Macromolecular complex of voltage gated-sodium channel $Na_v1.5$: α-α interactions in living cells

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1. Abstract

The $Na_v1.5$ sodium channel is encoded in chromosome 3 by the *SCN5A* gene. Many heart conditions are associated with $Na_v1.5$ malfunction. $Na_v1.5$ interacts with other proteins, such as 14-3-3. These proteins are part of the macromolecular complex with $Na_v1.5$ and thus might affect its function, gating or localization. Previous studies have shown that α subunits of $Na_v1.5$ can dimerize with each other. Interestingly, some mutants of $Na_v1.5$ that form dimers with wild-type $Na_v1.5$ exhibited dominant-negative effect: mutated α subunit affected activity of the wild-type channel. Since $Na_v1.5$ is an essential part of the action potential, mutants exhibiting dominant-negative effect would severely affect cardiac conduction system in patients with heterozygous *SCN5A* variants, Thus, understanding of the mechanisms behind $Na_v1.5$ dimerization could lead to the discovery of molecular targets that could prevent α-α subunit interactions. The aim of this project is to understand the mechanism behind $Na_v1.5$ α-α subunit interactions.

2. Introduction

$Na_v1.5$ actively affects the conduction velocity through dictation of the slope and the amplitude of phase 0 in the cardiac action potential [1]. From previous studies, it is hypothesized that 14-3-3 plays an essential mediator role in interaction with $Na_v1.5$ and its α subunits. Although it has highly been suggested on the importance of orientation of two interacting α subunits towards each other [2], authors have not shown sufficient biochemical evidence to support their conclusion regarding the role of 14-3-3.

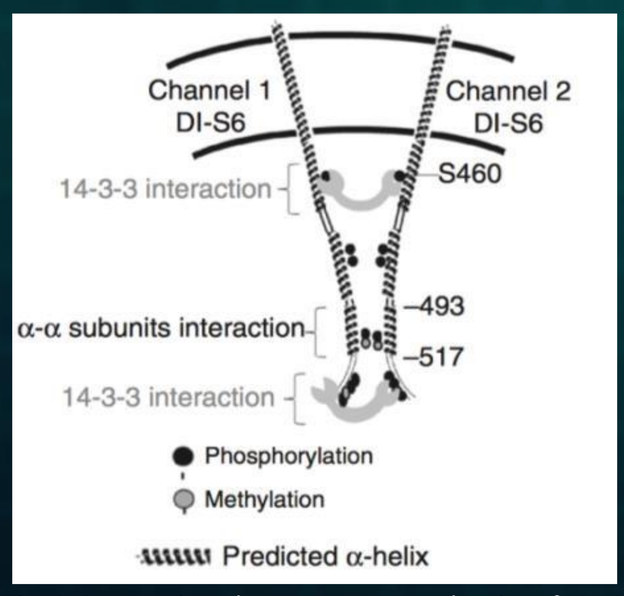

Fig. 1: Interactions between α-α – subunits of $Na_v1.5$ sodium channels to 14-3-3 mediator protein. (Clatot et al. 2017)

3. Aims and Leading Questions

The aim of this thesis is to investigate the macromolecular complex of $Na_v1.5$ sodium channels with the focus on their dimerization and involvement of the 14-3-3 protein.

1) Can $Na_v1.5$ dimers be formed in living cells?
2) Which scenario of protein – protein interaction is the most likely for dimer formation (*e.g.* N-N, N-C or C-C termini)?
3) Do 14-3-3 proteins play role of essential mediators in dimerization of $Na_v1.5$?

4. Material, Methods and procedures

NanoLuc® Binary Technology was used for this project. TsA201 and HEK293 cells are transfected with corresponding plasmids to produce $Na_v1.5$ fused with two NanoLuc® luciferase subunits [3]. When two $Na_v1.5$ proteins interact/dimerize, the tagged subunits on the NH_2 and the COOH region (illustrated in Fig.2), of the active luciferase enzyme generate a luminescent signals in cells that is proportional to dimerization activity.

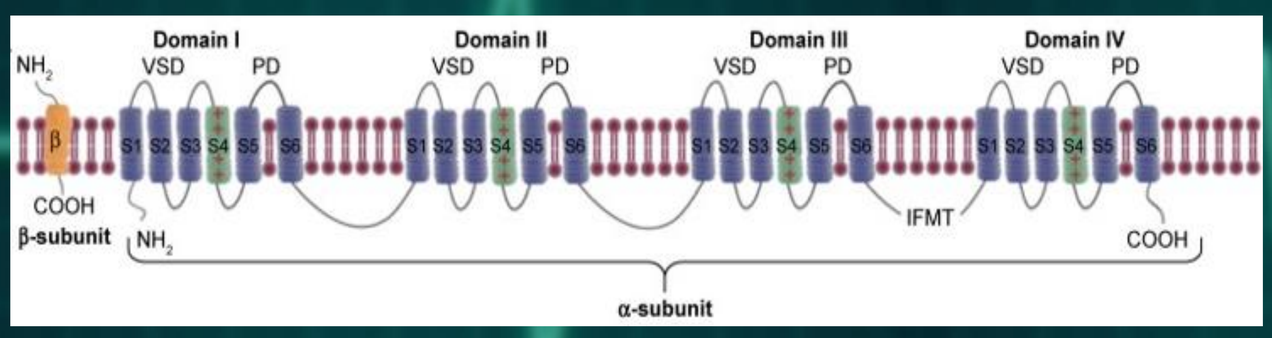

Fig. 2. An illustration of a complete voltage gated sodium channel model including the domains DI to DIV. (Ahmed et al. 2017)

