molecules

Supplementary Materials

Chemometric Models of Differential Amino Acids at the Na$_V\alpha$ and Na$_V\beta$ Interface of Mammalian Sodium Channel Isoforms

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Supplementary Materials

The Supplementary Material (SM) was necessary to document the essential findings as a synopsis taken from an even larger number of systematically conducted chemometric studies on the Sodium channels:

1. **Multiple Sequence Alignments (MSA): 9 (isoforms) x 3 (species) x 16 (ECLs) = 432 primary sequences.**
2. **3D models of 3D templates and homology models: 9 x 3 x 2 (β1,β3) = 54 PDB files.**
3. **Calculations of surfaces: 9 x 3 x 16 = 432 surface models.**
4. **Calculations of polar and non-polar surfaces: (9 x 3 x 16) x 2 (PSA, nonPSA) = 432 x 2 properties.**
5. **Calculations of molecular volumes: 9 x 3 x 16 = 432 volume patterns.**
6. **Interface area calculation (buried area): 9 x 3 x 2 (α / β) x 4 (ECLs)= 216 numbers of buried regions between subunits α and β.**
7. **Calculations of IF area of nine isoforms by three species by four IF-ECLs: 9 x 3 x 2 (β1,β3) x 4 (IF-ECLs) x 2 (α / β) = 432 total number of surface patterns.**
8. **Calculations of IF area concerning the polar and nonpolar atoms that form the interface: 9 x 3 x 2 (β1,β3) x 4 (IF-ECLs) x 2 (α / β) = 432 polar and 432 nonpolar atomic patterns.**
9. **Calculations of the MEPS area (red = negative, blue = positive) based on the vertices of the solvent-accessible surface of the atoms that make up the interface: 9 x 3 x 2 (β1,β3) x 4 (IF-ECLs) x 2 (α / β) = 432 positive charge patterns and 432 negative charge patterns.**
10. **Counting of the loop lengths: 9 (isoforms) x 3 (species) x 16 (ECLs) = 432 counts.**
11. **Counting of the polar residues: 9 (isoforms) x 3 (species) x 16 (ECLs) = 432 counts.**
12. **Counting of the cysteine residues as potential formation of S-S bridges: 9 (isoforms) x 3 (species) x 16 (ECLs) = 432 counts.**
13. **Counting of the aromatic, positively or negatively charged residues: 9 (isoforms) x 3 (species) x 16 (ECLs) x 3 = 432 x 3 counts.**

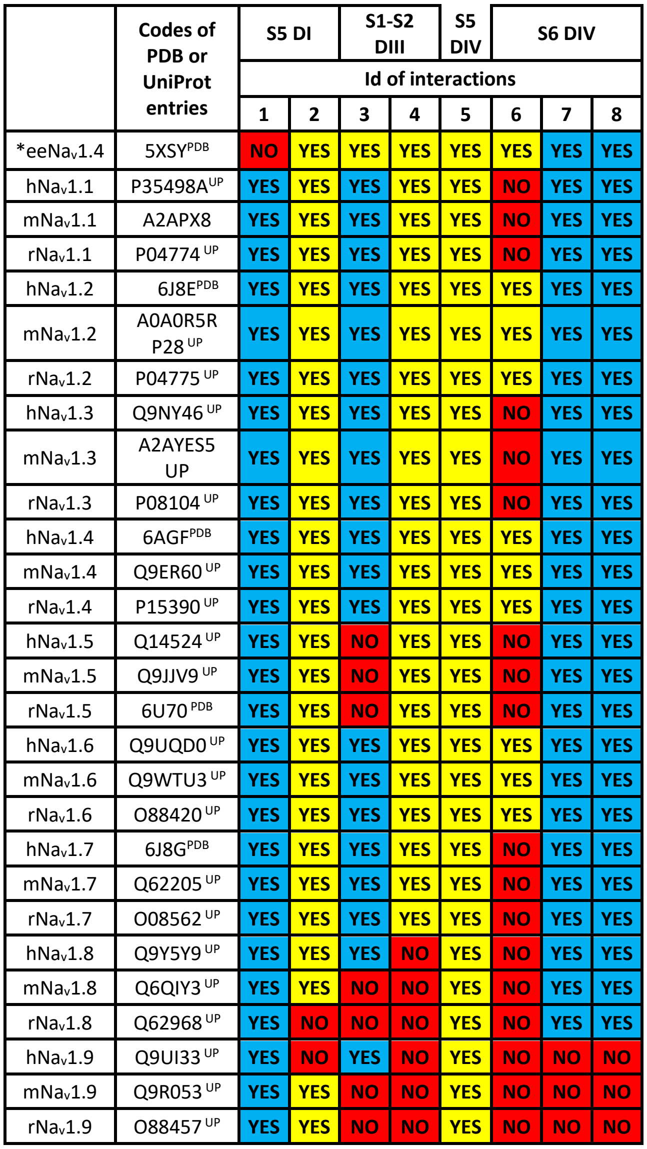
Table S1. PPI of the residues of the Na$_V\alpha$ and Na$_V\beta$ subunits

Isoform	**ECL**	**S5 DI**		**S1-S2 DIII**		**S5 DIV**	**S6 DIV**		
	PPI Id[3]	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
hNa$_v$1.1[7]	P35498[2]	agq**C**peg(O)	qcp**E**gym(O)	yid**Q**rkt(N)	dqr**K**tik(N)	gid**D**mfn(O)	Pnk**V**npg[NI]	nkv**N**pgs(O)	kvn**P**gss(O)
mNa$_v$1.1[7]	A2APX8[2]	agq**C**peg(O)	qcp**E**gym(O)	yid**Q**rkt(N)	dqr**K**tik(N)	gid**D**mfn(O)	pnk**V**npg[NI]	nkv**N**pgs(O)	kvn**P**gss(O)
rNa$_v$1.1[7]	P04774[2]	agq**C**peg(O)	qcp**E**gym(O)	yid**Q**rkt(N)	dqr**K**tik(N)	gid**D**mfn(O)	pnk**V**npg[NI]	nkv**N**pgs(O)	kvn**P**gss(O)
hNa$_v$1.2[5]	6J8E[1]	agq**C**peg(O)	qcp**E**gyi(O)	yie**Q**rkt(N)	eqr**K**tik(N)	gid**D**mfn(O)	pdk**D**hpg(O)	dkd**H**pgs(N)	kdh**P**gss(O)
mNa$_v$1.2[7]	B1AWN6	agq**C**peg(O)	qcp**E**gyi(O)	yie**Q**rkt(N)	eqr**K**tik(N)	gid**D**mfn(O)	pek**D**hpg(O)	ekd**H**pgs(N)	kdh**P**gss(O)
rNa$_v$1.2[7]	P04775[2]	agq**C**peg(O)	qcp**E**gyi(O)	yie**Q**rkt(N)	eqr**K**tik(N)	gid**D**mfn(O)	pek**D**hpg(O)	ekd**H**pgs(N)	kdh**P**gss(O)
hNa$_v$1.3[7]	Q9NY46[2]	agq**C**peg(O)	qcp**E**gyi(O)	yie**Q**rkt(N)	eqr**K**tik(N)	gid**D**mfn(O)	pdt**I**hpg[NI]	dti**H**pgs(N)	tih**P**gss(O)
mNa$_v$1.3[7]	A2ASI5[2]	agq**C**peg(O)	qcp**E**gyi(O)	yie**Q**rkt(N)	eqr**K**tik(N)	gid**D**mfn(O)	pda**I**hpg[NI]	dai**H**pgs(N)	aih**P**gss(O)
rNa$_v$1.3[7]	P08104[2]	agq**C**peg(O)	qcp**E**gyi(O)	yie**Q**rkt(N)	eqr**K**tik(N)	gid**D**mfn(O)	pda**I**hpg[NI]	dai**H**pgs(N)	aih**P**gss(O)
eeNa$_v$1.4[5]	5XSY[1]	agk**C**peg[NI]	kcp**E**gyt(O)	yiw**R**rrv(N)	wrr**R**vik(N)	gvd**D**ifn(O)	pdv**E**npg(O)	dve**N**pgt(O)	ven**P**gtd(O)
hNa$_v$1.4[5]	6AGF[1]	agh**C**peg(O)	hcp**E**gye(O)	yie**Q**rrv(N)	eqr**R**vir(N)	gid**D**mfn(O)	pnl**E**npg(O)	nle**N**pgt(O)	len**P**gts(O)
mNa$_v$1.4[7]	Q9ER60[2]	agh**C**peg(O)	hcp**E**gye(O)	yie**Q**rrv(N)	eqr**R**vir(N)	gid**D**mfn(O)	ptl**E**npg(O)	tle**N**pgt(O)	len**P**gtn(O)
rNa$_v$1.4[7]	P15390[2]	agh**C**peg(O)	hcp**E**gye(O)	yie**Q**rrv(N)	eqr**R**vir(N)	gid**D**mfn(O)	ptl**E**npg(O)	tle**N**pgt(O)	len**P**gtn(O)
hNa$_v$1.5[7]	Q14524[2]	agt**C**peg(O)	tcp**E**gyr(O)	yle**E**rkt[NI]	eer**K**tik(N)	gid**D**mfn(O)	ptl**P**nsn[NI]	tlp**N**sng(O)	lpn**S**ngs(O)
mNa$_v$1.5[7]	Q9JJV9[2]	agt**C**peg(O)	tcp**E**gyr(O)	yle**E**rkt[NI]	eer**K**tik(N)	gid**D**mfn(O)	pnl**P**nsn[NI]	nlp**N**sng(O)	lpn**S**ngs(O)
rNa$_v$1.5[5]	6U70[1]	agt**C**peg(O)	tcp**E**gyr(O)	yle**E**rkt[NI]	eer**K**tik(N)	gid**D**mfn(O)	pnl**P**nsn[NI]	nlp**N**sng(O)	lpn**S**ngs(O)
hNa$_v$1.6[7]	Q9UQD0	agq**C**peg(O)	qcp**E**gyq(O)	yie**Q**rkt(N)	eqr**K**tir(N)	gid**D**mfn(O)	ldk**E**hpg(O)	dke**H**pgs (N)	keh**P**gsg(O)
mNa$_v$1.6[7]	Q9WTU3	agq**C**peg(O)	qcp**E**gfq(O)	yie**Q**rkt(N)	eqr**K**tir(N)	gid**D**mfn(O)	ldk**E**hpg(O)	dke**H**pgs(N)	keh**P**gsg(O)
rNa$_v$1.6[7]	O88420[2]	agq**C**peg(O)	qcp**E**gfq(O)	yie**Q**rkt(N)	eqr**K**tir(N)	gid**D**mfn(O)	ldk**E**hpg(O)	dke**H**pgs(N)	keh**P**gsg(O)
hNa$_v$1.7[5]	6J8G[1]	sgq**C**peg(O)	qcp**E**gyt(O)	yie**R**kkt(N)	erk**K**tik(N)	gin**D**mfn(O)	pkk**V**hpg[NI]	kkv**H**pgs(N)	kvh**P**gss(O)
mNa$_v$1.7[7]	Q62205[2]	sgq**C**peg(O)	qcp**E**gye(O)	yie**K**kkt(N)	ekk**K**tik(N)	gin**D**mfn(O)	pkk**V**hpg[NI]	kkv**H**pgs(N)	kvh**P**gss(O)
rNa$_v$1.7[7]	O08562[2]	sgq**C**peg(O)	qcp**E**gyi(O)	yie**K**kkt(N)	ekk**K**tik(N)	gin**D**mfn(O)	pkk**V**hpg[NI]	kkv**H**pgs(N)	kvh**P**gss(O)
hNa$_v$1.8[7]	Q9Y5Y9[2]	sgh**C**pdg(O)	hcp**D**gyi(O)	yld**Q**kpt(N)	dqk**P**tvk[NI]	gid**D**mfn(O)	pnl**P**nsn[NI]	nlp**N**sng(O)	lpn**S**ngt(O)
mNa$_v$1.8[7]	Q6QIY3[2]	agh**C**pnd(O)	hcp**N**dyv(O)	yle**E**kpr[NI]	eek**P**rvk[NI]	gid**D**mfn(O)	pnr**P**nsn[NI]	nrp**N**sng(O)	rpn**S**ngs(O)
rNa$_v$1.8[7]	Q62968[2]	agh**C**pgg(O)	hcp**G**gyv[NI]	yle**E**kpr[NI]	eek**P**rvk[NI]	gid**D**mfn(O)	pnl**P**nsn[NI]	nlp**N**sng(O)	lpn**S**ngs(O)
hNa$_v$1.9[7]	Q9UI33[2]	nsa**C**siq(O)	acs**I**qye[NI]	hle**N**qpk(N)	enq**P**kiq[NI]	gid**D**ifn(O)	rsk**E**scn [NI]	ske**S**cns [NI]	kes**C**nss [NI]
mNa$_v$1.9[7]	Q9R053[2]	rrs**C**pdg(O)	scp**D**gst(O)	nlp**S**rpq [NI]	psr**P**qve[NI]	gid**D**ifn(O)	esk**A**scn [NI]	ska**S**cns[NI]	kas**C**nss [NI]
rNa$_v$1.9[7]	O88457[2]	srp**C**png(O)	pcp**N**gst(O)	nlp**S**rpq [NI]	psr**P**qve[NI]	gid**D**ifn(O)	eak**E**hcn [NI]	ake**H**cns [NI]	keh**C**nss [NI]
PPI Id[4]		**1b**	**2b**	**3b**	**4b**	**5b**	**6b**	**7b**	**8b**
eeNa$_v\beta$1[5]	5XSY[1]	sck**M**rge[NI]	yfd**R**tlt(N)	acv**E**vds(O)	vds**D**tea(O)	ckm**R**gev(N)	mgs**K**ntf(N)	sng**A**cve(N)	gac**V**evd(N)
hNa$_v\beta$1[5]	6AGF[1]	sck**R**rse(N)	hvy**R**llf(N)	gcv**E**vds(O)	vds**E**tea(O)	ckr**R**set(N)	ngs**R**gtk(N)	acg**G**cve(N)	ggc**V**evd(N)
mNa$_v\beta$1[7]	P97952[2]	sck**R**rse(N)	hvy**R**llf(N)	gcv**E**vds(O)	vds**D**tea(O)	ckr**R**set(N)	ngs**R**gtk(N)	awg**G**cve(N)	ggc**V**evd(N)
rNa$_v\beta$1[7]	Q00954[2]	sck**R**rse(N)	hvy**R**llf(N)	gcv**E**vds(O)	vds**E**tea(O)	ckr**R**set(N)	ngs**R**gtk(N)	awg**G**cve(N)	ggc**V**evd(N)
hNa$_v\beta$3[6]	4L1D[1]	scm**K**ree(N)	nvs**R**efe(N)	vcv**E**vps(O)	vps**E**tea(O)	cmk**R**eev(N)	ngs**K**dlq(N)	cfp**V**cve(N)	pvc**V**evp(N)
mNa$_v\beta$3[7]	Q8BHK2	scm**K**ree(N)	nvs**R**efe(N)	vcv**E**vps(O)	vps**E**tea(O)	cmk**R**eev(N)	ngs**K**dlq(N)	cfp**V**cve(N)	pvc**V**evp(N)
rNa$_v\beta$3[7]	Q9JK00[2]	scm**K**ree(N)	nvs**R**efe(N)	vcv**E**vps(O)	vps**E**tea(O)	cmk**R**eev(N)	ngs**K**dlq(N)	cfp**V**cve(N)	pvc**V**evp(N)

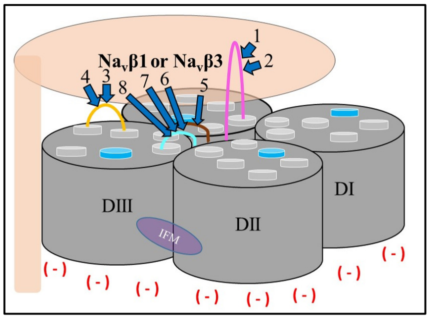
[1]PDB code (http://www.rcsb.org/), [2]UniProt code (https://www.uniprot.org/), [3]PPI-Id for positions of computed polar interactions the residues in Na$_v\alpha$ (1 – 8), [4]Id for positions of computed polar interactions of the residues in Na$_v\beta$1 and Na$_v\beta$3 (1b – 8b) with respect to Na$_v\alpha$, templates ([5]Cryo-EM structures, [6]crystal structures), [7]models, [NI] no interaction; residues that form computed polar interactions (bold capital letters); amino acids adjacent in sequence to residues that form computed polar interactions (lowercase letters); positively and negatively charged residues (blue and red, respectively), polar residues (cyan), non-polar residues (orange).

