A functional $Na_V1.7$- Na_VAb chimera with a reconstituted high affinity ProTx-II binding site

Ramkumar Rajamani, Sophie Wu, Iyoncy Rodrigo, Mian Gao, Simon Low, Lisa Megson, David Wensel, Rick L. Pieschl, Debra J. Post-Munson, John Watson, David R. Langley, Michael K. Ahlijanian, Linda J. Bristow, and James Herrington

Address for correspondence:

Ramkumar Rajamani, Ph.D.

Bristol-Myers Squibb

5 Research Parkway

Wallingford, CT 06492

Ramkumar.Rajamani@bms.com

Supplemental Figure 1. Chimeric NavAb-DII-S1-S4 and NavAb-DII-S3-S4 amino acid sequence of channels expressed in Sf9 cells.

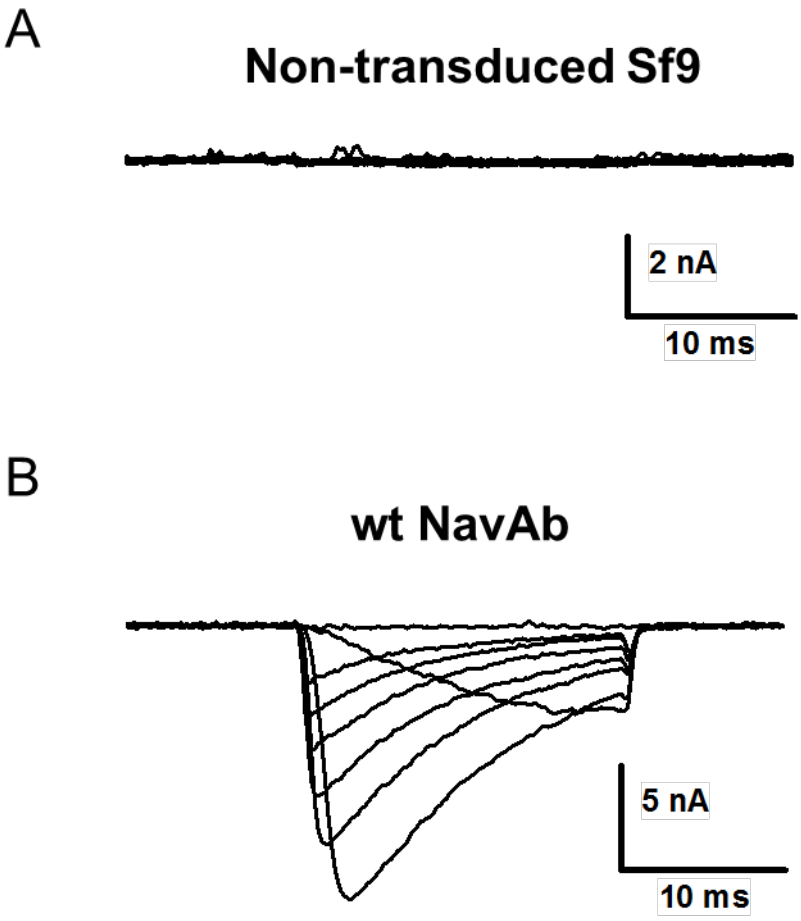
NavAb-DII-S1-S4

MDYKDDDDKGSLVPRGSHMYLRITNIVESSFFTKFITI
CIVLNTLFMAMEHHPMTEEFKNVLAIGNLVFTGIFAAE
IILRIYVHRISFFKDPWSLFDFFVVTLSLVELFLADVE
GLSVLRSFRLLRLFRLVTAVPQMRKIVSALISVIPGML
SVIALMTLFFYIFAIMATQLFGERFPEWFGTLGESFYT
LFQVMTLESWSMGIVRPLMEVYPYAWVFFIPFIFVVTF
VMINLVVAIIVDAMAILNQKEEQHIIDEVQSHEDNINN
EIIKLREEIVELKELIKTSLKN

