



Phosphoproteomics of cardiac Nav1.5 channels



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Phosphoproteomics of cardiac Nav1.5 channels

Research experiences: Céline Marionneau

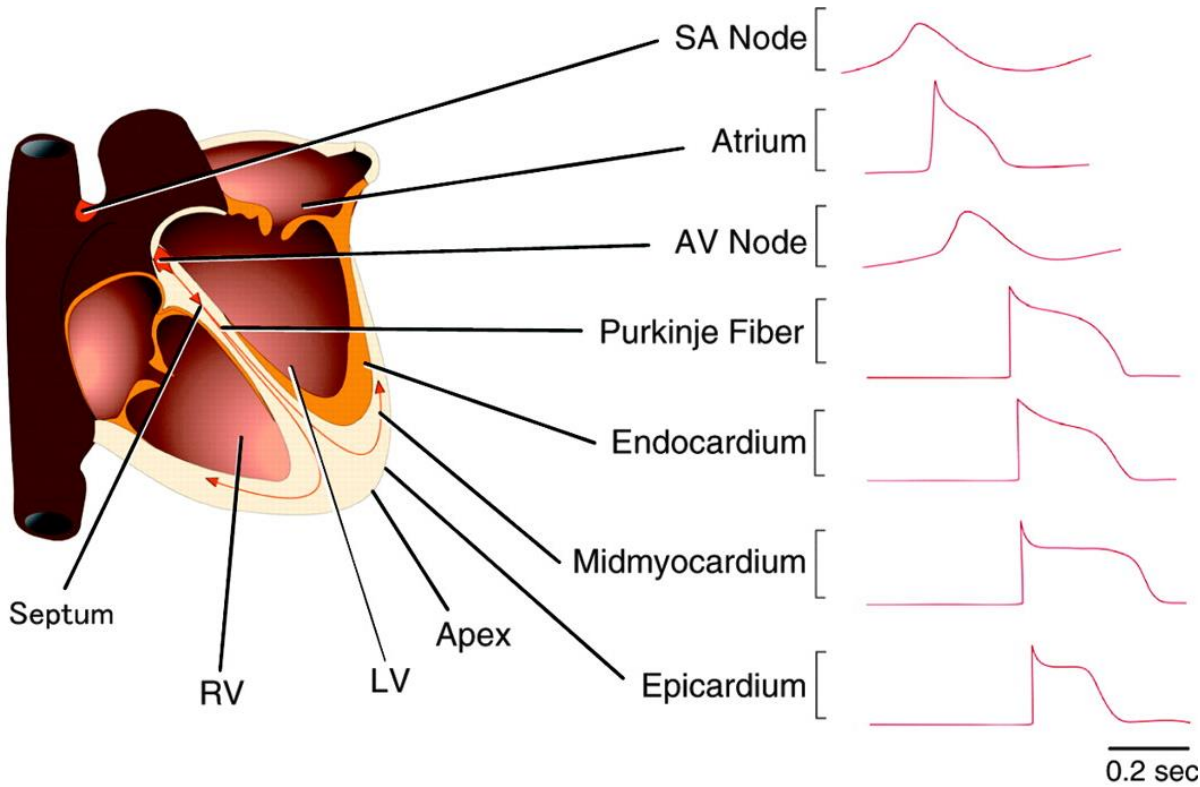
- 2001-2005: PhD (University of Nantes, *l'institut du thorax, Nantes*)
Functional genomics of cardiac ion channels
- 2005-2009 : Postdoctoral fellow (Washington University, Saint Louis, MO, USA)
Post-translational regulation of neuronal Kv4.2 channels
- 2009-2010 : Postdoctoral fellow (*l'institut du thorax, Nantes*)
- Since Nov 2010 : Chargée de recherche au CNRS (*Equipe II, l'institut du thorax, Nantes*)
Post-translational regulation of cardiac Nav1.5 channels

Phosphoproteomics of cardiac Nav1.5 channels

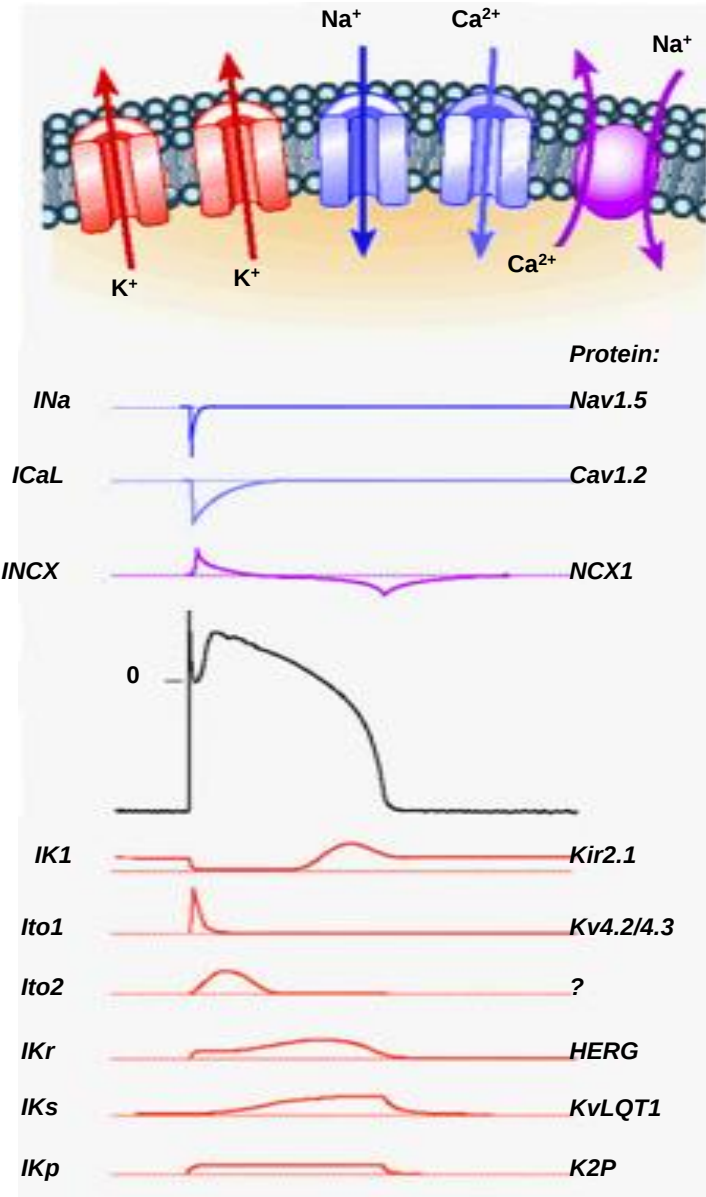
From this conference, you should know:

- Context : Cardiac electrophysiology, Nav1.5 channels
- Proteomics : goals, methods
- Apprehend the research developed

Cardiac excitability

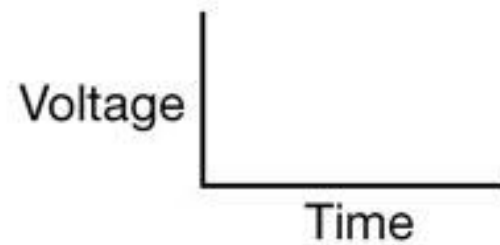
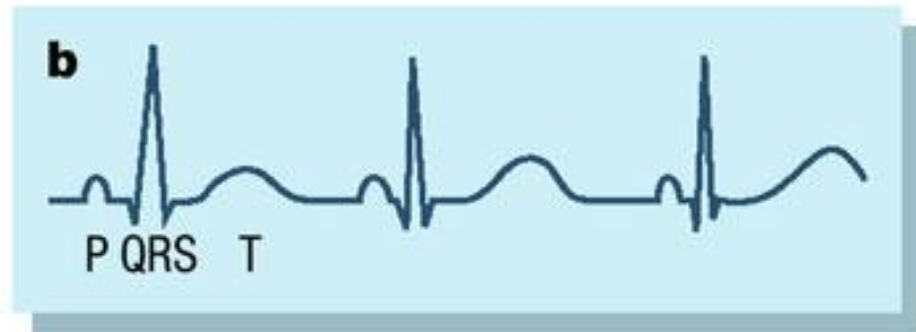
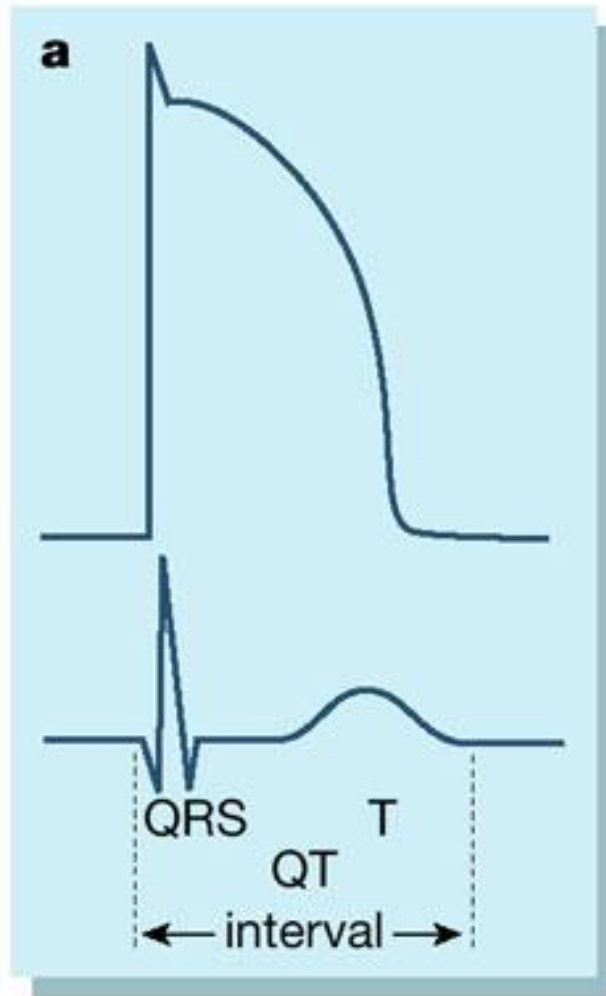


