Supplementary File

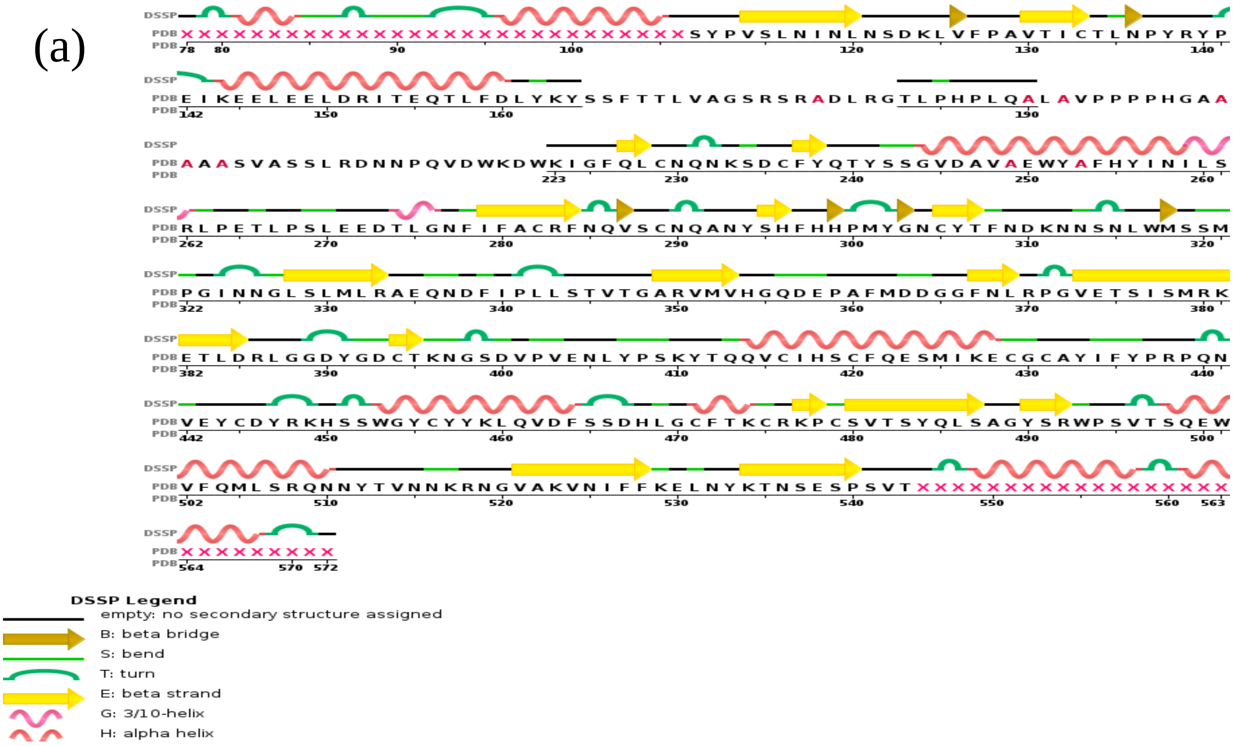
An epithelial sodium channel (ENaC)-specific aptamer determined through structure-based virtual screening for the development of hypertension early detection system

Dina Ratna Komala[1], Ari Hardianto[1], Shabarni Gaffar[1], Yeni Wahyuni Hartati[1]

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Indonesia

(Email: dina15004@mail.unpad.ac.id)

(a)



