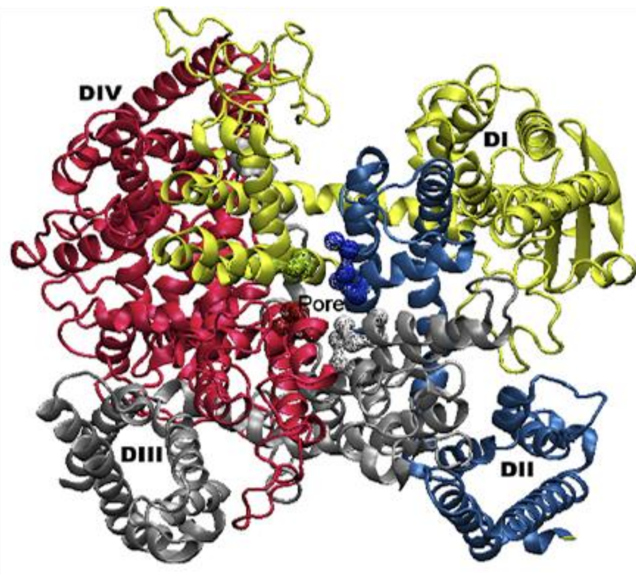
Molecular mechanism of Na$_v$1.5 oligomerization



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1. Abstract

Na$_v$1.5 is a voltage-gated sodium channel and is crucial for generating an action potential (electrical impulse) in the cardiomyocytes. It consists of α-subunit and an auxiliary β-subunit. Studies have shown that the α-subunits of Na$_v$1.5 interact with each other, by forming dimer. However, it is still unclear how they interact, and which molecular mechanism is involved in this oligomerization. The aim of the study is to investigate the role of the β-subunits in the process of Na$_v$1.5 oligomerization. Therefore, TsA201 cells are transient transfected with Na$_v$1.5 and co-expressed with β-subunit. Then Co-immunoprecipitation (Co-IP) and NanoBiT assay is performed to see if there is a difference in Na$_v$1.5 interaction. The Co-IP results and the NanoBiT results cannot be interpreted, due to inconclusive results.

2. Introduction

Na$_v$1.5

Na$_v$1.5 is encoded by SCN5A gene and is responsible for a rapid upstroke of cardiac action potential during the phase 0, by generating an Inward sodium current (I_{Na}). The main function is taken by the α-subunit of Na$_v$1.5. The auxiliary subunit β interacts with α-subunit and finely regulates its function. Furthermore, the β-subunit is further divided into β1, β2, β3, β4 and β1b, which is encoded by SCN1b-4b gene. Mutations in Na$_v$1.5 are associated with various types of cardiovascular diseases and can cause life-threatening arrythmias. [1]

Forming dimers within Na$_v$1.5

Studies have reported that co-expressing of one Na$_v$1.5 wild type and one non functional Na$_v$1.5 mutant, produces less than 50% of the I_{Na}. This effect has been named dominant negative effect (DNE), leading to a stronger decrease of the sodium current. Since it is known that Na$_v$1.5 are present as monomer and not oligomerize, the discovery of DNE was unexpected. So far it has been shown that the α-subunit of Na$_v$1.5 form dimer, but the exact mechanism of the oligomerization is not fully understood. [2] Interestingly, a study had suggested that the auxiliary β1-subunit of Na$_v$1.5, might play a role in this DNE by modulating the Na$_v$1.5 oligomerization mechanism. [3]

3. Aims and leading questions

The aim of this project is to investigate the role of the auxiliary β-subunits in the process of Na$_v$1.5 oligomerization.

Leading question:

- Does any of the β-subunits of Na$_v$1.5 play a role in the Na$_v$1.5 oligomerization?

