



Phosphoproteomics of cardiac Nav1.5 channels



Céline Marionneau

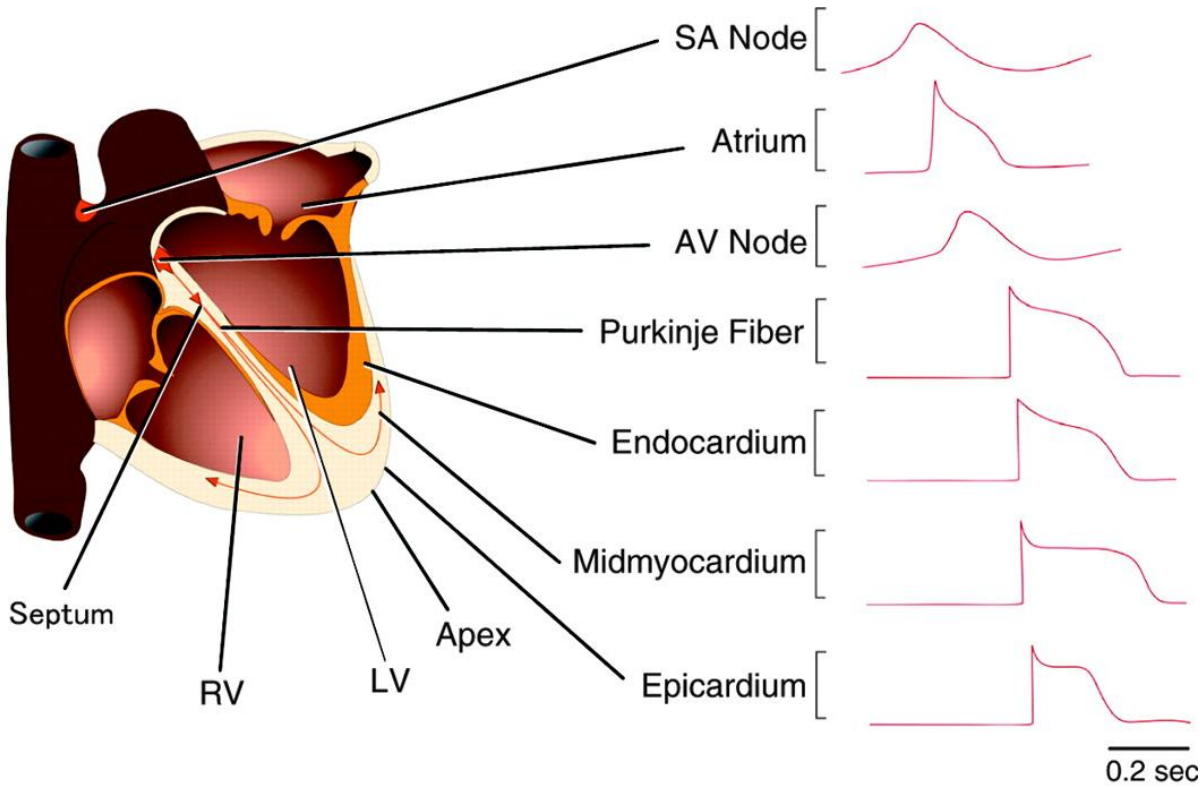
L'unité de recherche de l'institut du thorax
Inserm UMR 1087 / CNRS UMR 6291
Nantes, France

Phosphoproteomics of cardiac Nav1.5 channels

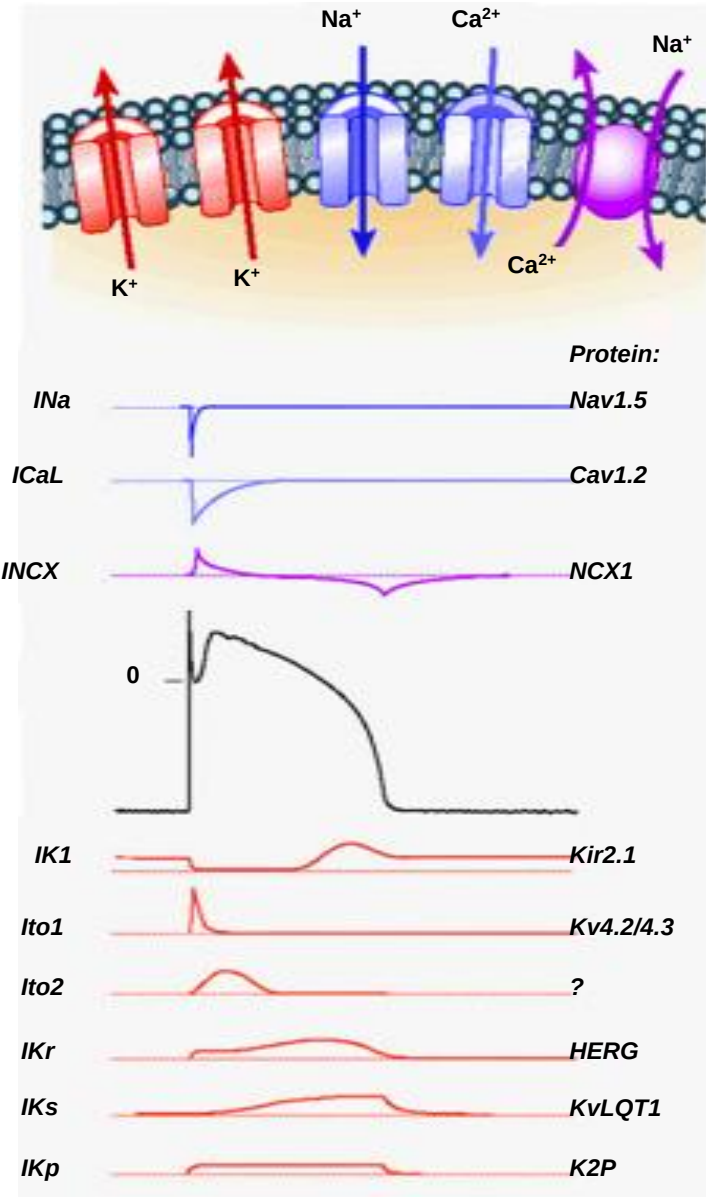
From this conference, you should know:

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

Cardiac excitability

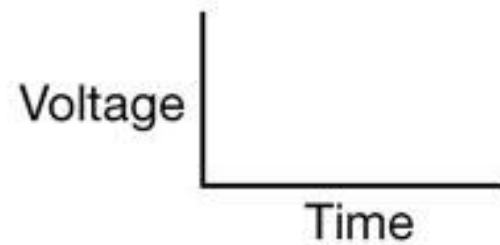
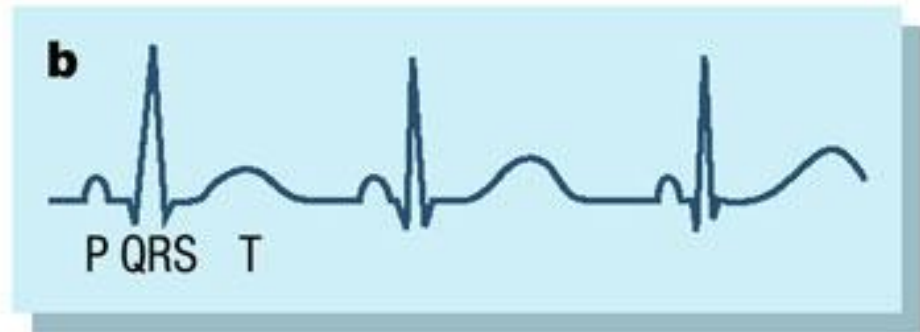
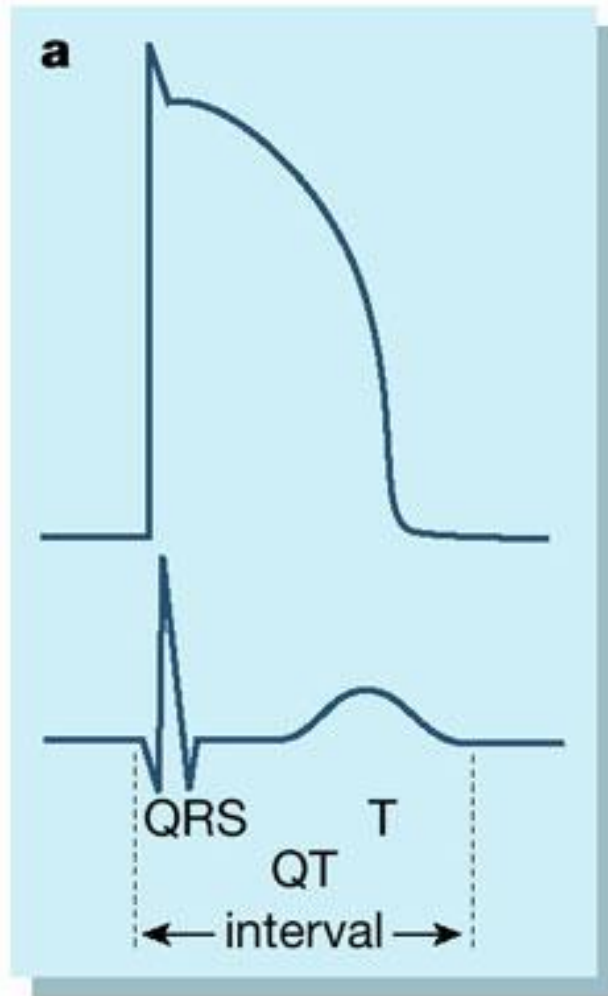