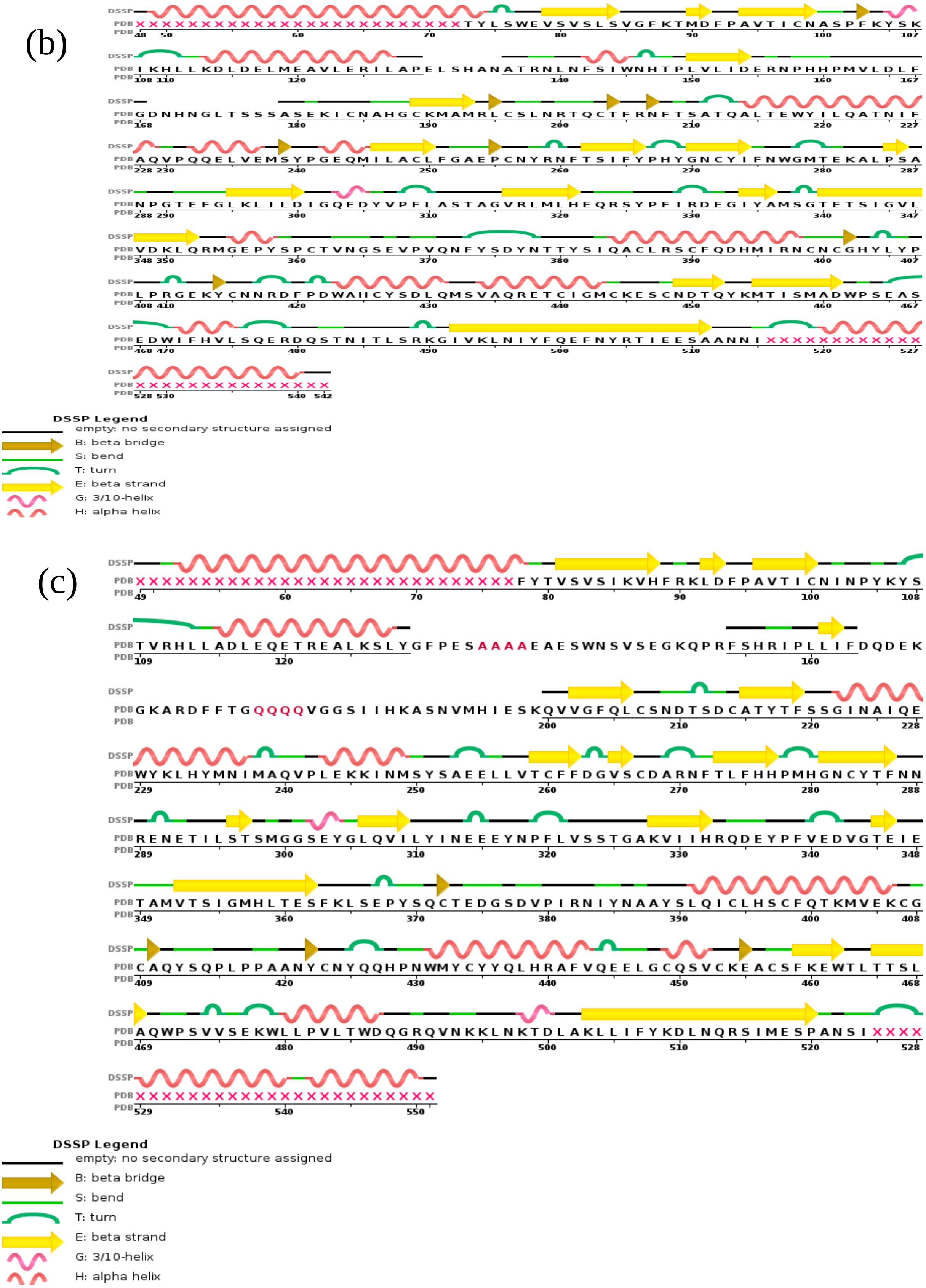
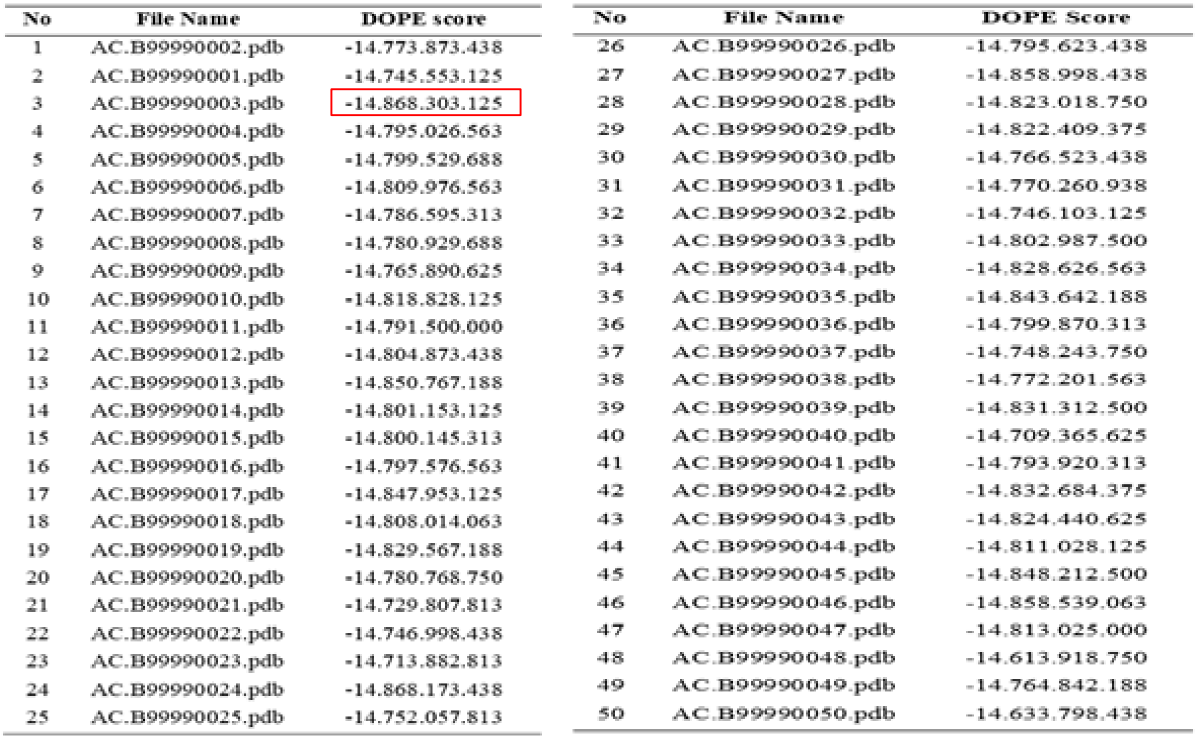