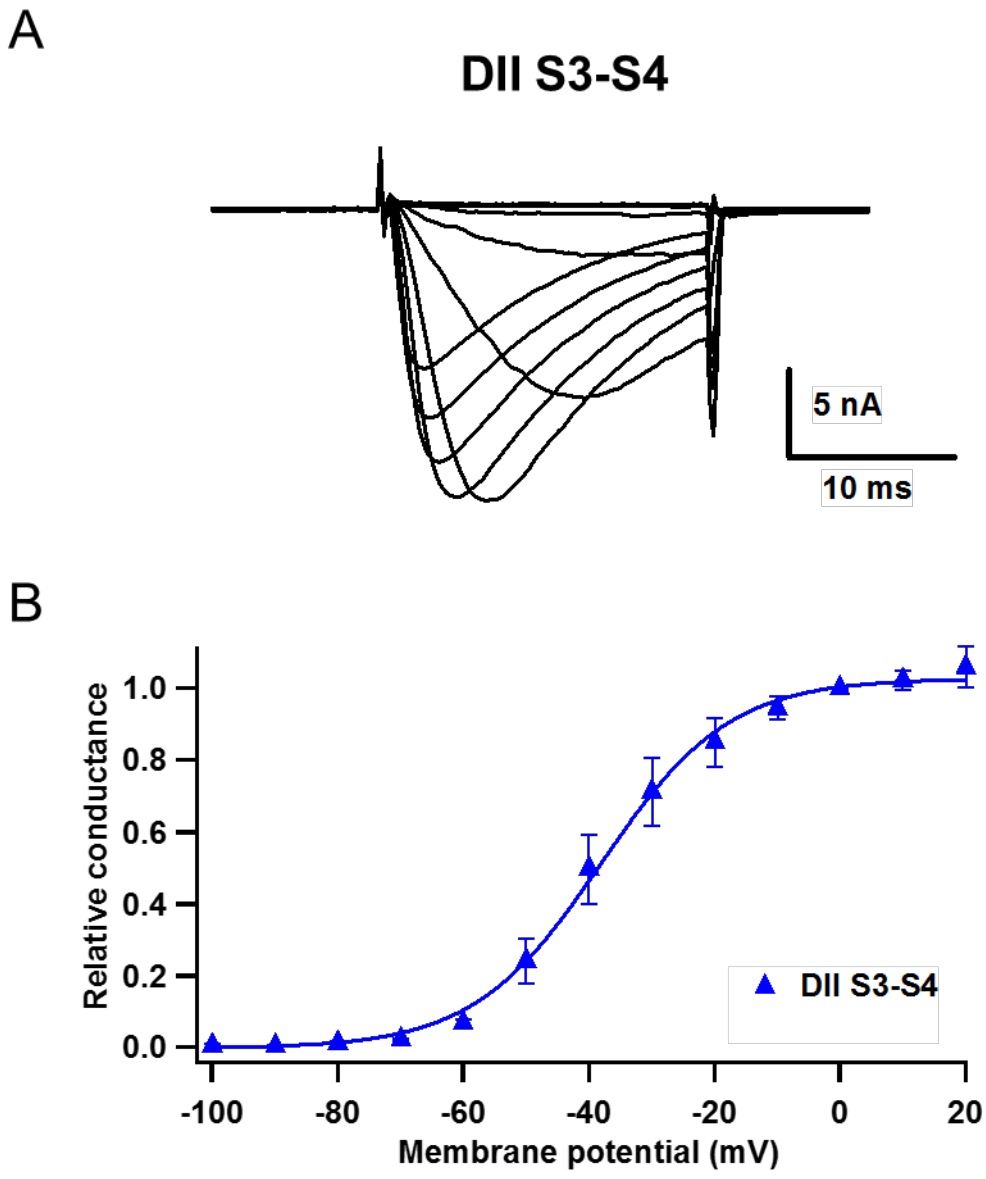
NavAb-DII-S3-S4

MDYKDDDDKGSLVPRGSHMYLRITNIVESSFFTKFIIY
LIVLNGITMGLETSKTFMQSFGVYTTLFNQIVITIFTI
EIILRIYVHRISFFKDPWSLFDFFVVTLSLVELFLADV
EGLSVLRSFRLLRLFRLVTAVPQMRKIVSALISVIPGM
LSVIALMTLFFYIFAIMATQLFGERFPEWFGTLGESFY
TLFQVMTLESWSMGIVRPLMEVYPYAWVFFIPFIFVVT
FVMINLVVAIIVDAMAILNQKEEQHIIDEVQSHEDNIN
NEIIKLREEIVELKELIKTSLKN