Nerbonne and Kass, *Physiol Rev* 2005

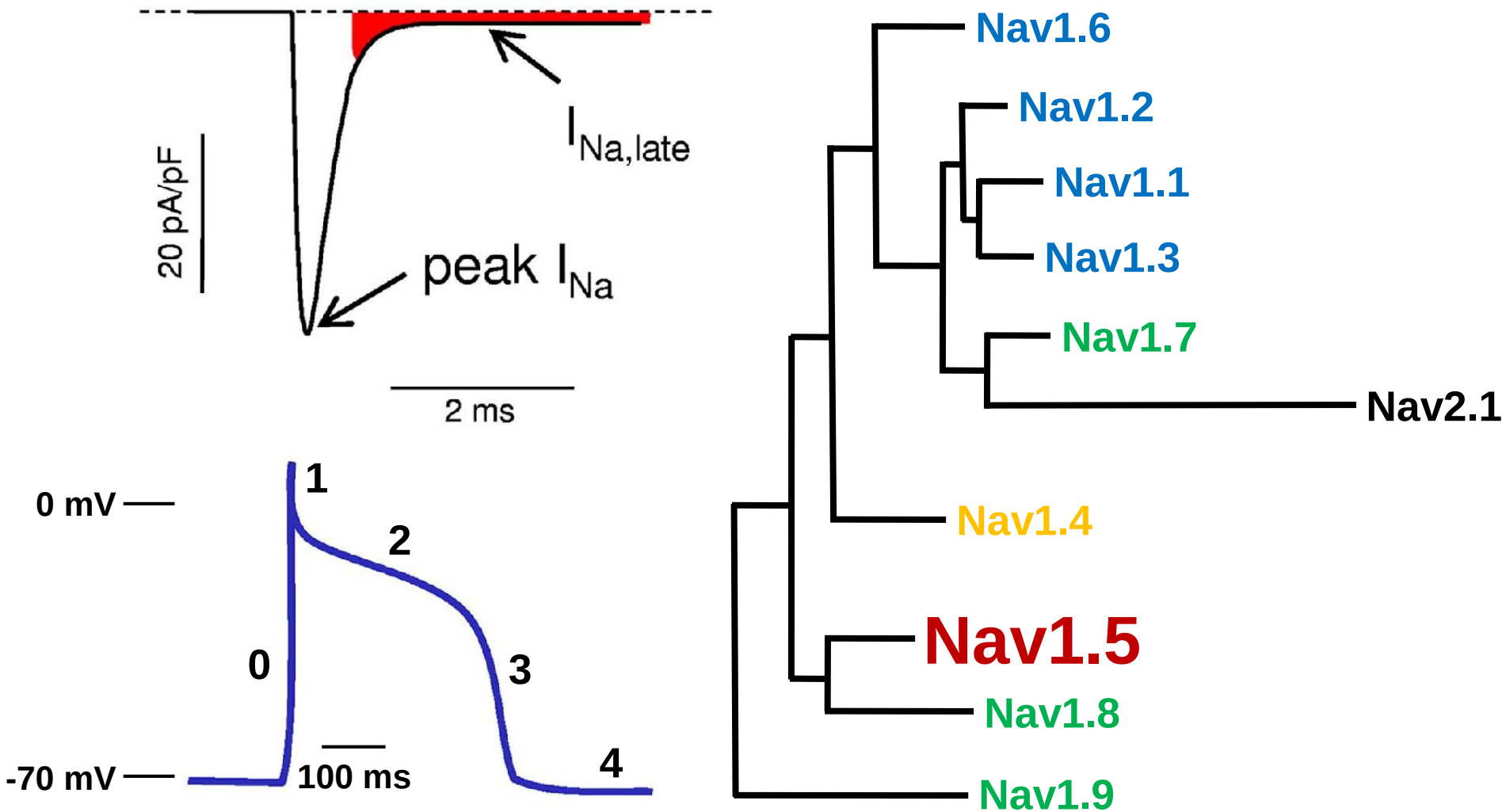


Marban, *Nature* 2002

Electrocardiograms : ECG



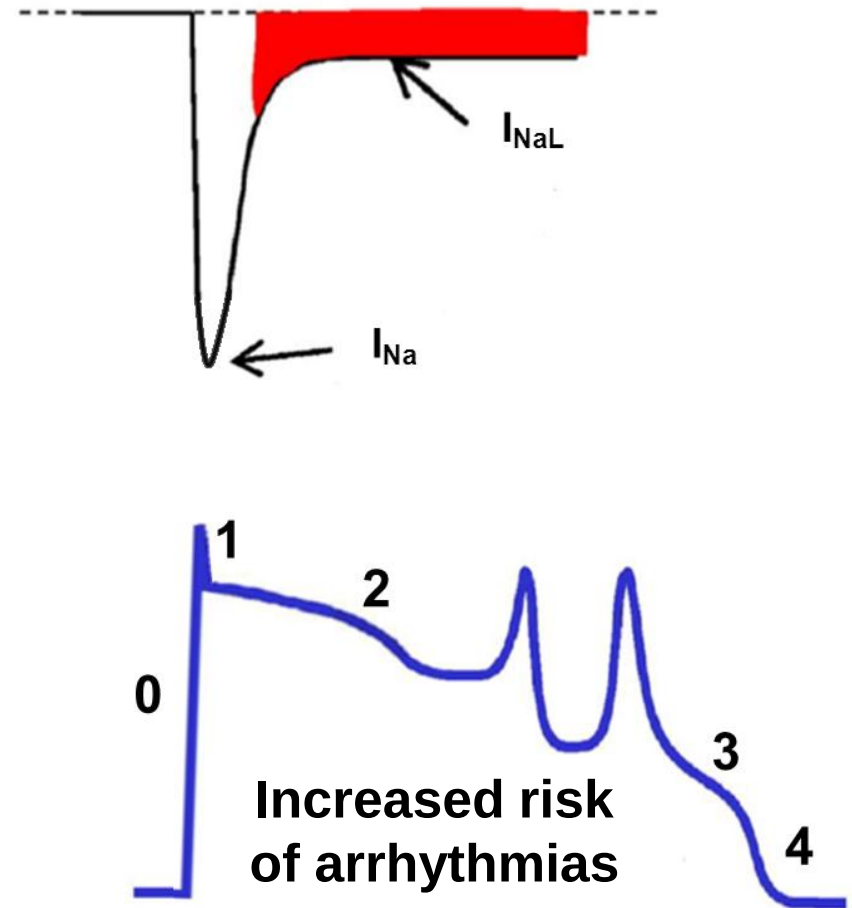
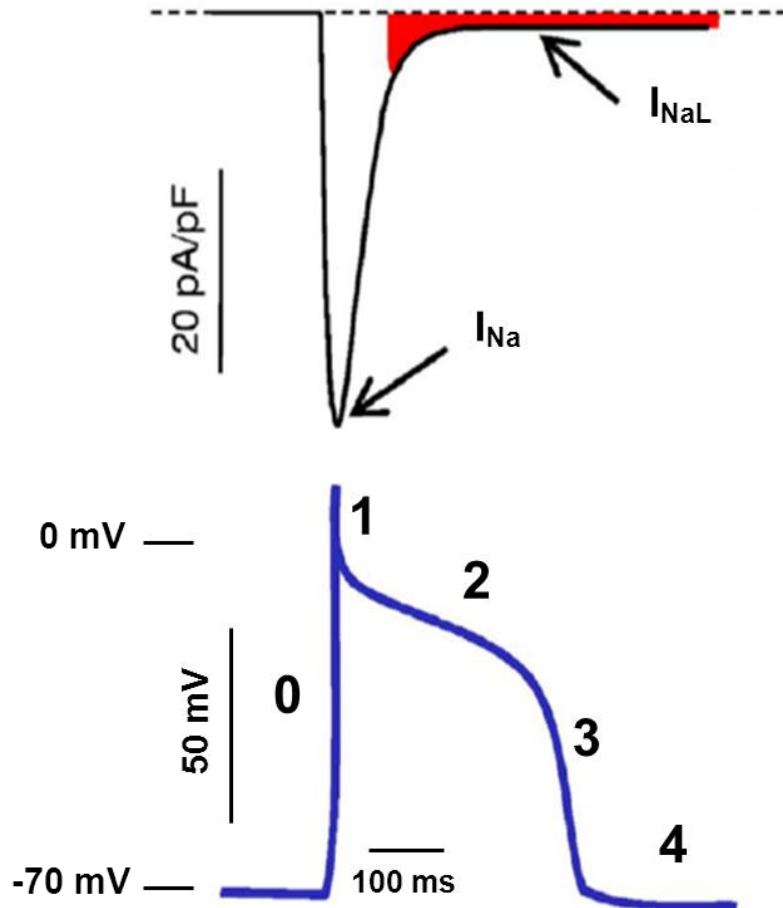
Cardiac voltage-gated Na⁺ channels



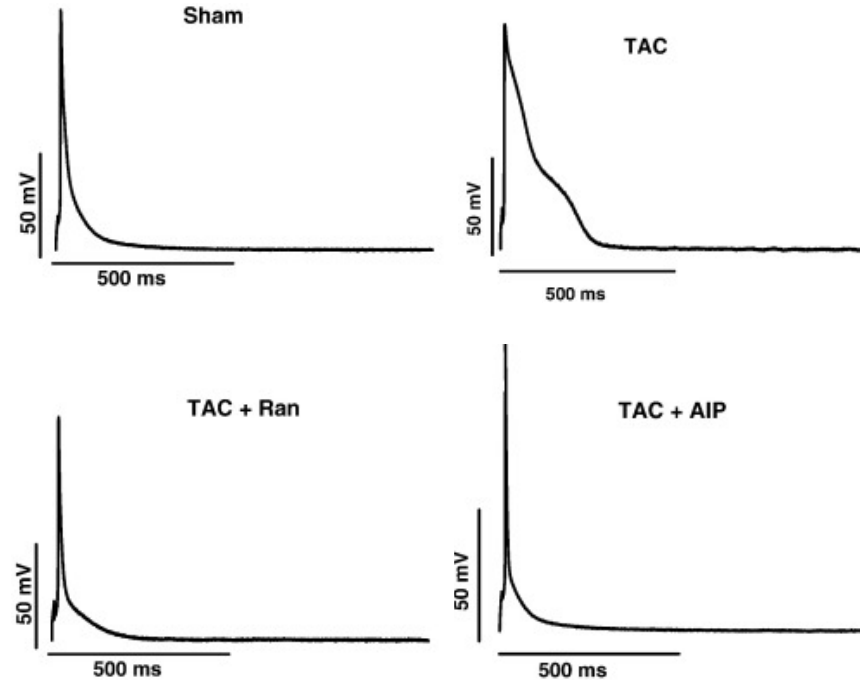
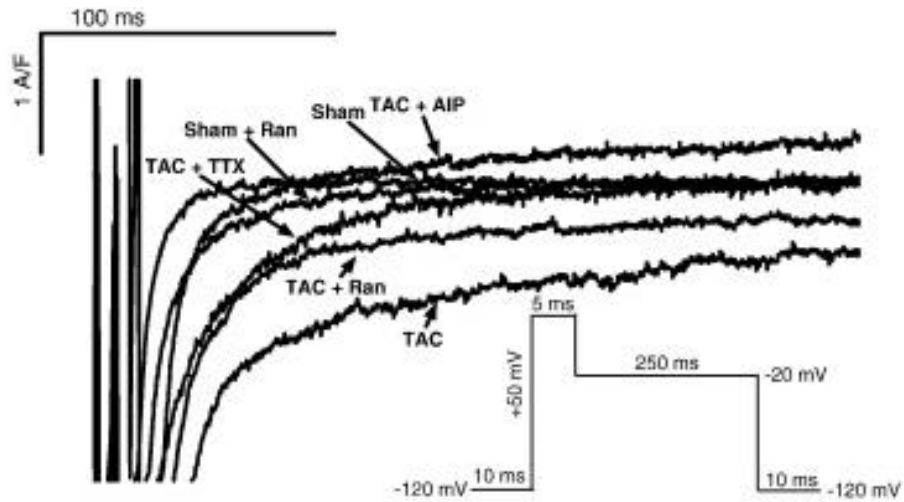
Nav1.5 channels in heart failure