TABLE S1 – alternative presentation. Symbols: YES means there is a contribution to the PPI. NO means there is none. See also, Table 1 in the manuscript, MS.

Isoform		**Extracelular loop**



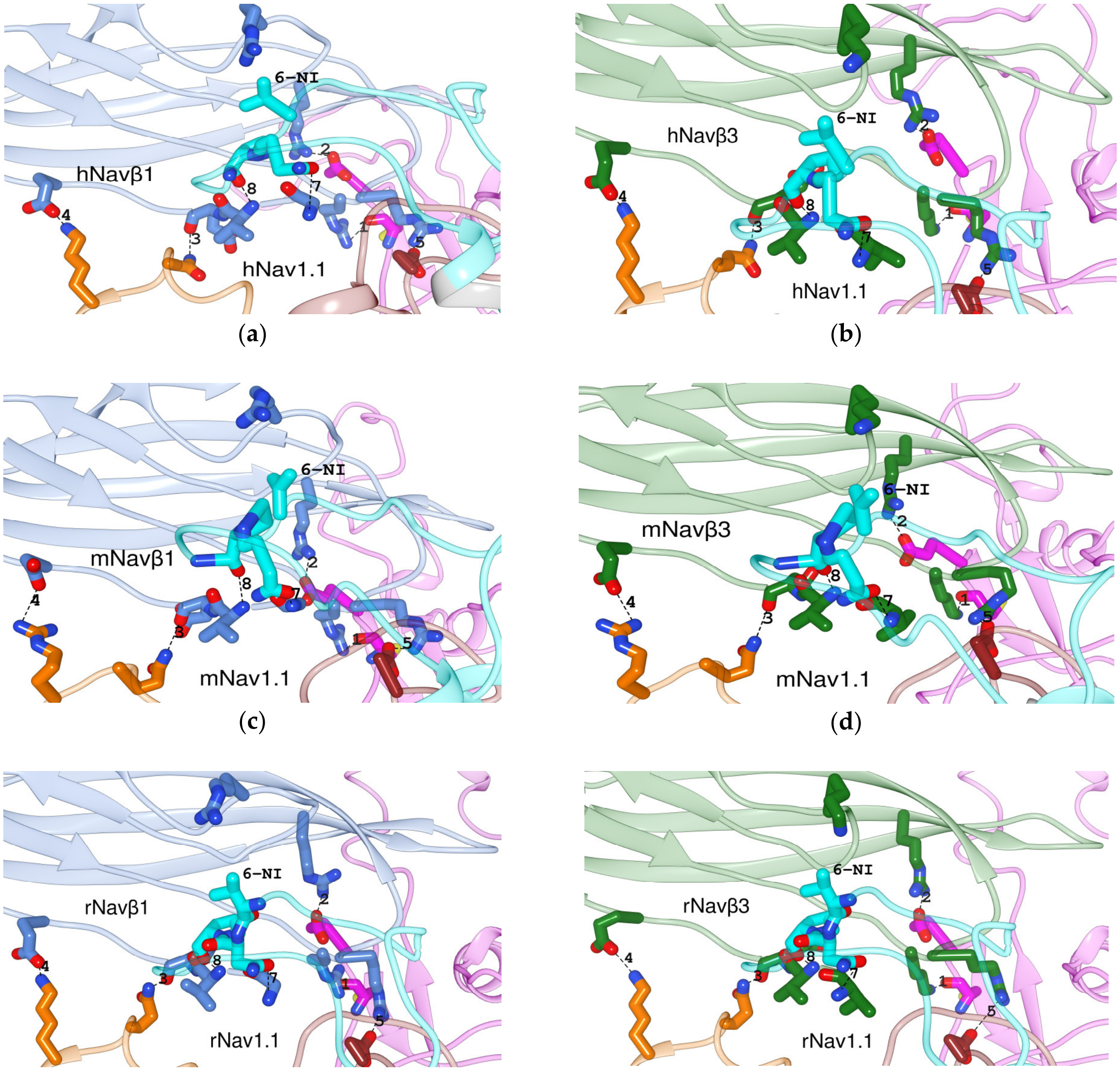
	Codes of PDB or UniProt entries	S5 DI		S1-S2 DIII		S5 DIV	S6 DIV		
		Id of interactions							
		1	2	3	4	5	6	7	8
*eeNa$_v$1.4	5XSY^{PDB}	NO	YES	YES	YES	YES	YES	YES	YES
hNa$_v$1.1	P35498A^{UP}	YES	YES	YES	YES	YES	NO	YES	YES
mNa$_v$1.1	A2APX8	YES	YES	YES	YES	YES	NO	YES	YES
rNa$_v$1.1	P04774^{UP}	YES	YES	YES	YES	YES	NO	YES	YES
hNa$_v$1.2	6J8E^{PDB}	YES	YES	YES	YES	YES	YES	YES	YES
mNa$_v$1.2	A0A0R5R P28^{UP}	YES	YES	YES	YES	YES	YES	YES	YES
rNa$_v$1.2	P04775^{UP}	YES	YES	YES	YES	YES	YES	YES	YES
hNa$_v$1.3	Q9NY46^{UP}	YES	YES	YES	YES	YES	NO	YES	YES
mNa$_v$1.3	A2AYES5 UP	YES	YES	YES	YES	YES	NO	YES	YES
rNa$_v$1.3	P08104^{UP}	YES	YES	YES	YES	YES	NO	YES	YES
hNa$_v$1.4	6AGF^{PDB}	YES	YES	YES	YES	YES	YES	YES	YES
mNa$_v$1.4	Q9ER60^{UP}	YES	YES	YES	YES	YES	YES	YES	YES
rNa$_v$1.4	P15390^{UP}	YES	YES	YES	YES	YES	YES	YES	YES
hNa$_v$1.5	Q14524^{UP}	YES	YES	NO	YES	YES	NO	YES	YES
mNa$_v$1.5	Q9JJV9^{UP}	YES	YES	NO	YES	YES	NO	YES	YES
rNa$_v$1.5	6U70^{PDB}	YES	YES	NO	YES	YES	NO	YES	YES
hNa$_v$1.6	Q9UQD0^{UP}	YES	YES	YES	YES	YES	YES	YES	YES
mNa$_v$1.6	Q9WTU3^{UP}	YES	YES	YES	YES	YES	YES	YES	YES
rNa$_v$1.6	O88420^{UP}	YES	YES	YES	YES	YES	YES	YES	YES
hNa$_v$1.7	6J8G^{PDB}	YES	YES	YES	YES	YES	NO	YES	YES
mNa$_v$1.7	Q62205^{UP}	YES	YES	YES	YES	YES	NO	YES	YES
rNa$_v$1.7	O08562^{UP}	YES	YES	YES	YES	YES	NO	YES	YES
hNa$_v$1.8	Q9Y5Y9^{UP}	YES	YES	YES	NO	YES	NO	YES	YES
mNa$_v$1.8	Q6QIY3^{UP}	YES	YES	NO	NO	YES	NO	YES	YES
rNa$_v$1.8	Q62968^{UP}	YES	NO	NO	NO	YES	NO	YES	YES
hNa$_v$1.9	Q9UI33^{UP}	YES	NO	YES	NO	YES	NO	NO	NO
mNa$_v$1.9	Q9R053^{UP}	YES	YES	NO	NO	YES	NO	NO	NO
rNa$_v$1.9	O88457^{UP}	YES	YES	NO	NO	YES	NO	NO	NO



Blue = H-Bond

Yellow = Salt Bridge

Red = No interaction

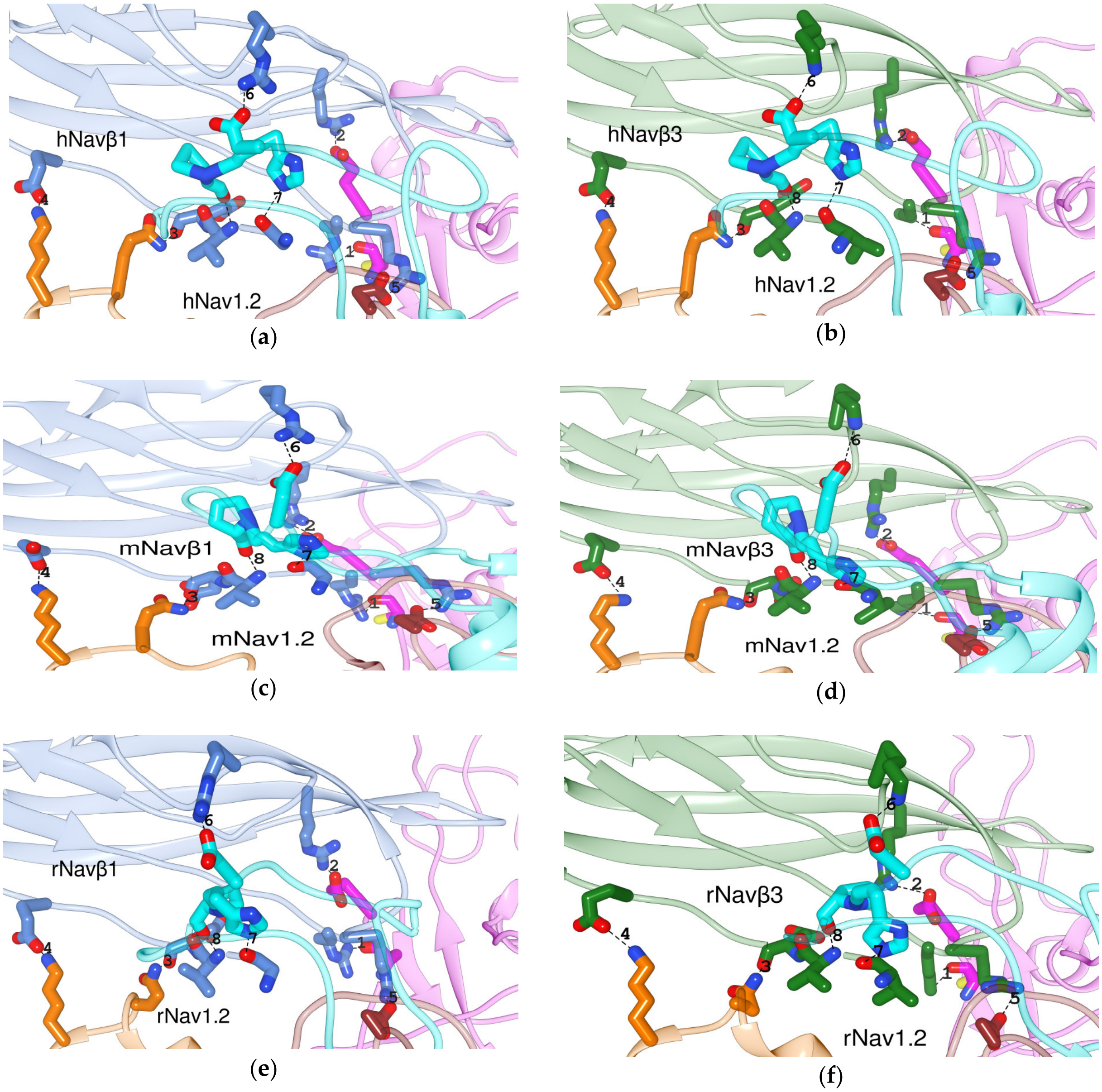


(**a**) (**b**)

(**c**) (**d**)

(**e**) (**f**)

Figure S1. PPIs of the three h,m,rNa$_v$1.1 isoforms in complex with Na$_v$β1 and Na$_v$β3. (**a**, **b**) complexes of human; (**c**, **d**) mouse; (**e**, **f**) rat; residues that do not participate in the interface: gray; S5 DI: magenta; S1-S2 DIII: orange; S5 DIV: brown; S6 DIV: cyan; Na$_v$β1: cornflower blue; Na$_v$β3: forest green; numbers: PPI Id according to the Table S1; NI: no interaction.

