Figures and figure supplements

Atomic mutagenesis in ion channels with engineered stoichiometry

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**Figure 1.** Conformational dynamics of wild type and Trp434Ind Shaker channel. (A) Position of the Trp434–Asp447 H-bond. (B) Time series of dihedral angles $\chi^1(\text{N–C}^{\alpha}\text{–C}^{\beta}\text{–C}^{\gamma})$ of Arp447 for wild type (left) and Trp434Ind (right) demonstrating the flipping of the Arp447 side-chains during the simulations. (C) Time series of distance between Asp447 and Trp434 in four subunits characterizing the local conformational dynamics during equilibrium simulations for wild type (left) and Trp434Ind (right) of Shaker. The distance is measured between Asp447 ($C^g$) and Trp434 ($N^{e1}$) or equivalent atom in the same position in Trp434Ind. (D) Top panels, typical conformations for protein and water molecules and occupancy (20%) map of water molecules around selectivity filter of subunit C. Bottom panels, 2D average occupancy map for all four subunits during 500ns MD simulations. The x-axis describes the radius to the center of the selectivity filter, and the y-axis is the z-coordinate of water molecules. In all simulations the selectivity filter in the conductive state. Videos 1 and 2 indicate 150 ns vignettes of the simulation for the W434-D447 and W434Ind-D447 in the presence shown water molecules. In each case, a subunit of Shaker (residues 433 to 447) is shown in new-cartoon representation, and side chains of W434 and D447 are shown in sticks, and water molecules are in VDW representations.

DOI: 10.7554/eLife.18976.002
Figure 2. Attachment of ER retention/retrieval motifs paired with split inteins results in co-dependent surface membrane expression of Shaker channels. (A) Expression of either Shaker channel with C-terminal appended Kir6.2 ER retention/retrieval motif (ER\text{ret}, yellow) with N-intein (red) (A, left) or Shaker channel with N-terminal appended Kir6.2 ER retention/retrieval motif (yellow) with C-intein (green) (A, right), resulted in negligible ionic current (A, lower panels). Representative currents in lower panel A, are raw capacitance and ionic currents that are blanked above and below 25 and \(-5\mu\text{A}\), respectively. P/4 subtracted currents are shown in lower panel B to display normal channel kinetics observed following expression of engineered ER\text{ret}-intein Shaker channels. See Materials and methods for ER-retention/retrieval and intein sequences. (B) Co-expression of Shaker N- and C-terminal ER\text{ret}-intein channels resulted in surface membrane trafficking of Shaker channels and robust ionic current (lower panel) measured by TEVC. (C) Injection of increased amounts of C-terminal (filled circles) or N-terminal (open circles) ER\text{ret}-intein-tagged Shaker cRNA resulted in insignificant surface expression of Shaker channels as measured with TEVC. Co-injection of increased amounts of N- and C-terminal ER\text{ret}-intein cRNA resulted in saturated ionic current (filled triangles). Number of observations where two Shaker monomers are expressed are indicated in parentheses above symbols. Average values where only one Shaker monomer was expressed were composed of 2–10 observations.

