

Supplementary material

Table S1. Collocation conditions of buffer A and B.

Name	H ₂ O (%)	Acetonitrile (%)	TFA (%)
Buffer A	90	10	0.05
Buffer B	10	90	0.05

Table S2. Determination of peptide quality used by Circular Dichroism Spectra (CD).

peptide	quality
PIIIA*	1mg
PIIIA*-1	2.4mg
PIIIA*-2	2.1mg
PIIIA*-3	1mg

Table S3. Test the concentration and supply of the compounds. The concentration of the test compound is 3mM. Soluble in dimethyl sulfoxide (DMSO). On the day of the test, the cells were dissolved in extracellular fluid. Compound PIIIA* was sent to the sample before, and the concentration was 0.9mM.

Compound	Molecular weight	Purity	Test concentration	Storage conditions
----------	------------------	--------	--------------------	--------------------

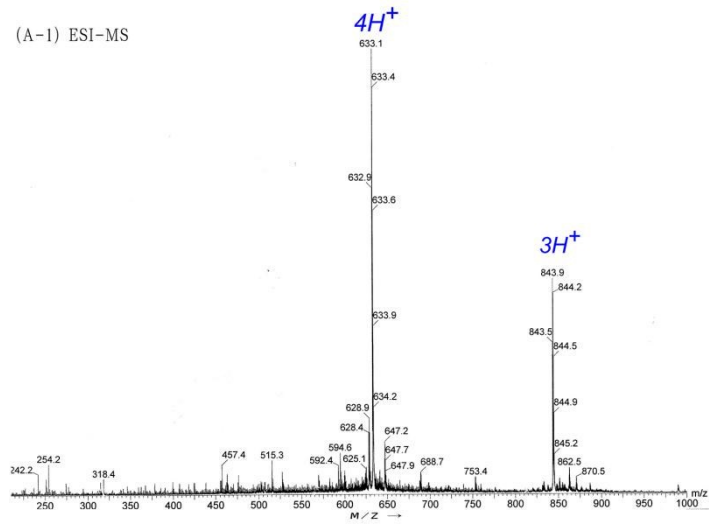
PIIIA*	2591.13	95%	0.1, 0.3 1	-20°C
PIIIA*-1	2529.00	95%	1 ,3 ,10	-20°C
PIIIA*-2	2529.00	95%	1 ,3 ,10	-20°C
PIIIA*-3	2529.00	95%	1 ,3 ,10	-20°C

Table S4. Molecular weight and yield of peptide synthesized. Molecular weight include theoretical molecular weight (TMW) and determination of molecular weight (DMW). Yield refers to the output of the final product.

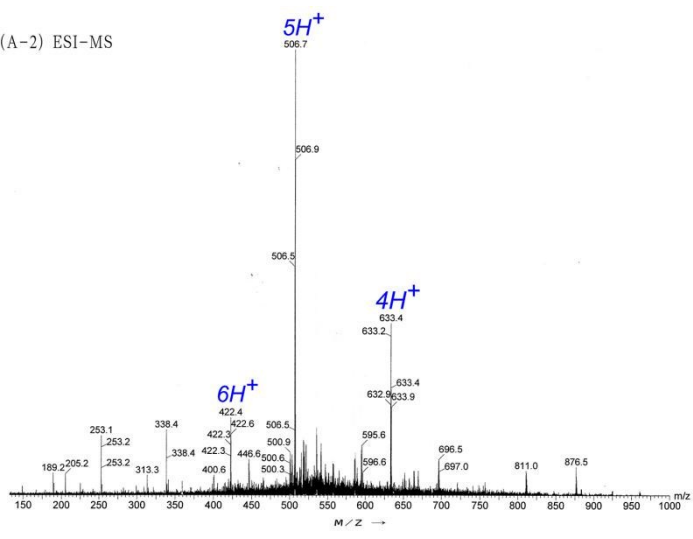
Peptide	TMW[M+3]	DMW[M+3]	TMW[M+4]	DMW[M+4]	TMW[M+5]	DMW[M+5]	TMW[M+6]	DMW[M+6]	Yield
PIIIA*-1	844.0	843.9	633.2	633.1	506.8	-	422.5	-	4.6 mg
PIIIA*-2	844.0	-	633.2	633.4	506.8	506.7	422.5	-	3.6 mg
PIIIA*-3	844.0	844.2	633.2	633.2	506.8	506.7	422.5	422.4	3.0 mg