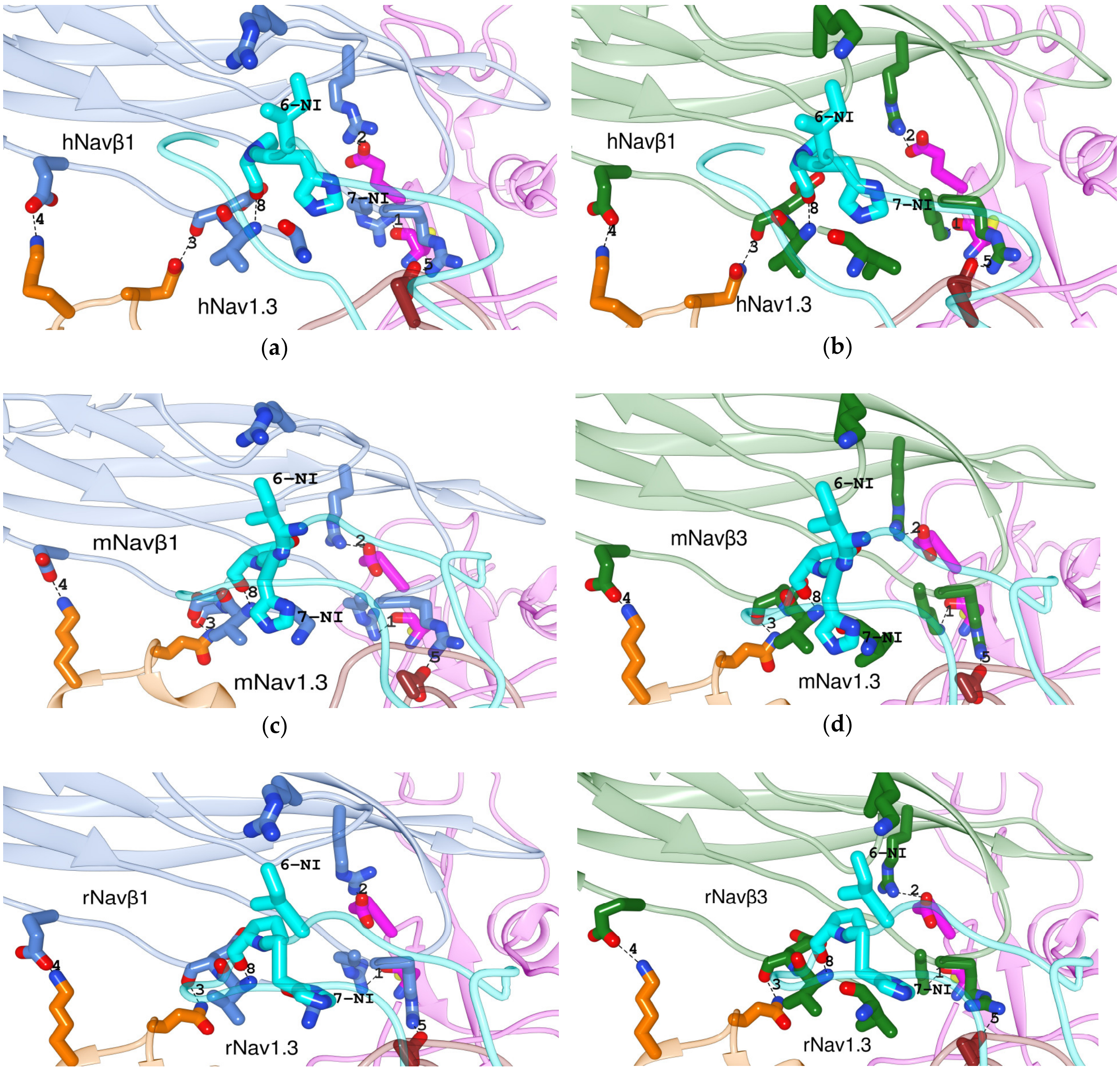


(**a**) (**b**)

(**c**) (**d**)

(**e**) (**f**)

Figure S2. PPIs of the three h,m,rNa$_v$1.2 isoforms in complex with Na$_v$β1 and Na$_v$β3. (**a**, **b**) complexes of human; (**c**, **d**) mouse; (**e**, **f**) rat; residues that do not participate in the interface: gray; S5 DI: magenta; S1-S2 DIII: orange; S5 DIV: brown; S6 DIV: cyan; Na$_v$β1: cornflower blue; Na$_v$β3: forest green; numbers: PPI Id according to the Table S1; NI: no interaction.

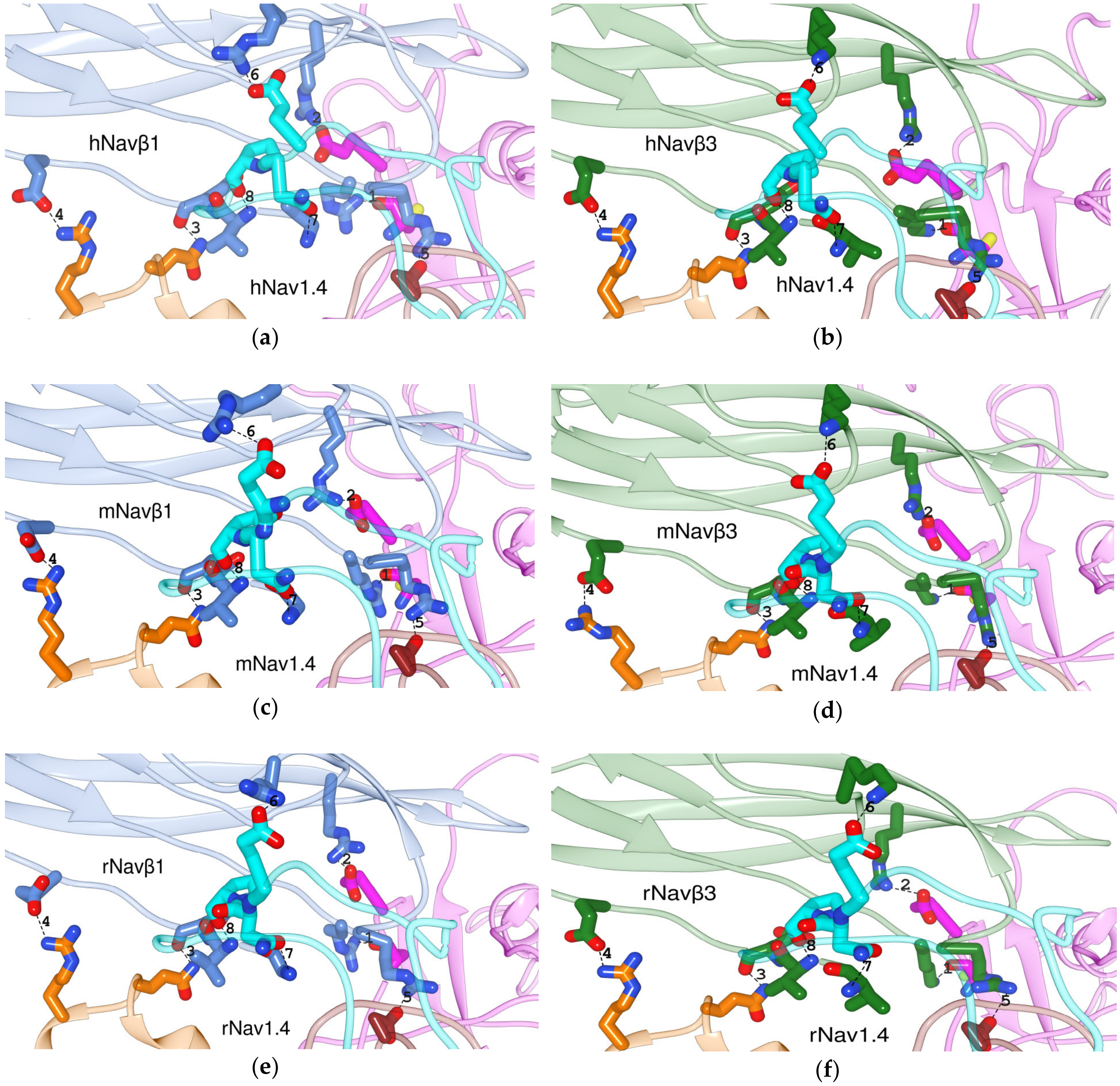


(**a**) (**b**)

(**c**) (**d**)

(**e**) (**f**)

Figure S3. PPIs of the three Na$_v$1.3 isoforms in complex with Na$_v$β1 and Na$_v$β3. (**a**, **b**) complexes of human; (**c**, **d**) mouse; (**e**, **f**) rat; residues that do not participate in the interface: gray; S5 DI: magenta; S1-S2 DIII: orange; S5 DIV: brown; S6 DIV: cyan; Na$_v$β1: cornflower blue; Na$_v$β3: forest green; numbers: PPI Id according to the Table S1; NI: no interaction.

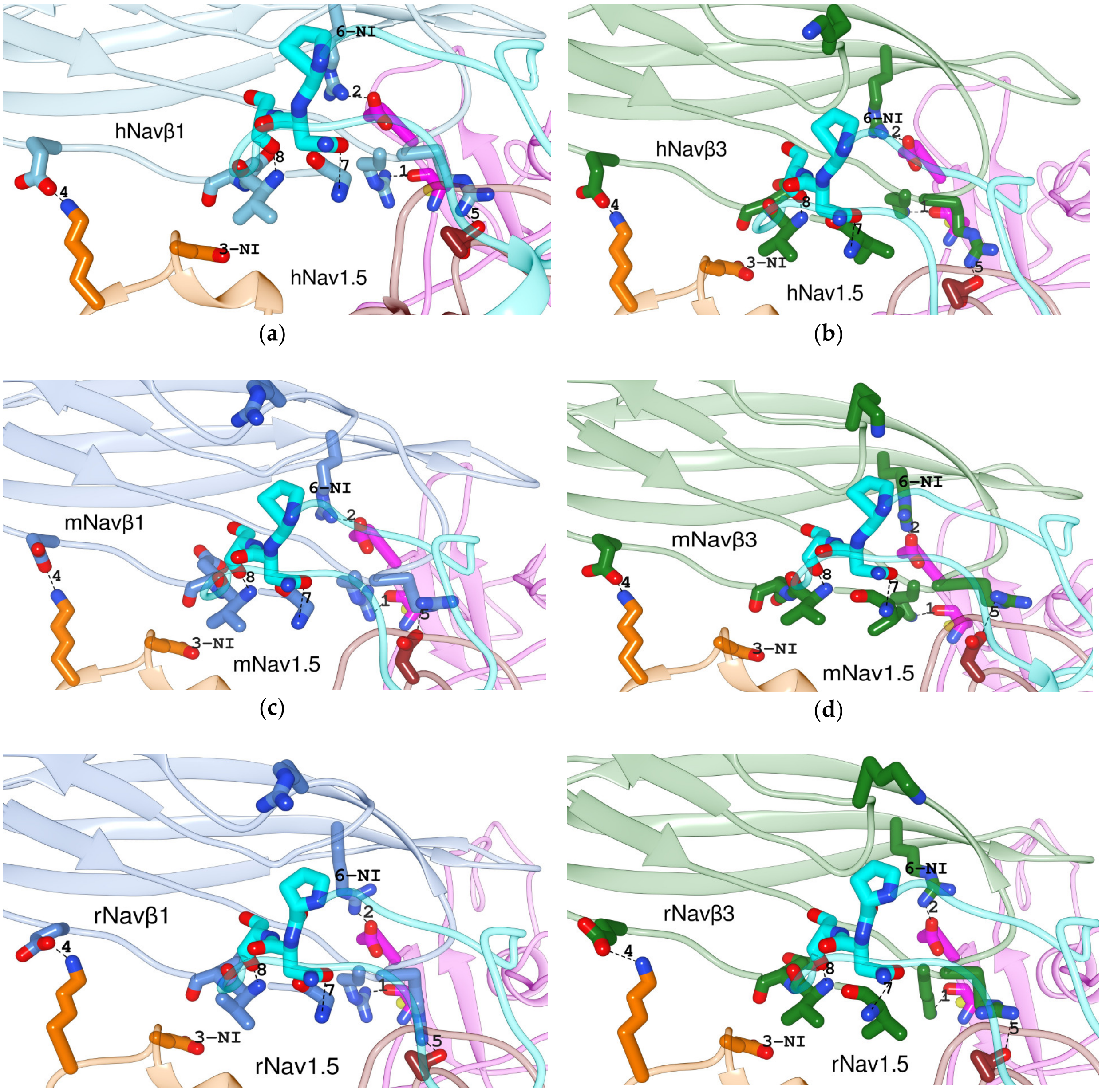


(**a**) (**b**)

(**c**) (**d**)

(**e**) (**f**)

Figure S4. PPIs of the three h,m,rNa$_v$1.4 isoforms in complex with Na$_v$β1 and Na$_v$β3. (**a**, **b**) complexes of human; (**c**, **d**) mouse; (**e**, **f**) rat; residues that do not participate in the interface: gray; S5 DI: magenta; S1-S2 DIII: orange; S5 DIV: brown; S6 DIV: cyan; Na$_v$β1: cornflower blue; Na$_v$β3: forest green; numbers: PPI Id according to the Table S1; NI: no interaction.

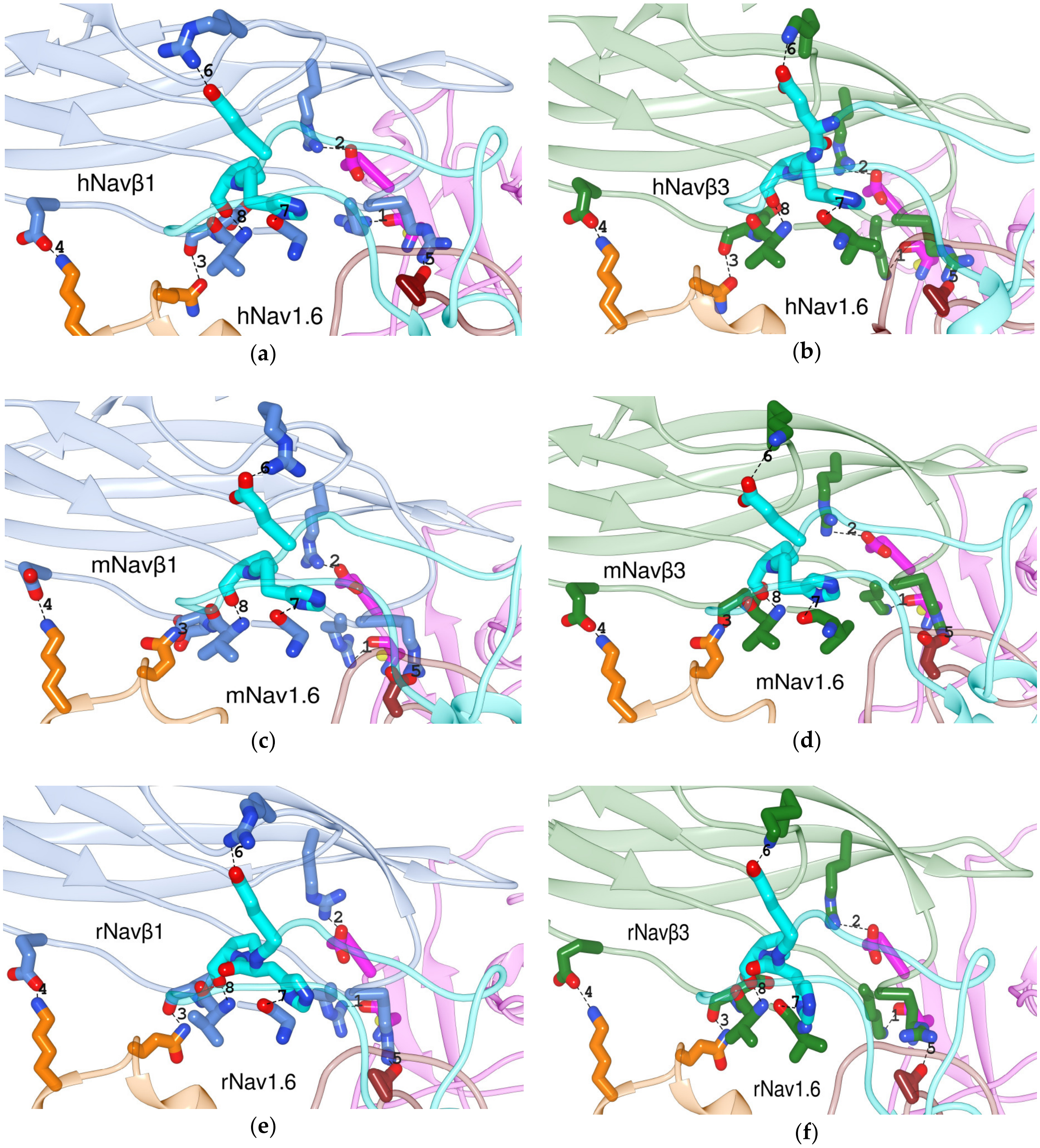


(**a**) (**b**)