Nerbonne and Kass, *Physiol Rev* 2005

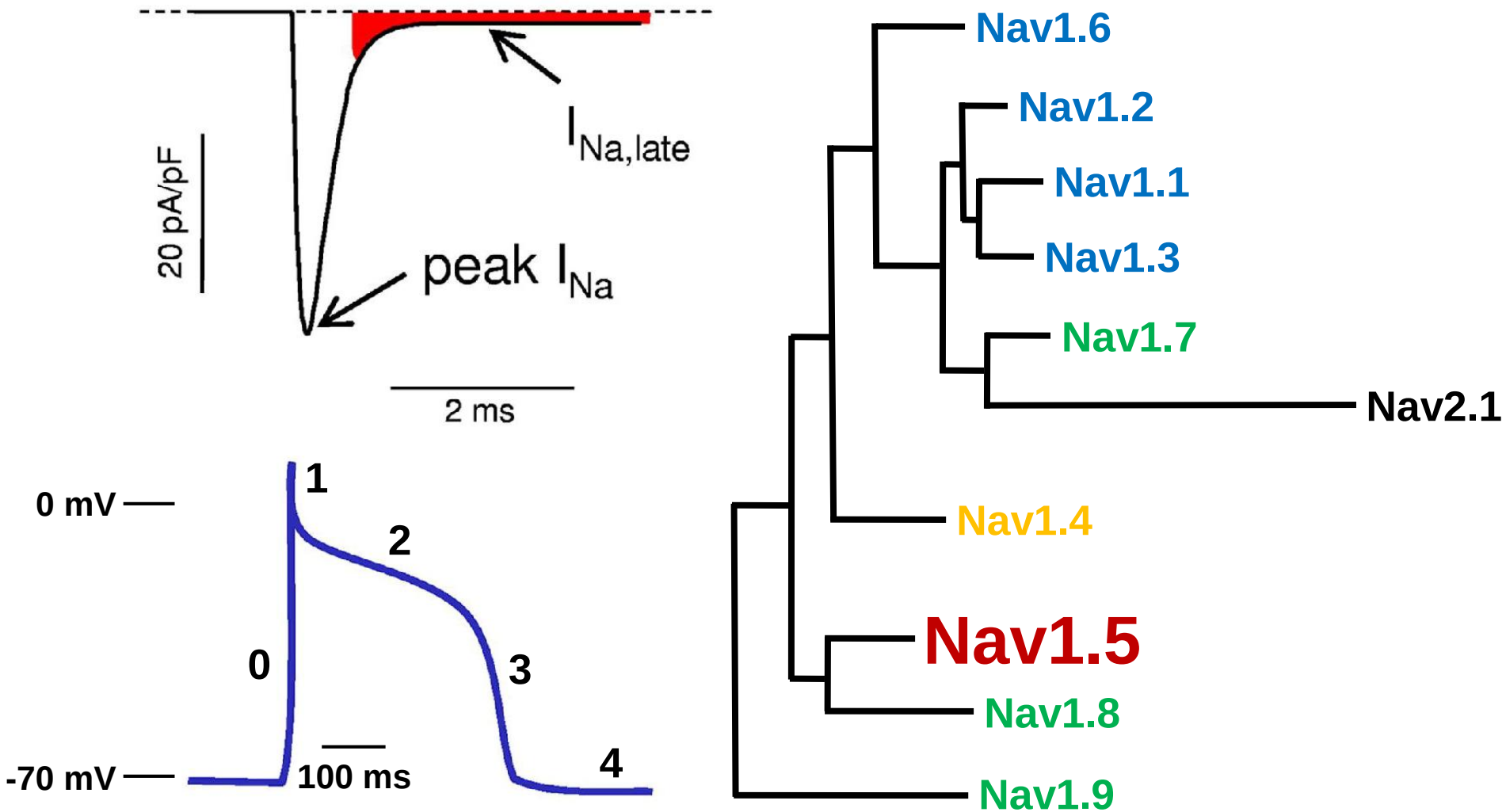


Marban, *Nature* 2002

Electrocardiograms : ECG



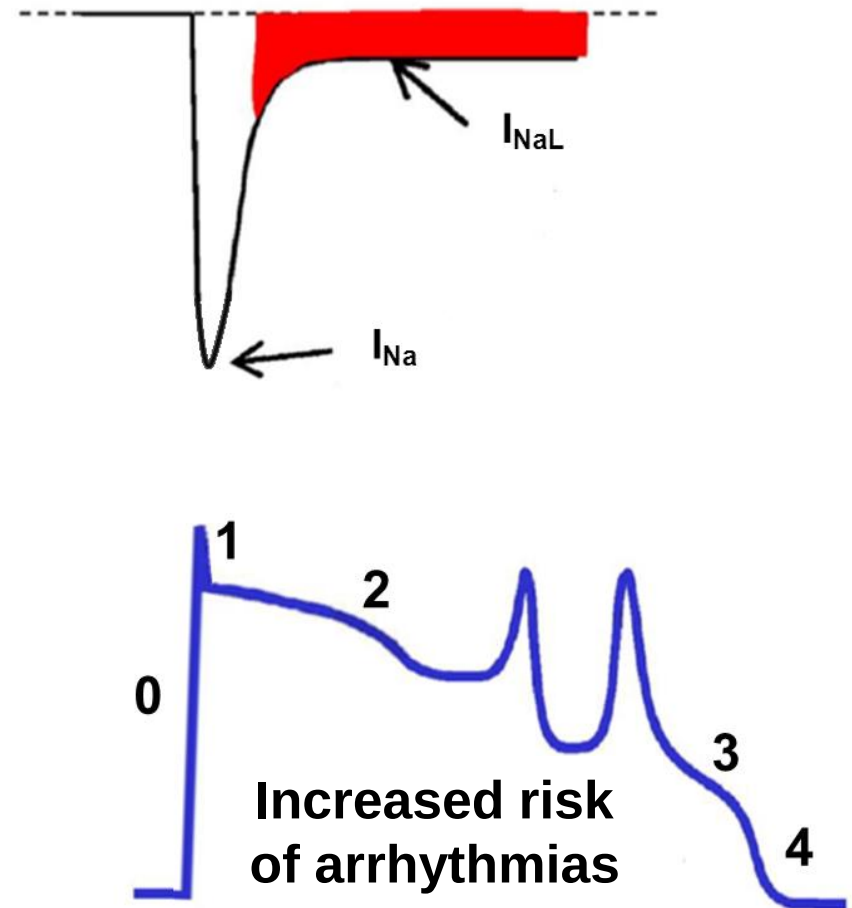
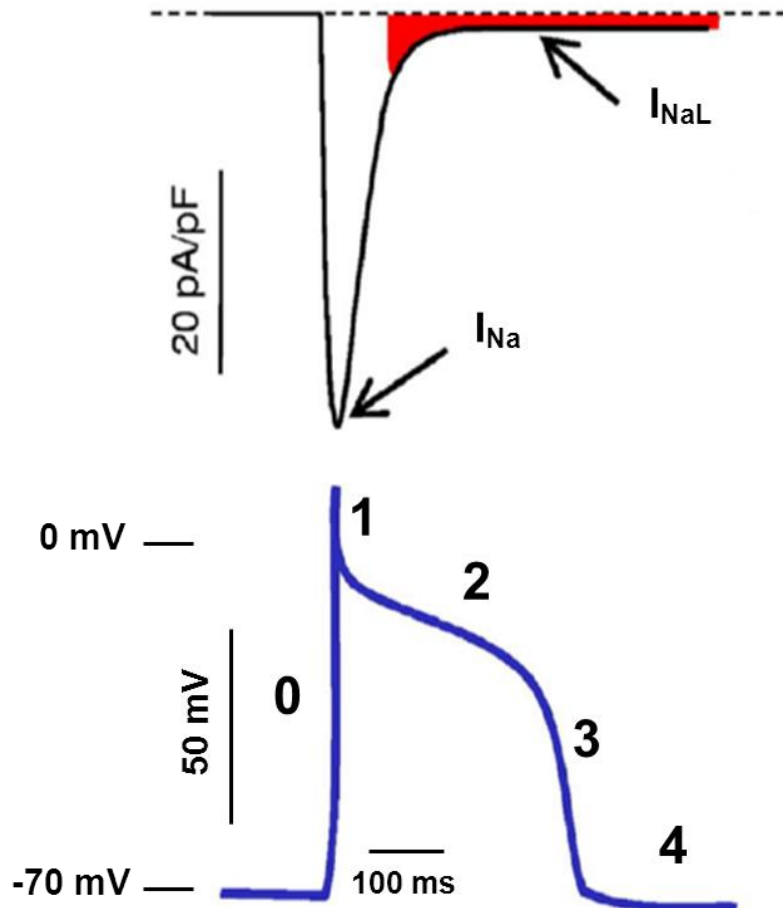
Cardiac voltage-gated Na⁺ channels



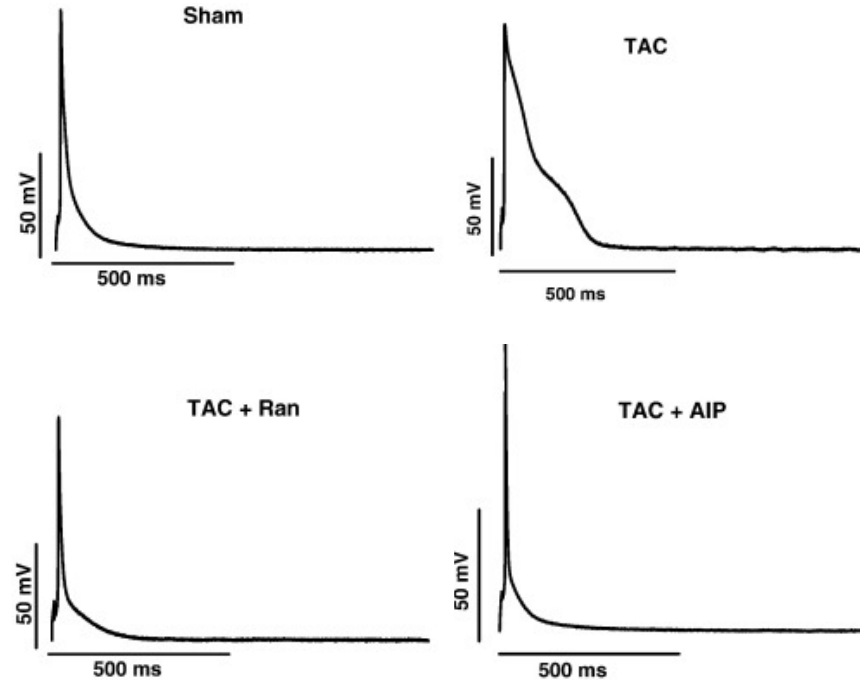
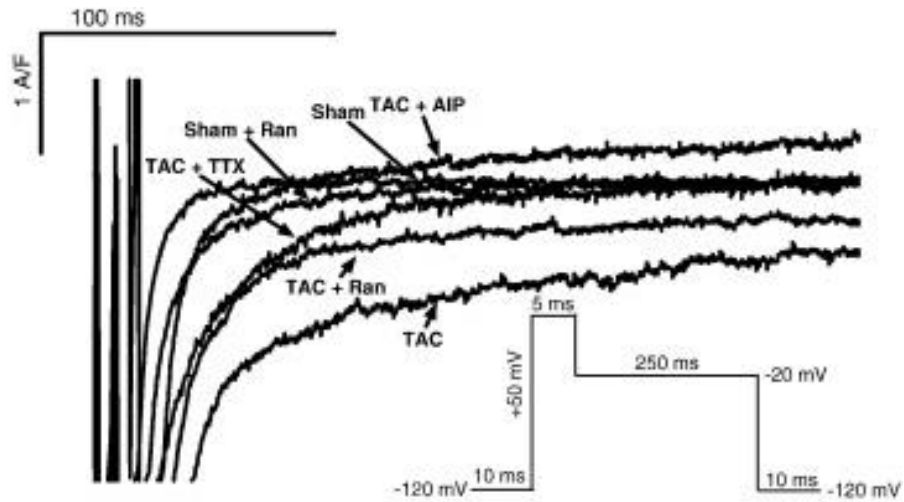
Nav1.5 channels in heart failure

Normal

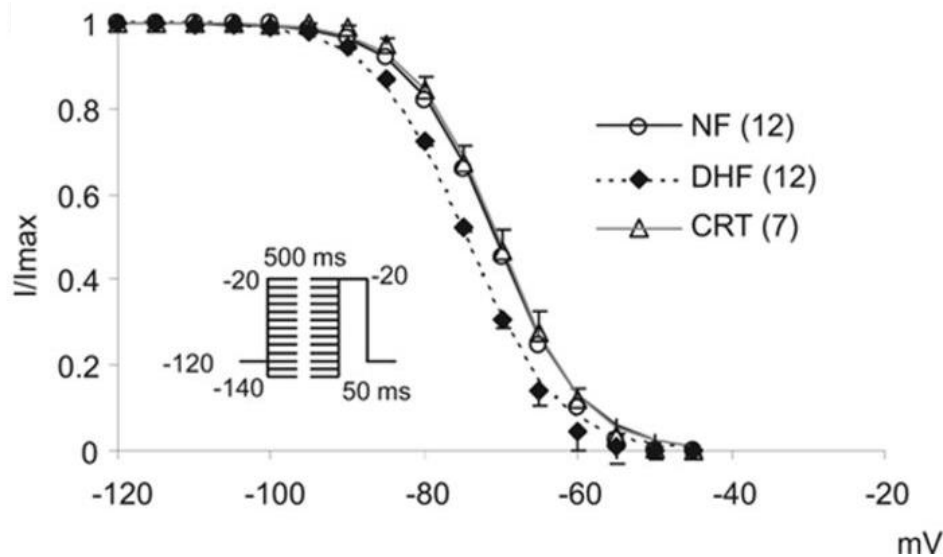
Disease



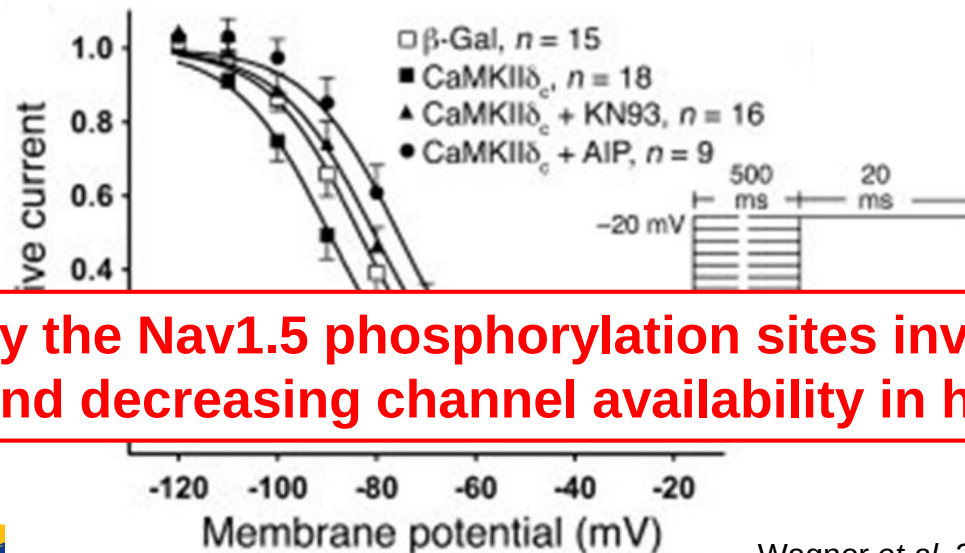
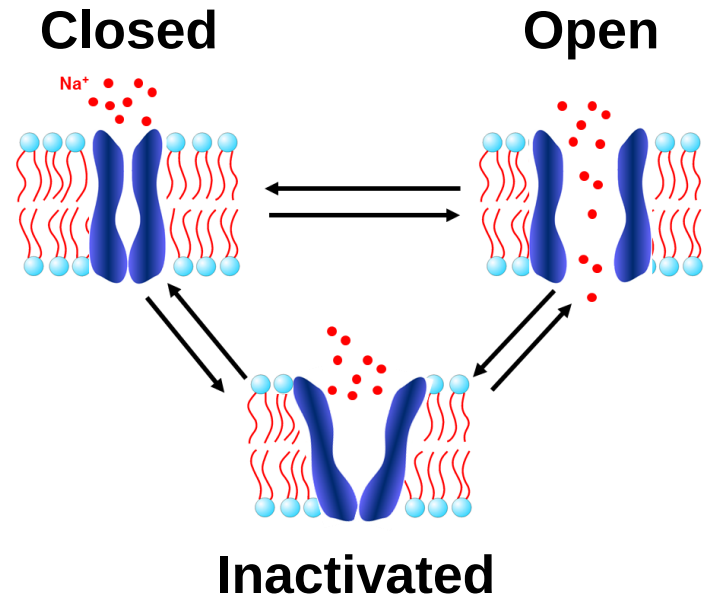
CaMKII increases I_{NaL} in heart failure



