Development of peptide modulators targeting protein-protein interactions of the Sodium Leak Channel Complex

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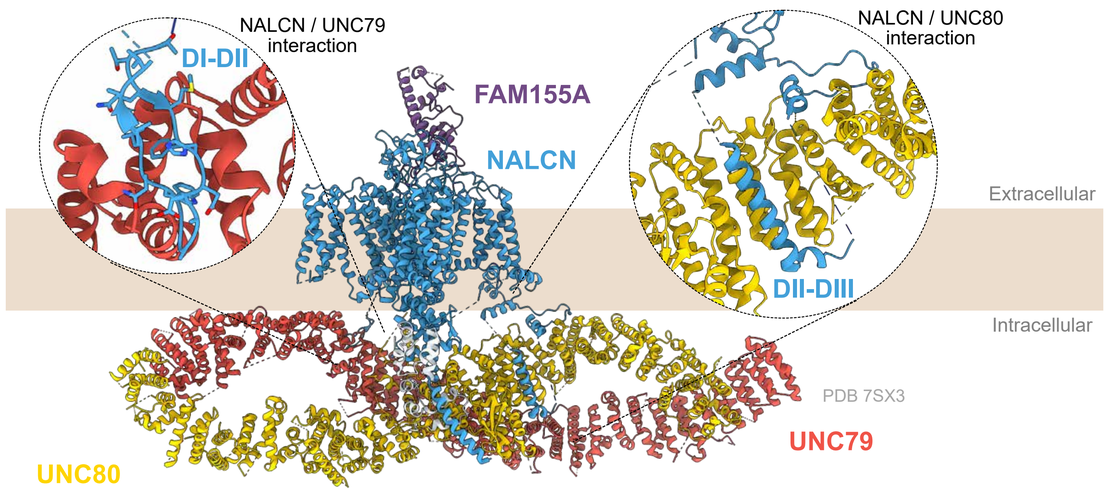
1 The Sodium Leak Channel (NALCN) Complex

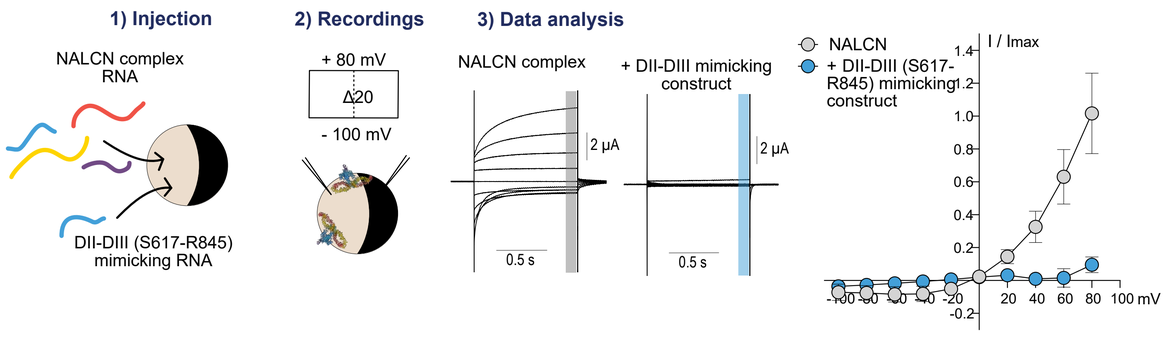
The Sodium leak channel, non-selective (NALCN) conducts a current of monovalent cations into neurons thereby adjusting the membrane potential and excitability. (PMID: 17448995)

NALCN consists of 4 domains connected by intracellular linkers. The DI-DII linker forms a loop which binds to a hydrophobic surface of uncoordinated protein-79 (UNC79). The DII-DIII linker forms alpha helical structures which interact with uncoordinated protein-80 (UNC80). (PMID: 32494638).