(**c**) (**d**)

(**e**) (**f**)

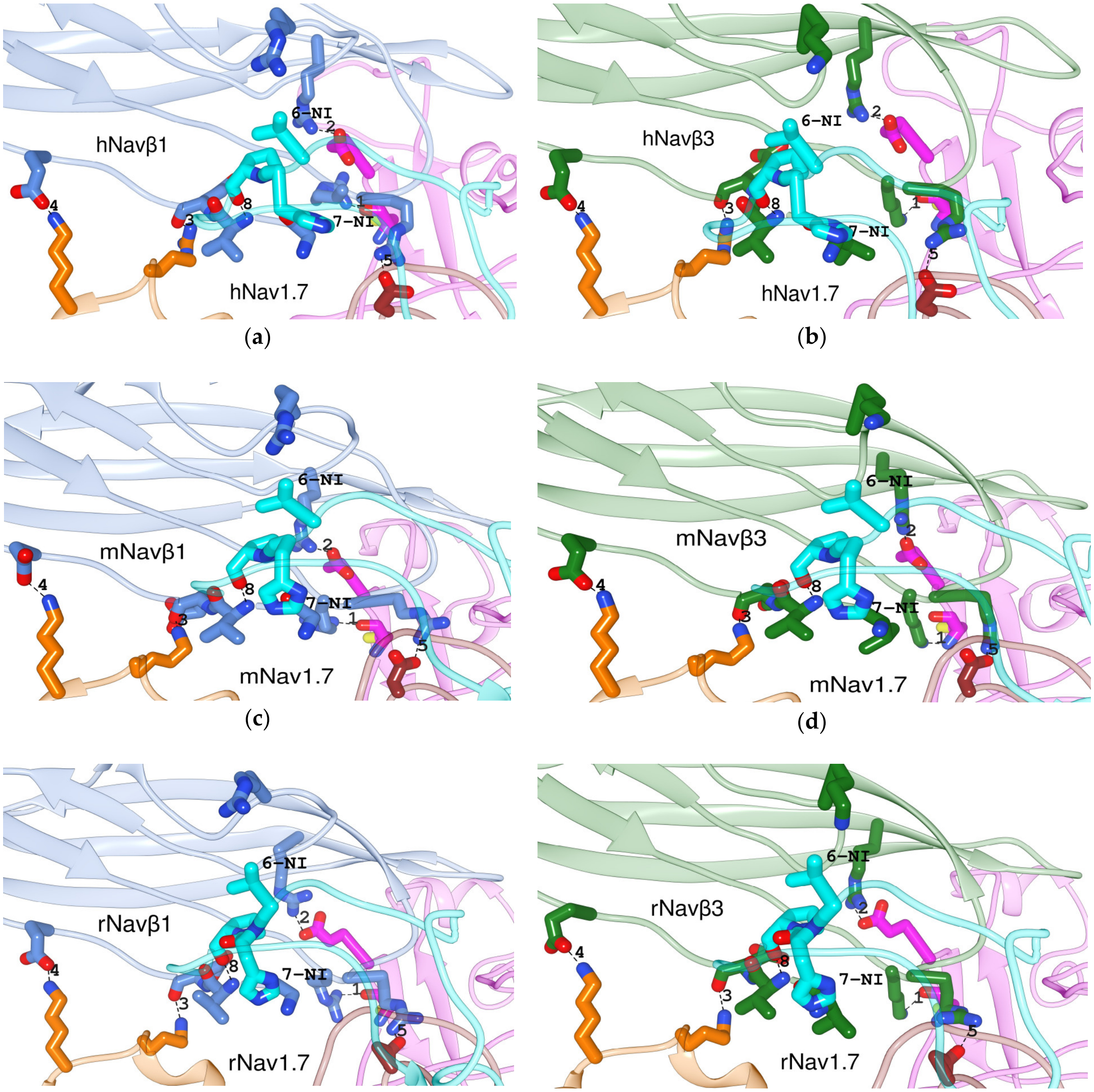
Figure S5. PPIs of the three h,m,rNa$_v$1.5 isoforms in complex with Na$_v$β1 and Na$_v$β3. (**a**, **b**) complexes of human; (**c**, **d**) mouse; (**e**, **f**) rat; residues that do not participate in the interface: gray; S5 DI: magenta; S1-S2 DIII: orange; S5 DIV: brown; S6 DIV: cyan; Na$_v$β1: cornflower blue; Na$_v$β3: forest green; numbers: PPI Id according to the Table S1; NI: no interaction.


(**a**) (**b**)

(**c**) (**d**)

(**e**) (**f**)

Figure S6. PPIs of the three h,m,rNa$_v$1.6 isoforms in complex with Na$_v$β1 and Na$_v$β3. (**a**, **b**) complexes of human; (**c**, **d**) mouse; (**e**, **f**) rat; residues that do not participate in the interface: gray; S5 DI: magenta; S1-S2 DIII: orange; S5 DIV: brown; S6 DIV: cyan; Na$_v$β1: cornflower blue; Na$_v$β3: forest green; numbers: PPI Id according to the Table S1; NI: no interaction.



(**a**) (**b**)

(**c**) (**d**)

(**e**) (**f**)

Figure S7. PPIs of the three h,m,rNa$_{\text{v}}$1.7 isoforms in complex with Na$_{\text{v}}$β1 and Na$_{\text{v}}$β3. (**a**, **b**) complexes of human; (**c**, **d**) mouse; (**e**, **f**) rat; residues that do not participate in the interface: gray; S5 DI: magenta; S1-S2 DIII: orange; S5 DIV: brown; S6 DIV: cyan; Na$_{\text{v}}$β1: cornflower blue; Na$_{\text{v}}$β3: forest green; numbers: PPI Id according to the Table S1; NI: no interaction.

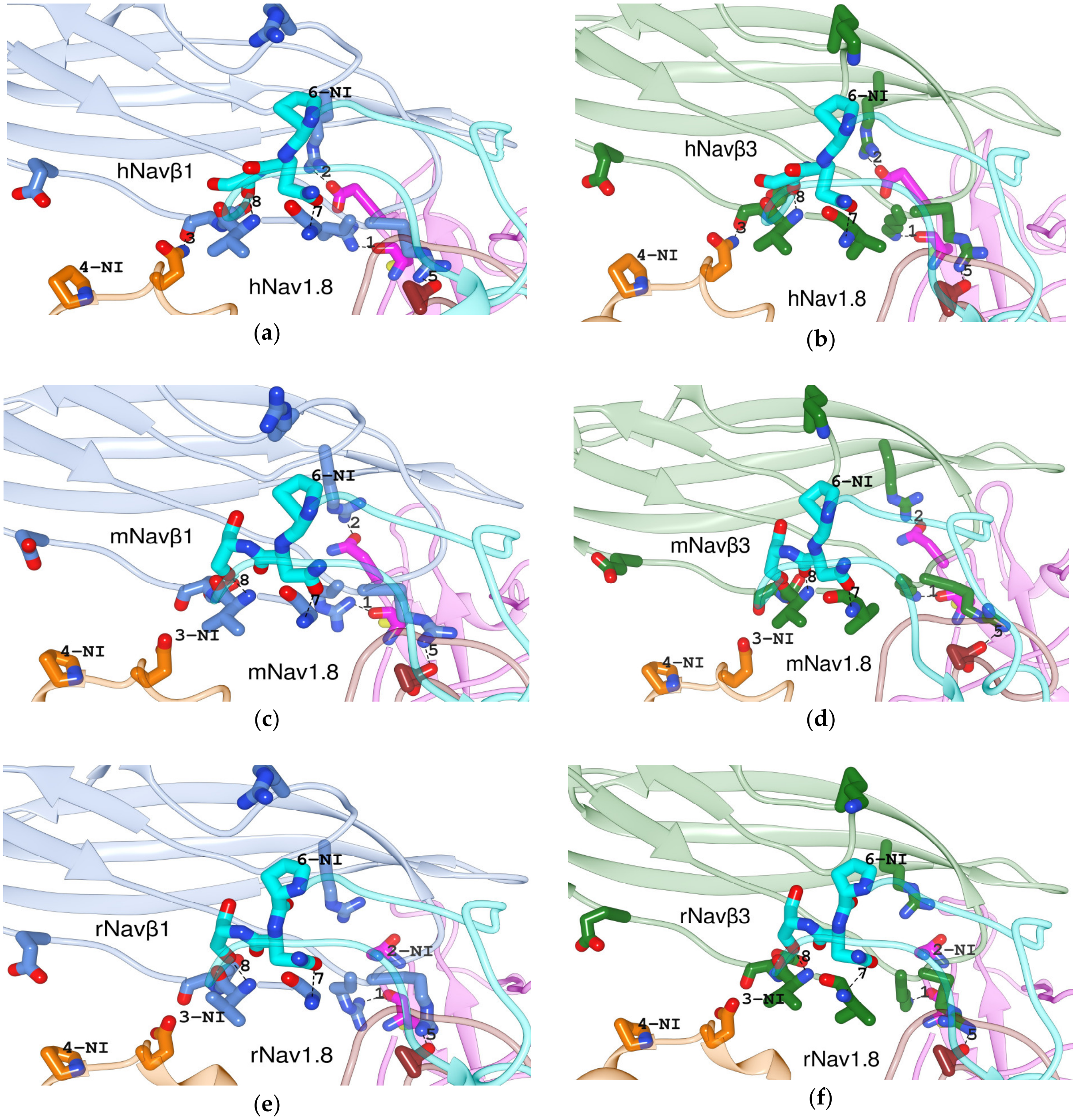


Figure S8. PPIs of the three h,m,rNa$_v$1.8 isoforms in complex with Na$_v$β1 and Na$_v$β3. (**a**, **b**) complexes of human; (**c**, **d**) mouse; (**e**, **f**) rat; residues that do not participate in the interface: gray; S5 DI: magenta; S1-S2 DIII: orange; S5 DIV: brown; S6 DIV: cyan; Na$_v$β1: cornflower blue; Na$_v$β3: forest green; numbers: PPI Id according to the Table S1; NI: no interaction.

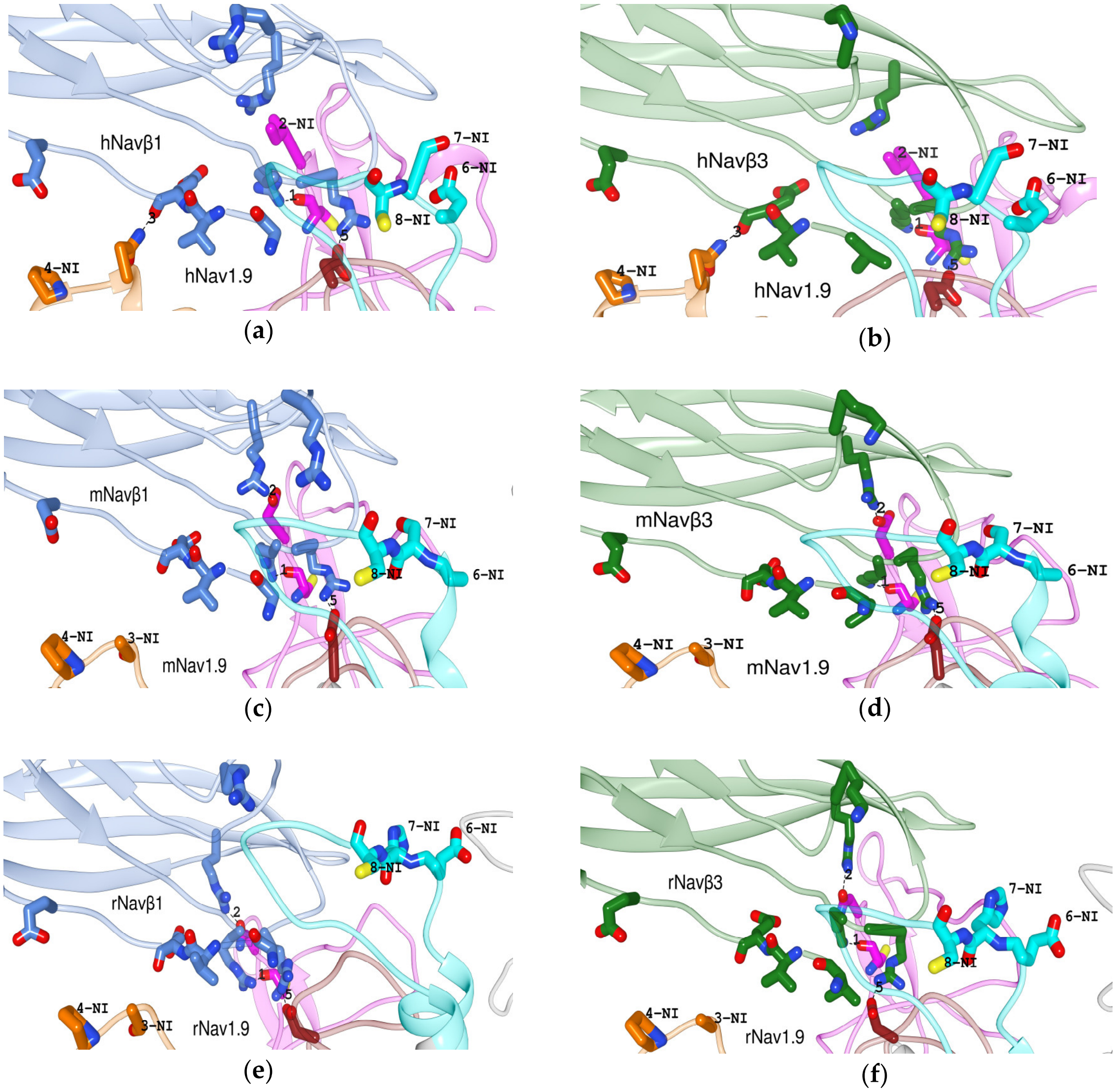


Figure S9. PPIs of the three h,m,rNa$_v$1.9 isoforms in complex with Na$_v$β1 and Na$_v$β3. (**a**, **b**) complexes of human; (**c**, **d**) mouse; (**e**, **f**) rat; residues that do not participate in the interface: gray; S5 DI: magenta; S1-S2 DIII: orange; S5 DIV: brown; S6 DIV: cyan; Na$_v$β1: cornflower blue; Na$_v$β3: forest green; numbers: PPI Id according to the Table S1; NI: no interaction.

