Molecular Pharmacology

Supplemental Data

Rational engineering defines a molecular switch that is essential for activity of spider-venom peptides against the analgesics target $Na_V1.7$

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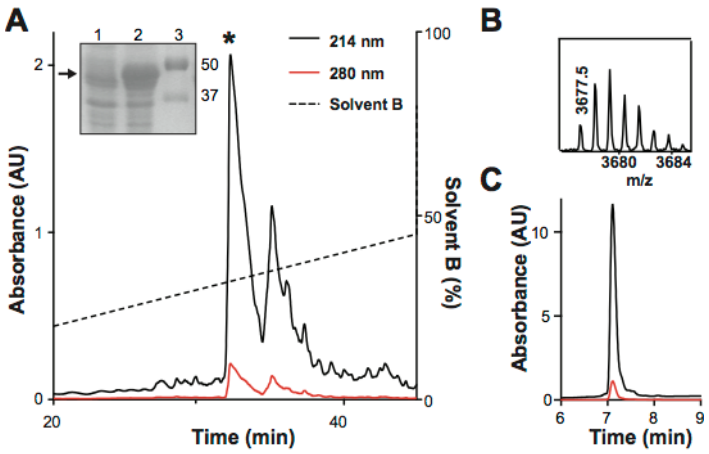
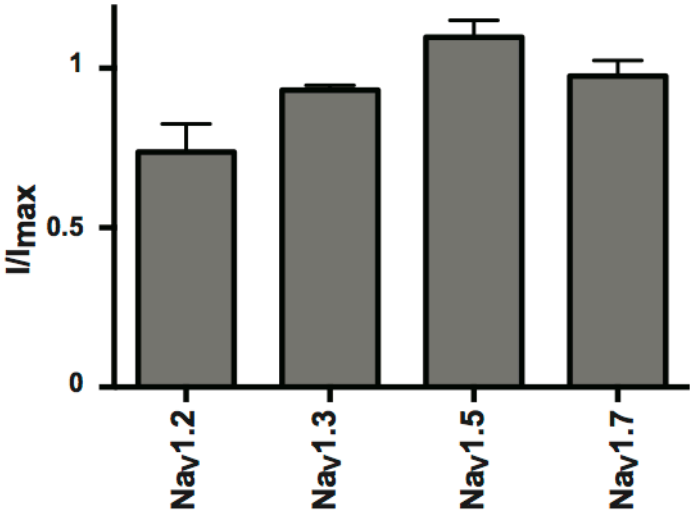


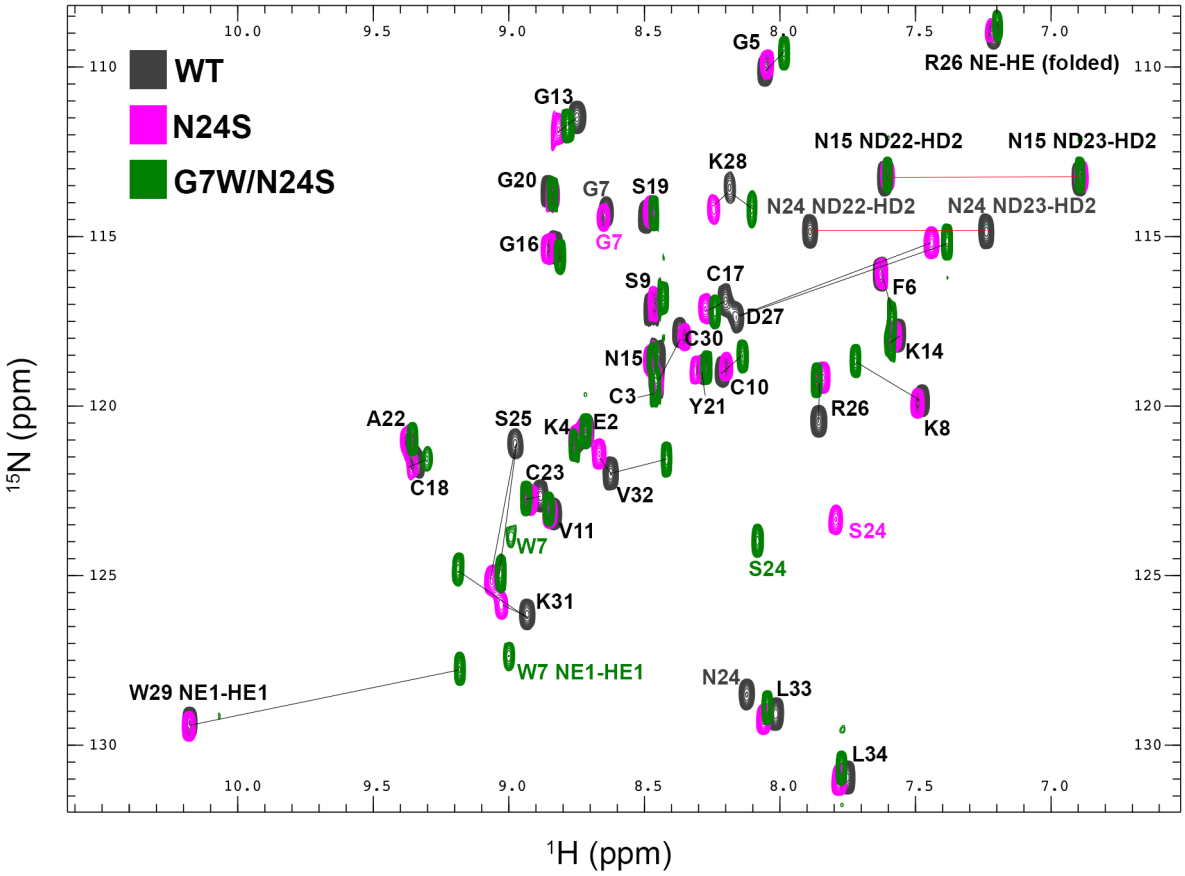
Supplemental Fig. S1. Expression and purification of wild-type Hhn2b. (A) RP-HPLC chromatogram showing purification of recombinant Hhn2b after removal of the His_6-MBP fusion tag by TEV protease. The peak corresponding to Hhn2b is highlighted with an asterisk. Inset, SDS-PAGE gel showing *E. coli* cells before (lane 1) and after (lane 2) IPTG induction. Lane 3 contains molecular mass standards, with masses indicated in kDa on the right of the gel. The arrow indicates the position of the MBP-peptide fusion protein. (B) MALDI-TOF MS spectrum showing the $M+H^+$ ion for the purified fully oxidized recombinant Hhn2b: observed 3677.5 Da, calculated 3677.7 Da. (C) RP-HPLC chromatogram of purified Hhn2b showing high purity as judged by one single uniform peak.