CaMKII decreases Nav1.5 channel availability



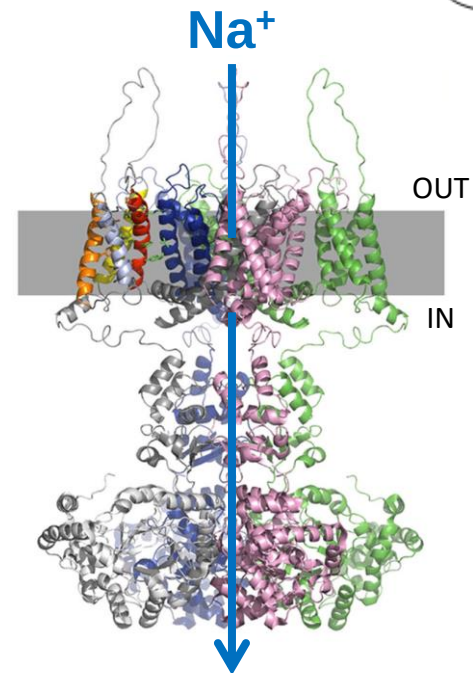
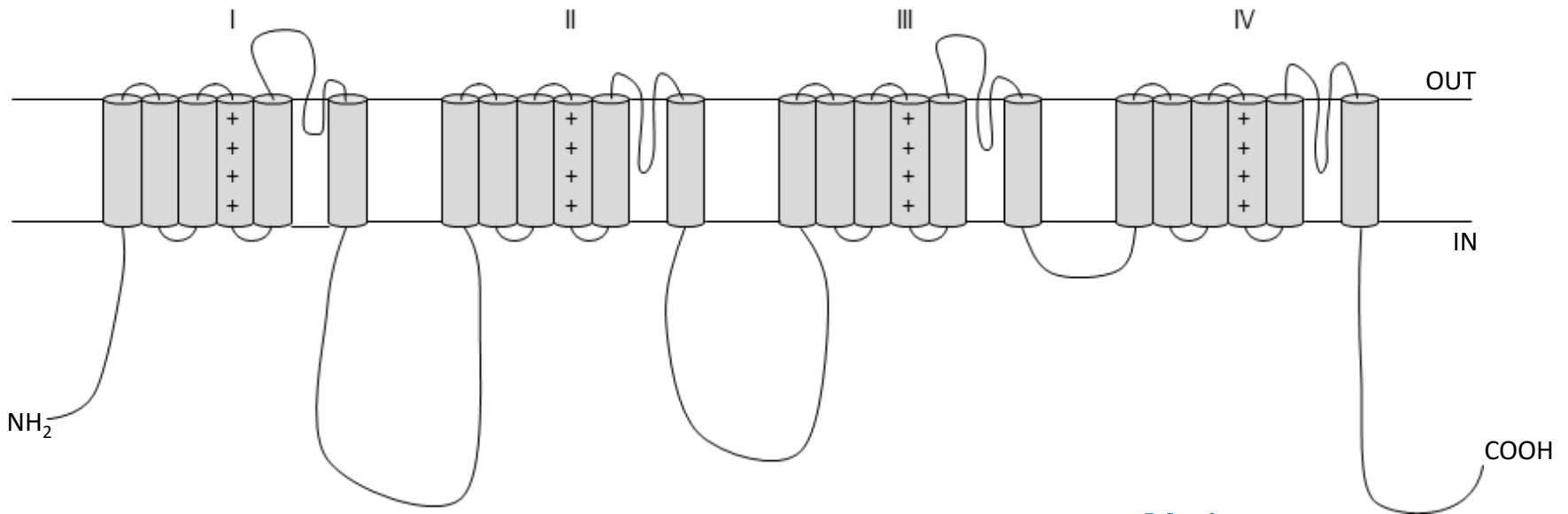
Aiba et al, 2013



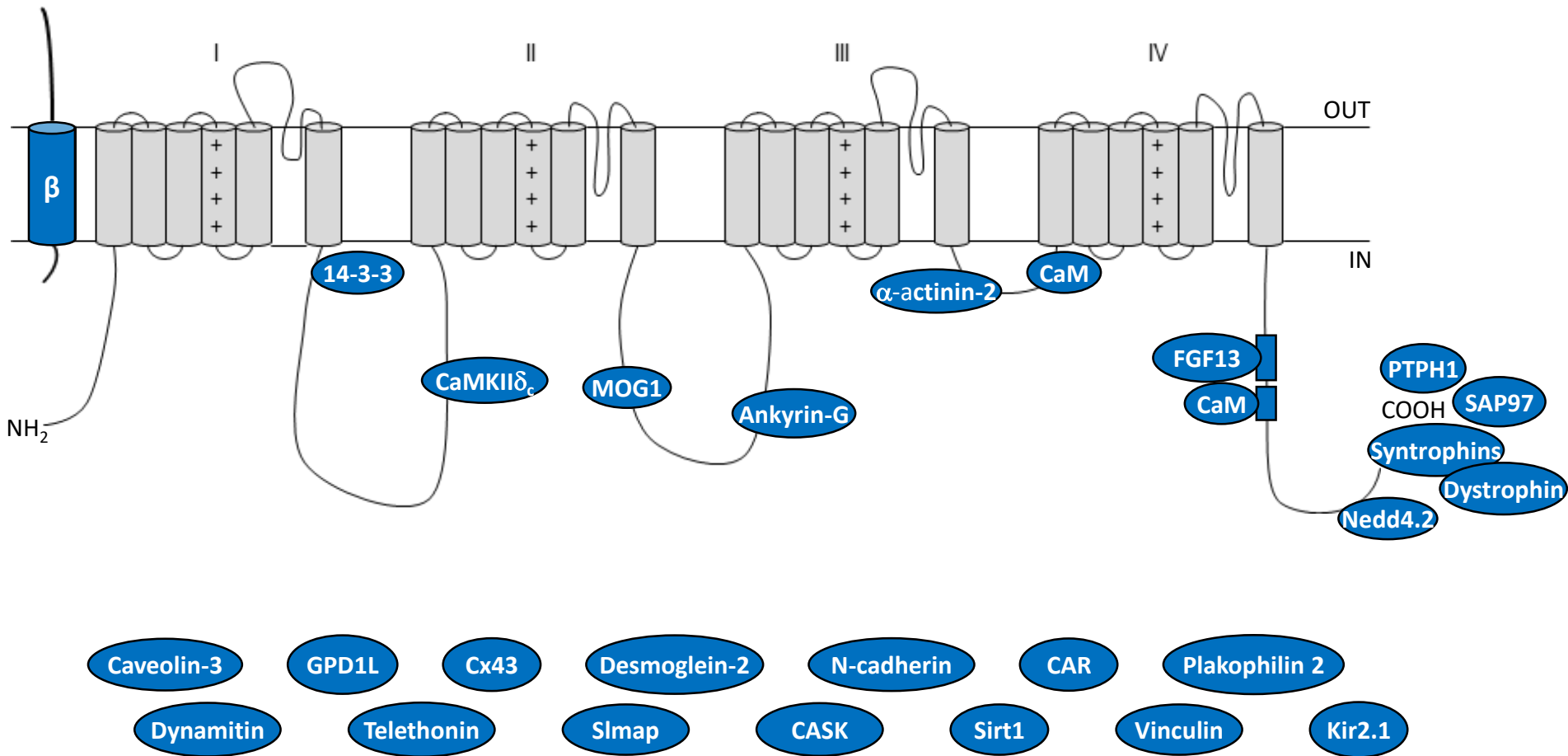
Goals: to identify the Nav1.5 phosphorylation sites involved in increasing I_{NaL} and decreasing channel availability in heart failure

Wagner et al, 2006

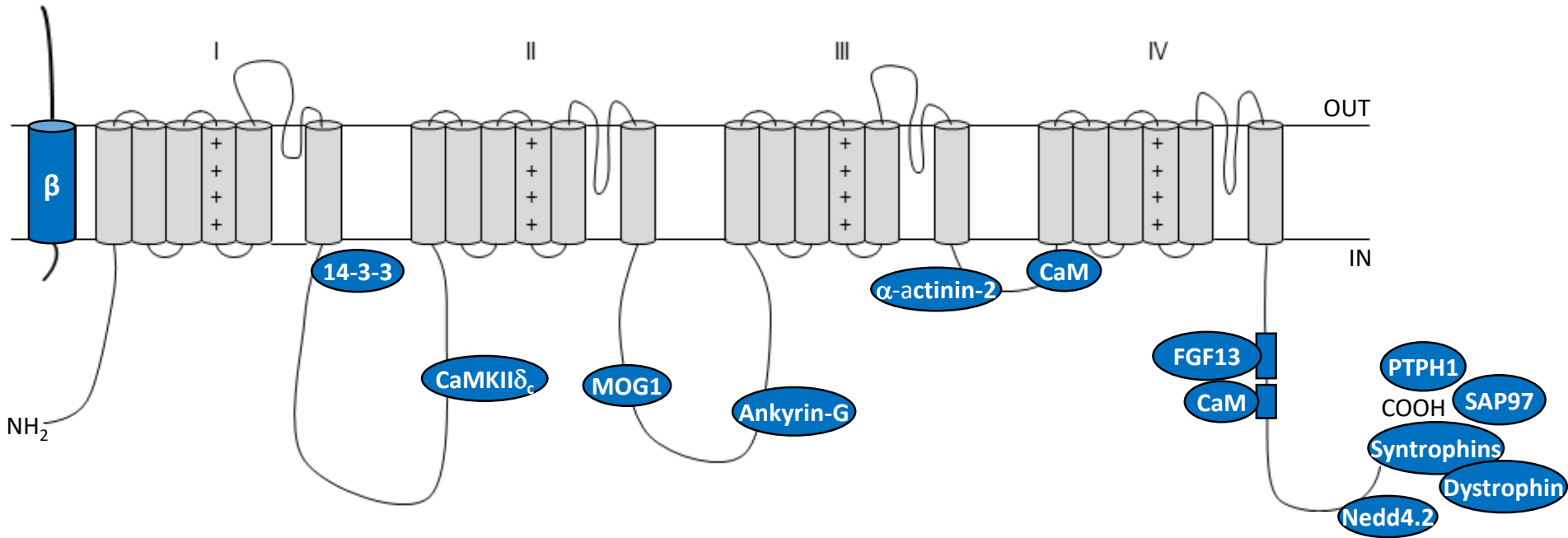
Cardiac Nav1.5 channel complexes



Cardiac Nav1.5 channel complexes



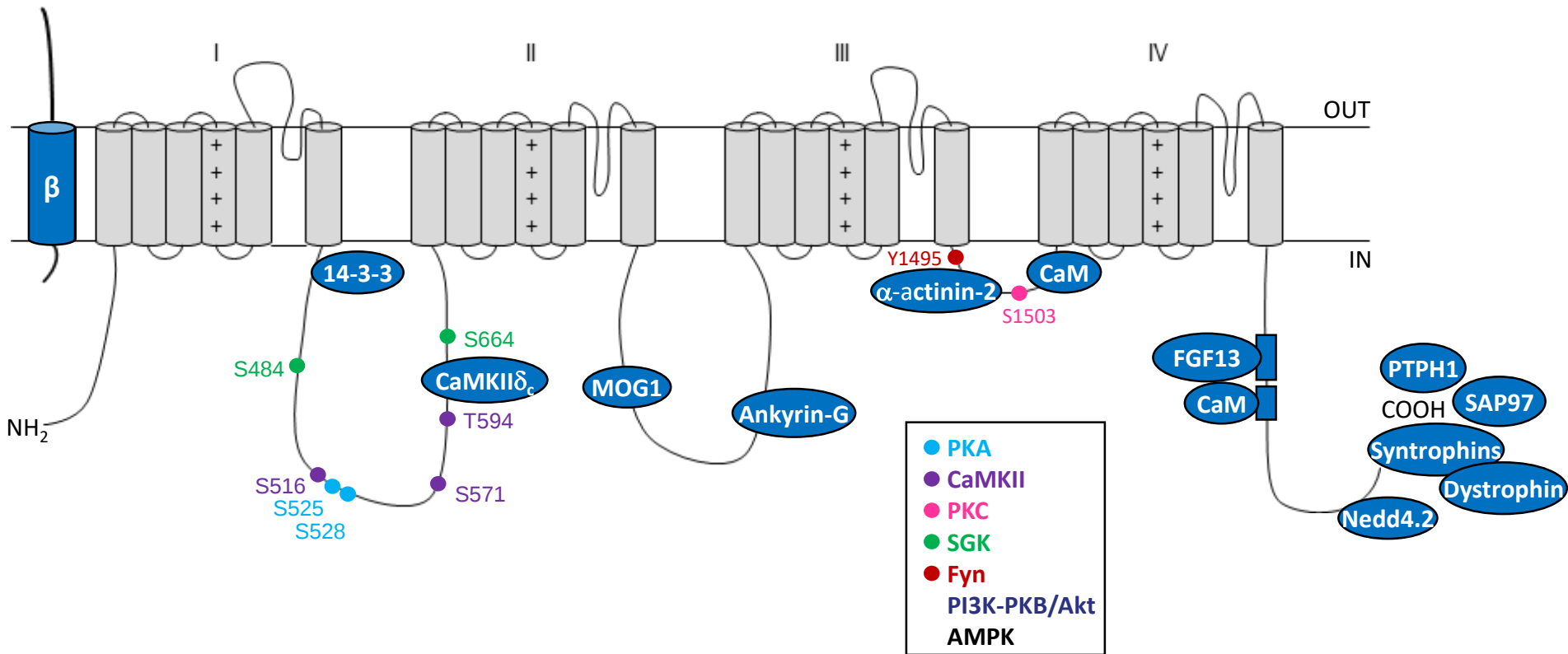
Cardiac Nav1.5 channel complexes



O-GlcNAcylation Glycosylation Phosphorylation Methylation Acetylation
 Oxidation Nitrosylation Lipoxidation Ubiquitylation Palmitoylation SUMOylation