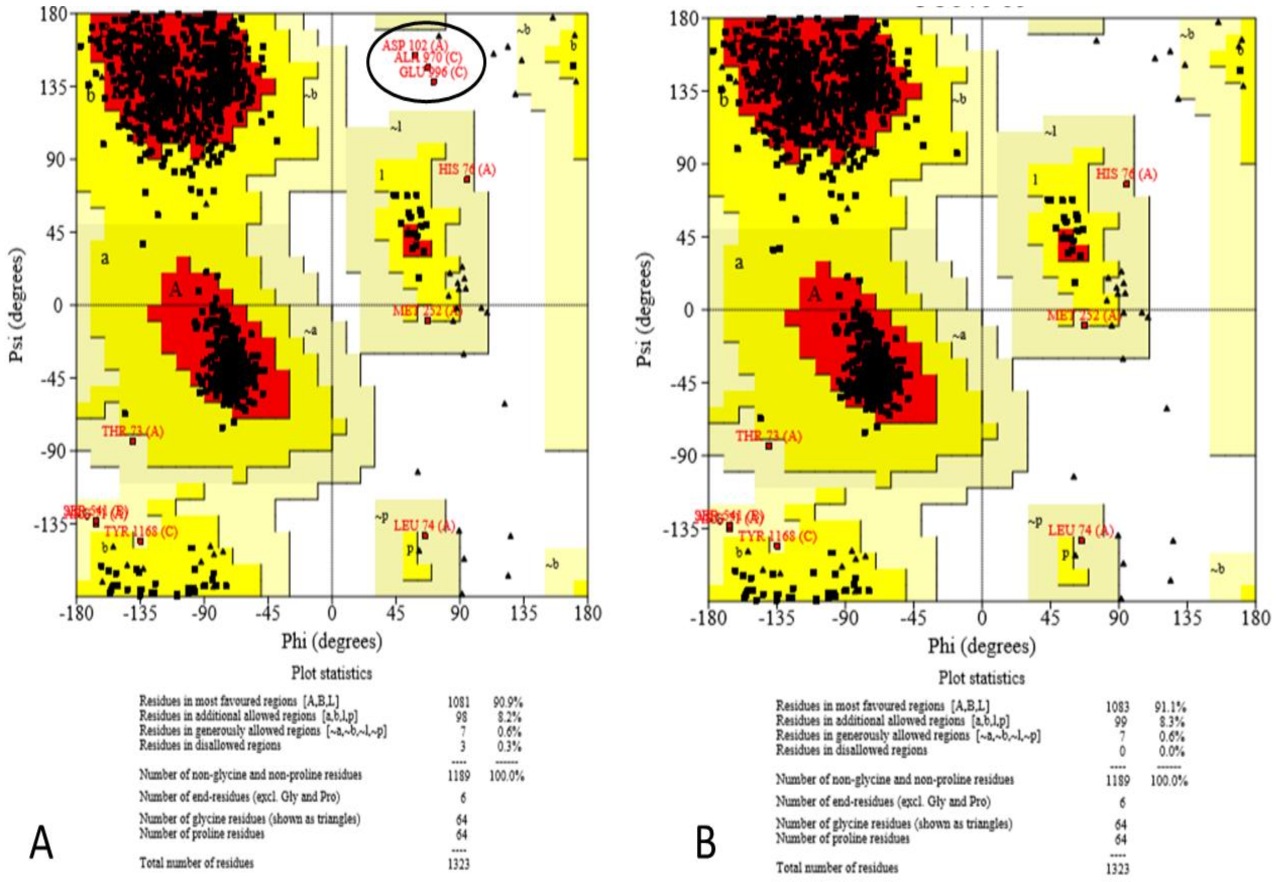
Figure S1. Subunits (a) α, (b) β, (c) γ sequences of ENaC protein (PDB ID: 6BQN) retrieved from RCSB Protein Data Bank (PDB). Each subunit has missing residues in the middle of sequence which is indicated by the empty DSSP sign at the secondary structure.