Table S2. Residues of contact of Na$_v$s S4 DIII with the Na$_v$β1 and Na$_v$β3 subunits

Na$_v$α			**Na$_v$β**					
Isoform	**Code**	**[1]Residues S4 DIII**	**Subunit**	**Code**	**[2]Residues**	**Subunit**	**Code**	**[2]Residues**
hNa$_v$1.1	[4]P35498	gai**K**sl**R**tlr						
hNa$_v$1.2	[3]6J8E	gai**K**sl**R**tlr	hNa$_v$β1	[3]6AGF	mtf**K**ilc	hNa$_v$β3	[3]4L1D	npm**K**lrc
hNa$_v$1.3	[4]Q9NY46	gai**K**sl**R**tlr						

hNa$_v$1.4	[3]6AGF	gpi**K**sl**R**tlr						
hNa$_v$1.5	[4]Q14524	gpi**K**sl**R**tlr						
hNa$_v$1.6	[4]Q9UQD0	gai**K**sl**R**tlr						
hNa$_v$1.7	[3]6J8G	gpi**K**sl**R**tlr						
hNa$_v$1.8	[4]Q9Y5Y9	api**K**al**R**tlr						
hNa$_v$1.9	[4]Q9UI33	mel**K**sf**R**tlr						
mNa$_v$1.1	[4]A2APX8	gai**K**sl**R**tlr	mNa$_v\beta$1	[4]P97952	mtf**K**ilc	mNa$_v\beta$3	[4]Q8BHK2	nsm**K**lrc
mNa$_v$1.2	[4]B1AWN6	gai**K**sl**R**tlr						
mNa$_v$1.3	[4]A2ASI5	gai**K**sl**R**tlr						
mNa$_v$1.4	[4]Q9ER60	gpi**K**sl**R**tlr						
mNa$_v$1.5	[4]Q9JJV9	gpi**K**sl**R**tlr						
mNa$_v$1.6	[4]Q9WTU3	gai**K**sl**R**tlr						
mNa$_v$1.7	[4]Q62205	gpi**K**sl**R**tlr						
mNa$_v$1.8	[4]Q6QIY3	asi**K**al**R**tlr						
mNa$_v$1.9	[4]Q9R053	pnl**K**sf**R**nlr						
rNa$_v$1.1	[4]P04774	gai**K**sl**R**tlr	rNa$_v\beta$1	[4]Q00954	mtf**K**ilc	rNa$_v\beta$3	[4]Q9JK00	npm**K**lrc
rNa$_v$1.2	[4]P04775	gai**K**sl**R**tlr						
rNa$_v$1.3	[4]P08104	gai**K**sl**R**tlr						
rNa$_v$1.4	[4]P15390	gpi**K**sl**R**tlr						
rNa$_v$1.5	[4]P15389	gpi**K**sl**R**tlr						
rNa$_v$1.6	[4]O88420	gai**K**sl**R**tlr						
rNa$_v$1.7	[4]O08562	gpi**K**sl**R**tlr						
rNa$_v$1.8	[4]Q62968	asi**K**al**R**tlr						
rNa$_v$1.9	[4]O88457	psl**K**sf**R**tlr						

[1] S4 DIII residues that interact with Na$_v\beta$1 and Na$_v\beta$3 subunits; [2] Residues of Na$_v\beta$1 and Na$_v\beta$3 subunits that interact with S4 DIII; [3] PDB code: http://www.rcsb.org/; [4] UniProt code: https://www.uniprot.org/. Of note, table is in black&white. The interacting amino acids are in bold face and capital letters, e.g. K and R.

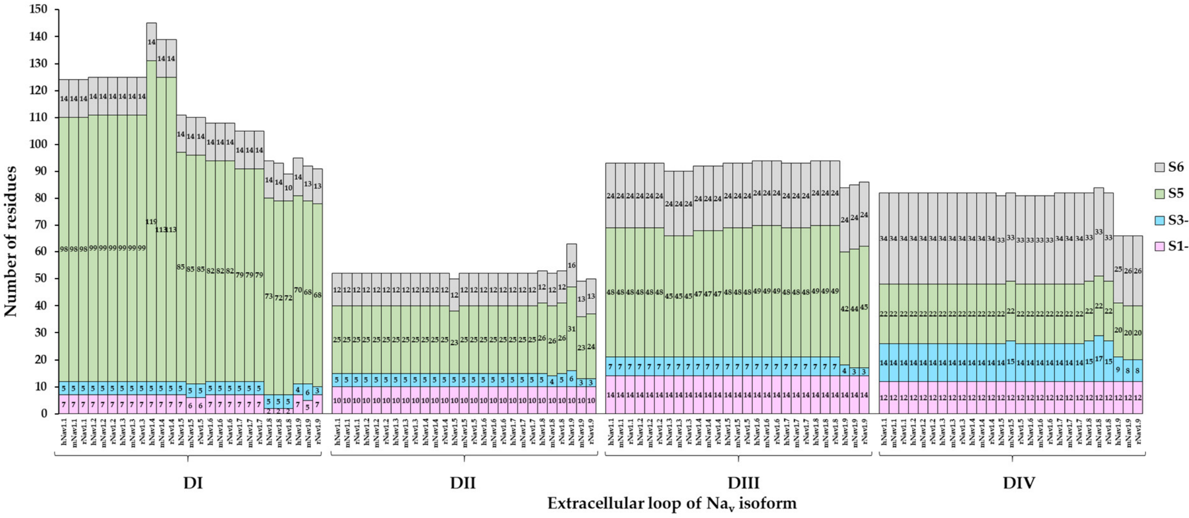
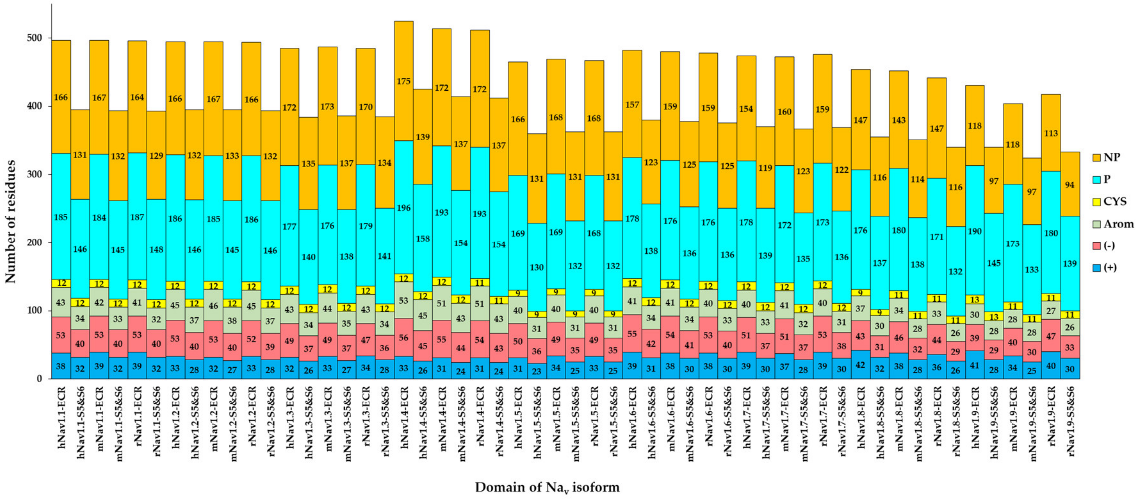
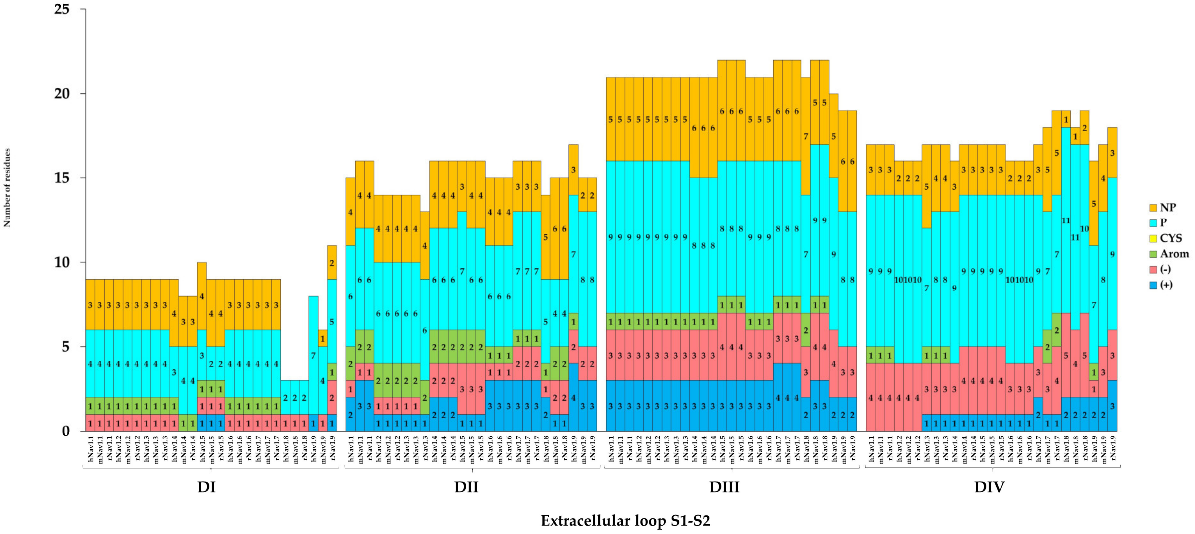


Figure S10. Length of extracellular loops of the NavS; extracellular loops: S1-S2, S3-S4, S5 and S6.

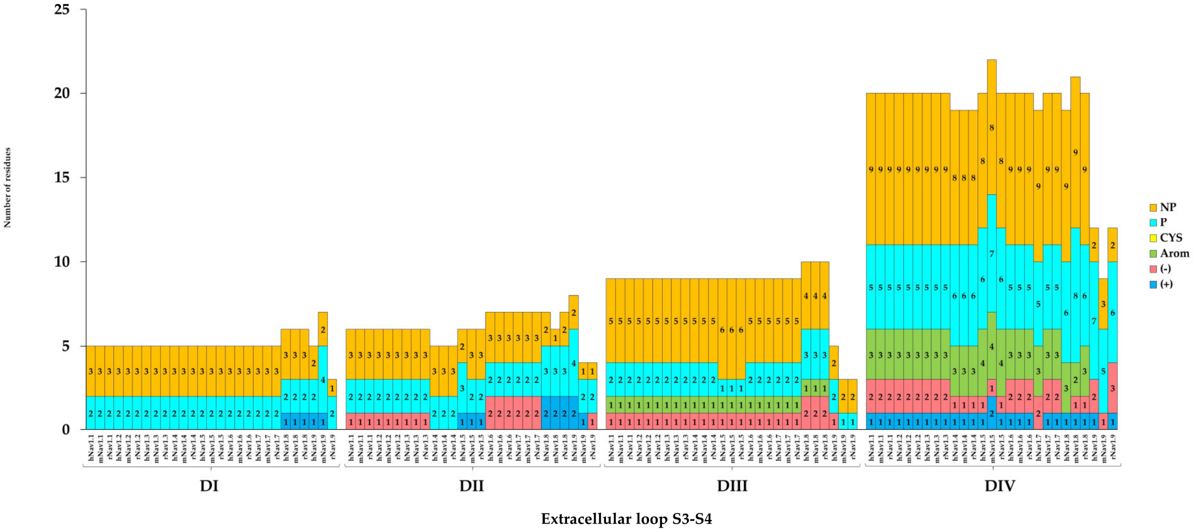


(**a**)

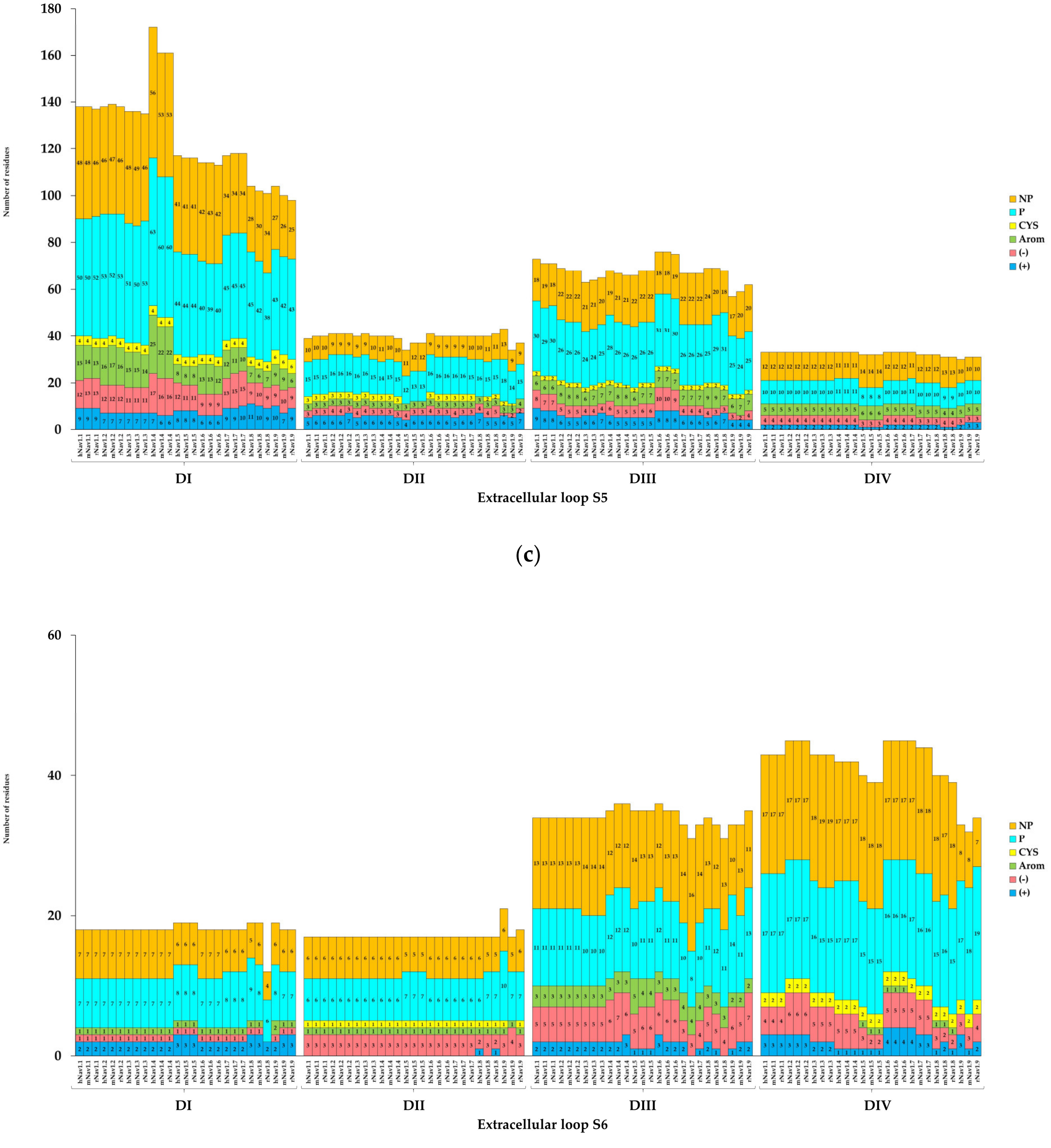
Figure S11. Properties of the residues of the extracellular region of the Na$_v$; (**a**) number of residues; ECR: extracellular region; S5 & S6: S5 and S6 extracellular loops; NP: nonpolar, P: polar; CYS: cysteines; Arom: aromatic; (-): negatively charged; (+): positively charged. The polar and non-polar residues represent the total residues of the analyzed region.