Figure S2



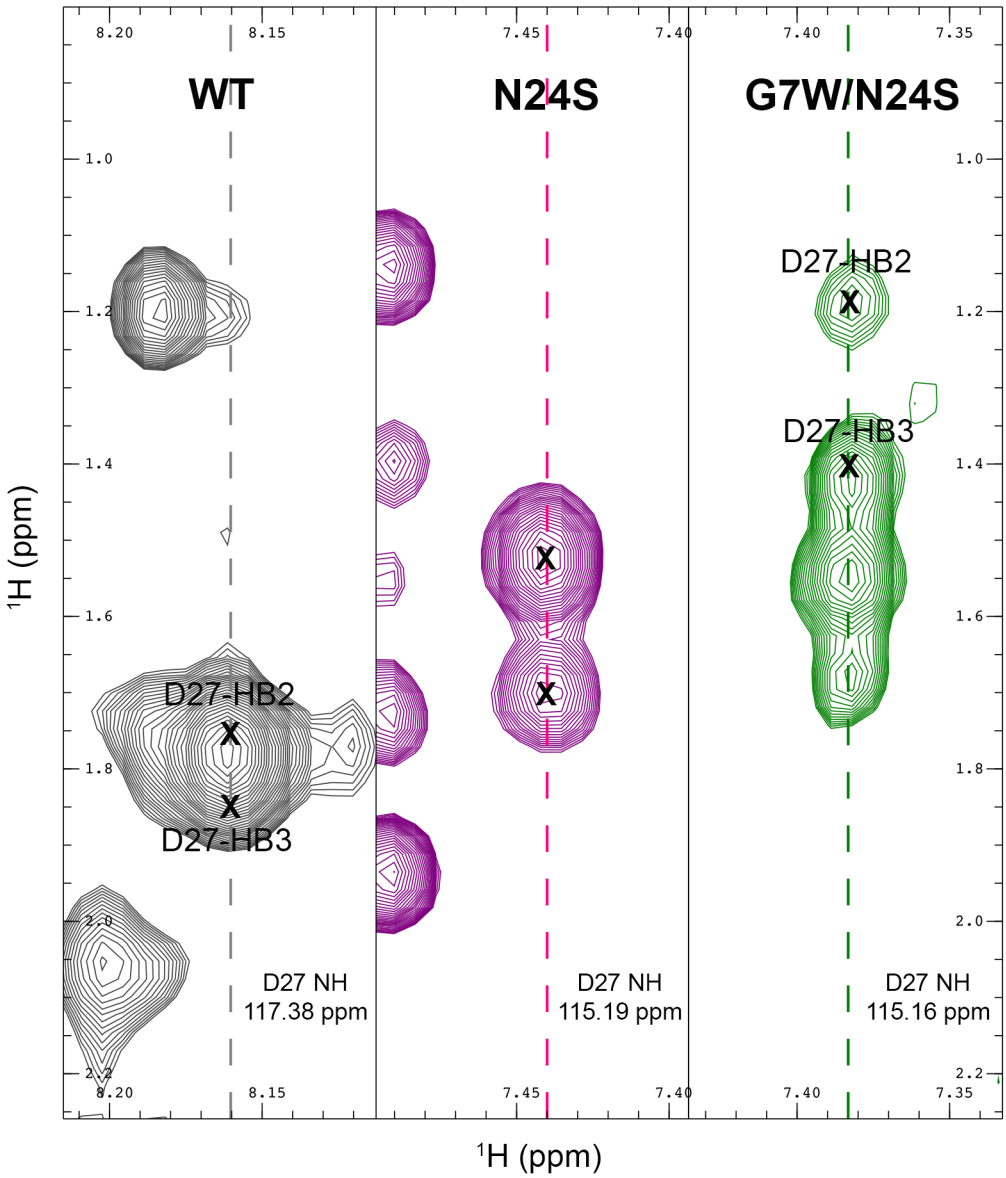
Supplemental Fig. S2. Wild-type recombinant Hhn2b has no or little effect on human Na_V channels. Currents were recorded from channels heterologously expressed in *X. laevis* oocytes using two-electrode voltage-clamp. I/I_{max} in the presence of 1 μM of Hhn2b (*n* = 3–4; error bars represent s.e.m.). Hhn2b had no effect on human $Na_V1.3$, $Na_V1.5$ or $Na_V1.7$, and gave 25% inhibition of $Na_V1.2$. Currents were evoked by depolarization to –20 mV from a holding potential of –90 mV. All channels were co-expressed with the β1 subunit.