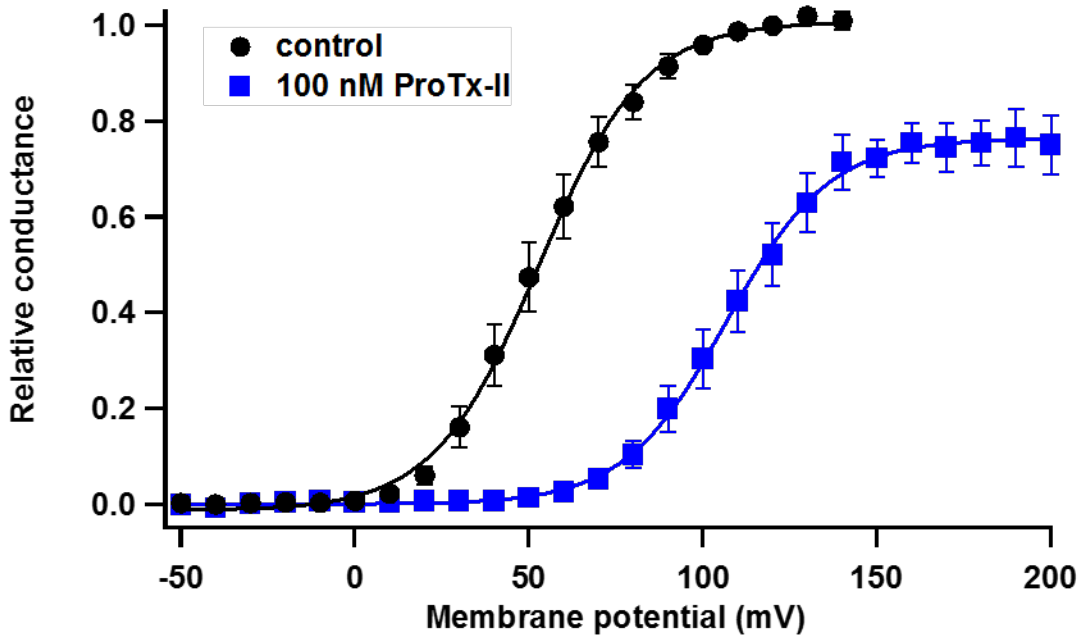
Supplemental Figure 2. Functional expression of NavAb channels in Sf9 cells. A. Representative recording from a non-transduced Sf9 cell. Currents in response to 20 ms step depolarizations between -120 mV and +120 mV in 10 mV increments are shown. B. Representative recording from a Sf9 cell transduced with BacMam encoding wild-type NavAb. Currents were evoked by 20 ms step depolarizations between -140 mV and 0 mV. For clarity, step depolarizations in 20 mV increments are shown.



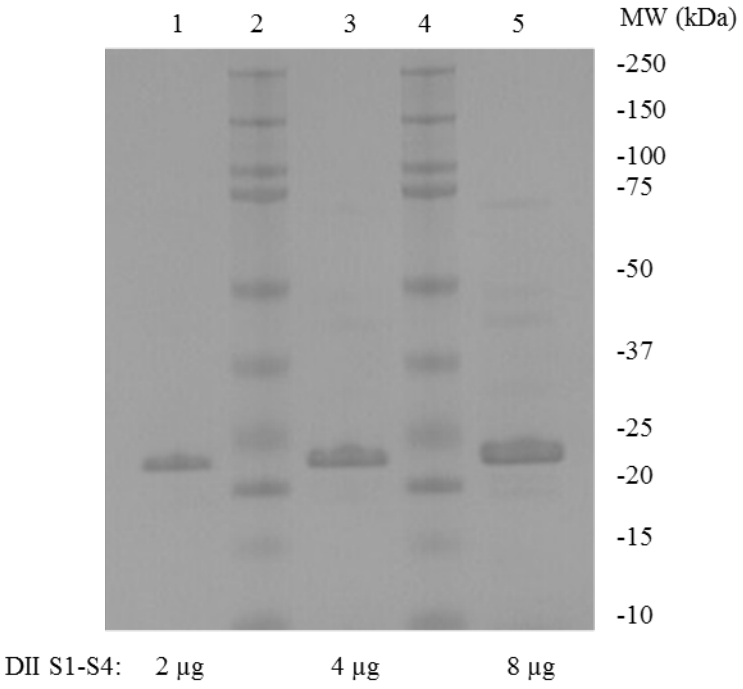
Supplemental Figure 3. Functional expression of DII S3-S4 chimera in Sf9 cells. A. Representative recording from a Sf9 cell transduced with BacMam encoding DII S3-S4 chimera. Currents in response to 20 ms step depolarizations between -140 mV and 0 mV in 10 mV increments are shown. B. Plot of mean relative conductance (± S.E.M.) versus step potential for DII S3-S4 chimera channels estimated from tail current analysis. Tail current amplitudes were normalized to the amplitude following the step to 0 mV for each cell (n=7). The solid line is a fit of the Boltzmann equation to the data. Parameters of the fit are given in Table 1.