Normal

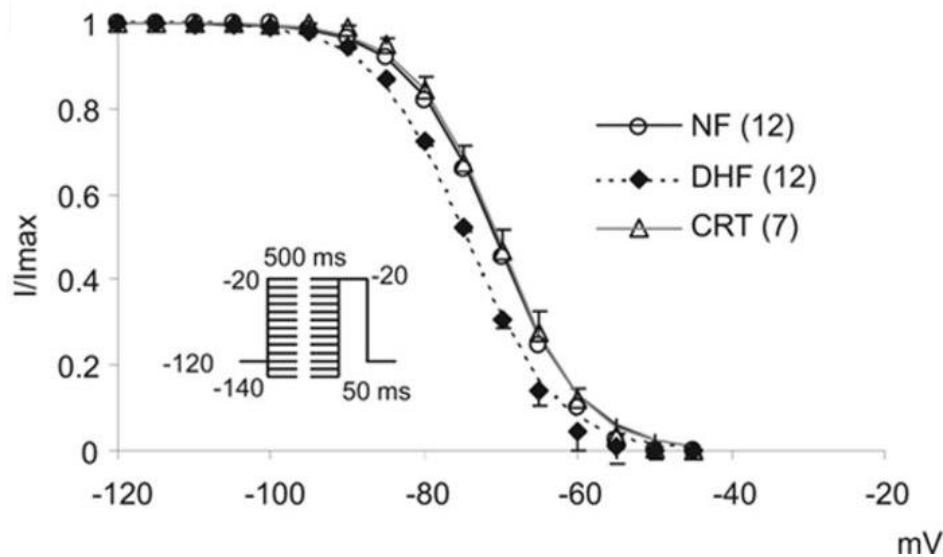
Disease



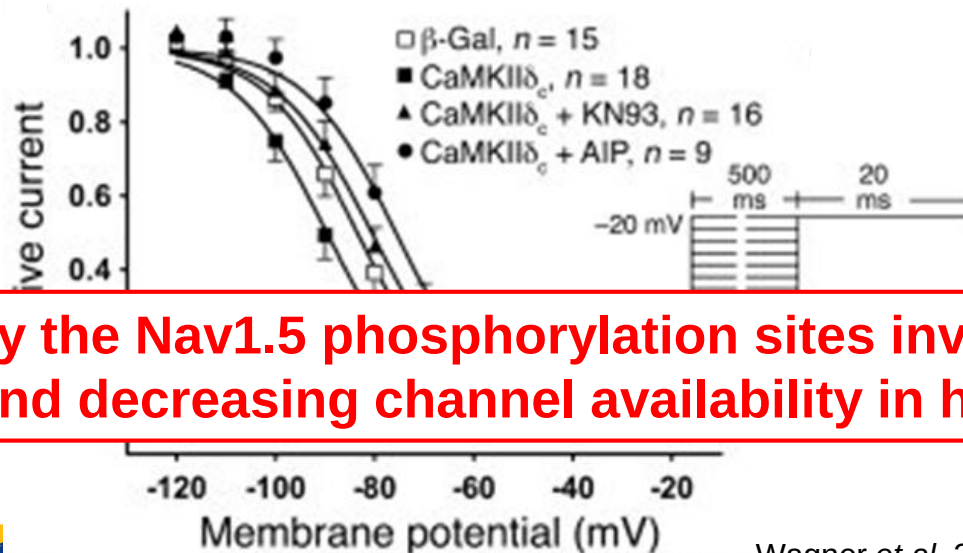
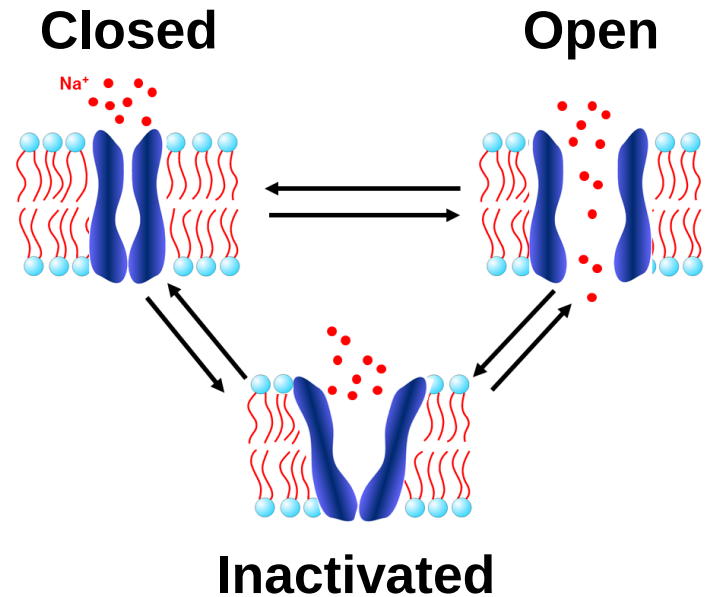
CaMKII increases I_{NaL} in heart failure



CaMKII decreases Nav1.5 channel availability



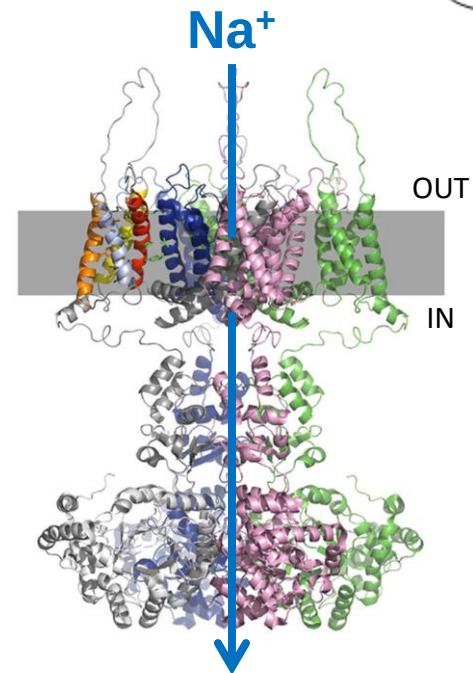
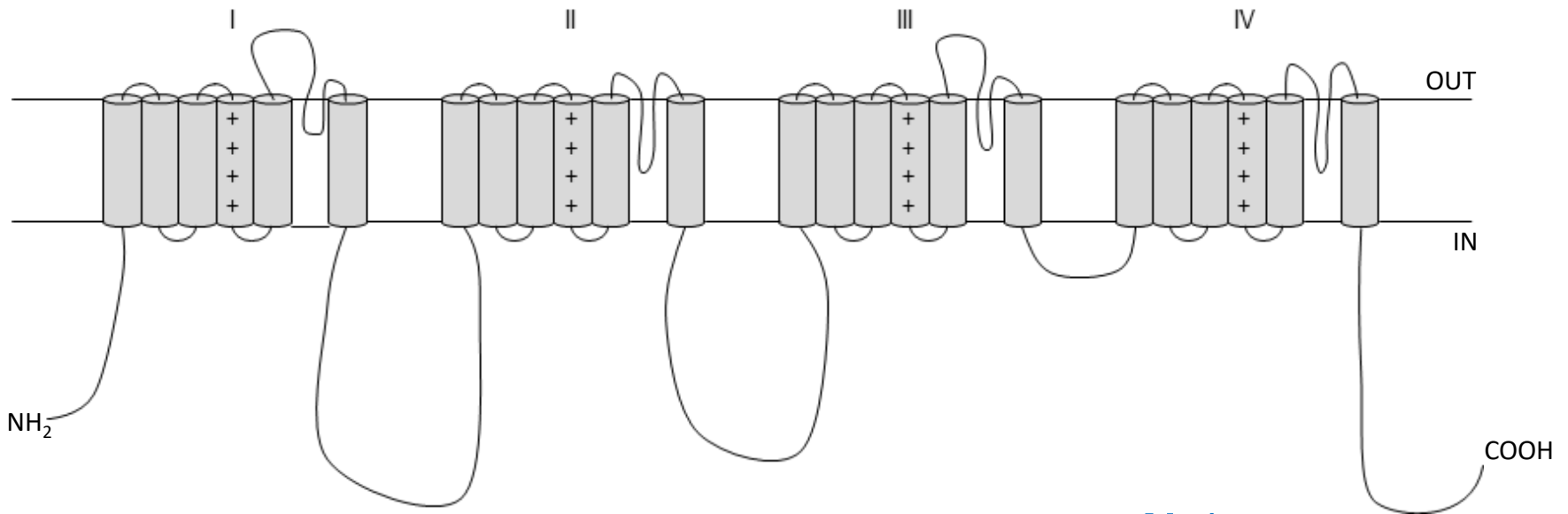
Aiba et al, 2013