Figure S1. (A-1), (A-2) and (A-3) represent high-resolution ESI-MS analysis of final product PIIIA*-1, PIIIA*-2, and PIIIA*-3, respectively. (B-1), (B-2) and (B-3) represent RP-HPLC traces of PIIIA*-1, PIIIA*-2, and PIIIA*-3.

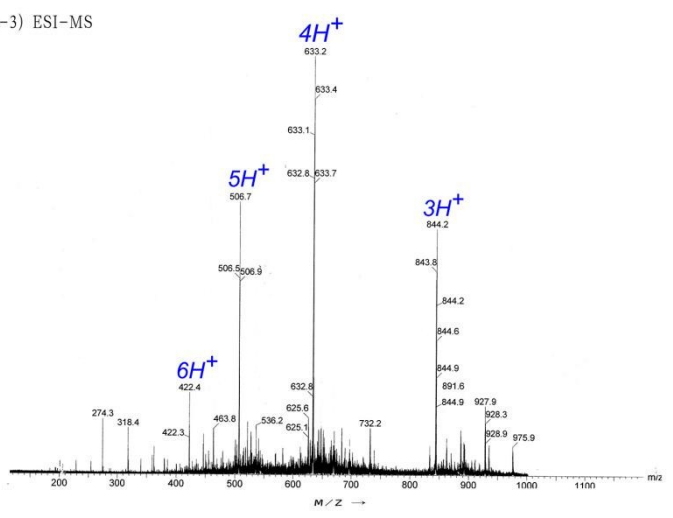
(A-1) ESI-MS



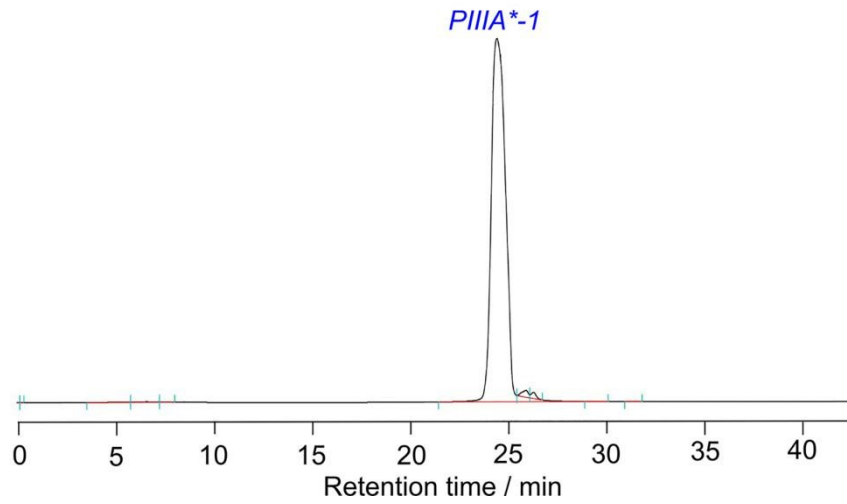
(A-2) ESI-MS