Caveolin-3 GPD1L Cx43 Desmoglein-2 N-cadherin CAR Plakophilin 2
 Dynaminin Telethonin Slmap CASK Sirt1 Vinculin Kir2.1

Before 2012 : 9 phosphorylation sites



O-GlcNAcylation

Glycosylation

Phosphorylation

Methylation

Acetylation

Oxidation

Nitrosylation

Lipoxidation

Ubiquitylation

Palmitoylation

SUMOylation

Caveolin-3

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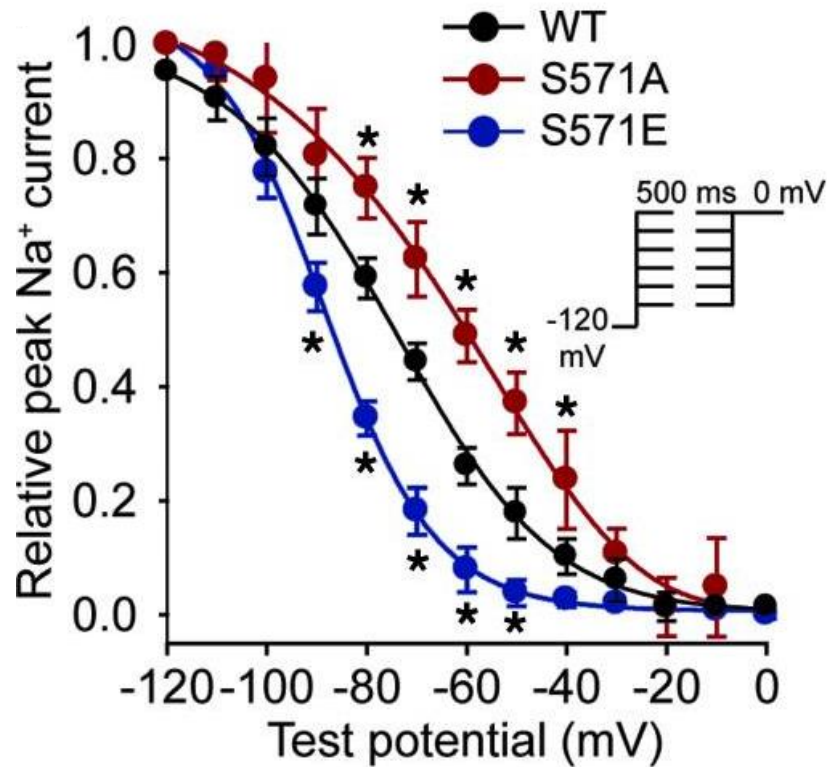
Kir2.1

Methods to identify phosphorylation sites

- *In silico analyses*
 - phosphorylation consensus sequence (CaMKII: RXX[S/T])
 - *In vitro analyses*
 - kinase assay + mass spectrometry
 - *In situ analyses*
 - purification of proteins + mass spectrometry
- ➡ Functional analyses :
- directed mutagenesis (Ser/Thr > Glu/Asp ; Ser/Thr > Ala)

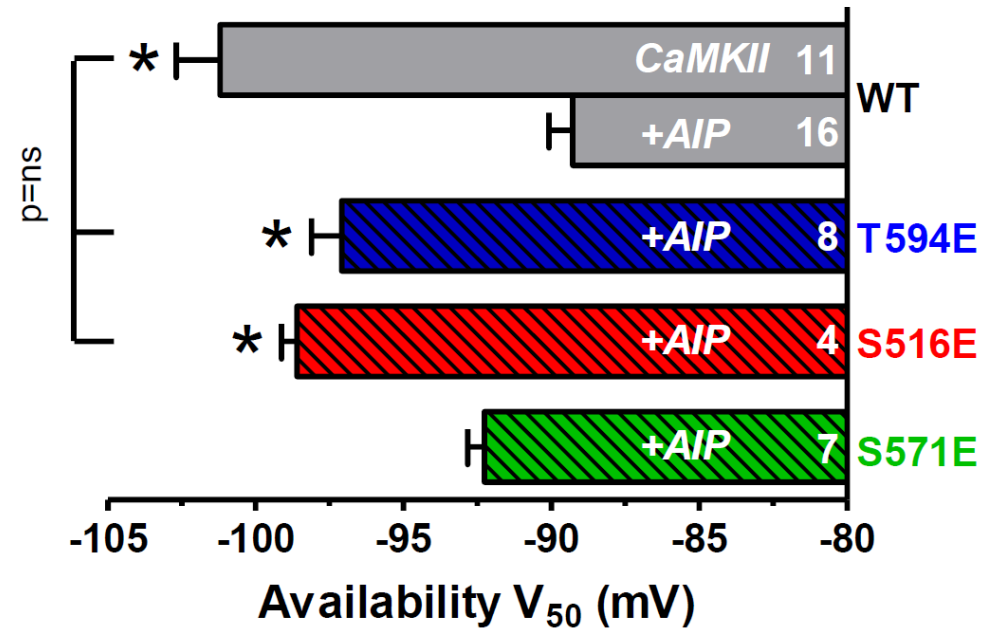
CaMKII phosphorylation sites

In silico



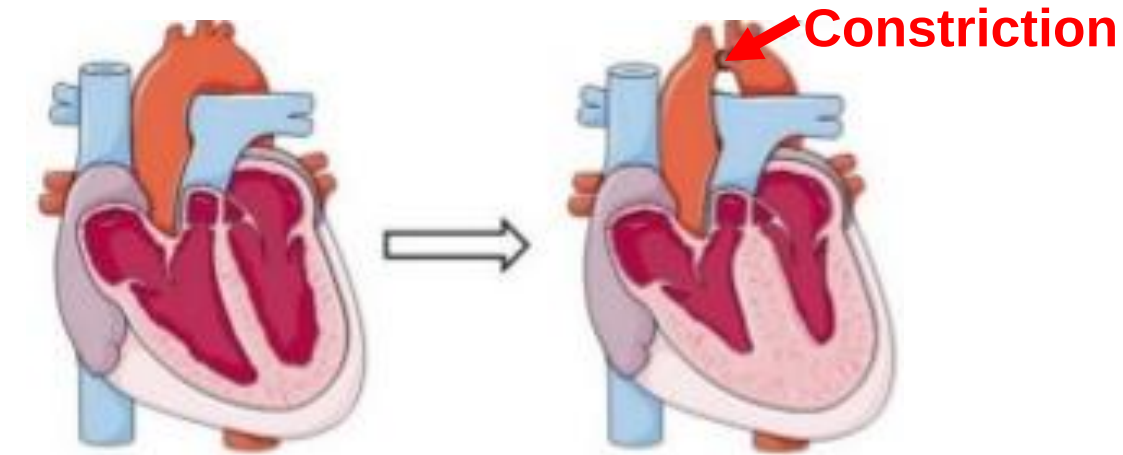
Hund *et al*, 2010

In vitro



Ashpole *et al*, 2012

Phosphoproteomics of cardiac Nav1.5 channels

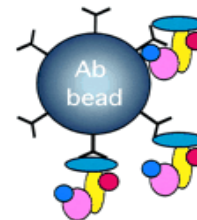
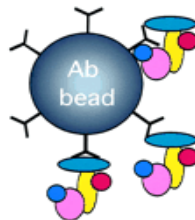


**Sham
Ventricles**

**TAC
Ventricles**

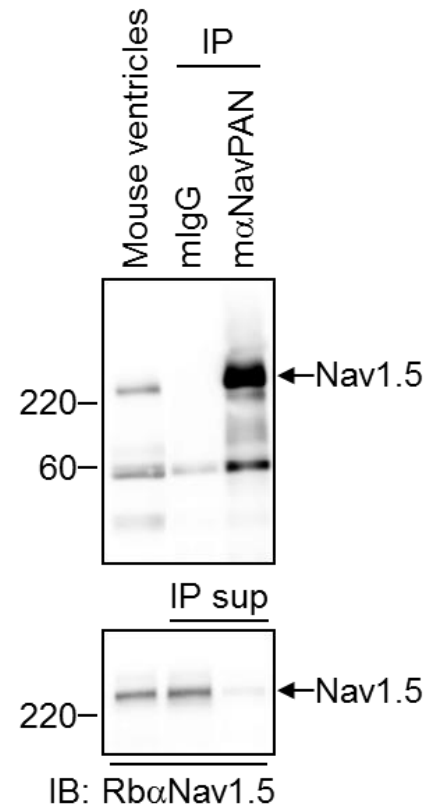
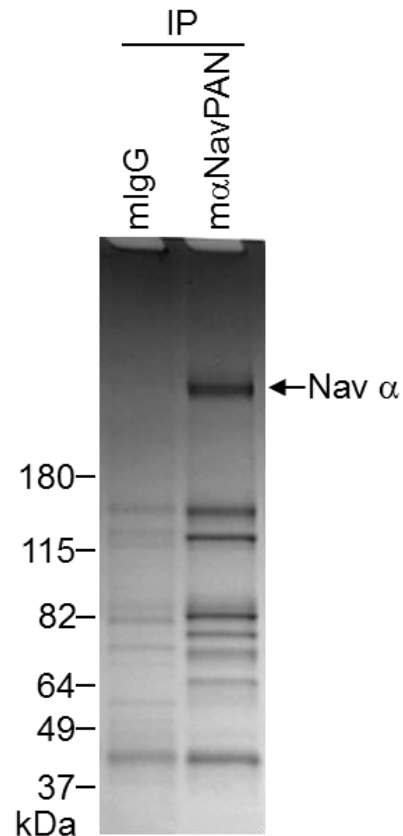
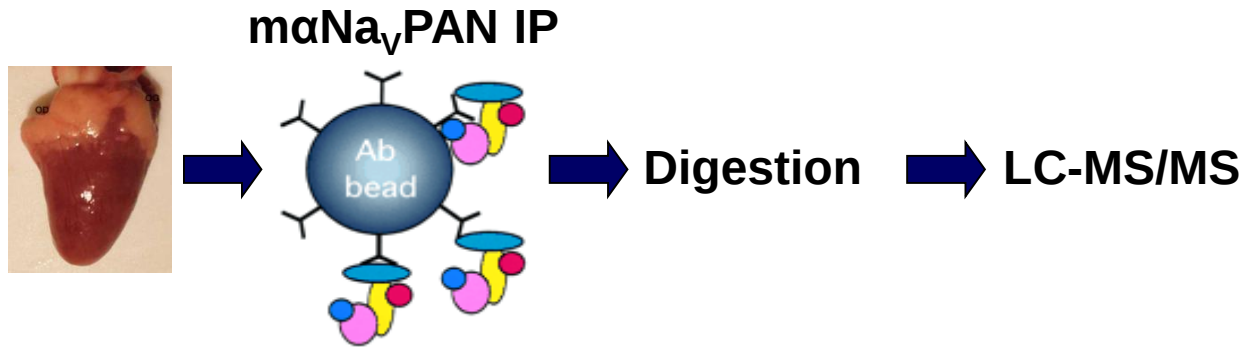
m α NavPAN-IPs