5. Results

1) The results of the NanoBiT assay showed that there is a live protein – protein interaction between α subunits in living cells due to protein – protein interactions.
2) The likelihood of dimerization of $Na_v1.5$ is depending on the different termini interaction of the α subunits. We concluded that NH_2 – COOH (B33B+B33C) isn't as likely to form dimers as COOH – COOH (B33A+B33C) orientation of Nav1.5 α subunits.
3) Difopein, did not affect dimerization of $Na_v1.5$ in live cells since functional and dysfunctional mut. difopein showed similar luminescence signals.

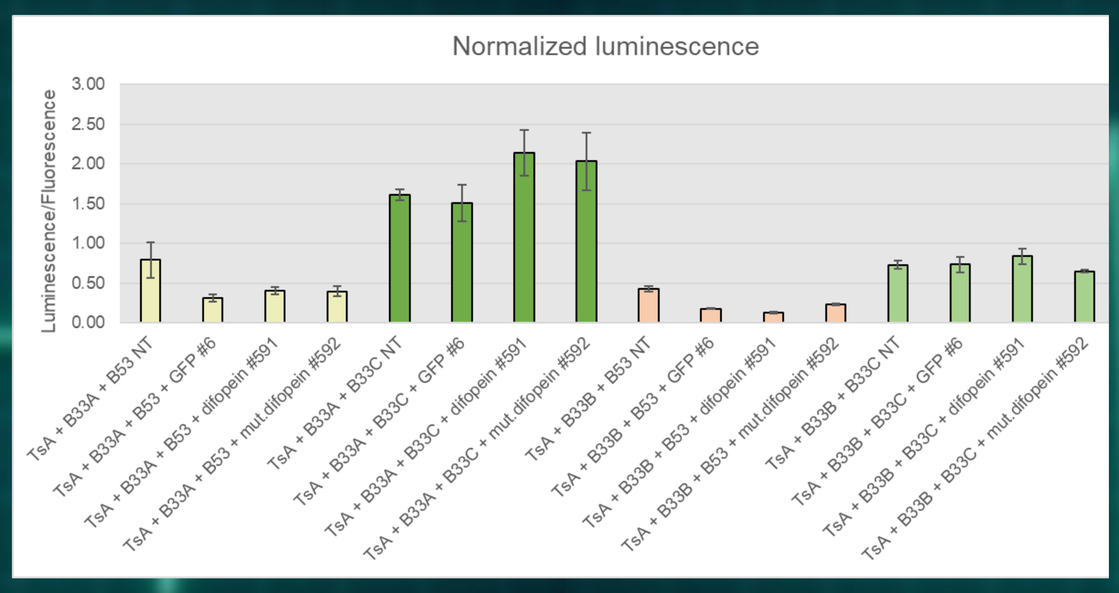

Fig. 3. NanoBit assay luminescence results of protein – protein interactions of combinations (A.Seljmani 2021)

6. Discussion

The luminescence intensity comparing the different possibilities is linked to the relevance of dimerization combinations. We showed that both intracellular termini interact in different scenarios which is a previously unknown state of $Na_v1.5$ dimerization. C-C termini showed the highest likelihood of interaction. In our case (Fig.3) the usage of 14-3-3 inhibitor showed that difopein did not affect dimerization of Nav1.5 in live cells as expected. In this project, the TsA201 cells which express endogenous levels of 14-3-3 proteins are not responsible for the physical dimerization of Nav1.5. NH_2 - NH_2 interaction yet remains to be tested.

References

[1] Park, Fishman et al.,2011 "The cardiac conduction system"
[2] Clatot et al.,2017 "Voltage-gated sodium channels assemble and gate as dimers"
[3] promega.com - NanoBiT® PPI Starter Systems - promega.com/products/protein-interactions/live-cell-protein-interactions/nanobit-ppi-starter-systems

Figures

Fig.1 Clatot et al.,2017 Interactions between α-α – subunits to 14-3-3 mediator protein. "Voltage-gated sodium channels assemble and gate as dimers"
Fig.2 Ahmed et al., 2017 An illustration of a complete voltage gated sodium channel model including the domains DI to DIV. ""Drug Design, Development and Therapy Dove press Modeling the Human Na v 1.5 Sodium Channel: Structural and Mechanistic Insights of Ion Permeation and Drug Blockade."
Fig.3 A.Seljmani (2021) NanoBit assay luminescence results of protein – protein interactions of combinations
Background picture : https://www.pinterest.com/pin/813462751428268183/