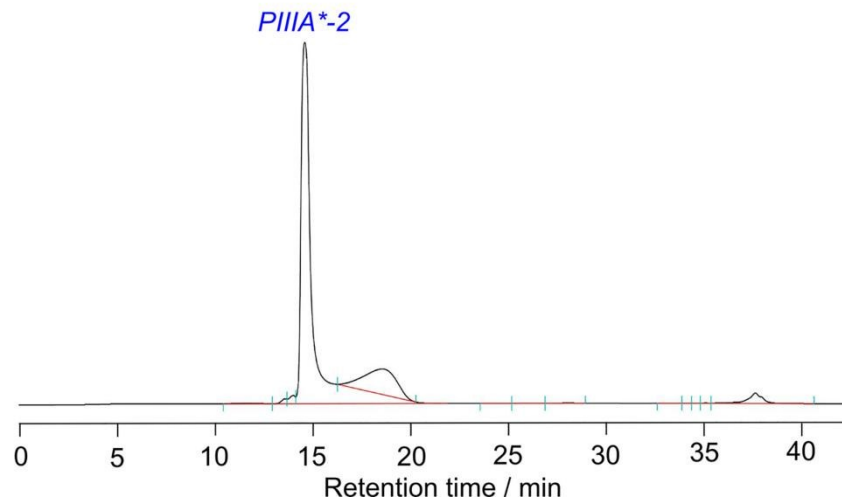
(A-3) ESI-MS



(B-1) HPLC traces (214nm)



(B-2) HPLC traces (214nm)



(B-3) HPLC traces (214nm)

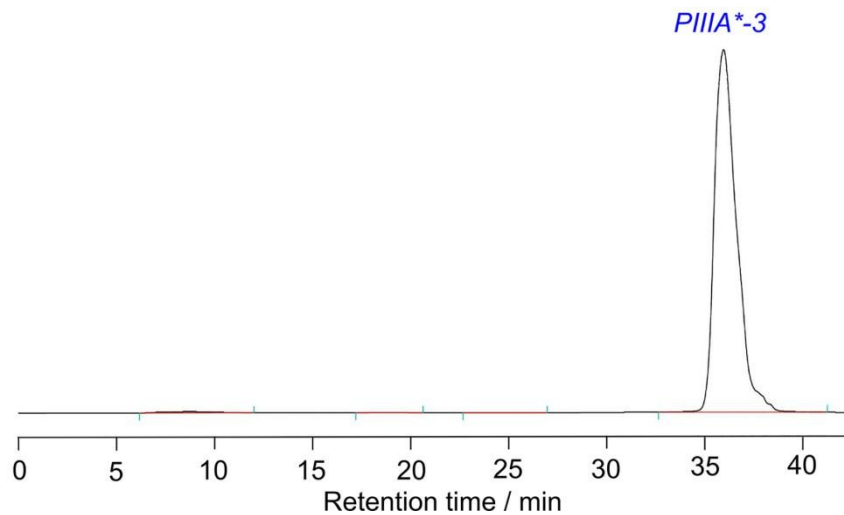


Figure S2. The Supplementary electrophysiological recording. A, B, C and D represent the supplementary electrophysiological recording of PIIIA*, PIIIA*-1, PIIIA*-2, and PIIIA*-3, respectively.