No	File Name	DOPE score
1	AC.B99990002.pdb	-14.773.873.438
2	AC.B99990001.pdb	-14.745.553.125
3	AC.B99990003.pdb	-14.868.303.125
4	AC.B99990004.pdb	-14.795.026.563
5	AC.B99990005.pdb	-14.799.529.688
6	AC.B99990006.pdb	-14.809.976.563
7	AC.B99990007.pdb	-14.786.595.313
8	AC.B99990008.pdb	-14.780.929.688
9	AC.B99990009.pdb	-14.765.890.625
10	AC.B99990010.pdb	-14.818.828.125
11	AC.B99990011.pdb	-14.791.500.000
12	AC.B99990012.pdb	-14.804.873.438
13	AC.B99990013.pdb	-14.850.767.188
14	AC.B99990014.pdb	-14.801.153.125
15	AC.B99990015.pdb	-14.800.145.313
16	AC.B99990016.pdb	-14.797.576.563
17	AC.B99990017.pdb	-14.847.953.125
18	AC.B99990018.pdb	-14.808.014.063
19	AC.B99990019.pdb	-14.829.567.188
20	AC.B99990020.pdb	-14.780.768.750
21	AC.B99990021.pdb	-14.729.807.813
22	AC.B99990022.pdb	-14.746.998.438
23	AC.B99990023.pdb	-14.713.882.813
24	AC.B99990024.pdb	-14.868.173.438
25	AC.B99990025.pdb	-14.752.057.813

No	File Name	DOPE Score
26	AC.B99990026.pdb	-14.795.623.438
27	AC.B99990027.pdb	-14.858.998.438
28	AC.B99990028.pdb	-14.823.018.750
29	AC.B99990029.pdb	-14.822.409.375
30	AC.B99990030.pdb	-14.766.523.438
31	AC.B99990031.pdb	-14.770.260.938
32	AC.B99990032.pdb	-14.746.103.125
33	AC.B99990033.pdb	-14.802.987.500
34	AC.B99990034.pdb	-14.828.626.563
35	AC.B99990035.pdb	-14.843.642.188
36	AC.B99990036.pdb	-14.799.870.313
37	AC.B99990037.pdb	-14.748.243.750
38	AC.B99990038.pdb	-14.772.201.563
39	AC.B99990039.pdb	-14.831.312.500
40	AC.B99990040.pdb	-14.709.365.625
41	AC.B99990041.pdb	-14.793.920.313
42	AC.B99990042.pdb	-14.832.684.375
43	AC.B99990043.pdb	-14.824.440.625
44	AC.B99990044.pdb	-14.811.028.125
45	AC.B99990045.pdb	-14.848.212.500
46	AC.B99990046.pdb	-14.858.539.063
47	AC.B99990047.pdb	-14.813.025.000
48	AC.B99990048.pdb	-14.613.918.750
49	AC.B99990049.pdb	-14.764.842.188
50	AC.B99990050.pdb	-14.633.798.438

Figure S2. Results of modeling of ENaC protein with subunits A, B and C (PDB ID: 6BQN) using Modeller 9.20 program



Plot statistics (A)

Residues in most favoured regions [A,B,L]	1081	90.9%
Residues in additional allowed regions [a,b,l,p]	98	8.2%
Residues in generously allowed regions [~a,~b,~l,~p]	7	0.6%
Residues in disallowed regions	3	0.3%
	----	------
Number of non-glycine and non-proline residues	1189	100.0%
Number of end-residues (excl. Gly and Pro)	6	
Number of glycine residues (shown as triangles)	64	
Number of proline residues	64	

Total number of residues	1323	

Plot statistics (B)

Residues in most favoured regions [A,B,L]	1083	91.1%
Residues in additional allowed regions [a,b,l,p]	99	8.3%
Residues in generously allowed regions [~a,~b,~l,~p]	7	0.6%
Residues in disallowed regions	0	0.0%
	----	------
Number of non-glycine and non-proline residues	1189	100.0%
Number of end-residues (excl. Gly and Pro)	6	
Number of glycine residues (shown as triangles)	64	
Number of proline residues	64	

Total number of residues	1323	

Figure S3. Evaluation of ENaC protein model subunits A, B and C using the PROCHECK program (A) before loop optimization, (B) after loop optimization.

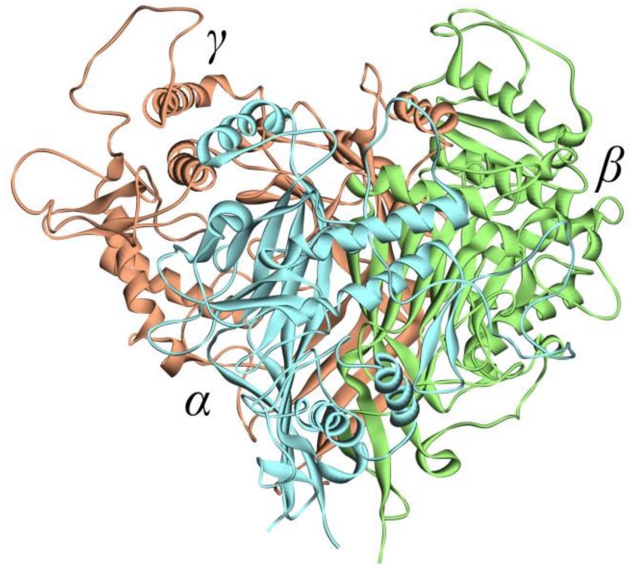


Figure S4. Three-dimensional structure of ENaC after re-modelling using Modeller 9.20 program.