Number of residues
0
5
10
15
20
25
DI
DII
DIII
DIV
Extracellular loop S1-S2
NP
P
CYS
Arom
(-)
(+)

(**a**)


Number of residues
0
5
10
15
20
25
DI
DII
DIII
DIV
Extracellular loop S3-S4
NP
P
CYS
Arom
(-)
(+)

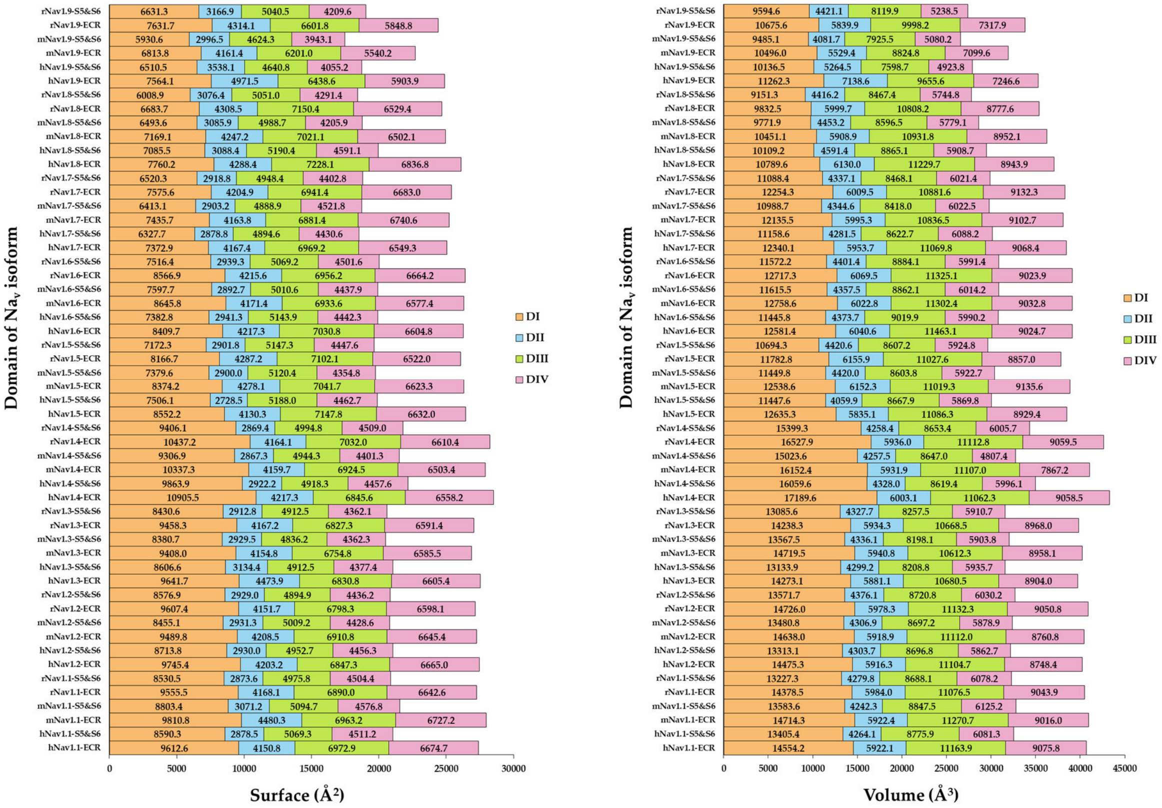
(**b**)



(**c**)

(**d**)

Figure S12. Properties of residues of Naᵥ extracellular loops; (**a**) S1-S2 extracellular loop; (**b**) S3-S4 extracellular loop; (**c**) S5 extracellular loop; (**d**) S6 extracellular loop; NP: nonpolar, P: polar, CYS: cysteines; Arom: aromatic; (-): negatively charged; (+): positively charged. The polar and non-polar residues represent the total residues of the analyzed region.



(**a**) (**b**)

Figure S13. Surface and volume properties of ECR and S5 and S6 extracellular loops (ECLs) of the Na$_v$s (**a**) SAA and (**b**) molecular volume; S5 & S6: S5 and S6 extracellular loops; DI – DIV: domains I – IV, respectively.

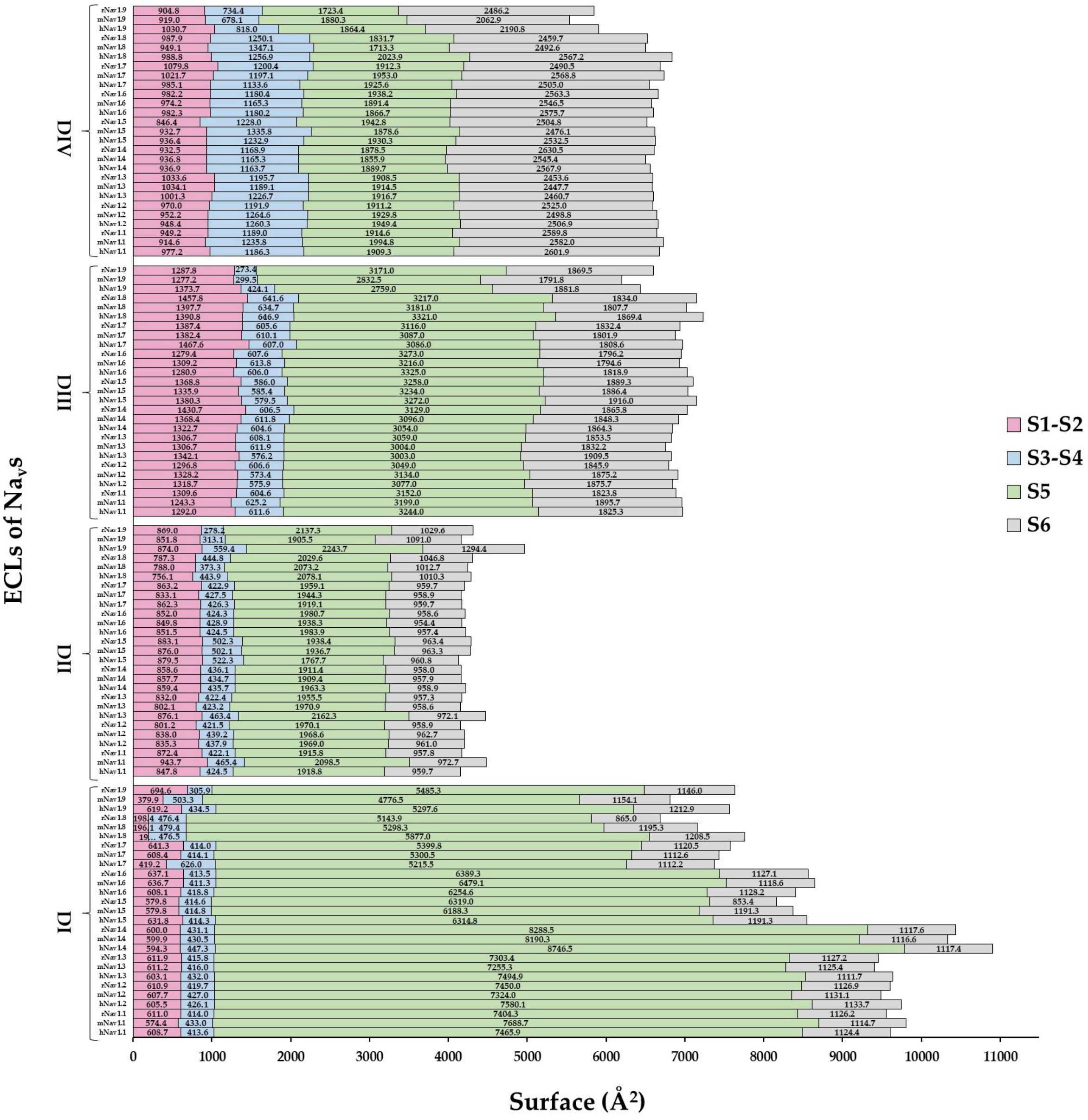


Figure S14. SAA of ECLs of the Na$_V$s; DI – DIV: domains I – IV, respectively.

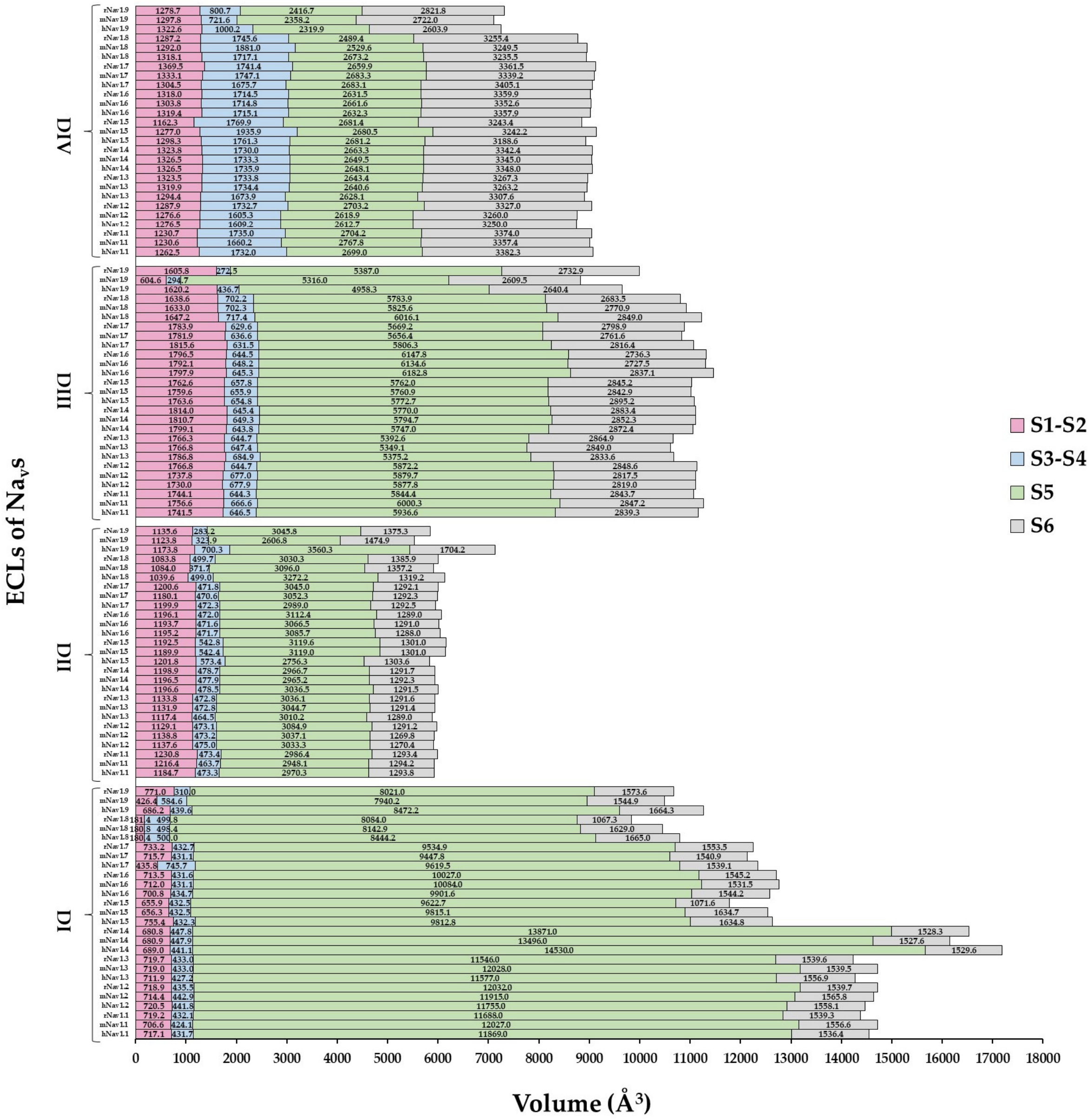


Figure S15. Molecular volume of ECLs of the Na_Vs; DI – DIV: domains I – IV, respectively.

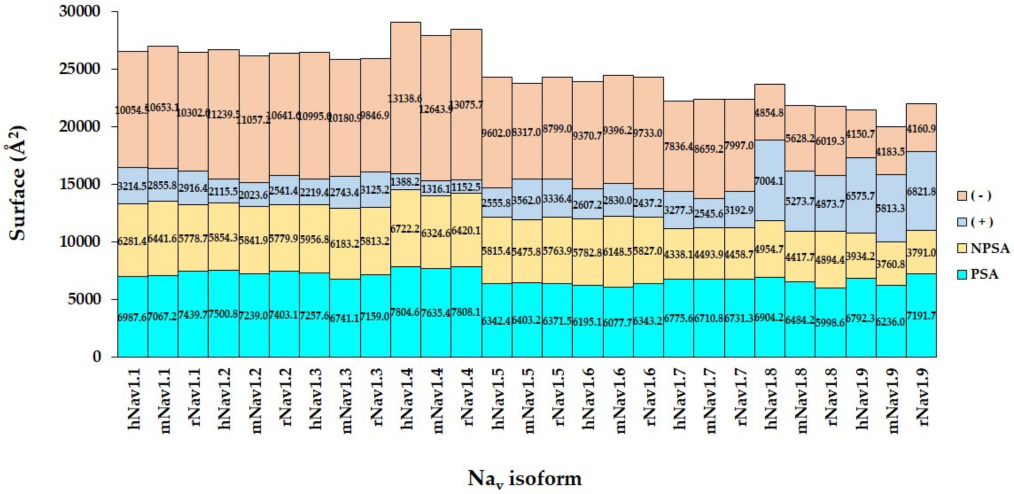
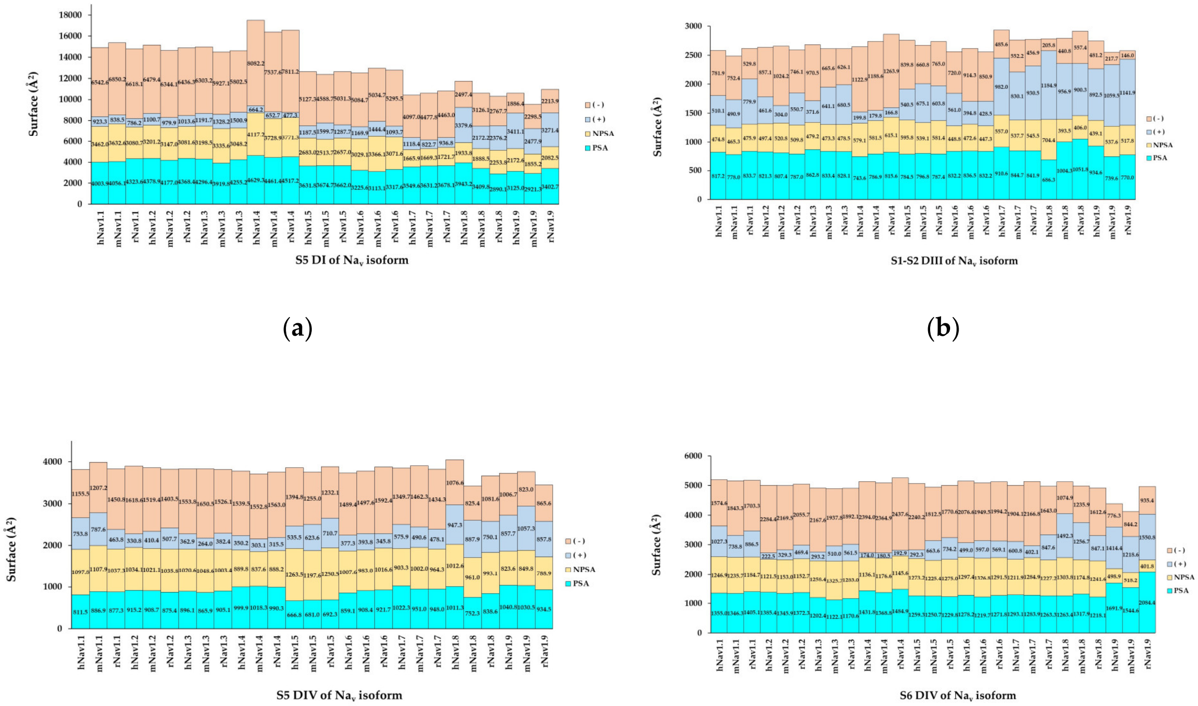


Figure S16. Properties of total SAA of IF-ECLs of Navs; PSA: Polar surface area; NPSA: non-polar surface area; (+): P-MEPS; (-): N-MEPS. The PSA and NPSA represents the total area of the analyzed region.