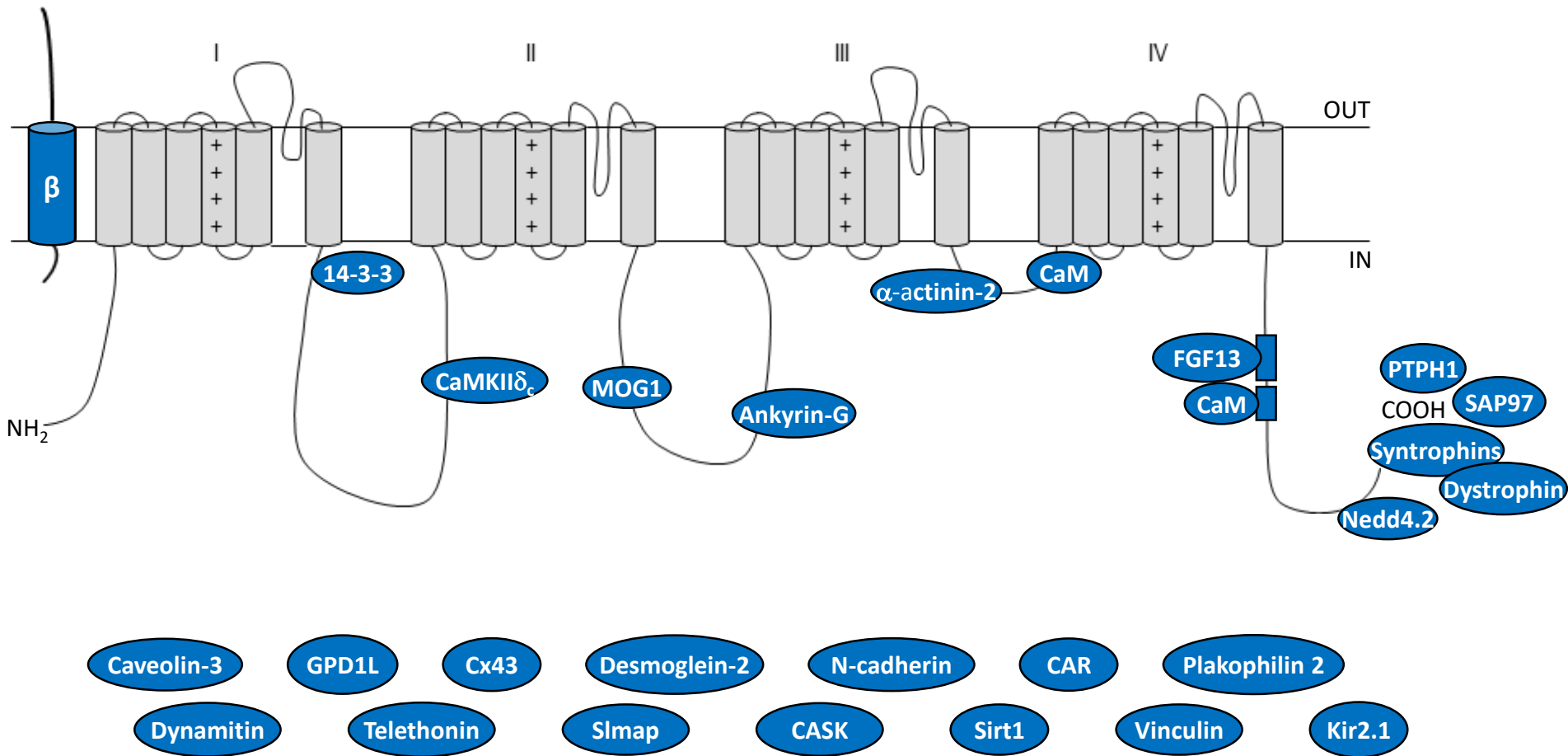
Goals: to identify the Nav1.5 phosphorylation sites involved in increasing I_{NaL} and decreasing channel availability in heart failure