Table S1. Unfavorable interactions in the iDE-ENaC complex obtained from molecular docking

No.	iDE		ENaC			Interaction
	Atom Type	Nucleotide	Subunit	Residue	Atom Type	
1	NE	C69	α	ARG386	O5'	Unfavorable Bump
2	OD2	C67	α	ASP393	OP1	Unfavorable Negative-Negative
3	O	G56	β	THR280	N2	Unfavorable Bump
4	O	G56	β	THR280	H22	Unfavorable Bump
5	O	G56	β	THR280	H21	Unfavorable Bump
6	HH22	G57	β	ARG324	H21	Unfavorable Donor-Donor

Table S2. Analysis of iDE-creatinine interactions using Biovia Discovery Studio obtained from molecular docking

No	iDE		Creatinine		Interaction
	Nucleotide	Atom Type*	Residue	Atom Type*	
1	T43	OP2	ARG96	HH22	Hydrogen Bond;Electrostatic
2	T59	OP1	LYS196	HZ1	Hydrogen Bond;Electrostatic
3	T59	OP1	LYS196	HZ2	Hydrogen Bond;Electrostatic
4	G46	OP2	LYS319	HZ3	Hydrogen Bond;Electrostatic
5	G41	OP1	LYS32	NZ	Electrostatic
6	T43	OP2	ARG96	NH1	Electrostatic
7	T17	OP1	ARG96	NH2	Electrostatic
8	T43	OP1	ARG96	NH2	Electrostatic
9	A45	OP1	LYS319	NZ	Electrostatic
10	G15	OP1	ARG320	NH1	Electrostatic
11	G16	OP1	ARG320	NH1	Electrostatic
12	G16	OP2	ARG320	NH2	Electrostatic
13	T43	OP2	ARG341	NH2	Electrostatic
14	G12	H21	ASP195	OD1	Hydrogen Bond
15	G12	H22	ASP195	OD1	Hydrogen Bond
16	C60	O3'	THR180	HG1	Hydrogen Bond
17	T17	O4	SER285	HG	Hydrogen Bond
18	G44	OP2	THR313	HG1	Hydrogen Bond
19	T43	OP2	GLN318	HE22	Hydrogen Bond
20	T43	Pi-Orbitals	ALA339	O	Pi-Lone Pair
21	C23	Pi-Orbitals	PHE68	Pi-Orbitals	Hydrophobic
22	T43	Pi-Orbitals	ALA339	Amide	Hydrophobic
23	T17	Pi-Orbitals	VAL72	Alkyl	Hydrophobic
24	T42	Pi-Orbitals	ILE69	Alkyl	Hydrophobic
25	T43	Pi-Orbitals	ALA339	Alkyl	Hydrophobic
26	G56	Pi-Orbitals	PRO200	Alkyl	Hydrophobic
27	G57	Pi-Orbitals	VAL198	Alkyl	Hydrophobic
28	C58	Pi-Orbitals	VAL198	Alkyl	Hydrophobic

*Atom type may refer to orbital systems or moieties for hydrophobic or pi-lone pair interactions.

Table S3. Analysis of iDE-ENaC interactions using Biovia Discovery Studio obtained from molecular docking.

No	iDE		ENaC			Interaction
	Nucleotide	**Atom Type**	**subunit**	**Residue**	**Atom Type**	
1	C69	OP1	*α*	ARG386	HH21	Hydrogen Bond;Electrostatic
2	C69	OP2	*α*	ARG386	NH1	Electrostatic
3	C68	OP1	*α*	ARG386	NH2	Electrostatic
4	T6	OP1	*β*	LYS282	NZ	Electrostatic
5	A7	OP2	*β*	LYS282	NZ	Electrostatic
6	C58	OP1	*β*	LYS282	NZ	Electrostatic
7	A4	OP1	*β*	LYS350	NZ	Electrostatic
8	C69	OP1	*α*	ARG386	HE	Hydrogen Bond
9	C69	OP2	*α*	ASP393	HN	Hydrogen Bond
10	C68	OP1	*α*	LYS477	HN	Hydrogen Bond
11	C67	O2	*β*	LYS88	HZ1	Hydrogen Bond
12	C67	O2	*β*	LYS88	HZ3	Hydrogen Bond
13	A13	N3	*β*	ASN378	HD22	Hydrogen Bond
14	G56	H1	*β*	MET279	O	Hydrogen Bond
15	G56	H22	*β*	MET279	O	Hydrogen Bond
16	G57	H21	*β*	MET279	SD	Hydrogen Bond
17	C69	HO3'	*β*	LEU83	O	Hydrogen Bond
18	G57	Pi-Orbitals	*β*	MET279	SD	Pi-Sulfur