Figure S3



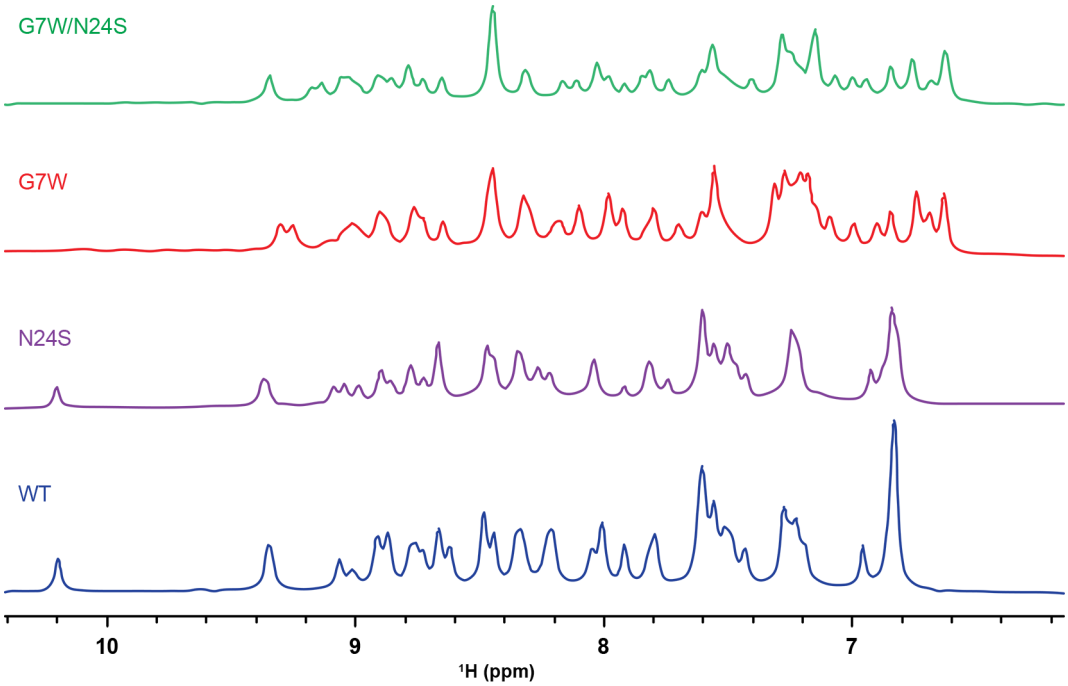
Supplemental Fig. S3. **Overlay of the 2D ^{1}H-^{15}N-HSQC spectra of WT, N24S and G7W/N24S mutants of Hhn2b.** Spectra are shown in grey, magenta and green for the WT, N24S and G7W/N24S mutants, respectively. Red lines connect the two peaks corresponding to the sidechain amide group of Asn15/24 and black lines connect peaks that have shifted upon the mutations.

Figure S4



Supplemental Fig. S4. ^{15}N-NOESY strips showing the intramolecular NOE signals of the β-protons of Asp27 in WT, N24S and G7W/N24S mutants of Hhn2b. The signals of the D27 sidechain protons are consistently shifted to lower frequencies as the two mutations are introduced, indicating increased proximity to an aromatic ring.

Figure S5



Supplemental Fig. 5. NH region of 1D ^{1}H-NMR spectra of Hhn2b (WT) and mutants in the presence of excess amount of POPC liposomes. In the presence of POPC the spectra of all Hhn2b and mutants remain well resolved. In all experiments the peptide:POPC ratio was 1:30.