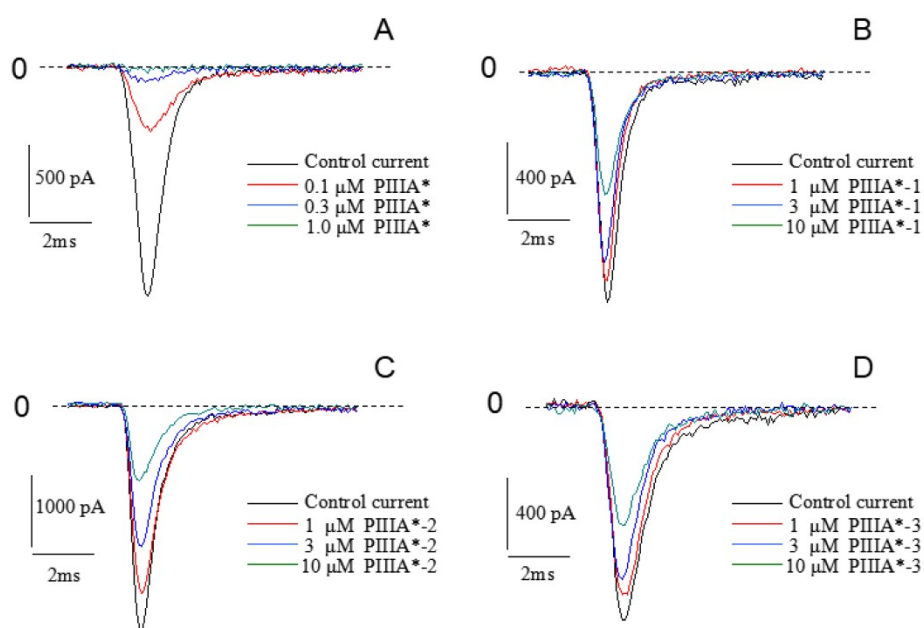
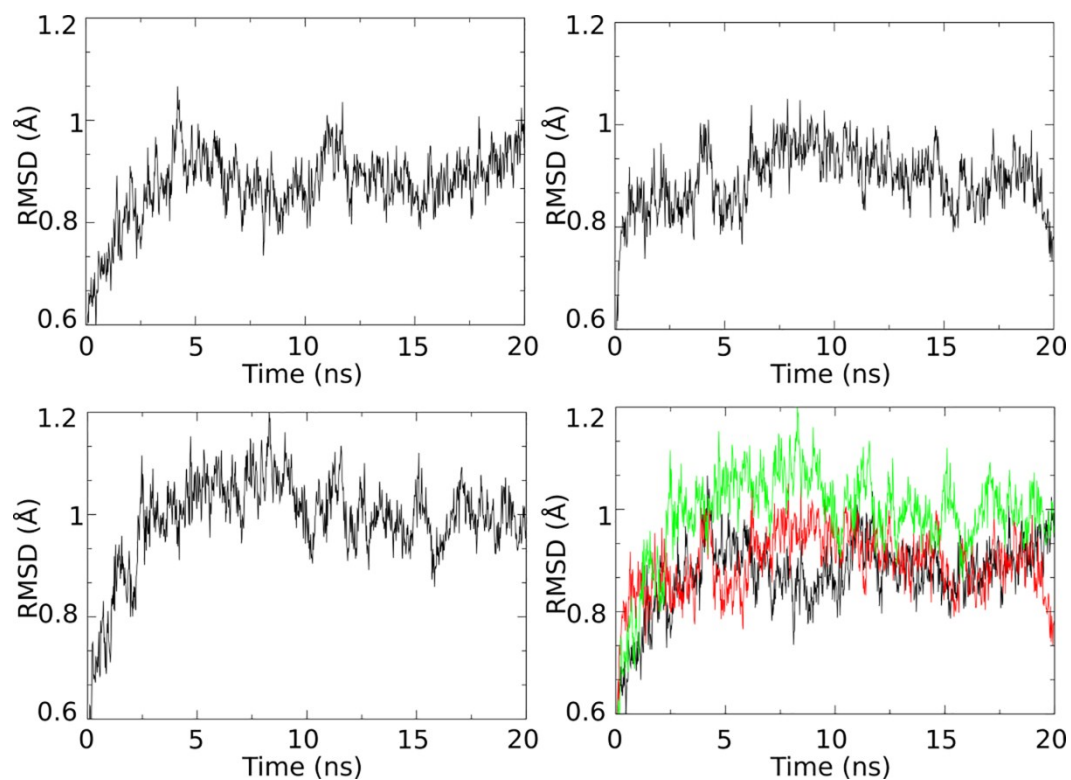


Figure S3. The RMSD value of the Nav1.4/PI3A*-1, 2, and 3 system. (A), (B) and (C) denote RMSD graphs of Nav1.4/PI3A*-1, Nav1.4/PI3A*-2 and Nav1.4/PI3A*-3 respectively. (D) is a superposition diagram for three complex RMSD. In the D diagram, black, red and green represent Nav1.4/PI3A*-1, Nav1.4/PI3A*-2 and Nav1.4/PI3A*-3, respectively.



^a The RMSD value was averaged on five MD trajectories.

Figure S4. The RMSD value of the PIIIA*-1. To determine whether PIIIA*-1 reached equilibrium at 100 ns, we carried out two MD in 150 ns. According to the RMSD diagram, PIIIA*-1 had reached equilibrium at 100 ns.

