is key to their function for large molecules and relies on other, weaker interactions than covalent bonds. This renders these compounds extremely sensitive to their environment, and necessitating low storage temperatures (-20°C or -80°C), gentle handling conditions and reduced handling times and temperatures (e.g. on ice).

3. Solubility

Where Wnt is water-soluble, other large molecule compounds are of a highly lipophilic nature and can be difficult to keep in an aqueous solution. However, with an organic solvent such as DMSO and/or pluronic acid, it is possible to ensure the compound is maintained in solution when applied to the recording environment. Pluronic acid can form micelles, a process that has been shown in literature to be dependent on the pluronic acid variant, temperature, and concentration[5].

For more information on large molecule handling optimisation for APC recordings, please contact your application specialist or email: info@sophion.com.

Wnt 5A and 10B signal activation

Not only Wnt 9B, but also the two Wnt proteins, 5A and 10B, were shown to activate currents in PC3 cells (see Fig. 4A and 4B, respectively).

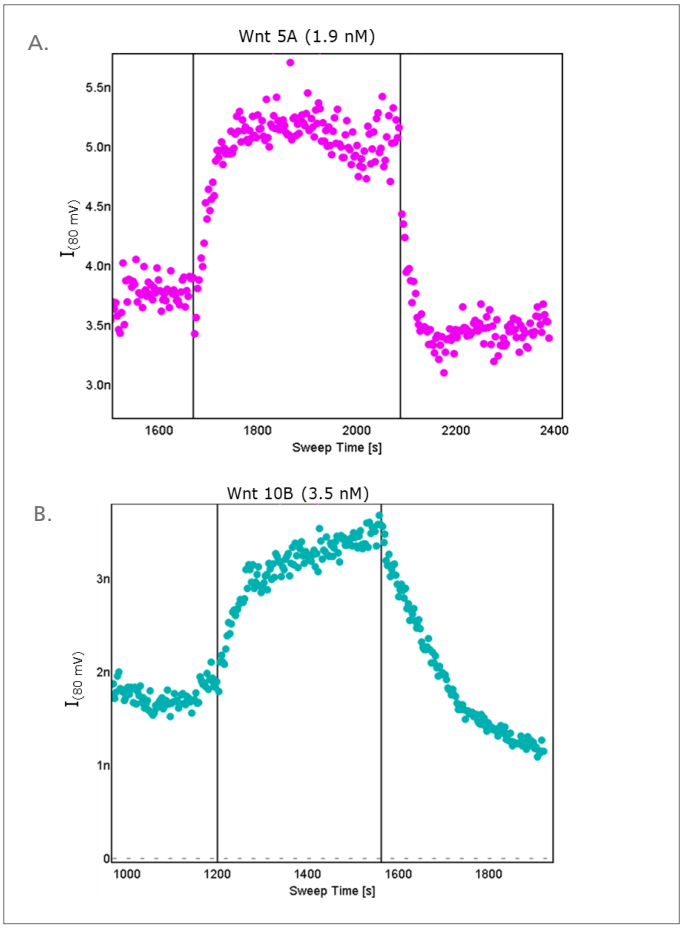


Fig. 4: Wnt 5A and 10B on QPatch 48. PC3 current amplitude over time in the absence or presence of A) 1.9 nM Wnt 5A and B) 3.5 nM Wnt 10B. As for Figure 2, a ramp protocol was applied with peak currents measured at +80 mV.

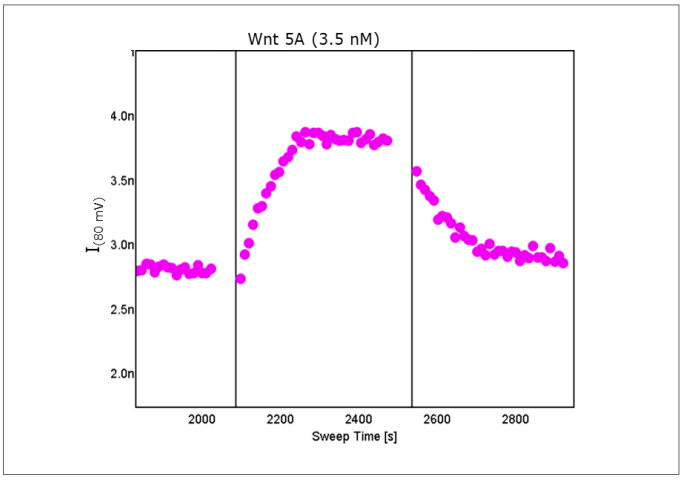


Fig. 5: Qube 384 recordings of PC3 cells activated by Wnt proteins after optimisation of compound handling. PC3 current amplitude over time in the absence or presence of 3.5 nM Wnt 5A. As for Figure 2, a ramp protocol was applied. Currents measured at +80mV.

Wnt on Qube 384

As for the QPlate, the microfluidic channels of the QChip have been optimised to minimise compound adsorption. Consequently, it was also possible to monitor Wnt 5A signal activation on the Qube 384. See Fig. 5.

Methods

Cells

PC3 is a prostate cancer cell line obtained from the American Type Culture Collection (ATCC) and cultured in RPMI 1640 (Invitrogen, Paisley, UK) medium containing 5 mM L-glutamine and fetal bovine serum as described previously[6].

Manual patch clamp

From Ashmore *et al.*, 2019[7]. Cells were grown on sterile 13 mm-diameter glass coverslips (Thermo Fisher Scientific, Paisley, UK), and recordings were made using an Axon 200B amplifier (Molecular Devices, Sunnyvale, CA, USA). Cells were held at 0 mV, and no series resistance or capacitance compensation was used. The coverslips were placed in a 1.5 ml bath continuously perfused with extracellular solution at 37°C. For more info, see Ashmore *et al.*, 2019[7].

Automated patch clamp

QPatch: On the day of the experiment, cells were trypsinized according to Sophion standard procedures, and after harvest, the cell suspensions were prepared by the automated cell preparation. The cells were measured in parallel (with individual amplifiers), with a gigaseal achieved in >90% of the cells. The mean capacitance of a typical sample of 48 stably recorded (>20 min) cells was 29.1 pF, corresponding to a spherical cell 30 µm in diameter. For more info, see Ashmore *et al.*, 2019[7].

Qube 384: After minor adjustments, mainly in the whole-cell protocol, the QPatch assay was transferred to the Qube.

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Large molecules and APC in the literature

For more information about use of QPatch and Qube 384 for peptide/toxin characterisation also see below incomplete list of references, contact our application scientists or visit our publication database at https://sophion.com/knowledge-center/publications/.

1. Agwa AJ, Blomster L V, Craik DJ, King GF, Schroeder CI. Efficient Enzymatic Ligation of Inhibitor Cystine Knot Spider Venom Peptides: Using Sortase A To Form Double-Knottins That Probe Voltage-Gated Sodium Channel Na(V)1.7. Bioconjug Chem. 2018 Oct;29(10):3309–19.
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