Table S4. Unfavorable interactions in the iDE-creatinine complex obtained from molecular docking

No.	iDE		Creatinine		Interaction
	Nucleotide	**Atom Type**	**Residue**	**Atom type**	
1	T17	P	ILE69	CD1	Unfavorable Bump
2	T17	OP2	ILE69	CD1	Unfavorable Bump
3	T17	O4	VAL72	CB	Unfavorable Bump
4	T17	O4	VAL72	CG2	Unfavorable Bump
5	T17	C5	CYS283	SG	Unfavorable Bump
6	T17	C7	CYS283	CB	Unfavorable Bump
7	T17	C7	CYS283	SG	Unfavorable Bump
8	G20	P	HIS66	CB	Unfavorable Bump
9	G20	OP2	HIS66	CB	Unfavorable Bump
10	G20	OP2	HIS66	CG	Unfavorable Bump
11	T22	C7	PHE68	CE2	Unfavorable Bump
12	T42	C7	PHE68	C	Unfavorable Bump
13	T42	C7	PHE68	O	Unfavorable Bump
14	T42	C7	ILE69	N	Unfavorable Bump
15	T43	C5'	GLN318	NE2	Unfavorable Bump
16	T43	C5'	GLN318	HE21	Unfavorable Bump
17	T43	C2'	ALA339	CB	Unfavorable Bump
18	T43	C1'	ALA339	CB	Unfavorable Bump
19	T43	N3	ASP340	OD1	Unfavorable Bump
20	T43	H3	ASP340	OD1	Unfavorable Bump
21	A45	OP1	LYS319	CG	Unfavorable Bump
22	A45	OP1	LYS319	CD	Unfavorable Bump
23	A45	O5'	LYS319	CE	Unfavorable Bump
24	A45	C5'	LYS319	CE	Unfavorable Bump
25	A45	C4'	LYS319	CE	Unfavorable Bump
26	G46	C3'	GLY321	CA	Unfavorable Bump
27	G46	O3'	GLY321	N	Unfavorable Bump
28	G46	O3'	GLY321	CA	Unfavorable Bump
30	G46	O3'	GLY321	C	Unfavorable Bump
31	G46	O3'	THR322	N	Unfavorable Bump
32	T47	P	ARG320	C	Unfavorable Bump
33	T47	OP1	ARG320	C	Unfavorable Bump
34	T47	OP2	ARG320	C	Unfavorable Bump
35	T47	P	GLY321	N	Unfavorable Bump
36	T47	P	GLY321	CA	Unfavorable Bump
37	T47	P	GLY321	C	Unfavorable Bump
38	T47	P	GLY321	O	Unfavorable Bump
39	T47	P	GLY321	HN	Unfavorable Bump
40	T47	OP1	GLY321	C	Unfavorable Bump
41	T47	OP1	GLY321	O	Unfavorable Bump
42	T47	OP2	GLY321	N	Unfavorable Bump

43	T47	OP2	GLY321	CA	Unfavorable Bump
44	T47	OP2	GLY321	HN	Unfavorable Bump
45	T47	O5'	GLY321	CA	Unfavorable Bump
46	T47	O5'	GLY321	C	Unfavorable Bump
47	T47	O5'	GLY321	O	Unfavorable Bump
48	T47	C5'	GLY321	C	Unfavorable Bump
49	T47	C5'	GLY321	O	Unfavorable Bump
50	A48	OP1	THR322	O	Unfavorable Bump
51	G56	N2	PRO200	CG	Unfavorable Bump
52	G56	N2	PRO200	CD	Unfavorable Bump
53	G56	H21	PRO200	CG	Unfavorable Bump
54	G56	H21	PRO200	CD	Unfavorable Bump
55	G56	H22	PRO200	CD	Unfavorable Bump
56	C58	C4'	VAL198	CG1	Unfavorable Bump
57	C58	O4'	VAL198	CB	Unfavorable Bump
58	C58	O4'	VAL198	CG1	Unfavorable Bump
59	C58	O4'	VAL198	CG2	Unfavorable Bump
60	C60	C4'	GLU181	OE2	Unfavorable Bump
61	C60	OP1	GLU181	OE2	Unfavorable Negative-Negative
62	G26	OP1	GLU310	OE1	Unfavorable Negative-Negative
63	T17	H3	VAL72	HN	Unfavorable Donor-Donor
64	G46	H21	GLY321	HN	Unfavorable Donor-Donor
65	G19	O3'	GLY65	O	Unfavorable Acceptor-Acceptor
66	G20	OP1	GLY65	O	Unfavorable Acceptor-Acceptor
67	T59	O4'	ASP195	OD1	Unfavorable Acceptor-Acceptor

Table S5. Analysis of iDE-ENaC interactions using Biovia Discovery Studio obtained from the last frame of the 100-ns MD trajectory.