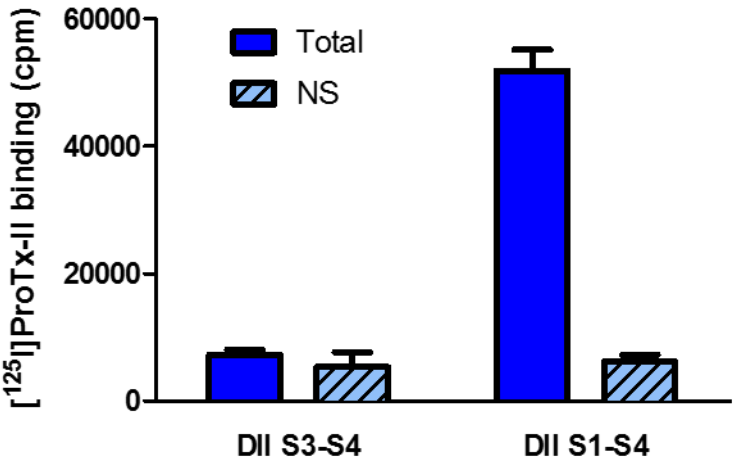
Supplemental Figure 4. Effect of ProTx-II on the voltage-dependent gating of DII S1-S4 chimera channels. Plot of mean relative conductance (± S.E.M.) versus step potential for DII S3-S4 chimera channels in control (circles) and following application of 100 nM ProTx-II (squares). Conductance was estimated from peak current measurements and normalized to the conductance following the control step to +120 mV for each cell (n=7). The solid line is a fit of the Boltzmann equation to the data. Parameters of the fit in Control are: $V_{1/2}$ = 52.7 mV, slope = 15.1 mV, Maximum conductance = 1.0. For the fit in 100 nM ProTx-II, the parameters are: $V_{1/2}$ = 106.8 mV, slope = 14.8 mV, maximum conductance = 0.76.



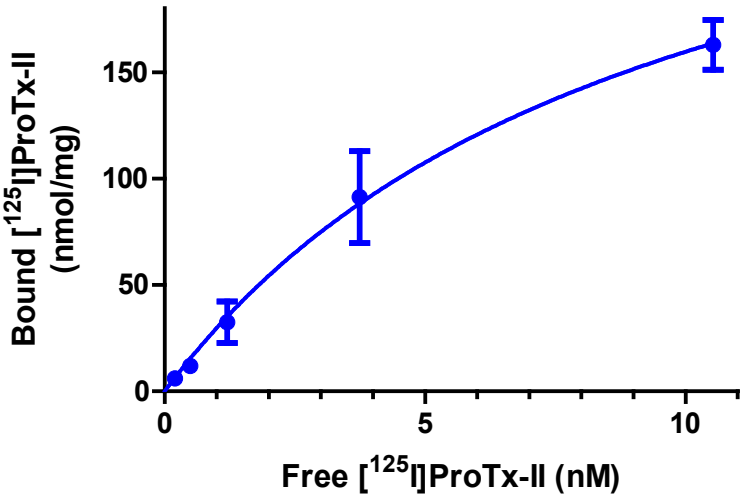
Supplemental Figure 5. Purified NavAb DII S1-S4 chimera. Representative SDS-PAGE gel of the final purified material stained with Instant Blue. Lanes 1, 3 and 5 were loaded with 2 µg, 4 µg or 8 µg protein, respectively. MW markers were loaded in lanes 2 and 4.



Supplemental Figure 6. Specific binding of [^{125}I]ProTx-II to purified DII S1-S4 chimera but not DII S3-S4 chimera. Biotinylated DII S3-S4 or DII S1-S4 protein (0.3 μg) in DDM were incubated with 1.67 nM [^{125}I]ProTx-II. To define non-specific (NS) binding, cold ProTx-II (2 μM) was included in the binding reaction. Mean cpms ± S.E.M. (n=3) are plotted.



Supplemental Figure 7. Saturation binding of [^{125}I]ProTx-II to purified DII S1-S4 chimera. DII S1-S4 protein was incubated with 0.2-10.5 nM [^{125}I]ProTx-II and specific binding determined. Mean bound [^{125}I]ProTx-II bound ± S.E.M. is plotted versus free [^{125}I]ProTx-II concentration. Each point represents the mean of five experiments measured in triplicate. The solid line is a fit of a single site model to the data with the parameters B_{max} = 309 nmol/mg, K_d = 9.8 nM.



Supplemental Table 1. Activation parameters of wild-type and chimeric channels. Values are derived from the fits of the Boltzmann equation to the normalized mean conductance versus membrane potential. For wt NavAb, wt $hNa_V1.7$ and DII S3-S4 chimera, conductance was estimated from peak current measurements. For the DII S1-S4 chimera, tail current amplitudes were used to estimate conductance.

Channel	$V_{1/2}$ (mV)	slope (mV)
wt Na_VAb	-93.4	7.7
wt $hNa_V1.7$	-19.4	6.9
DII S1-S4	+51.8	12.3
DII S3-S4	-38.1	10.2