4. Methods and materials

TsA201 cells were transient transfected with FLAG-tagged Na$_v$1.5, HA-tagged Na$_v$1.5 and were then co-expressed with the different β-subunits. For the negative control, HA-tagged Na$_v$1.5 and untagged Na$_v$1.5 were co-transfected with Myc-tagged β1b-subunit. Then they were incubated for 48h at 37°C. Afterwards Bradford assay is performed to determine the protein concentration. Then Co-IP is performed, to study protein-protein-interactions. Therefore, Anti-FLAG® M2 Magnetic Beads were added, which targets the FLAG-tagged Na$_v$1.5 to precipitate the protein complex. The samples were loaded to SDS-PAGE to separate the protein followed by western blotting. Different primary antibodies are used to target FLAG/HA-tagged Na$_v$1.5 and Myc-tagged β1b-subunit. For the detection, green and red fluorescent secondary antibodies were used to bind the primary antibodies and were scanned.

To compare the Co-IP results, another method called NanoBiT assay, is performed as well. NanoBiT assay detects intracellularly protein:protein interaction in real-time. This assay is based on NanoLuc® luciferase system, which is composed of two subunits, LargeBiT and SmallBiT. Each subunit is fused to the Na$_v$1.5, these constructs are transient transfected to TsA201 cells, then co-expressed with different β-subunits. During the protein expression, the LargeBiT and SmallBiT are brought to close proximity to build functional bright luminescent enzyme, which can be measured. All experiments were performed of a minimum three times (n=3)

5. Results

Figure 1 In Co-IP, FLAG and HA tagged Na$_v$1.5 are transfected, except the negative control (NC). This includes HA tagged Na$_v$1.5, Na$_v$1.5 without tag and Myc tagged β1b-subunit. The band intensity is normalized with unspecific band of red ponceau. The empty plasmid, is considered as reference and was compared with all conditions. For the comparison, Kruskal-Wallis test was performed are not significant.

Figure 2 For the normalization, the luminescence signals of the conditions are divided by fluorescence signal (TsA201 cells). All conditions contain Na$_v$1.5. The transfected HaloTag-SmBiT represent the background signal and the one with empty plasmid is considered as reference. Kruskal-Wallis test was performed, is not significant. Additionally, each condition was compared with the corresponding background signals with ordinary one-way ANOVA test and is not significant.

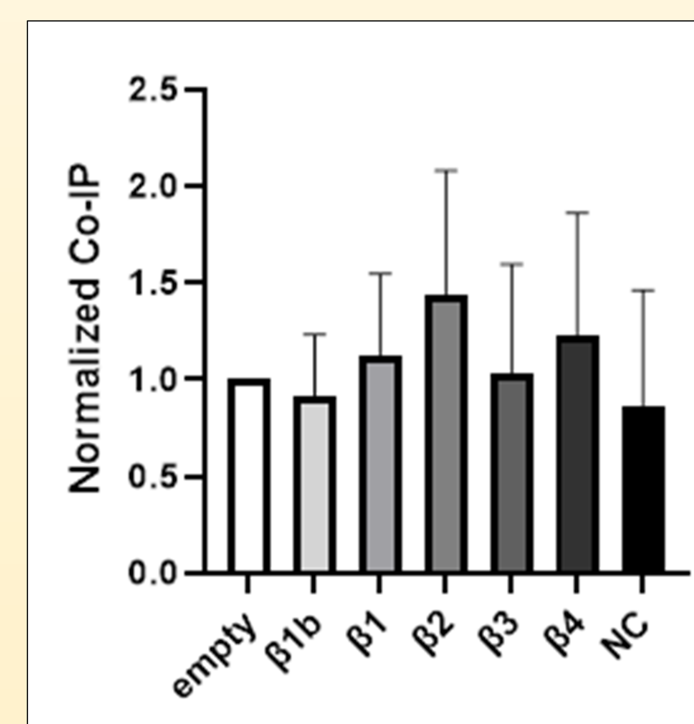

Figure 1 graphical summary of all quantified western blots of Co-IP (Sundaralingam, 2023)