No	iDE		ENaC			Interaction
	Atom Type	**Nucleotide**	**subunit**	**Residue**	**Atom Type**	
1	G21	OP1	α	LYS220	HZ2	Hydrogen Bond;Electrostatic
2	G21	OP1	α	LYS220	HZ3	Hydrogen Bond;Electrostatic
3	C68	OP2	α	ARG476	HH12	Hydrogen Bond;Electrostatic
4	C68	OP1	α	ARG476	HH22	Hydrogen Bond;Electrostatic
5	C69	OP2	α	LYS477	HZ3	Hydrogen Bond;Electrostatic
6	G57	OP1	β	LYS282	HZ2	Hydrogen Bond;Electrostatic
7	G20	OP2	β	ARG259	NH2	Electrostatic
8	T59	OP1	β	ARG324	NH1	Electrostatic
9	C60	OP1	β	ARG324	NH1	Electrostatic
10	A4	OP1	β	LYS350	NZ	Electrostatic
11	G57	OP1	α	ASN213	HD21	Hydrogen Bond
12	G55	N3	α	ASN214	HD21	Hydrogen Bond
13	G20	O3'	α	LYS220	HZ2	Hydrogen Bond
14	G20	O3'	α	LYS220	HZ3	Hydrogen Bond
15	C69	O2	β	SER82	HG	Hydrogen Bond
16	T43	O2	β	GLN233	HE22	Hydrogen Bond
17	G19	OP2	β	ASN260	HD22	Hydrogen Bond
18	T17	O2	β	SER263	HN	Hydrogen Bond
19	T59	O3'	β	ARG324	HH12	Hydrogen Bond
20	G3	O3'	β	GLN352	HE21	Hydrogen Bond
21	T17	H3	β	SER263	OG	Hydrogen Bond
22	T17	H3	β	SER263	O	Hydrogen Bond
23	G20	H22	α	GLN216	OE1	Hydrogen Bond
24	G56	H21	β	THR280	O	Hydrogen Bond
25	G56	H22	β	THR280	O	Hydrogen Bond
26	G57	H22	β	MET279	O	Hydrogen Bond
27	T43	Pi-Orbitals	β	PRO231	Alkyl	Hydrophobic

Table S6. Conservation of Hydrogen bonding in the binding of iDE to ENaC. The hydrogen bonding conservation was extracted from a 100-ns trajectory of molecular dynamics simulation of the complex.

ENaC			iDE		% Conservation
Subunit	**Residue**	**Atom**	**Nucleotide**	**Atom**	
alpha	ASN213	HD21	G56	O3'	8.2
		HD21	G57	OP1	5.3
	ASN214	ND2	G19	H22	0.2
		OD1	G19	H22	0.1
		HD21	G19	N2	0.1
		HD21	G55	N3	0.5
	GLN216	HE21	G20	N3	0.6
		HE21	G20	O3'	1.7
		NE2	G20	H22	0.2
		OE1	G20	H22	12.5
		HE21	G21	O4'	0.1
		HE21	G21	OP1	0.5
		HE22	G21	OP1	0.1
	LYS220	HZ2	G20	O3'	0.6
		HZ1	G20	O3'	0.3
		HZ3	G20	O3'	0.2
		HZ3	G21	OP1	6.1
		HZ1	G21	OP1	6.1
		HZ2	G21	OP1	4.9
		HZ1	G21	OP2	0.7
		HZ2	G21	OP2	0.4
		HZ3	G21	OP2	0.2
	ARG386	HH11	C69	O3'	0.4
		HH22	C69	O3'	0.1
		HH12	C69	O5'	7.1
		HE	C69	O5'	0.8
		HH22	C69	OP1	21
		HH12	C69	OP1	20.9
		HH21	C69	OP1	9.9
		HE	C69	OP1	5.7
		HH22	C69	OP2	2.4
		HH21	C69	OP2	0.4
	ASP393	H	C69	OP2	6.6
	ARG476	HH12	C68	OP1	15.7
		HH22	C68	OP1	14.7
		HE	C68	OP1	3.6
		HH11	C68	OP1	0.7
		HH21	C68	OP1	0.2
		HH21	C68	OP2	18.2
		HH22	C68	OP2	10.9
		HH12	C68	OP2	7.4
		HH11	C68	OP2	6.8
		HE	C68	OP2	6.7
		HH22	C69	OP2	0.3
	LYS477	HZ3	C68	OP1	7.1
		HZ2	C68	OP1	6.5
		HZ1	C68	OP1	6.2
		HZ3	C69	OP1	10.7
		HZ2	C69	OP1	10.7
		HZ1	C69	OP1	10.4
		HZ3	C69	OP2	7.9
		HZ2	C69	OP2	5.3
		HZ1	C69	OP2	4.2