(**a**) (**b**)

(**c**) (**d**)

Figure S17. Properties of SAA of IF-ECLs of Nav; (**a**) S5 DI; (**b**) S1-S2 DIII (**c**) S5 DIV; (**d**) S6 DIV; PSA: Polar surface area; NPSA non-polar surface area; (+): P-MEPS; (-): N-MEPS. The PSA and NPSA represents the total area of the analyzed region.

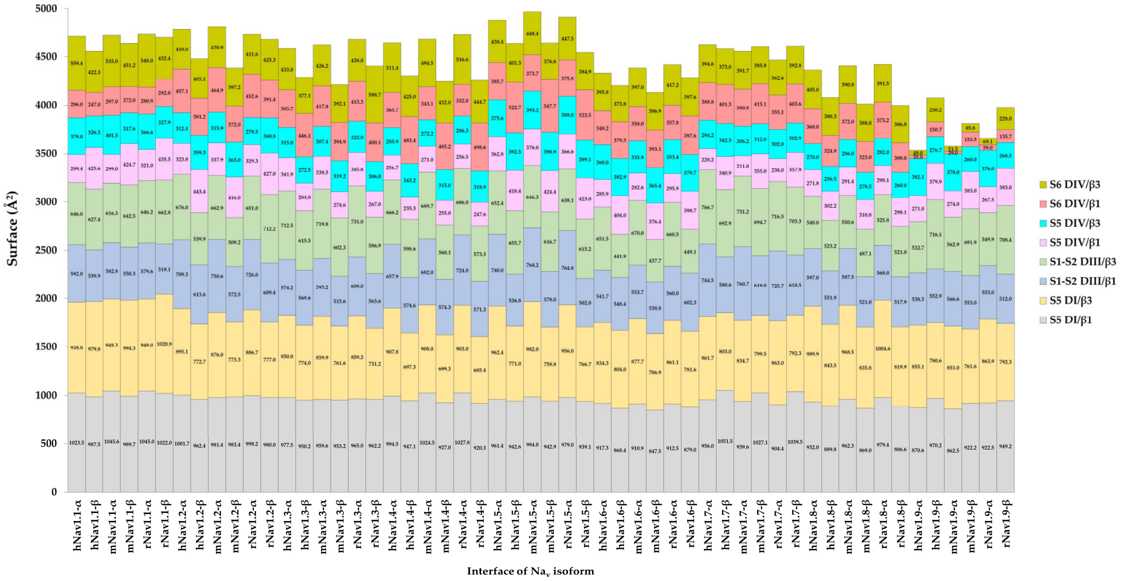


Figure S18. Total area of the atoms that form at the Na$_v$$\alpha$/Na$_v$β interface; α: area of the atoms in the α subunit that participate in the interface; β: area of the atoms in the β subunit that participate in the interface

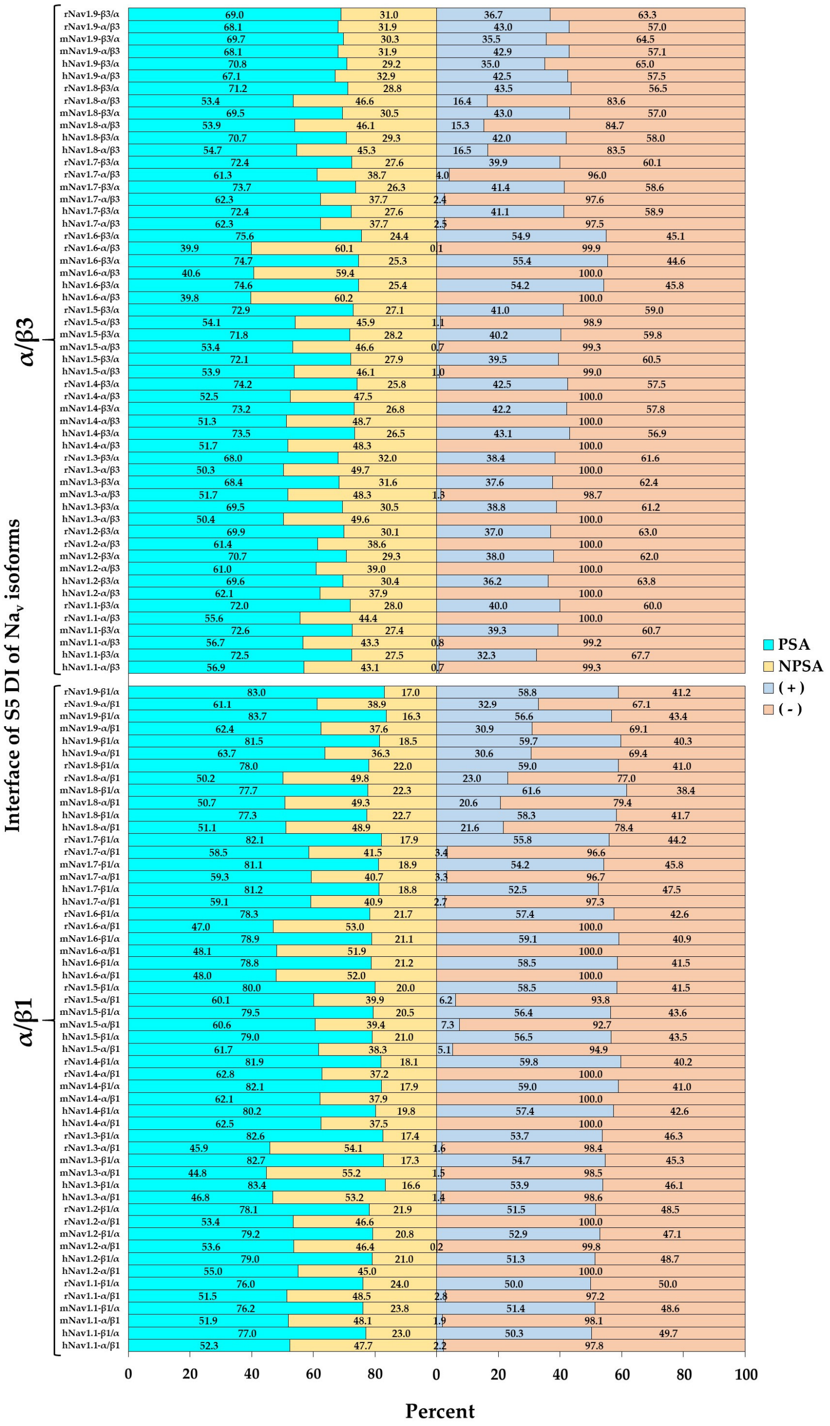


Figure S19. Percentage scores of the surface properties for atoms at Na$_v$α/Na$_v$β of ECL on S5 DI; α/ β: atoms in the α subunit that participate in the interface; β/α: atoms in the β subunit that participate in the interface; PSA: polar surface area; NPSA non-polar surface area; (+): P-MEPS; (-): N-MEPS. The PSA and NPSA represents the total area of the analyzed region.

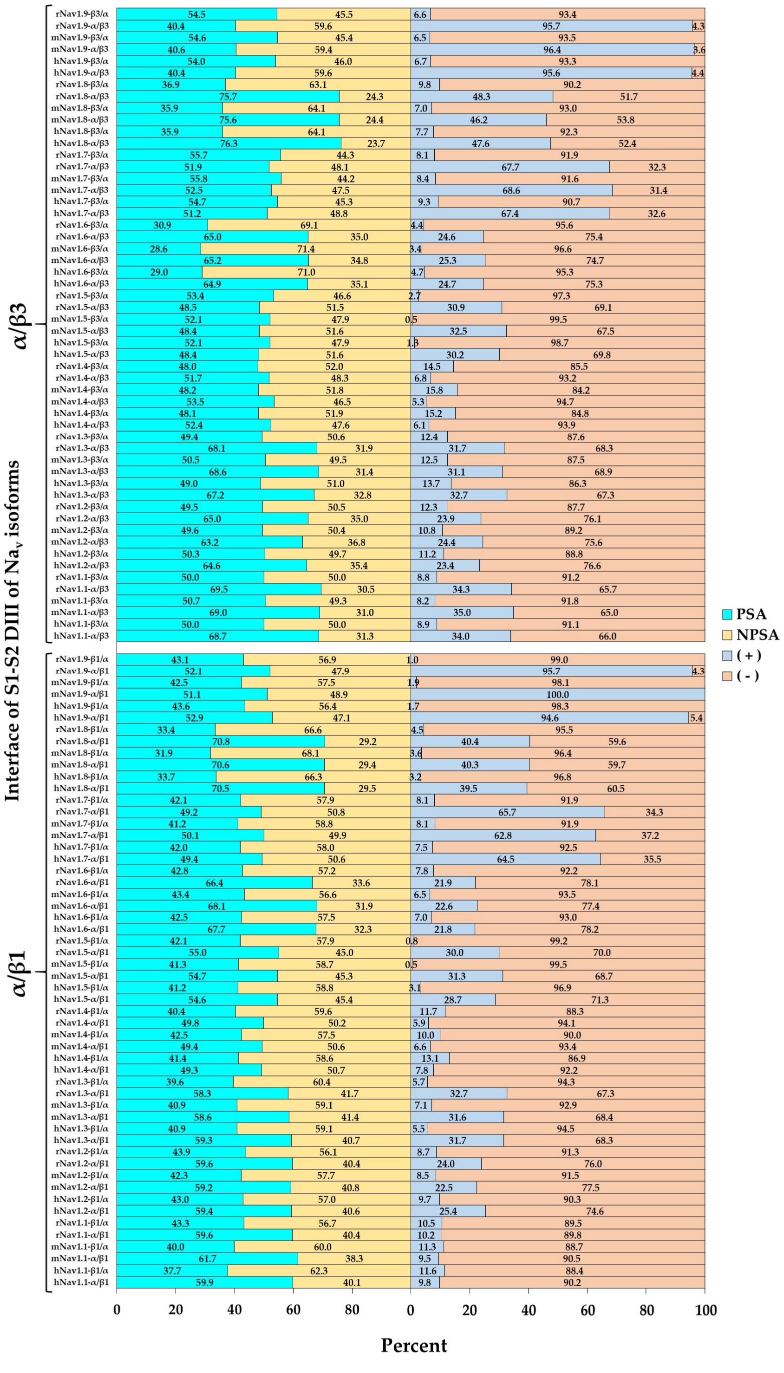


Figure S20. Percentage of the surface properties of atoms that form at the Na$_v$α/Na$_v$β interface of ECL S1-S2 DIII; α/ β: atoms in the α subunit that participate in the interface; β/α: atoms in the β subunit that participate in the interface; PSA: polar surface area; NPSA non-polar surface area; (+): P-MEPS; (-): N-MEPS. The PSA and NPSA represents the total area of the analyzed region.

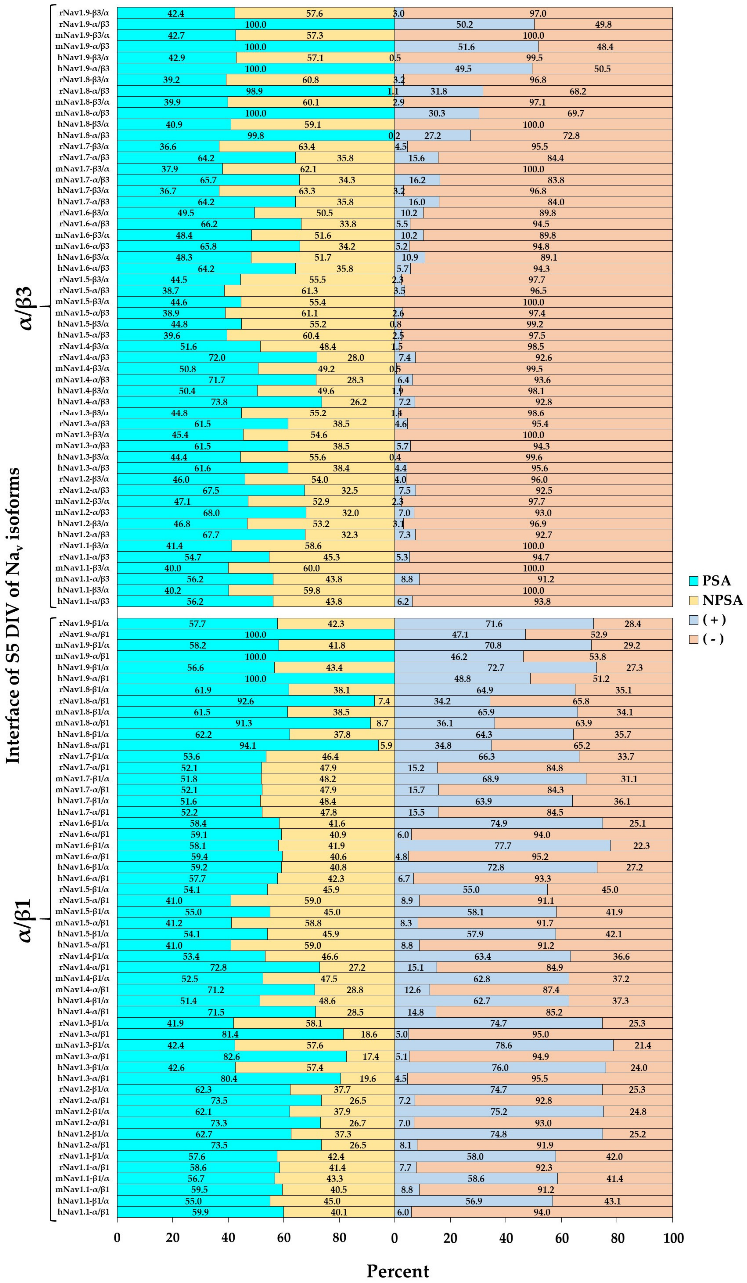


Figure S21. Percentage of the surface properties of atoms that form at the Na$_v$α/Na$_v$β interface of ECL S5 DIV; α/ β: atoms in the α subunit that participate in the interface; β/α: atoms in the β subunit that participate in the interface; PSA: polar surface area; NPSA non-polar surface area; (+): P-MEPS; (-): N-MEPS. The PSA and NPSA represents the total area of the analyzed region.