DOI: 10.7554/eLife.18976.005
Figure 3. Use of ER<sub>ret</sub>-intein tagged Shaker monomers allow for stoichiometric expression of Ind. (A) Normalized representative currents for Trp<sub>434</sub>Trp (WT) (left) and Trp<sub>434</sub>Ind (right). A schematic of the stoichiometry of the incorporated amino acids Trp (white) and Ind (black) is shown below the traces. (B) Average voltage dependence of normalized currents for Trp<sub>434</sub>Trp (WT) (open circles; n = 5) and Trp<sub>434</sub>Ind (filled circles, n = 9). (C) Average inactivation rates at +20 mV of WT Shaker, Trp<sub>434</sub>Phe (first monomer) four monomer concatemer, and Trp<sub>434</sub>Ind ER<sub>ret</sub>-intein constructs. DOI: 10.7554/eLife.18976.006
Figure 4. ERret-intein construction allows for expression of functional split WT Nav1.4. (A) Schematic of Na\textsubscript{v}1.4 protein with ER\textsubscript{ret}-intein sequence engineered on the C- (blue) and N-terminus (orange) of domains I-II and III-IV, respectively. (B) Western blot shows reconstitution and covalent linking of split-intein Na\textsubscript{v}1.4 proteins following expression in HEK293 cells. Lane 1, full-length WT Na\textsubscript{v}1.4; Lane 2, front half WT Na\textsubscript{v}1.4 I-II N-intein; Lane 3, back half WT Na\textsubscript{v}1.4 III-IV C-intein; Lane 4 front half WT Na\textsubscript{v}1.4 I-II N-intein + back half WT Na\textsubscript{v}1.4 III-IV C-intein expressed at 1:1; Lane 5, front half WT Na\textsubscript{v}1.4 I-II N-intein + back half WT Na\textsubscript{v}1.4 III-IV C-intein expressed at 2:1, respectively. (C) Representative Na\textsubscript{v}1.4 currents 12 hr following co-injection of front half WT Na\textsubscript{v}1.4 I-II N-intein + back half WT Na\textsubscript{v}1.4 III-IV C-intein. (D) Quantification of peak Na\textsuperscript{+} current @ -20 mV following injection of full-length WT Na\textsubscript{v}1.4 (circles; \(n = 10\)), front half WT Na\textsubscript{v}1.4 I-II N-intein (triangles; \(n = 6\)), back half WT Na\textsubscript{v}1.4 III-IV C-intein (squares; \(n = 7\)) and both front half WT Na\textsubscript{v}1.4 I-II N-intein + back half WT Na\textsubscript{v}1.4 III-IV C-intein (squares; \(n = 10\)). DOI: 10.7554/eLife.18976.008
Figure 4—figure supplement 1. Assembly of split-intein NaV1.4 constructs occurs within the cells. HEK293 cell lysates expressing either front half WT NaV1.4 I-II N-intein and back half WT NaV1.4 III-IV C-intein were mixed on ice for 10, 30 and 60 min and separated by acrylamide gels, transferred and immunoblotted. No higher order band was detected following incubation of split-intein constructs following cell-lysis indicating that assembly and intein-dependent peptide bond formation occurred within the cell.

DOI: 10.7554/eLife.18976.009
Figure 5. ER<sub>ret</sub>-intein construction allows for expression of functional split Na<sub>1.4</sub> with two suppressed codons. (A) Co-injection of 25 ng of cRNA encoding Na<sub>1.4</sub> I-II N-intein Tyr401TAG + Na<sub>1.4</sub> III-IV C-intein WT + tRNA(Phe) (left), Na<sub>1.4</sub> I-II N-intein WT + Na<sub>1.4</sub> III-IV C-intein F1304TAG + tRNA(Phe) (center) and Na<sub>1.4</sub> I-II N-intein Tyr401TAG + Na<sub>1.4</sub> III-IV C-intein Phe1304TAG + tRNA(Phe) (right), resulted in robust currents at 24 hr (lower panels). (B) Quantification of peak Na<sup>+</sup> current @ −20 mV following injection of 25 ng of cRNA of constructs in A with tRNA(Phe) (open symbols) and uncharged tRNA (closed symbols). (C) Representative Na<sub>1.4</sub> current following co-injection of 50 ng of cRNA encoding Na<sub>1.4</sub> I-II N-intein Y401TAG and Na<sub>1.4</sub> III-IV C-intein Phe1304TAG constructs. (D) Quantification of peak Na<sup>+</sup> current @ −20 mV following injection of 50 ng of cRNA encoding Na<sub>1.4</sub> I-II N-intein Tyr401TAG and Na<sub>1.4</sub> III-IV C-intein Phe1304TAG (orange) constructs with tRNA(Phe) (open symbols) and uncharged tRNA (closed symbols).

DOI: 10.7554/eLife.18976.010
Figure 5—figure supplement 1. Voltage dependence of Na\textsubscript{v}1.4 activation is unaltered by engineered split inteins. (A) Conductance-Voltage relationships of full-length WT Na\textsubscript{v}1.4 (solid line, circles) and front half WT Na\textsubscript{v}1.4 I-II N-intein + back half WT Na\textsubscript{v}1.4 III-IV C-intein (dashed line, diamonds). (B) Conductance-Voltage relationships of Na\textsubscript{v}1.4 I-II N-intein Y401TAG + Na\textsubscript{v}1.4 III-IV C-intein WT + tRNA(Phe) (circles), Na\textsubscript{v}1.4 I-II N-intein WT + Na\textsubscript{v}1.4 III-IV C-intein Phe1304TAG + tRNA(Phe) (triangles) and Na\textsubscript{v}1.4 I-II N-intein Tyr401TAG + Na\textsubscript{v}1.4 III-IV C-intein Phe1304TAG + tRNA(Phe) (squares).

DOI: 10.7554/eLife.18976.011