Mutations in NALCN, UNC79 and UNC80 are rare in humans but cause severe neurodevelopmental symptoms. (PMID: 30167850) Here, we aim to use the linker structures as a starting point to develop complex inhibiting peptides.



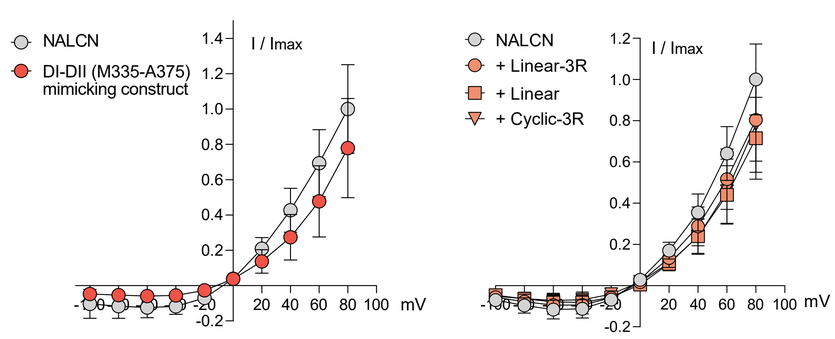
2 Two-electrode voltage clamp



We express the NALCN complex in *Xenopus leavis* oocytes and use two-electrode voltage clamp (TEVC) to measure NALCN activity. From these experiments, we found that a construct mimicking the DII-DIII linker fully inhibits NALCN currents.

3 DI-DII linker mimicking constructs

We also evaluated the inhibitory effect of DI-DII mimicking constructs and peptides in NALCN-expressing oocytes. However, none of these inhibited NALCN activity.

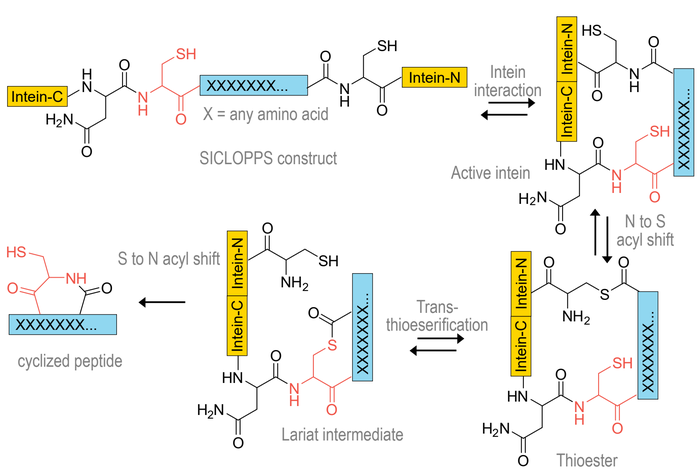


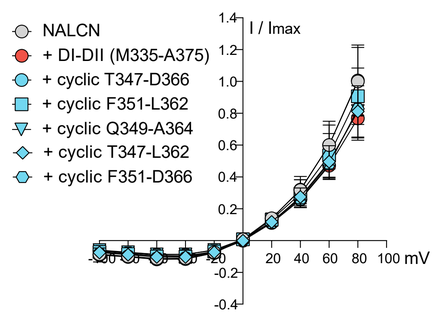
4 Membrane anchored DI-DII linker constructs

We added membrane anchors to the DI-DII loop to localize it at the membrane. One construct, where the DI-DII linker was coupled to a Nav β4 subunit, showed improved inhibition in the TEVC experiments. We are currently working to validate the effect of membrane anchoring.