m α NavPAN-IPs



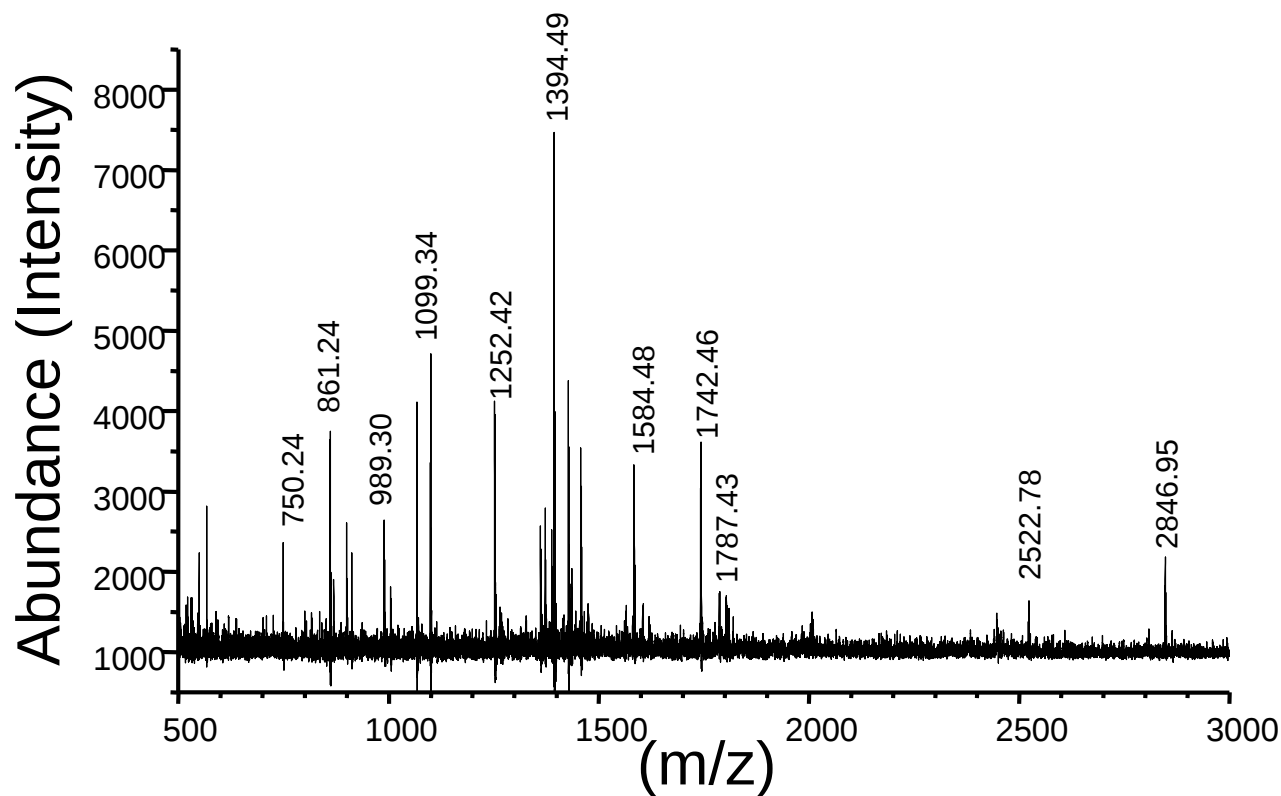
Quantitative phosphoproteomics

Purification of cardiac Nav1.5 channels



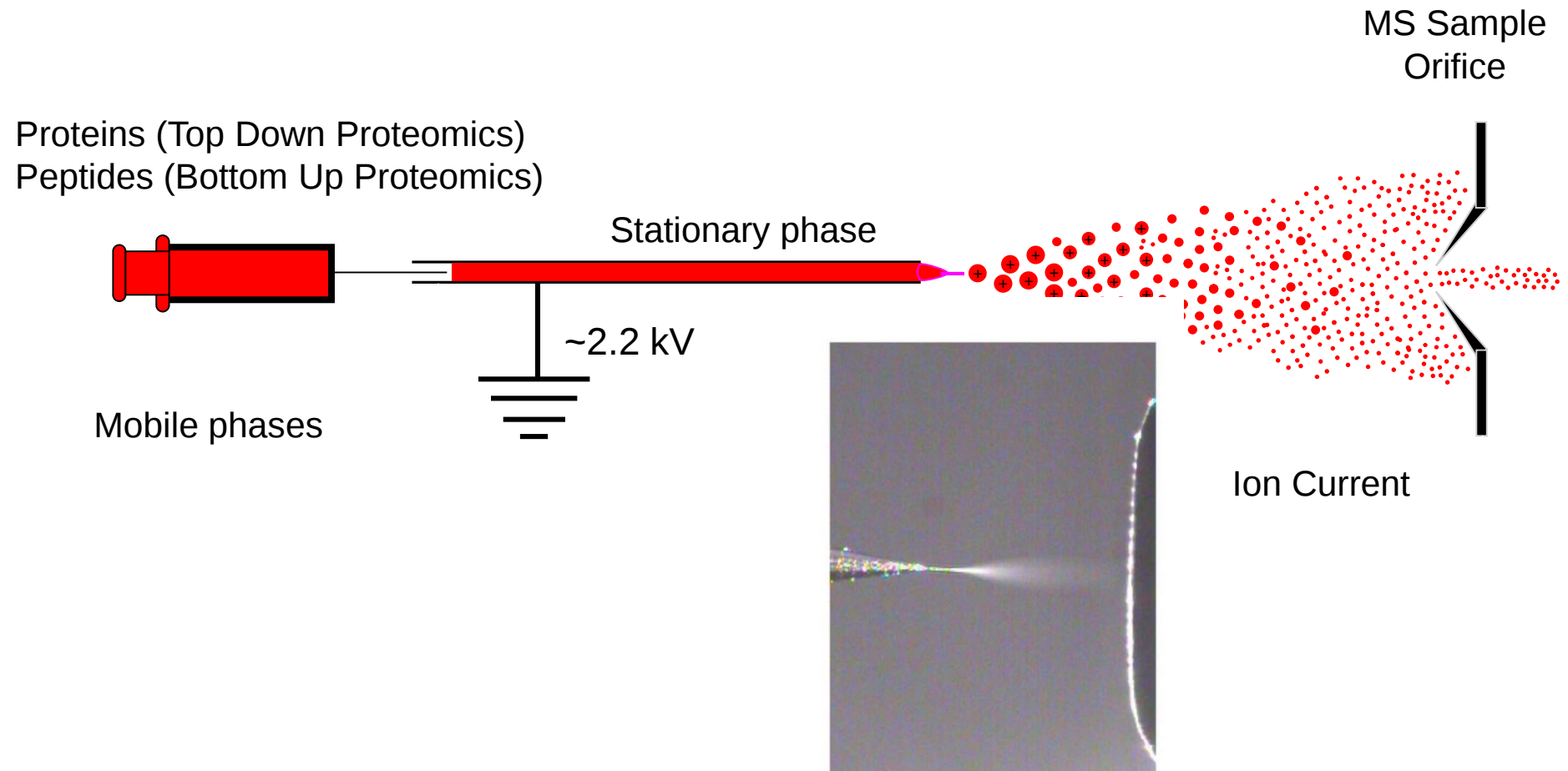
Mass Spectrometry – A Brief Definition

Mass Spectrometry is a technique that produces charged molecules, and separates them by magnetic and/or electric fields based on their mass to charge ratio (m/z).



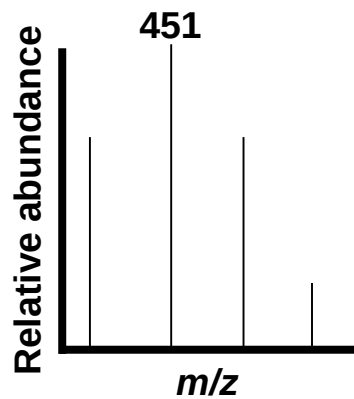
Electrospray ionization : ESI-MS

- Couples liquid chromatography to mass spectrometry
- Reversed-phase chromatography

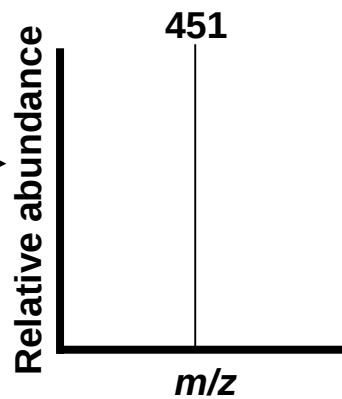


MS/MS analyses

MS1: mass spectrum of precursor ions

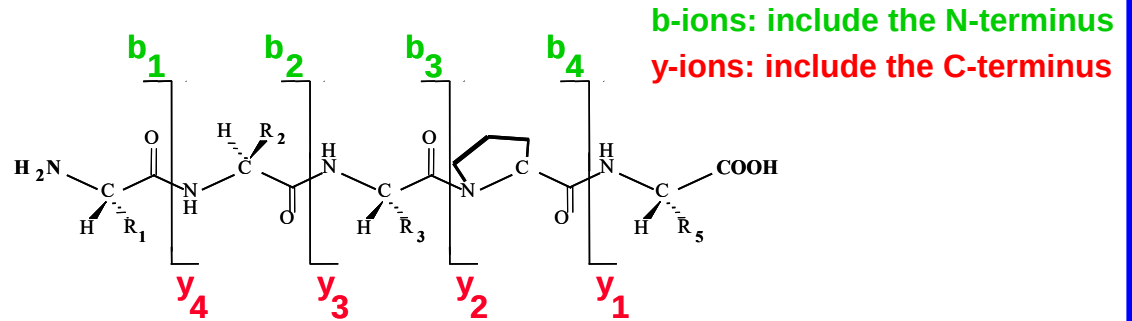
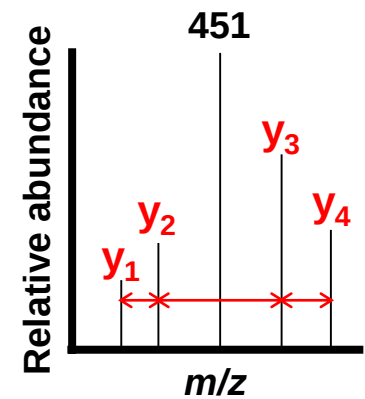


Isolation of the most abundant precursor ions



CID: Collision Induced Dissociation

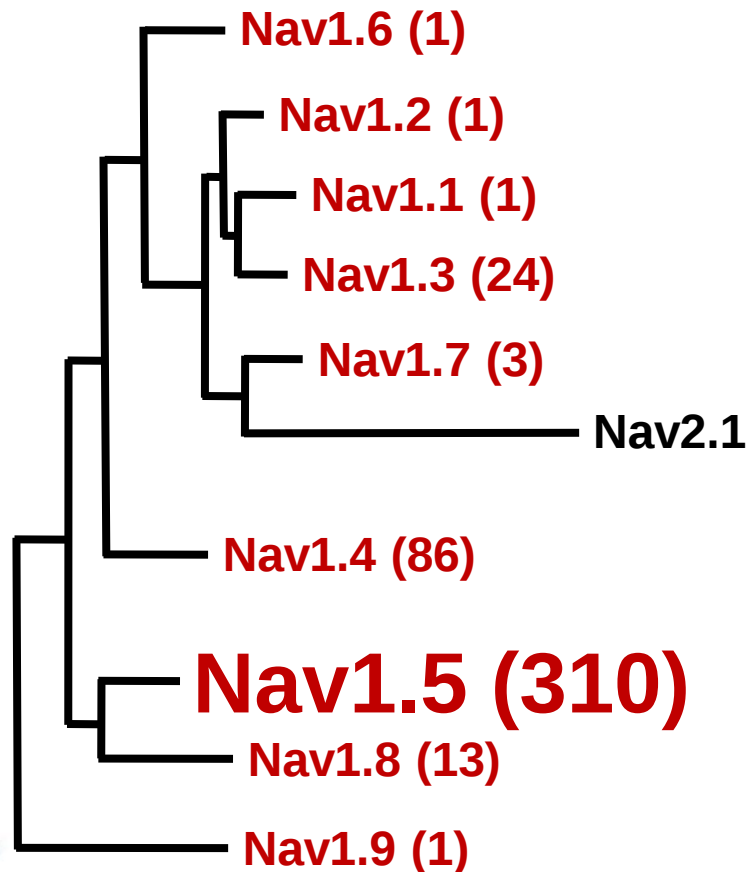
MS2 (MS/MS or tandem MS): fragmentation spectrum



Identification of Nav pore-forming subunits

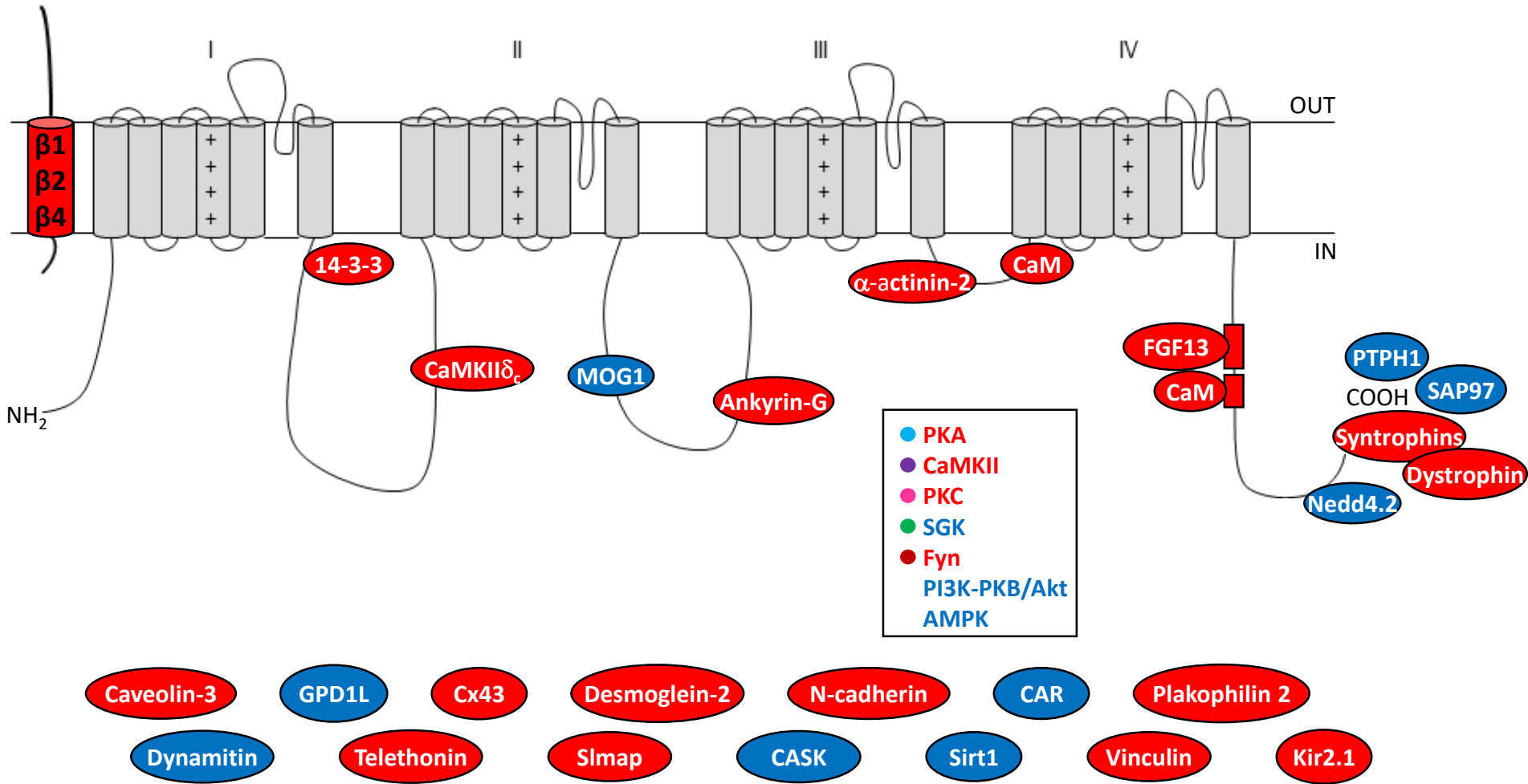
Nav_v1.5 :