Wagner et al, 2006

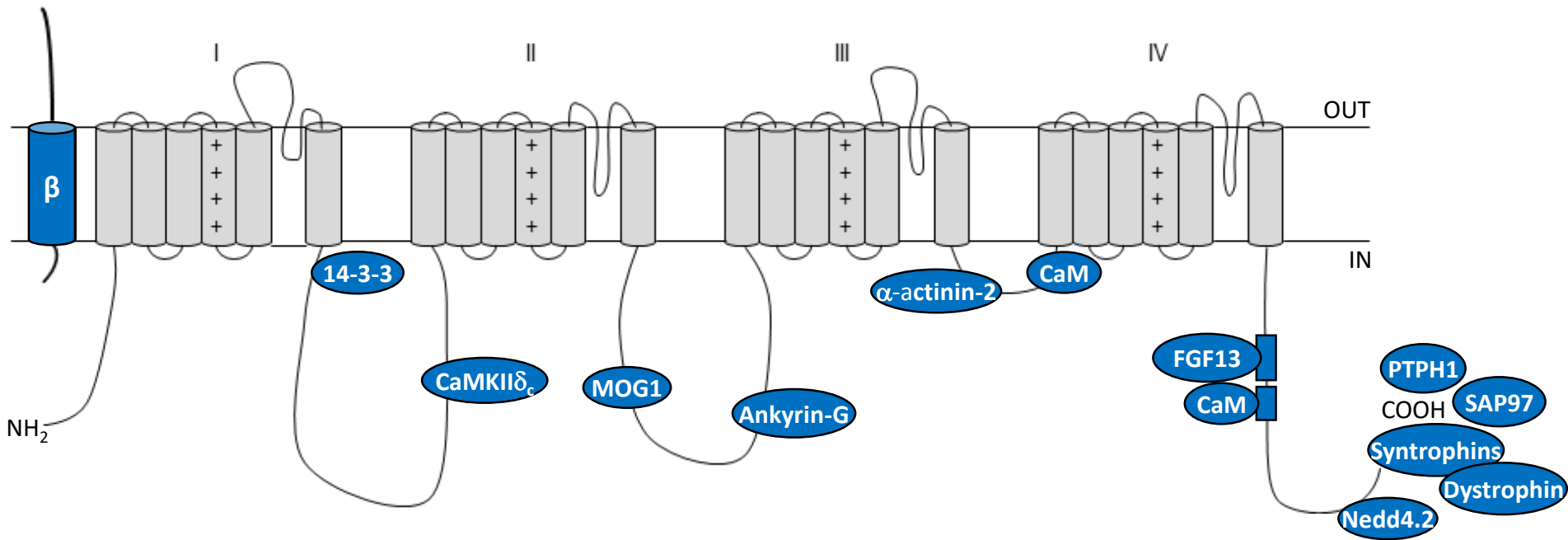
Cardiac Nav1.5 channel complexes



Cardiac Nav1.5 channel complexes



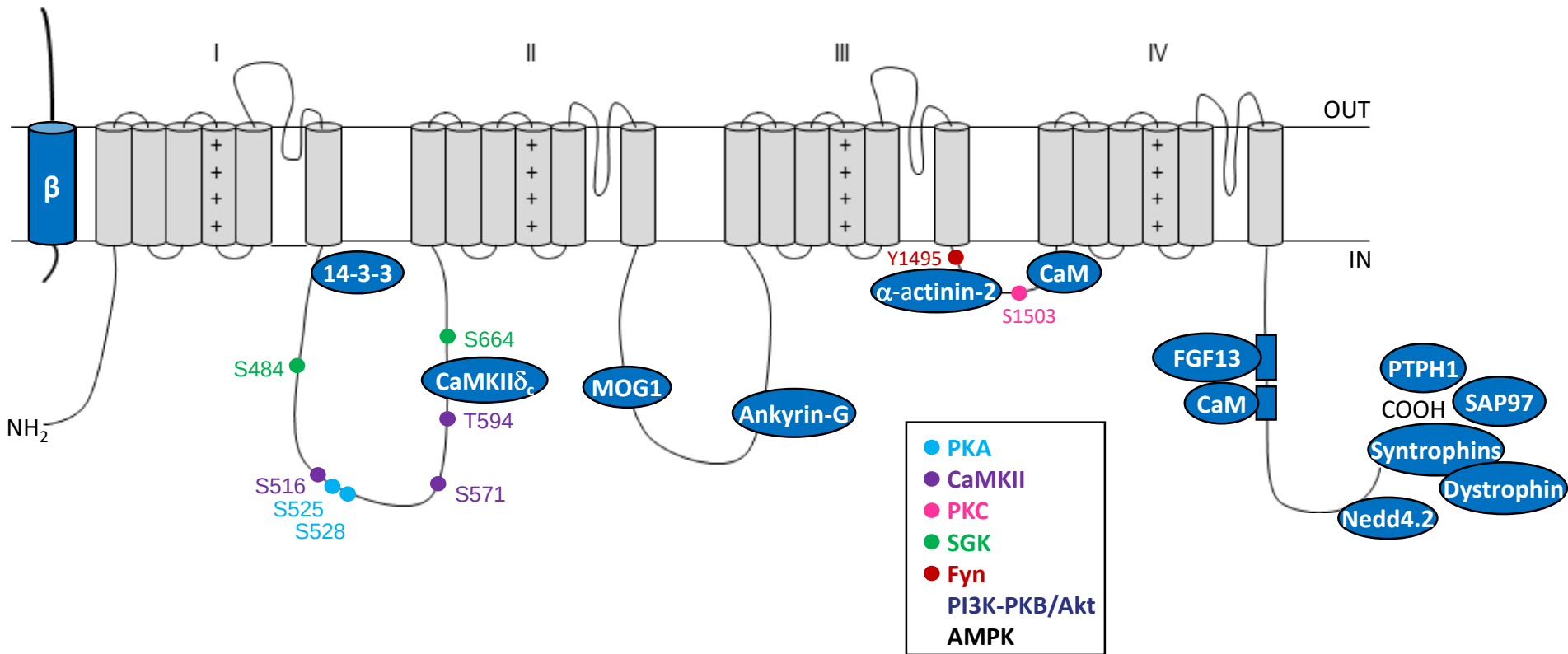
Cardiac Nav1.5 channel complexes



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

GPD1L

Cx43

Desmoglein-2

N-cadherin

CAR

Plakophilin 2

Dynamin

Telethonin

Slmap

CASK

Sirt1

Vinculin

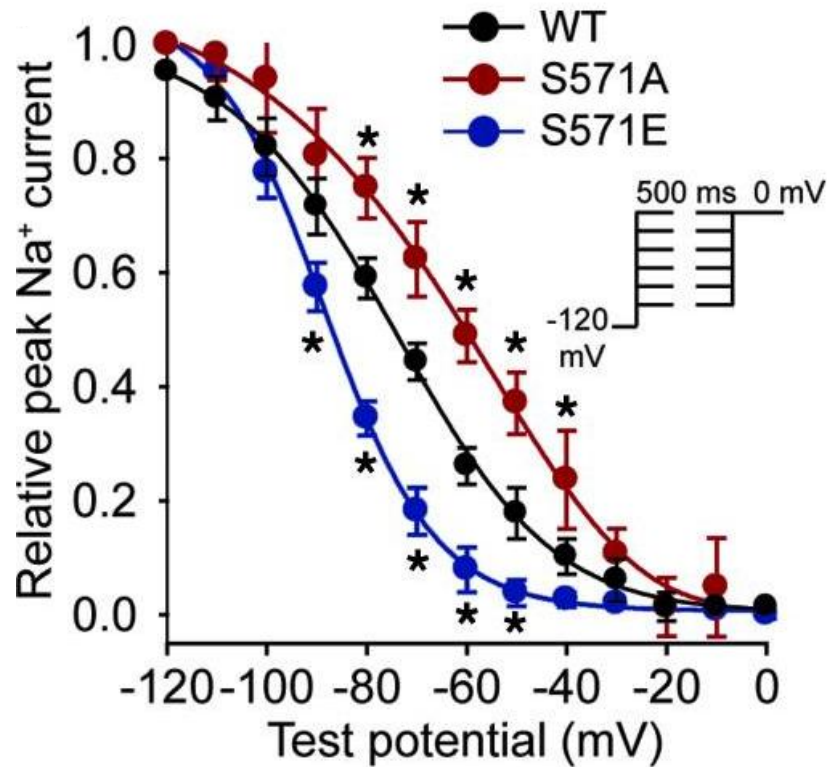
Kir2.1

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)