5 SICLOPPS (cyclic) DI-DII linker constructs

Split-intein circular ligation of peptides and proteins (SICLOPPS) is a method used to create circular proteins and peptides through the use of split inteins. (PMID: 28258013)

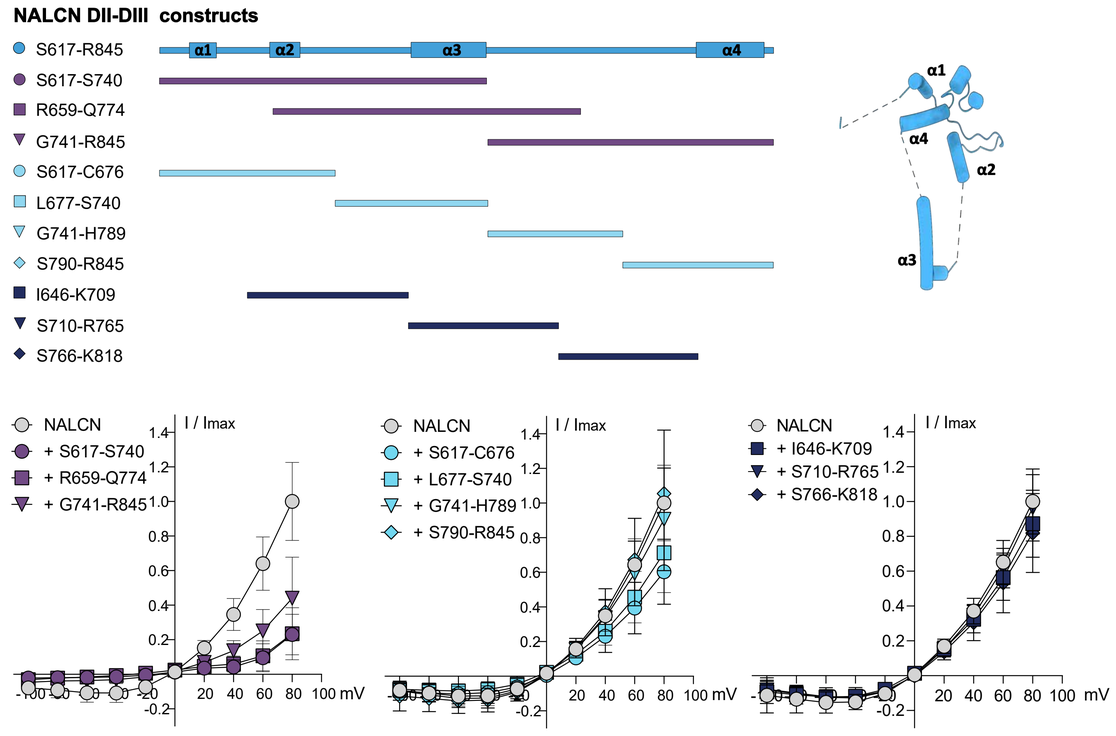




We designed and evaluated SICLOPPS constructs encoding cyclic peptides which mimic the DI-DII linker loop. However, none of the cyclic peptide inhibited NALCN activity.

6 DII-DIII linker mimicking constructs

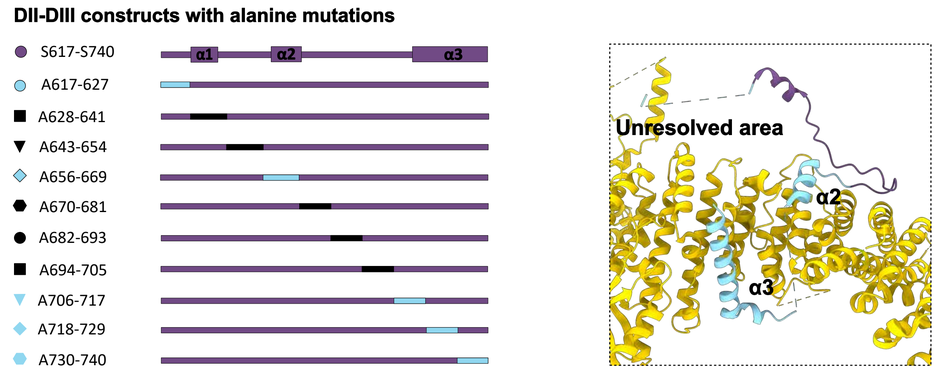
To identify key interaction sites of the DII-DIII mimicking construct, we shortened it by dividing it into halves and quarters.