- Q1080 and Q1080 del isoforms
- 310 unique peptides
- 56% amino acid coverage

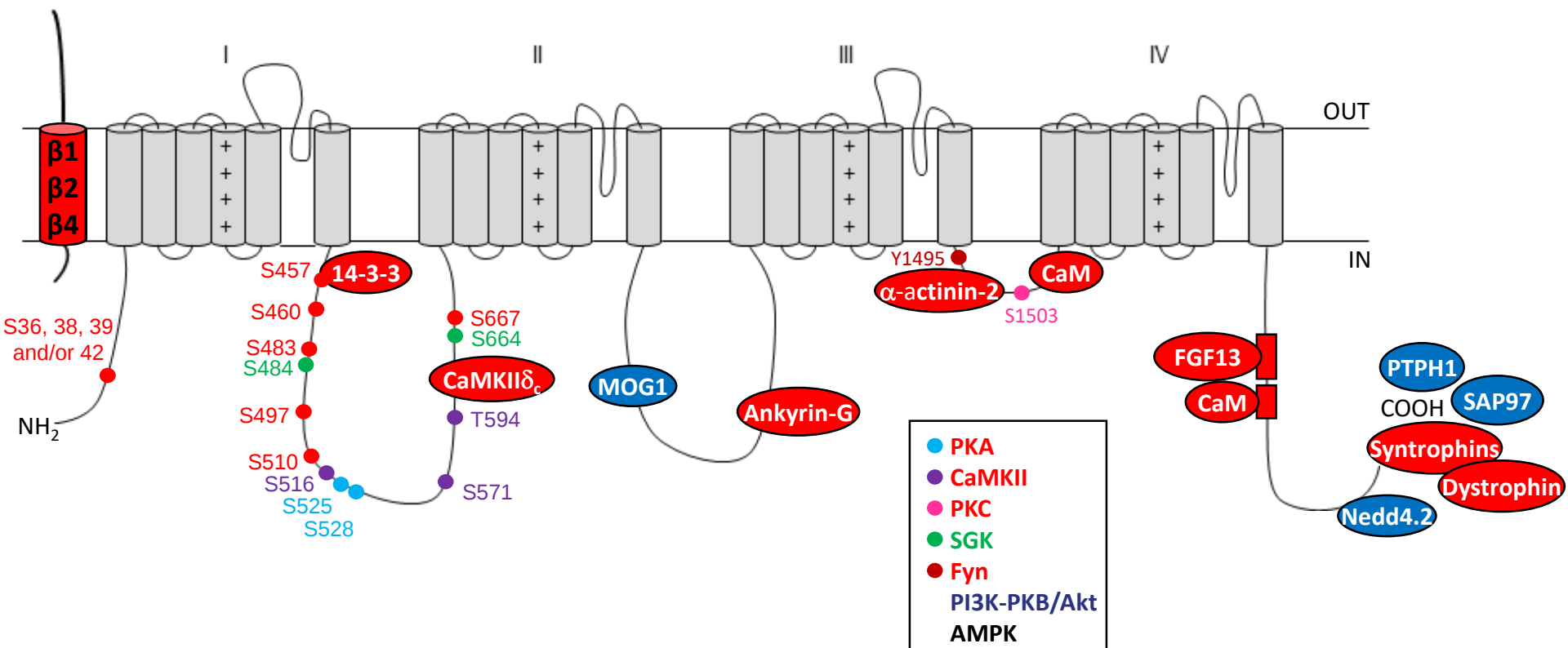


1 MANFLLPRGTS FRR FTRESLAAIEKR MAEKQARCSAT QE REGLP EEEAPRPQDLQASKKLPDLYGNPPREL
N-TERM
76 IGEPLEDLDPFYSTQKTFIVLNKGT IFR FSATNALYVLSPFHPVRRAAVKIIVHSLF SMLIMCTILTN CVFMAQ
N-TERM IS1
151 HDPPFWTKYVEYTFATYTFESLVKILAR GFCLHAFTFLRDPWNWLD FSVIVMAYTTEFVDLGNVSA LR TFRVLR
IS2 IS3 IS4
226 ALK TISVISGLK TIVGALIQSVKK LADVMVLTVF CLSVFALIGLQLFMGNLRHKCVRNFTELNGTNGSVEADGIV
IS5
301 WNSLDVYLNDPANYLLK NGTTDVL LCGN SSDAGTCPEGYR CLK AGENPDHGYTSFDSFAWAF LALFR LMTQDCWE
376 RLYQQT LRSAGKIY MIFFMLVIFLGSFYLVNLILAVVAMAYEEQNQATIAETEEKR FQ EAMEMLKKEHEALT I
IS6
451 RGVDTVSRS SLEMSPLAPVTNHERRSKRRKRLSS GTEDGGDDRLPKSD EDGPALNQL LTHGL RTSMRPRS
Loop I
526 RGSIFTFRRRDQGEADFADDENSTAGESESHRT LLVFWPLRRP TQGPFGFT APGHVNLGKRNSTVDCNGV
Loop I
601 VSLLGAGDAEATSPGSHLLRPVLRDPDPTTTPSEEPGGPQMLTPQAPCADGFEPEGARQRALSAVS VLTSALEE
Loop I
676 LEESHRCPPCWNRFQAHYLIWECCPLWMSIKQKV FVMDPFA DLTITMCIVLNTL FMALEHYNM TAEFEEMLO
IS1
751 VGNLVFTGIFTAETFKI IALDPYYFQCGWNIFDSIIVILS LIMEGLSRMGNLSVLR SFRLLRVFKLAKSWPTL
IIS2 IIS3 IIS4
826 NTLIKIIGNSVGALGNLTLVLAIIVFIFAVVGMQLFGK NYSELRRHRISDSGLLP WHMDDFFHAF LIIFRILCGE
IIS5
901 WIETMWDCMEVSGQSLCLLVFLVMVIGNLVVNLNLFALLLSFSADNL TAPDEGEMNNLQALARIQGRGLRV
IIS6
976 KRTTWDFCCGLLR RP KPAALAT ES QLFSCIAAPRSPPPPEVEKAPPARKETRFEEDKRPGQGT PGDTEPVCVF
Loop II
1051 IAVAESD DDQEDEENSLGTEEESSK [Q] QESQVVS GGHEPPQEPRAWSQVSET TS EAEASTSQADWQQERE
Loop II
1124 AEPRAPGCGETPEDSYSEGSTADMTNTADLLEQIPDLGEDVKDPEDCFTEGCVRRCPCCMVDTTQAPGVVWR LR
Loop II
1199 KTCYRIVEHWSWFETFI IFMILLSSGALAFEDIYLEERKTIKV LLEYADKMFTYV FVLEMLLKWVAYGFKKYFTNA
IIIS1 IIIS2
1274 WCWLD FLIVDVS LVS L VANTLGFAEMGP IKSRLTRLRALRPLRALS RFE GMRVVVNALVGAI PSIMNVLLVCLIFW
IIIS3 IIIS4
1349 LIFSIMGVNL FAGK FGRCINQTEGDLPLNYTIVNNKSECSFNV TGE LYTKVKVNFNDV GAGYLALLQVATFKG
IIIS5
1424 WMDIMYAAVDSRGYEEQPQWEDNLYMYIYFVVFI IFGSFETLNLFIGVII DNFNQKKLGGQDI FMTEEQKKYY
IIIS6
1499 NAMKKLGSK KPQKPIPRPLNKYQGFIFDI VTRQAFDVTIMFLICLNMTMMVETDDQSPEKVNILAKINLLFVAI
Loop III IVS1 IVS2
1574 FTGECIVKMAALRHYYFTNSWNIFDFVVLVSIVGTVLSDI IQYFFSPTLFRVIRLARIGRI LR LIRGAKGIRT
IVS3 IVS4
1649 LLFALMMSLPALFN IGLLLFLVMFIYSIFGMANFAYVKWEAGIDDMFNQTFAN SMLCLF QITTSAGWDGLLSPI
IVS5
1724 LNTGPPYCDPNLPNSNGSRGNCSPAVGILFETTYIIISFLIVNM YIAIILENF SVATEESTEPLSEDDFDMFY
IVS6
1799 EIWEKFDPEA QFIEYLALSDFADALSEPLRIAKPNQISL INMDLPMVSGDRIHCDILFAFKRVLGSGEMDA
C-TERM
1874 LKIQMEEK FMAANPSKISYEPITTTLRKHEEV SATVIQRAFRRHLLQRSVKHASFLFRQQACSGLSDEDA PER
C-TERM
1949 EGLIAYMMNENFSRSGPLS SSISS TSFPSPSYDSVTRATSNLPVRASDYSRSED LADFP PDRDRESIV
C-TERM