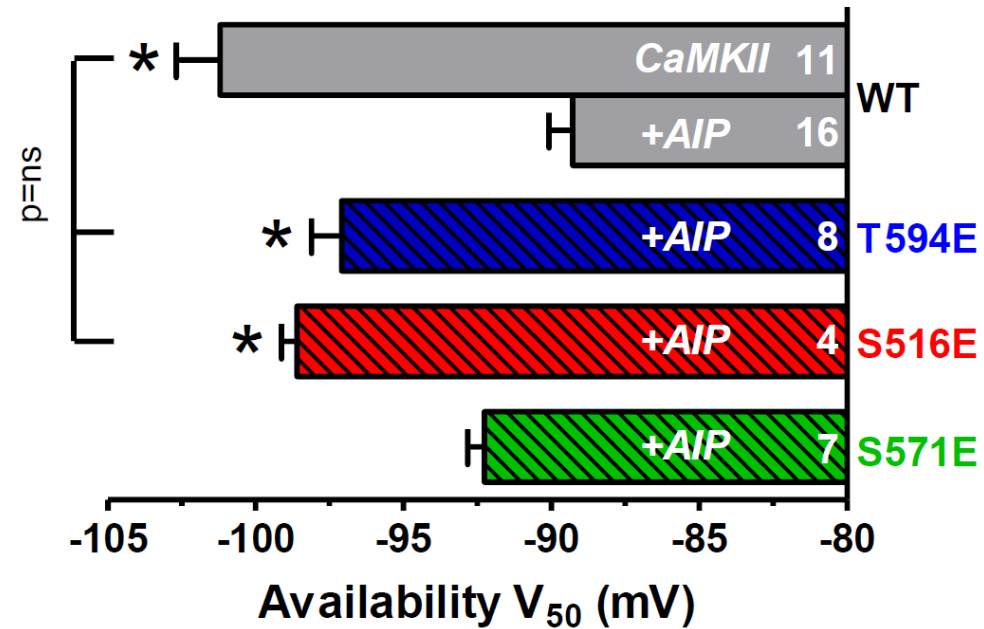
CaMKII phosphorylation sites

In silico



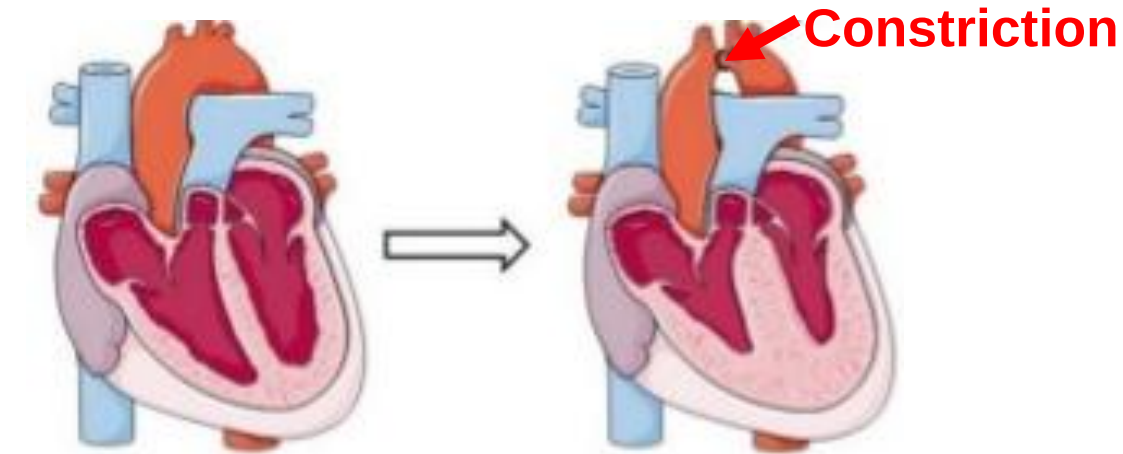
Hund *et al*, 2010

In vitro



Ashpole *et al*, 2012

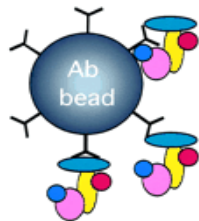
Phosphoproteomics of cardiac Nav1.5 channels



Sham Ventricle

↓

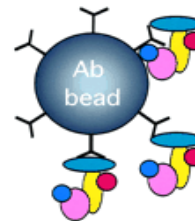
mαNavPAN-IPs



TAC Ventricle

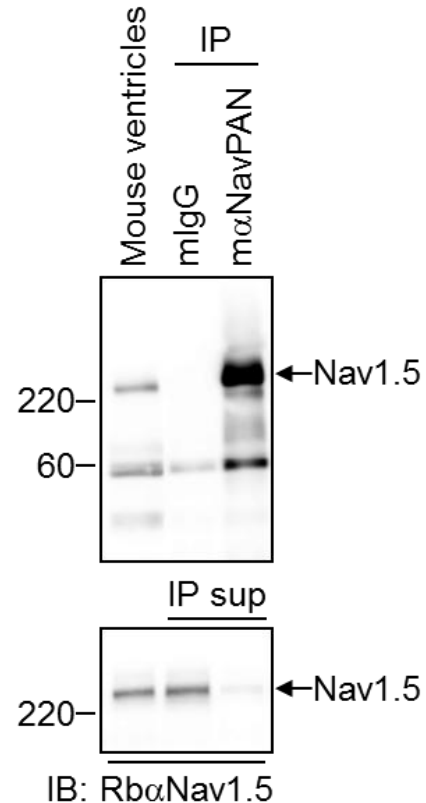
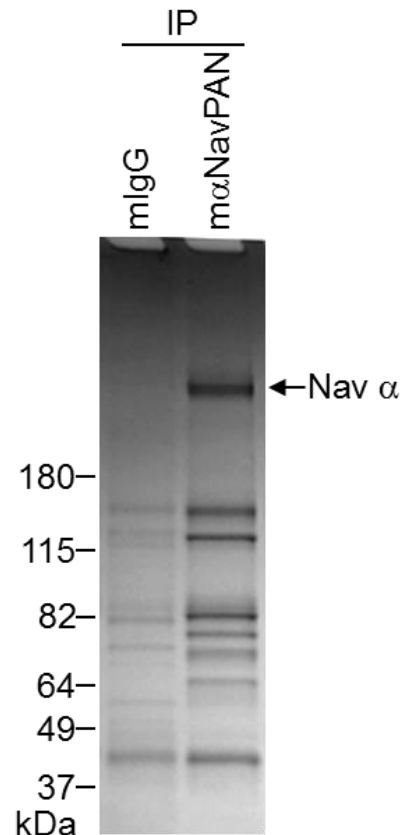
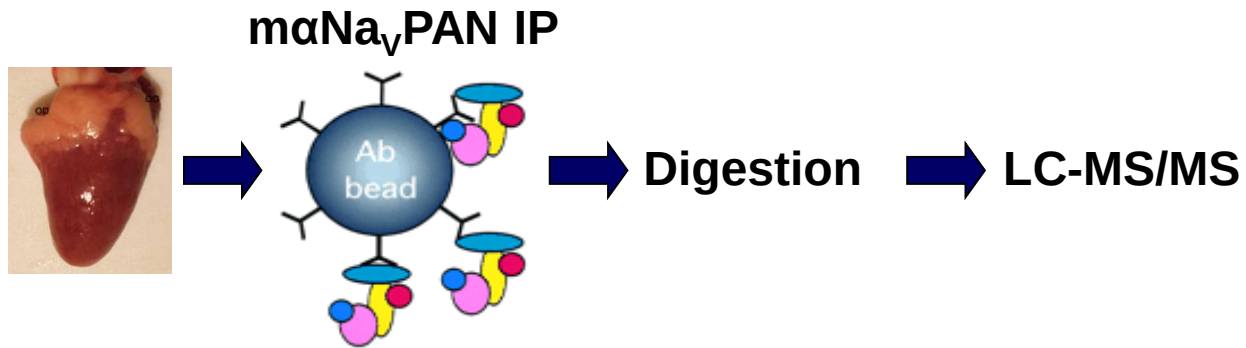
↓