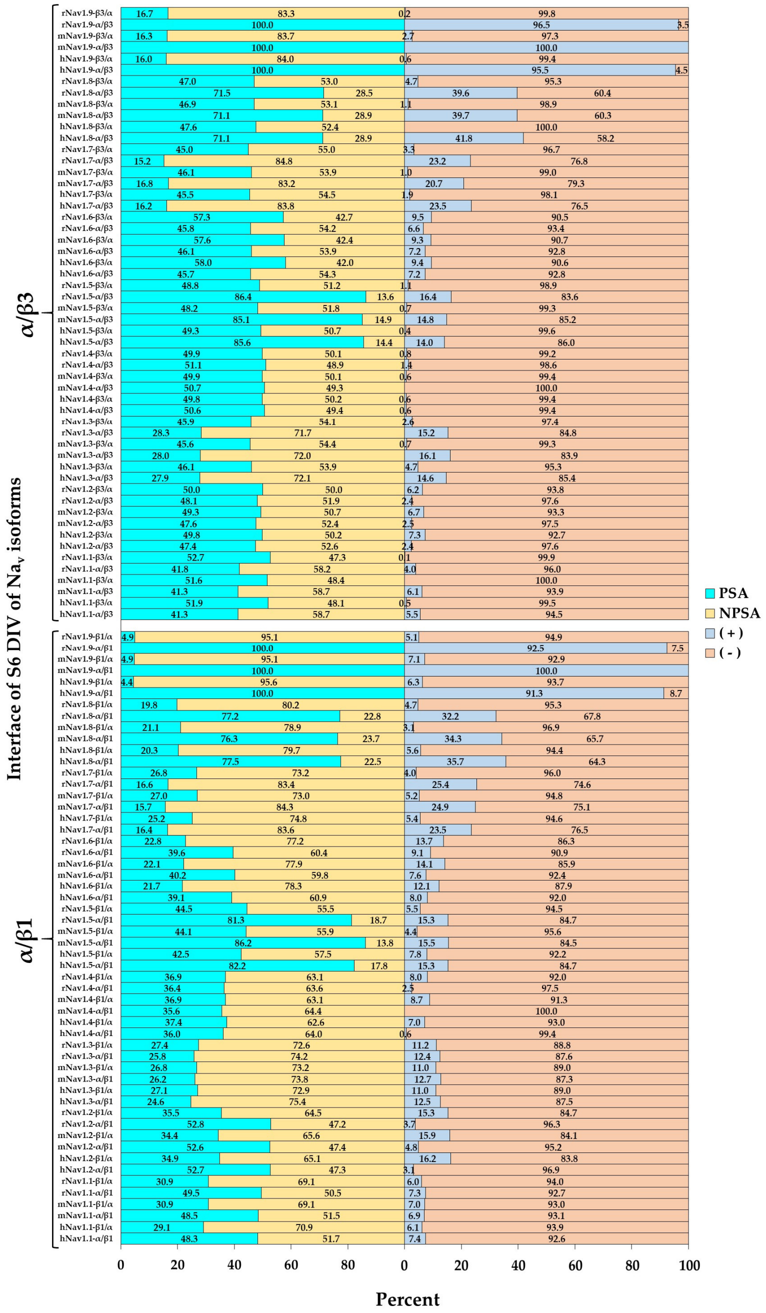
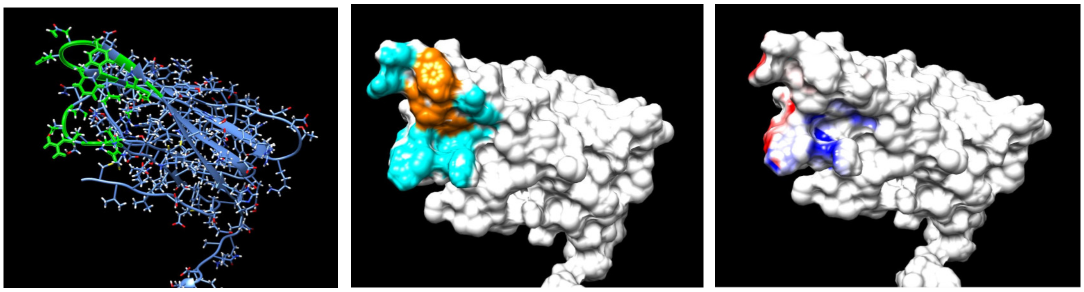


Figure S22. Percentage of the surface properties of atoms that form at the Na$_v$α/Na$_v$β interface of ECL S6 DIV; α/β: atoms in the α subunit that participate in the interface; β/α: atoms in the β subunit that participate in the interface; PSA: polar surface area; NPSA non-polar surface area; (+): P-MEPS; (-): N-MEPS. The PSA and NPSA represents the total area of the analyzed region.

Table S3. Interface area of the IF-ECLs of the Na$_v$s

Na$_v$ isoform	**IF-ECLs of Na$_v$s**	**HUMAN**		**MOUSE**		**RAT**	
		Na$_v$β1	**Na$_v$β3**	**Na$_v$β1**	**Na$_v$β3**	**Na$_v$β1**	**Na$_v$β3**
Na$_v$1.1	S5 DI	621.8	584.1	664.3	527.2	491.0	367.6
	S1-S2 DIII	294.4	339.8	308.4	299.6	263.0	332.6
	S5 DIV	120.2	151.9	163.1	170.8	137.1	164.6
	S6 DIV	217.5	264.1	270.0	295.9	210.0	327.5
Na$_v$1.2	S5 DI	587.7	552.4	612.8	485.6	535.8	490.1
	S1-S2 DIII	333.8	360.1	350.4	327.3	317.6	380.6
	S5 DIV	136.7	174.5	171.4	158.9	140.5	162.4
	S6 DIV	208.8	254.6	234.0	259.2	220.8	357.5
Na$_v$1.3	S5 DI	602.3	544.5	664.0	554.6	498.0	387.7
	S1-S2 DIII	293.1	330.3	313.2	309.4	315.0	378.9
	S5 DIV	137.9	183.6	171.8	162.6	130.7	135.3
	S6 DIV	187.1	251.5	249.4	272.4	215.4	312.2
Na$_v$1.4	S5 DI	578.6	550.6	559.4	441.0	549.9	434.7
	S1-S2 DIII	304.8	341.4	359.2	350.4	318.0	399.1
	S5 DIV	144.0	170.6	154.6	135.4	129.0	158.5
	S6 DIV	248.2	268.2	284.9	282.3	227.2	300.1
Na$_v$1.5	S5 DI	570.0	534.6	610.2	483.8	535.7	417.3
	S1-S2 DIII	324.7	360.3	368.6	362.0	293.9	357.9
	S5 DIV	175.3	200.0	189.5	184.6	170.9	197.0
	S6 DIV	249.3	281.2	280.7	274.9	218.7	314.3
Na$_v$1.6	S5 DI	525.5	471.6	552.9	440.3	465.3	388.4
	S1-S2 DIII	325.5	360.3	294.8	278.5	287.4	334.5
	S5 DIV	147.2	169.5	167.4	162.1	119.5	145.0
	S6 DIV	199.2	253.7	269.3	288.1	216.1	314.0
Na$_v$1.7	S5 DI	665.5	593.6	663.3	544.9	576.2	461.7
	S1-S2 DIII	381.9	425.8	389.6	380.2	376.9	428.6
	S5 DIV	108.5	141.4	143.9	140.4	89.0	122.7
	S6 DIV	203.5	236.8	232.6	252.1	183.5	261.1
Na$_v$1.8	S5 DI	585.5	551.0	623.7	510.7	583.5	464.8
	S1-S2 DIII	292.5	343.5	279.5	273.7	261.0	330.9
	S5 DIV	179.3	207.6	165.8	136.8	115.7	135.3
	S6 DIV	125.0	162.7	262.0	260.1	211.3	309.4
Na$_v$1.9	S5 DI	633.0	575.7	602.7	520.6	479.9	306.7
	S1-S2 DIII	317.7	385.5	266.2	271.1	298.4	368.2
	S5 DIV	141.6	186.1	138.8	133.1	96.4	128.7
	S6 DIV	17.7	24.9	14.7	25.7	30.0	34.9

The units of area are in Å^2. The interface surface area buried was calculated.



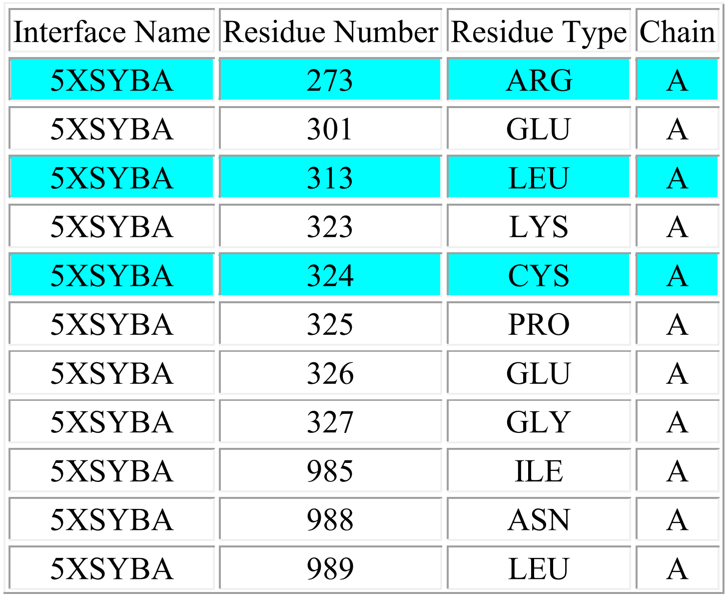
(**a**) (**b**) (**c**)

Figure S23. Demonstration example of the surface of atoms that form the interface in hNa$_v$β1 with the ECL S5 DI in the HMSR of hNa$_v$β1/hNa$_v$1.4 complex. (**a**) atoms that form the interface: green; (**b**) PSA: cyan and NPSA: orange interface area; (**c**) positive P-MEPS interface area: blue and N-MEPS: red; atoms and surface that don't form the interface: cornflower blue and white, respectively. MEPS was calculated for the individual structures and models using the Adaptive Poisson-Boltzmann Solver (APBS) [65] plug-in tool in Chimera Alpha 1.14 [61]. Of note, atom areas that contribute to the interface do not necessarily constitute buried areas (see Table S3).

Validation of the interface residue sequence between Na$_v$α subunit and Na$_v$β1 and Na$_v$β3 subunits

Finally we document the protein-protein interaction zone computed by HOTREGION for the eel template for sodium channel 3D models (PDB code: 5XSY) by a Web-based bioinformatic tool at http://prism.ccbb.ku.edu.tr/hotregion/ . The identified residues correspond to all those which can be studied by Chimera tool, too. For more details consult the software literature [110].

Table S4: The output list of interacting residues between both subunits for the eel sodium channel (PDB code: 5XSY) by HotRegion, a database of cooperative hot spots.



Interface Name	Residue Number	Residue Type	Chain
5XSYBA	273	ARG	A
5XSYBA	301	GLU	A
5XSYBA	313	LEU	A
5XSYBA	323	LYS	A
5XSYBA	324	CYS	A
5XSYBA	325	PRO	A
5XSYBA	326	GLU	A
5XSYBA	327	GLY	A
5XSYBA	985	ILE	A
5XSYBA	988	ASN	A
5XSYBA	989	LEU	A

5XSYBA	992	THR	A
5XSYBA	996	ILE	A
5XSYBA	1005	PHE	A
5XSYBA	1022	ILE	A
5XSYBA	1023	TYR	A
5XSYBA	1025	TRP	A
5XSYBA	1026	ARG	A
5XSYBA	1028	ARG	A
5XSYBA	1032	VAL	A
5XSYBA	1036	TYR	A
5XSYBA	1039	LYS	A
5XSYBA	1040	VAL	A
5XSYBA	1043	TYR	A
5XSYBA	1044	VAL	A
5XSYBA	1047	VAL	A
5XSYBA	1475	TYR	A
5XSYBA	1484	ASP	A
5XSYBA	1489	GLU	A
5XSYBA	1528	ASN	A
5XSYBA	1529	PRO	A
5XSYBA	1530	GLY	A
5XSYBA	1531	THR	A
5XSYBA	24	ALA	B
5XSYBA	25	CYS	B
5XSYBA	26	VAL	B
5XSYBA	27	GLU	B
5XSYBA	28	VAL	B
5XSYBA	31	ASP	B
5XSYBA	45	ILE	B
5XSYBA	47	CYS	B
5XSYBA	49	MET	B
5XSYBA	50	ARG	B
5XSYBA	105	GLN	B
5XSYBA	106	ASP	B
5XSYBA	132	PHE	B

5XSYBA	135	TYR	B
5XSYBA	137	PHE	B
5XSYBA	158	ALA	B
5XSYBA	159	SER	B
5XSYBA	162	SER	B
5XSYBA	166	MET	B
5XSYBA	170	ILE	B
5XSYBA	173	LEU	B
5XSYBA	174	GLN	B
5XSYBA	177	LEU	B
5XSYBA	180	GLU	B
5XSYBA	181	MET	B
5XSYBA	184	CYS	B
5XSYBA	185	TYR	B
5XSYBA	189	ALA	B

The Chimera scripts are documented here which were applied throughout the study.

< Protein – Protein Interface = PPI detection >

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measure buriedArea #0.1 #0.2
```

>Tools>Structure analysis>Attribute calculator

>Insert formula: sum(atom.areaSES)

Select and classif residues

<Superposition of PPIs by Match Maker >

open proteins 1 and 2

commands:

```
match #2 to #1 bring #3
swapaa (aminoacid name) #1 or #2:Number.(Chain)
```

```
mm #2 to #1/a pair bs alg sw matrix PAM-150 ss false cut 5.0
```

matrix similarity-matrix

The similarity-matrix can be any of: BLOSUM-30, BLOSUM-35, BLOSUM-40, BLOSUM-45, BLOSUM-50, BLOSUM-55, BLOSUM-60, BLOSUM-62 (default), BLOSUM-65, BLOSUM-70, BLOSUM-75, BLOSUM-80, BLOSUM-85, BLOSUM-90, BLOSUM-100, BLOSUM-N, PAM-40, PAM-120, PAM-150, PAM-250, SDM, HSDM, Nucleic.

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<Generation of PPI surface, buried surface area and calculate the area>

measure buriedArea #0.1 #0.2

>Tools>Structure analysis>Attribute calculator

>Insert formula: sum(atom.areaSES)

Interf

select @/buriedSESArea> 1

Aplly
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