Characterization of cardiac Nav1.5 channel complexes



2012 : 9 + 7 = 16 phosphorylation sites



Glycosylation

Phosphorylation

Méthylation

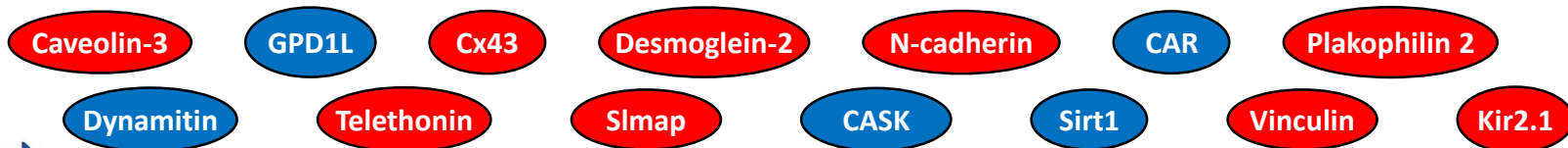
Acétylation

Oxidation

Nitrosylation

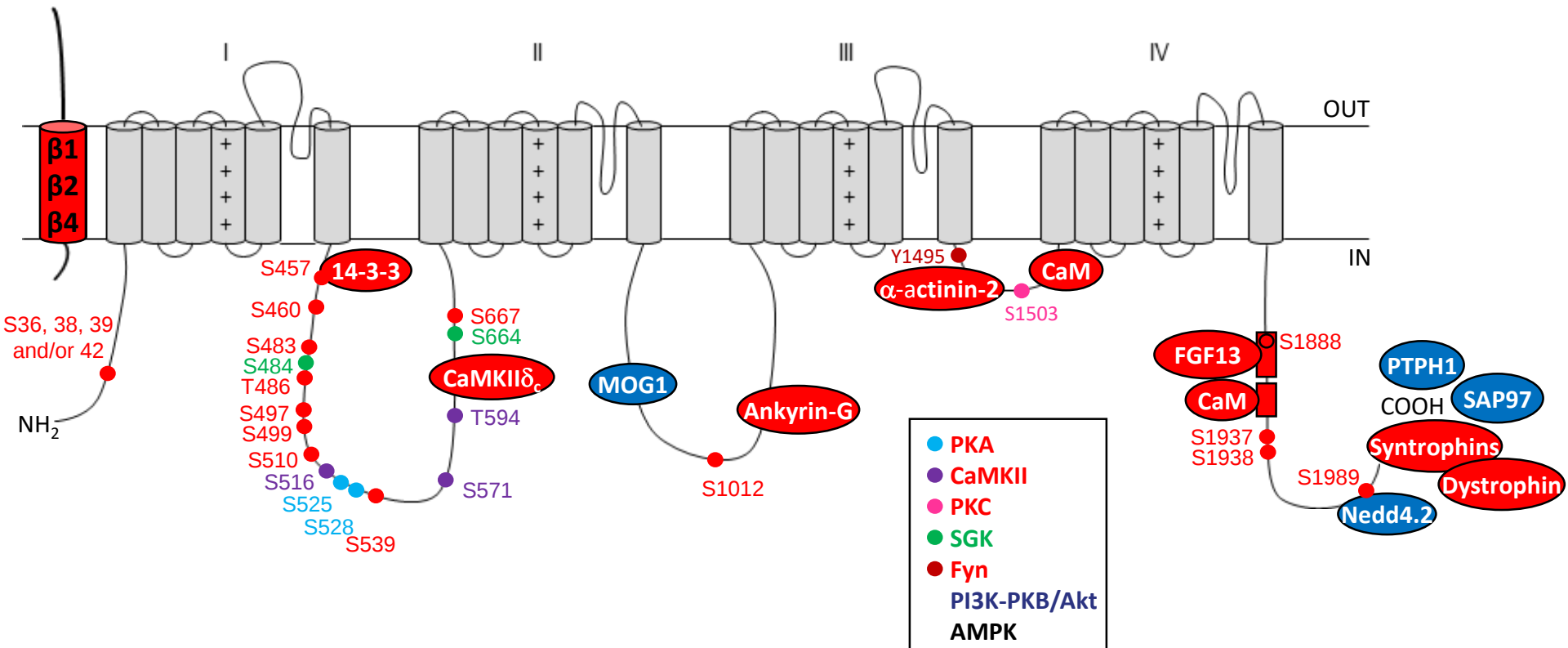
Lipoxidation

Ubiquitylation



Marionneau and Abriel, *JMCC* 2015
Marionneau et al, *JPR* 2012

2017 : 16 + 8 = 24 phosphorylation sites



Glycosylation

Phosphorylation

Méthylation

Acétylation

Oxidation

Nitrosylation

Lipoxidation

Ubiquitylation

Caveolin-3

GPD1L

Cx43

Desmoglein-2

N-cadherin

CAR

Plakophilin 2

Dynamitin

Telethonin

Slmap

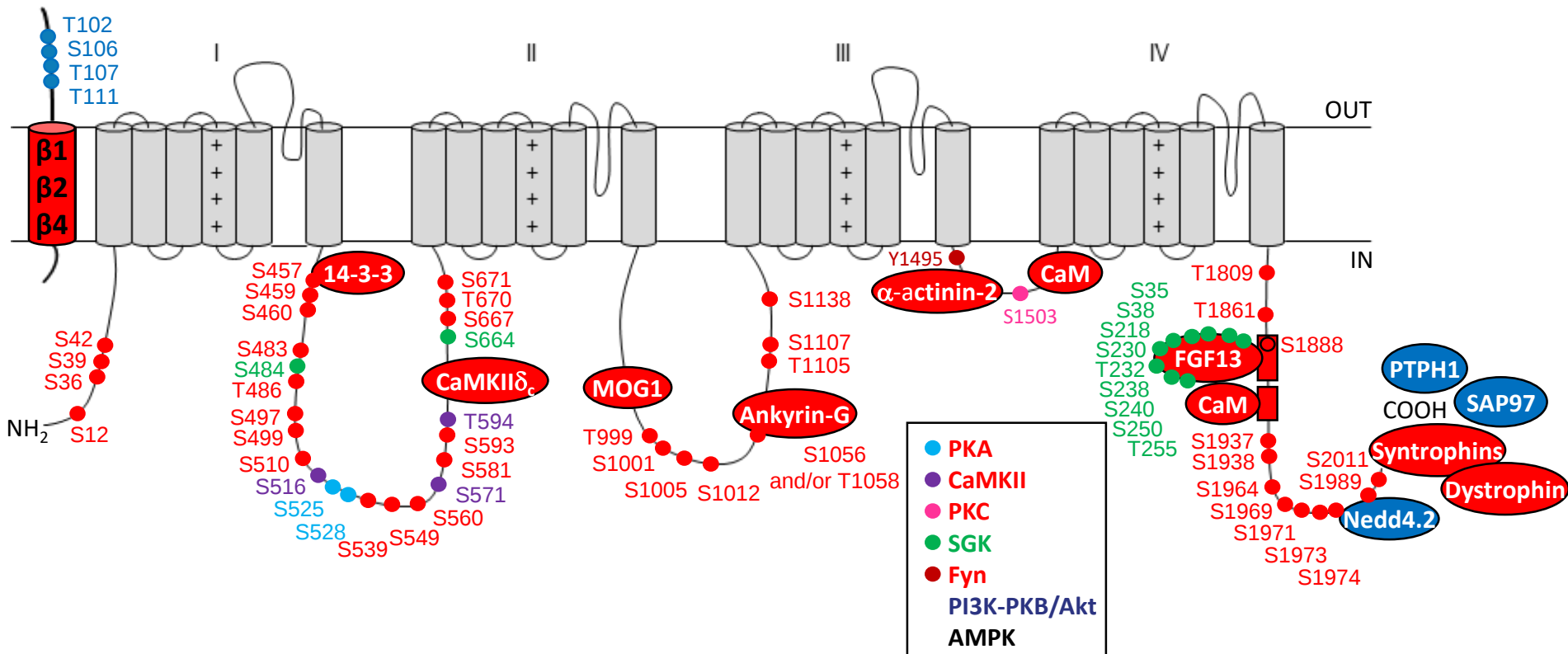
CASK

Sirt1

Vinculin

Kir2.1

2018 : 49 + 4 + 9 = 62 phosphorylation sites



Glycosylation

Phosphorylation

Méthylation

Acétylation

Oxidation

Nitrosylation

Lipoxidation

Ubiquitylation

Caveolin-3

GPD1L

Cx43

Desmoglein-2

N-cadherin

CAR

Plakophilin 2

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Telethonin

Slmap

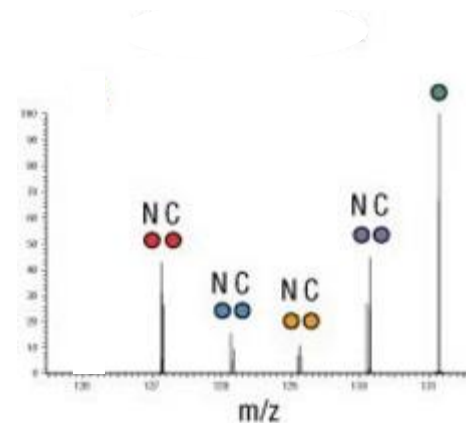
CASK

Sirt1

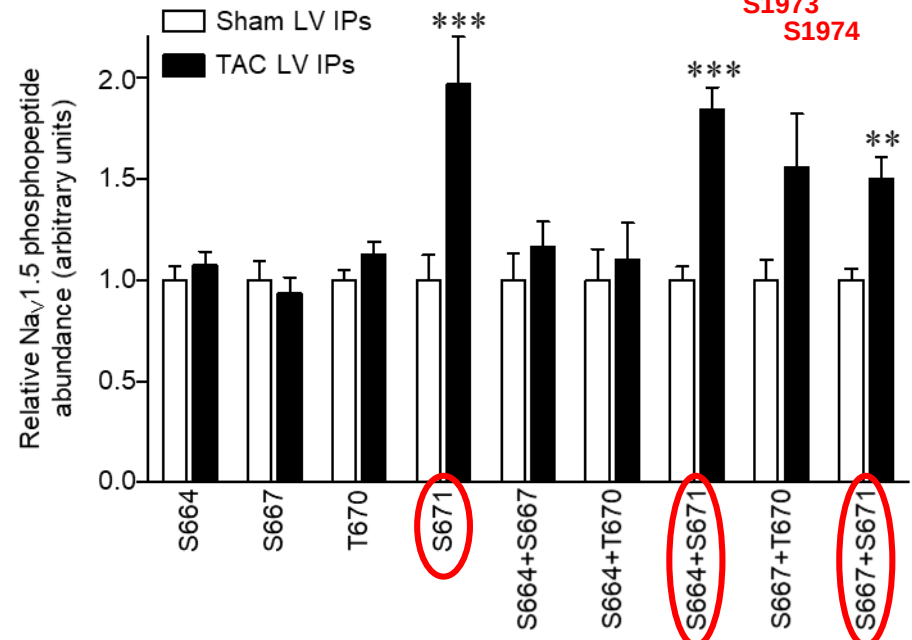
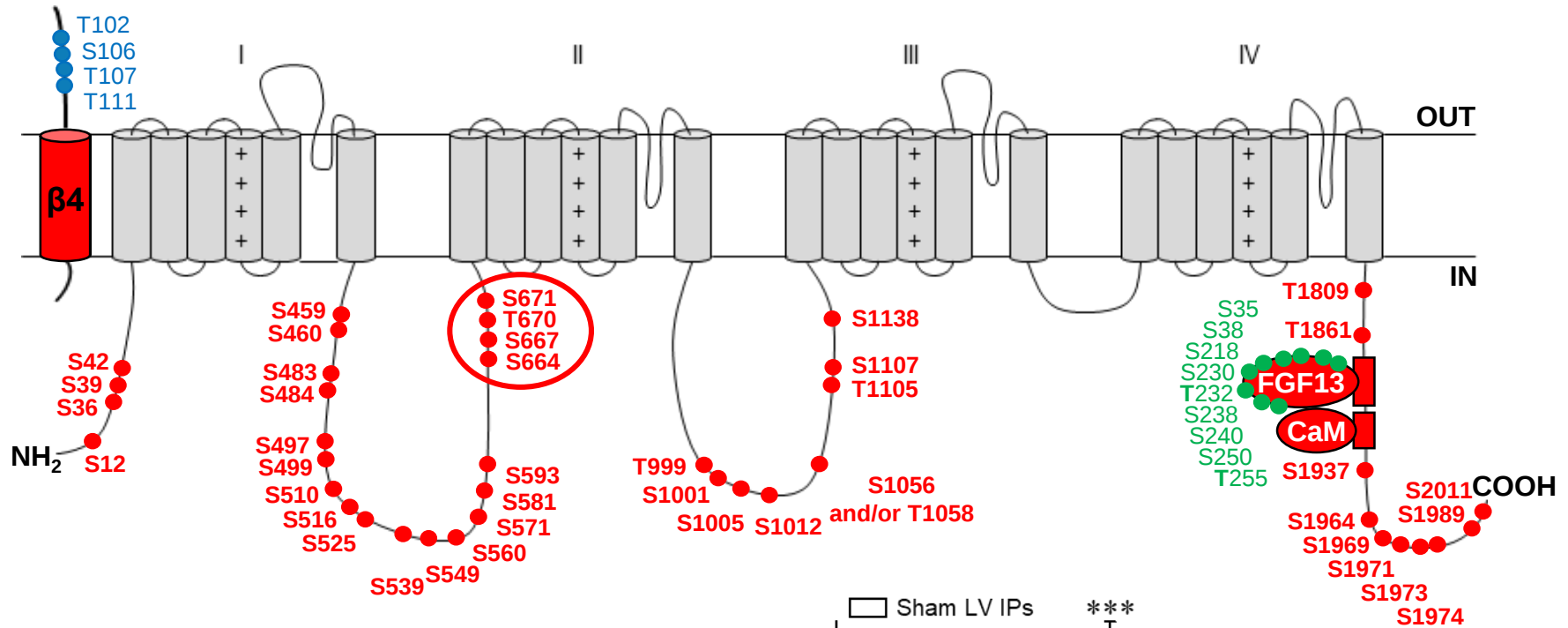
Vinculin

Kir2.1

Marionneau and Abriel, *JMCC* 2015
 Marionneau et al, *JPR* 2012
 Burel et al, *JBC* 2017
 Lorenzini et al, *JGP* 2021