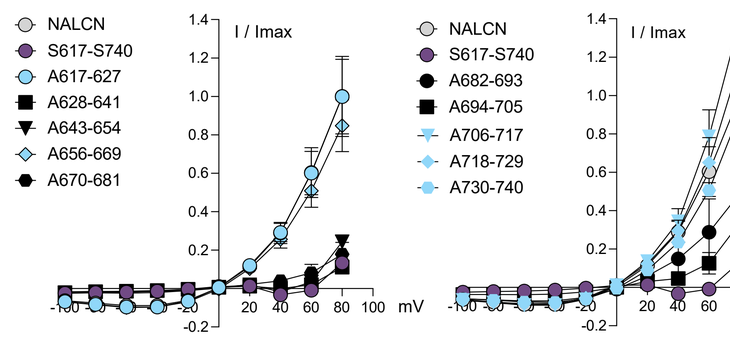


We found that the N-terminal and middle half (120 AA) of the DII-DIII linker also inhibited NALCN currents, while the C-terminal half was not as efficient. Shortening the construct further (60 AA) abolished the effect.

7 Alanine scan of the DII-DIII linker

To pinpoint important interaction sites in the N-terminal half of the DII-DIII linker (S617-S740), we introduced alanine mutations in stretches of 10-15 residues.





The alanine scan underlined the importance of an unresolved area in the N-terminus as well as the two alpha helices α2 and α3.

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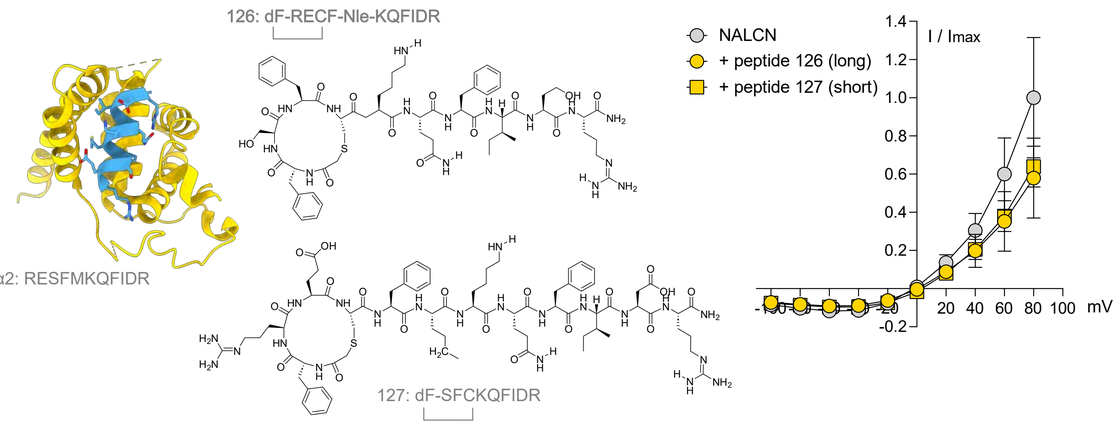
8 Stapled alpha helical peptides

We synthesized peptides with stapled alpha-helical folds to mimic the α2-helix of the DII-DIII linker. Two N-capped peptides inhibited outward currents at positive potentials. We are currently expanding our library of alpha-helical peptides which mimic structures of the α2- and α3-helices.



9 Future perspectives

The DI-DII / UNC79 interaction

Confirm the effect of membrane anchoring the DI-DII linker

Expand the library of membrane anchored DI-DII linker constructs

Other interaction partners

Identify and evaluate binding epitopes of other NALCN binding partners

The DII-DIII / UNC80 interaction

Narrow down binding epitopes by shortening alanine stretches

Expand library of stapled α2 and α3 mimicking peptides

Evaluate peptide stability in oocytes

Optimize conditions for injecting peptides