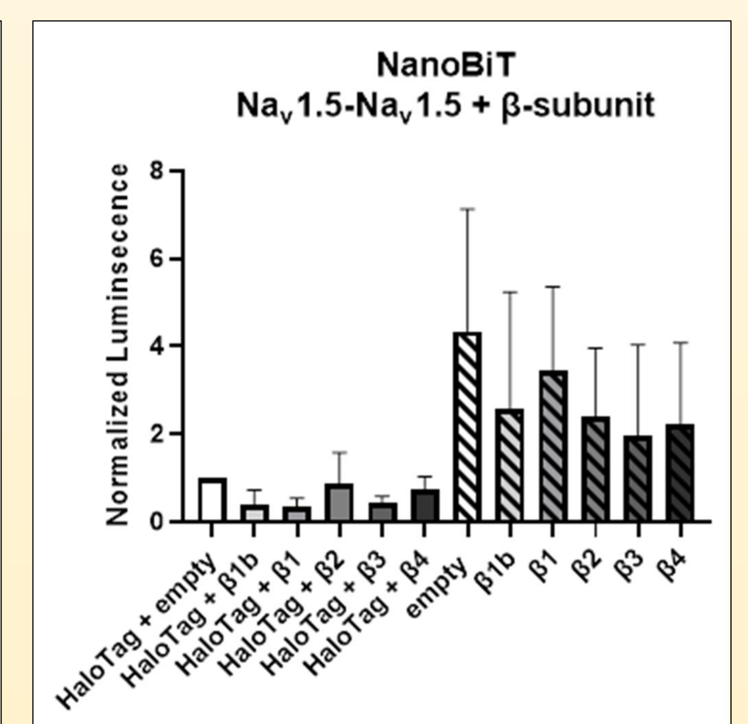

Figure 2 NanoBiT results of Na$_v$1.5-Na$_v$1.5 co-expressed with β-subunit. (Sundaralingam, 2023)

6. Discussion

In Figure 1 no increased Na$_v$1.5 interaction can be observed in Co-IP, when co expressed with β-subunits. The dimers weren't always present in the western blot. Also, the negative control is positive which makes the result inconclusive. It suggests an eventual unspecific binding of HA tagged Na$_v$1.5 with the Anti-FLAG® M2 Magnetic Beads. Therefore, the Co-IP was repeated using anti-HA beads instead of anti-FLAG beads. Similar conclusion can be draw from NanoBiT assays from figure 2. They also show no difference in the expression of Na$_v$1.5-Na$_v$1.5 interaction. However, the graphic should be also interpreted with caution because the standard deviation is wide.

References

[1] Iqbal, S. M., & Lemmens-Gruber, R. (2019). Phosphorylation of cardiac voltage-gated sodium channel: Potential players with multiple dimensions. Acta Physiologica (Oxford, England), 225(3), e13210. https://doi.org/10.1111/apha.13210

[2] Clatot, J., Coulombe, A., Deschênes, I., Guicheney, P., & Neyroud, N. (2022). Trafficking and Gating Cooperation Between Deficient Na$_v$1.5 –mutant Channels to Rescue I_{Na}. Frontiers in Bioscience (Landmark Edition), 27(7), 209. https://doi.org/10.31083/j.fbl2707209

[3] Mercier, A., Clement, R., Harnois, T., Bourmeyester, N., Faivre, J.-F., Findlay, I., Chahine, M., Bois, P., & Chatelier, A. (2012). The β1-subunit of Na$_v$1.5 cardiac sodium channel is required for a dominant negative effect through α-α interaction. PloS one, 7(11), e48690.

Figures

Figure 1 Sundaralingam, S. (2023). graphical summary of all quantified western blots of Co-IP from Na$_v$1.5 - Na$_v$1.5 + β-subunits. Bern

Figure 2 Sundaralingam, S. (2023). NanoBiT results of Na$_v$1.5 - Na$_v$1.5 co-expressed with β-subunit. Bern