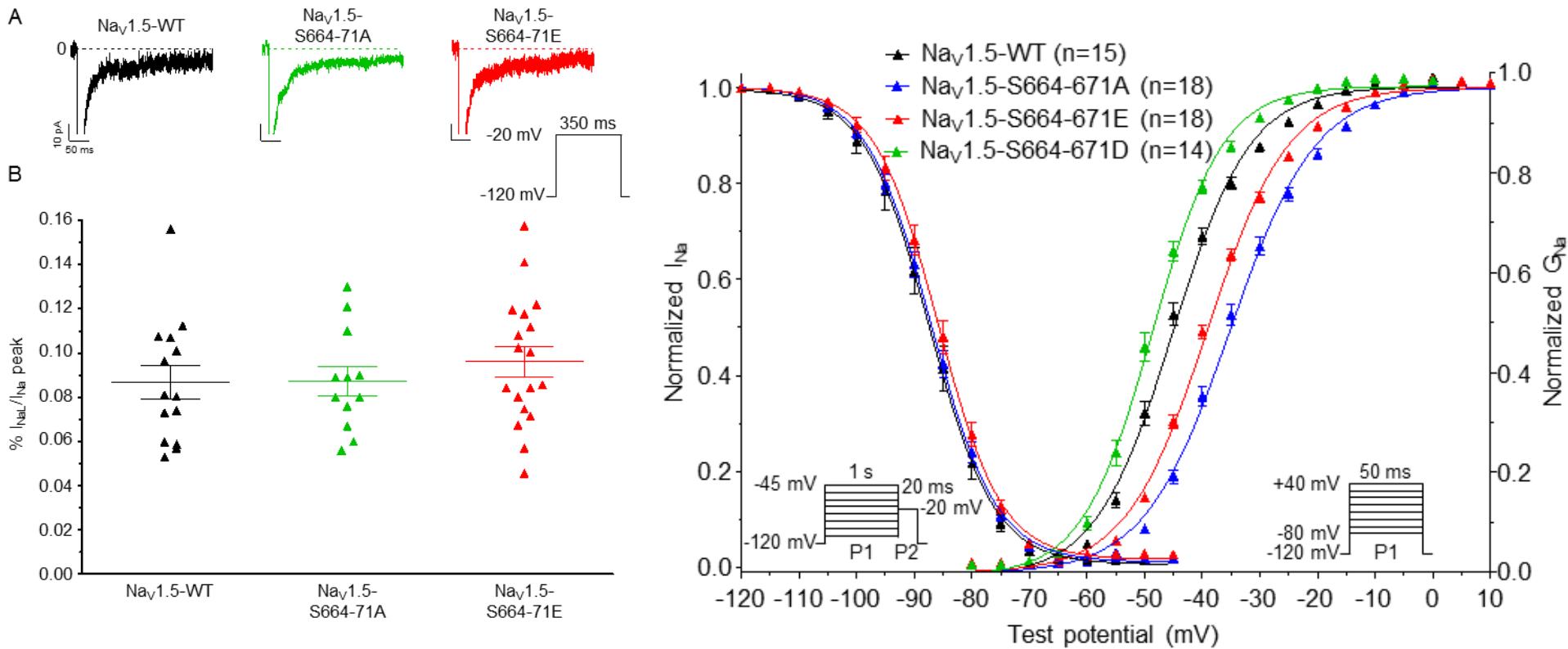


Quantitative phosphoproteomics in Sham/TAC mice

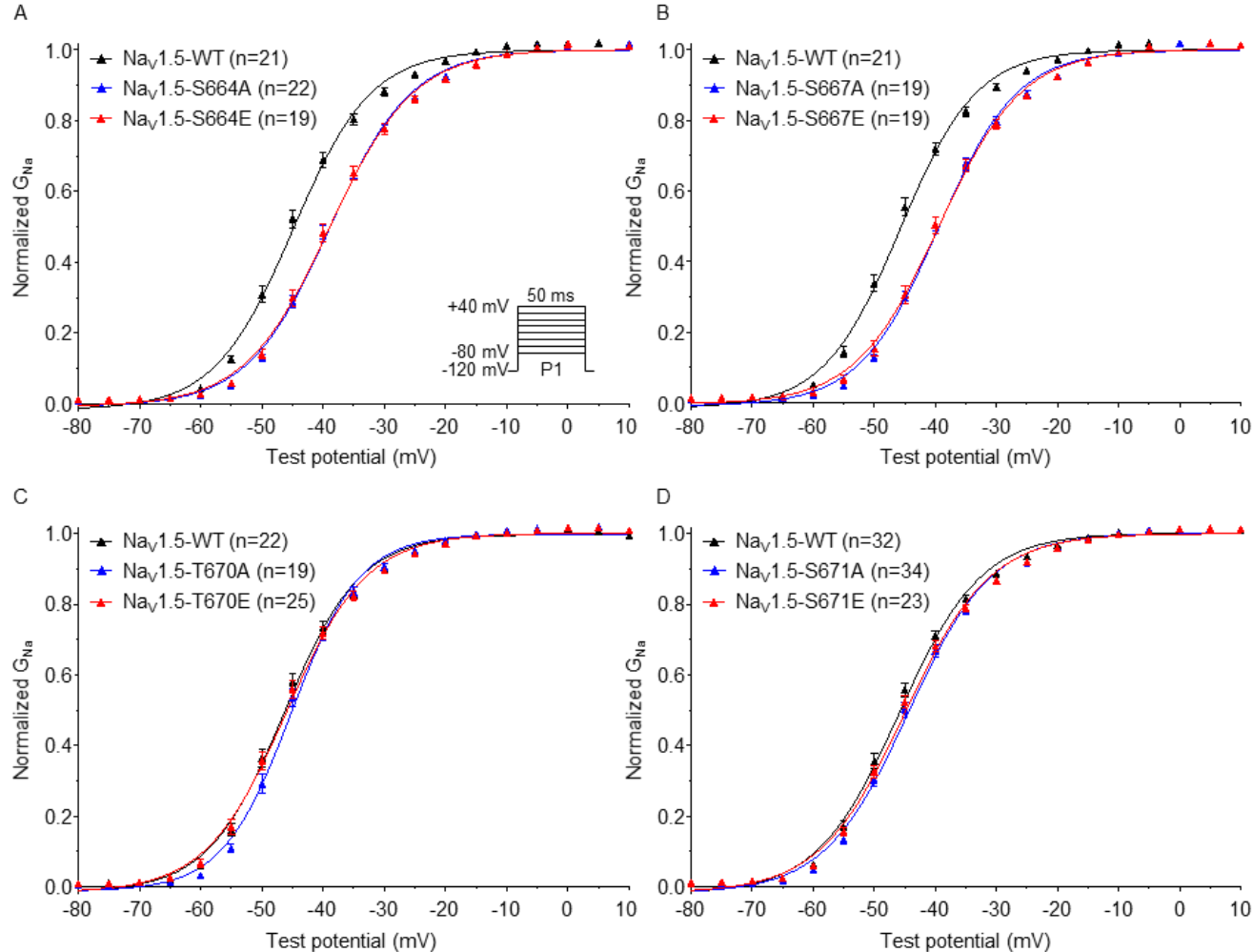


** $p < 0.01$, *** $p < 0.001$ in TAC vs Sham

Electrophysiological analysis of Nav1.5-S664-671A and E mutants

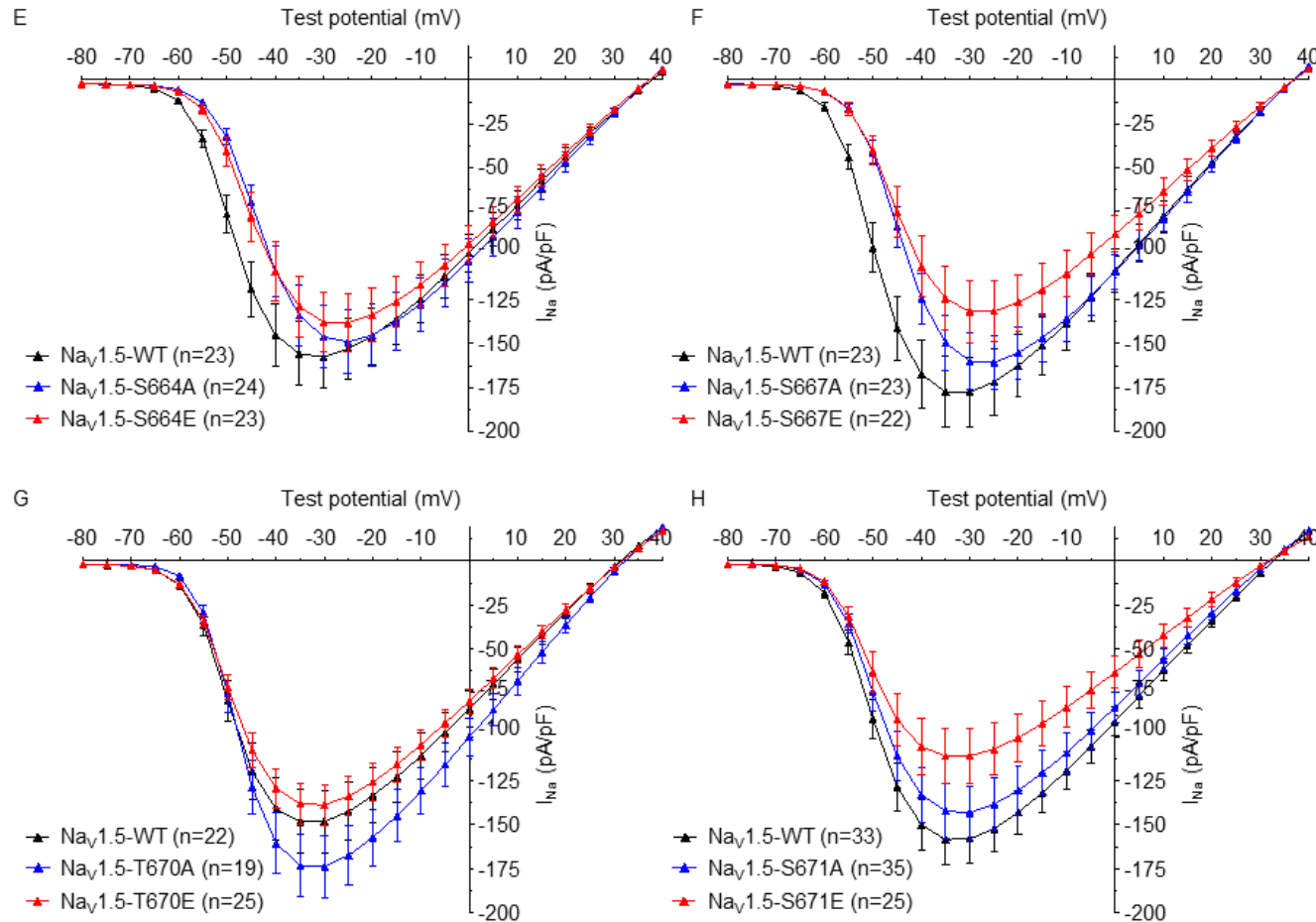


Electrophysiological analysis of simple Nav1.5 phosphomutants



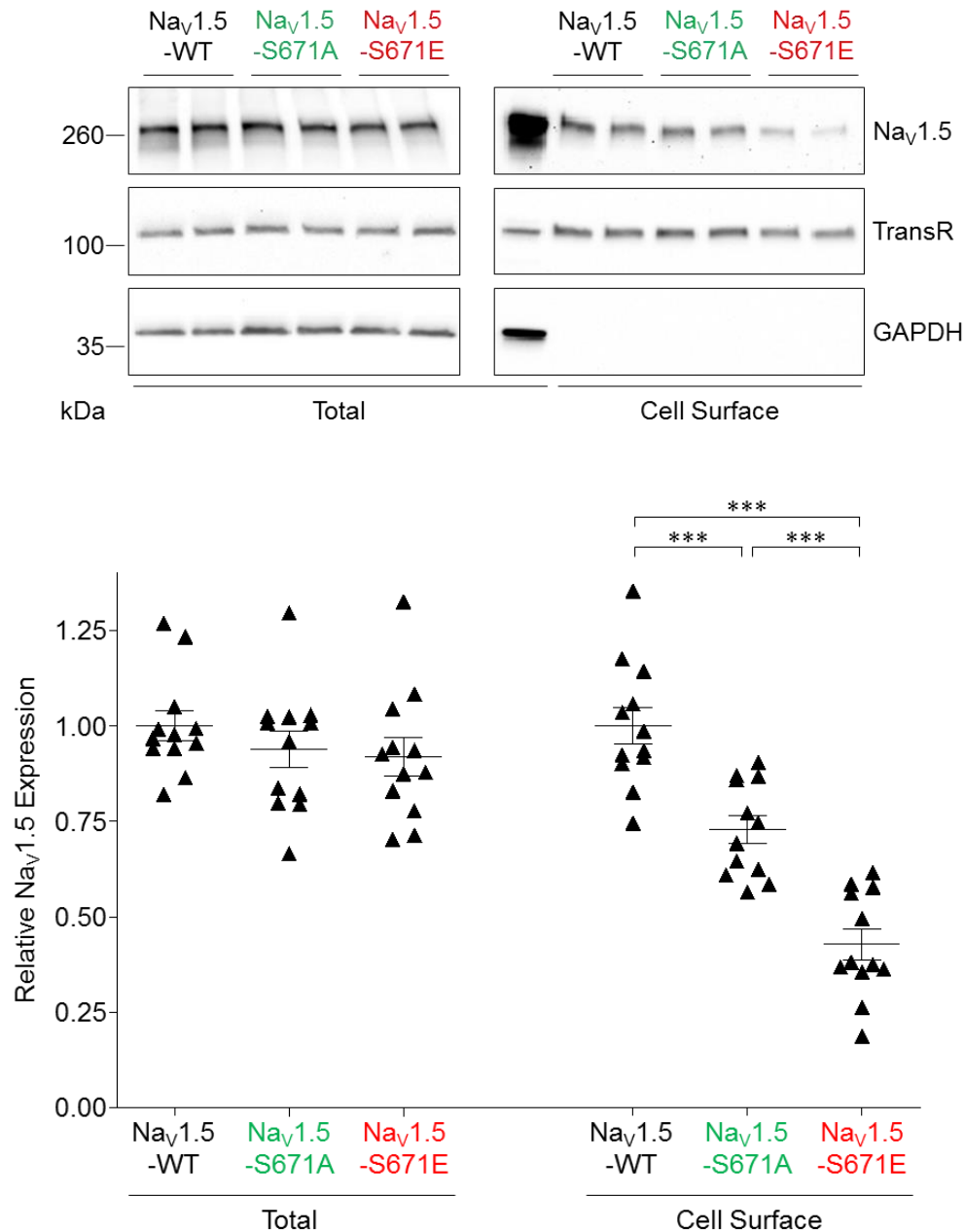
Main finding: Phosphorylation at S664 and S667 regulates Nav1.5 channel activation.

Electrophysiological analysis of simple Nav1.5 phosphomutants



Main finding: Phosphorylation at S671 decreases I_{Na} .

Phosphorylation at S671 decreases Nav1.5 cell surface expression



Take-home messages

- Cardiac electrophysiology, Nav1.5 channels
- Methods to analyze macromolecular complexes and PTM
- Identification of phosphorylation sites
 - *in silico*
 - *in vitro*
 - *in situ*
- Mass spectrometry
 - How does it work?
 - Advantages *versus* other approaches

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