ENaC			iDE		% Conservation
Subunit	**Residue**	**Atom**	**Nucleotide**	**Atom**	
β	SER82	HG	C69	O2	43.8
		HG	C69	O3'	4.5
	LEU83	H	C69	O3'	33.7
	LYS88	HZ1	A4	N3	0.5
		HZ3	A4	N3	0.4
		HZ2	A4	N3	0.1
		HZ3	A4	O3'	0.9
		HZ2	A4	O3'	0.9
		HZ1	A4	O3'	0.9
		HZ3	C67	O2	0.8
		HZ1	C67	O2	0.4
		HZ2	C67	O2	0.4
		HZ3	C69	OP1	0.1
	GLN233	HE22	T43	O2	1.4
		HE21	T43	O2	0.1
		HE21	T43	O4	0.4
		HE22	T43	O4	0.1
		HE22	T43	O4'	2.3
		HE21	T43	O4'	1.2
		HE21	T43	O5'	3.3
		HE21	T43	OP1	0.2
		HE22	T43	OP2	4.7
		HE21	T43	OP2	0.2
	ARG259	HH12	G20	OP1	14.2
		HH22	G20	OP1	3.1
		HH21	G20	OP1	0.7
		HE	G20	OP1	0.6
		HH11	G20	OP1	0.3
		HH22	G20	OP2	19.1
		HH12	G20	OP2	9.4
		HH21	G20	OP2	3
		HE	G20	OP2	0.7
		HH11	G20	OP2	0.5
	ASN260	HD22	G19	OP1	48.2
		HD22	G19	OP2	15.9
	SER263	H	T17	O2	56.2
		O	T17	H3	71.8
		OG	T17	H3	1.8
	MET279	O	G56	H21	0.1
		O	G57	H22	52.2
	THR280	O	G56	H22	26
	LYS282	HZ3	T6	O5'	0.2
		HZ2	T6	O5'	0.1
		HZ2	T6	OP1	0.4
		HZ3	T6	OP1	0.4
		HZ3	T6	OP2	0.2
		HZ2	A7	OP1	0.2
		HZ3	A7	OP1	0.1
		HZ1	A7	OP2	0.5
		HZ3	A7	OP2	0.3
		HZ2	A7	OP2	0.1
		HZ2	G56	O3'	0.3
		HZ1	G56	O3'	0.1
		HZ1	G57	OP1	5.6
		HZ2	G57	OP1	5.2
		HZ3	G57	OP1	4.3
		HZ2	G57	OP2	0.4
		HZ1	G57	OP2	0.3

ENaC			iDE		% Conservation
Subunit	**Residue**	**Atom**	**Nucleotide**	**Atom**	
		HZ3	G57	OP2	0.2
		HZ1	C58	OP1	6.5
		HZ3	C58	OP1	4.9
		HZ2	C58	OP1	4.5
		HZ2	C58	OP2	1.2
		HZ3	C58	OP2	0.5
		HZ1	C58	OP2	0.2
	ARG324	HH21	C58	O3'	0.7
		HH11	C58	O3'	0.3
		HH21	C58	OP1	0.1
		HH22	C58	OP1	0.1
		HH22	T59	O3'	0.1
		HH21	T59	O5'	0.1
		HH11	T59	OP1	47.2
		HE	T59	OP1	15.5
		HH21	T59	OP1	13.5
		HH21	T59	OP2	12.9
		HE	T59	OP2	3.1
		HH11	T59	OP2	0.5
		HH12	T59	OP2	0.1
		HH22	C60	OP1	1.7
		HH12	C60	OP1	1.4
		HH21	C60	OP1	0.1
		HH22	C60	OP2	38.7
		HH12	C60	OP2	37.2
	LYS350	HZ1	G3	O3'	2.4
		HZ3	G3	O3'	1.5
		HZ2	G3	O3'	0.9
		HZ1	A4	OP1	32.5
		HZ3	A4	OP1	21.5
		HZ2	A4	OP1	20.4
	GLN352	HE21	G3	O3'	20.6
		HE22	G3	O3'	0.2
		HE22	G3	OP1	22.3
		HE21	G3	OP1	0.2
		HE22	A4	OP1	4.9
		HE21	A4	OP1	1.7
		HE21	A4	OP2	3.6
		HE22	A4	OP2	0.2
	ASN378	HD21	A13	O3'	2.5
		HD21	A13	O4'	0.1
		HD22	T59	O3'	4
		HD21	T59	O3'	0.2
		HD21	T59	O4'	0.2
		HD22	T59	OP1	0.6
		HD22	C60	OP1	8.5
		HD21	C60	OP1	3
		HD21	C60	OP2	0.8