mαNavPAN-IPs



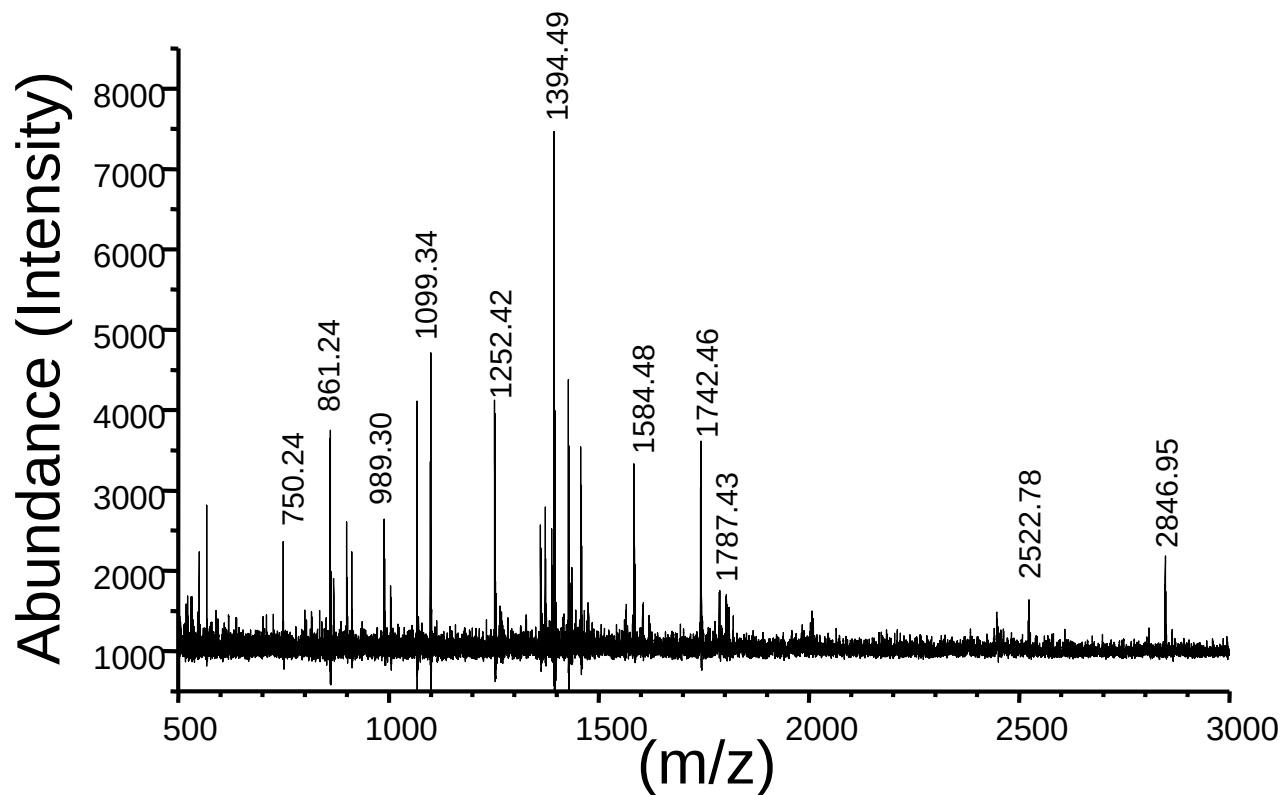
Quantitative phosphoproteomics

Purification of cardiac Nav1.5 channels



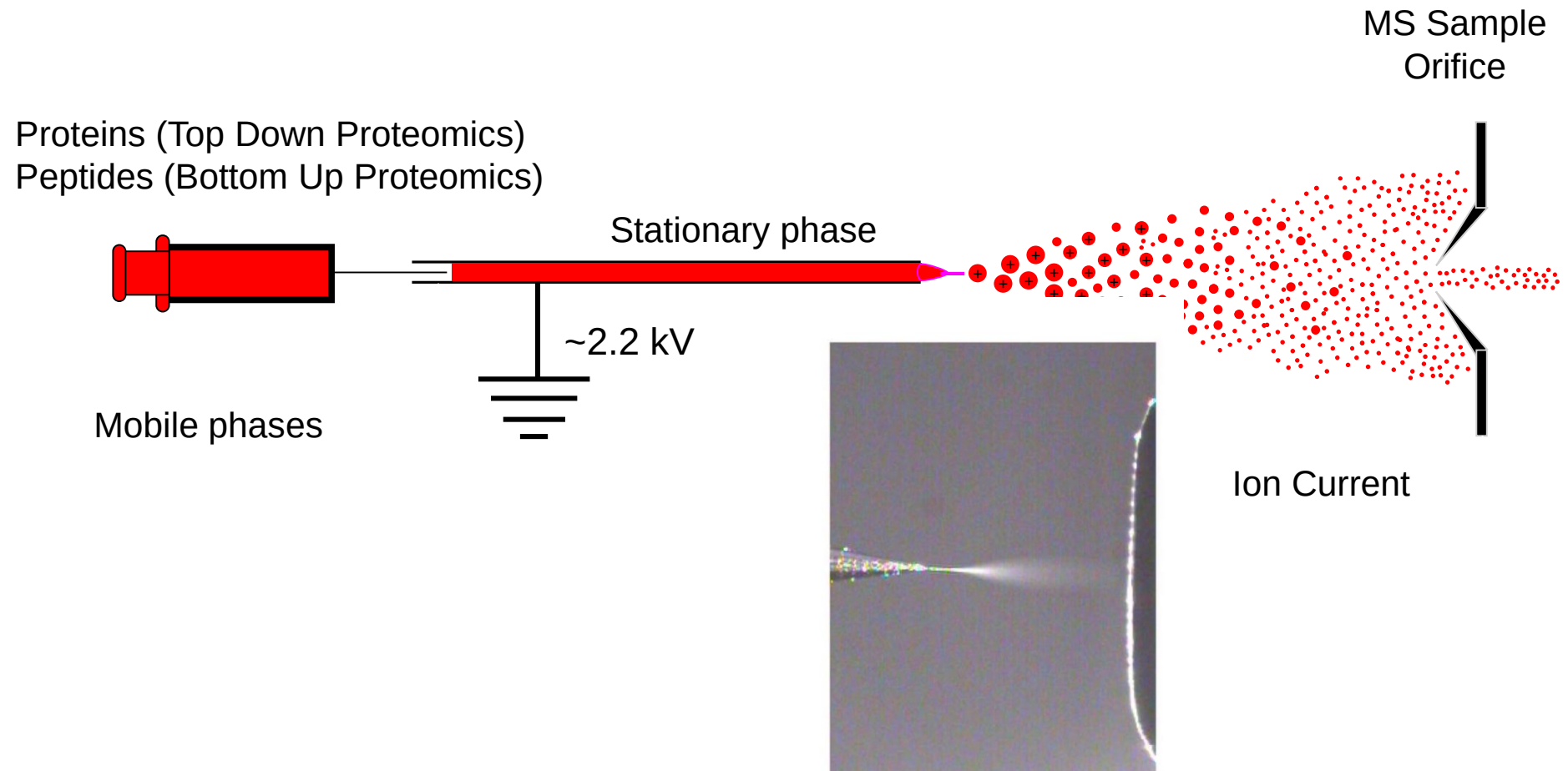
Mass Spectrometry – A Brief Definition

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).



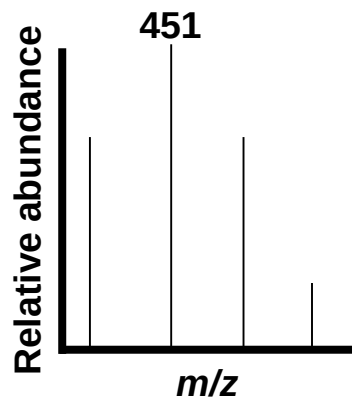
Electrospray ionization : ESI-MS

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

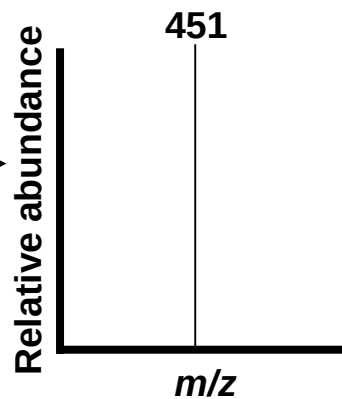


MS/MS analyses

MS1: mass spectrum of precursor ions

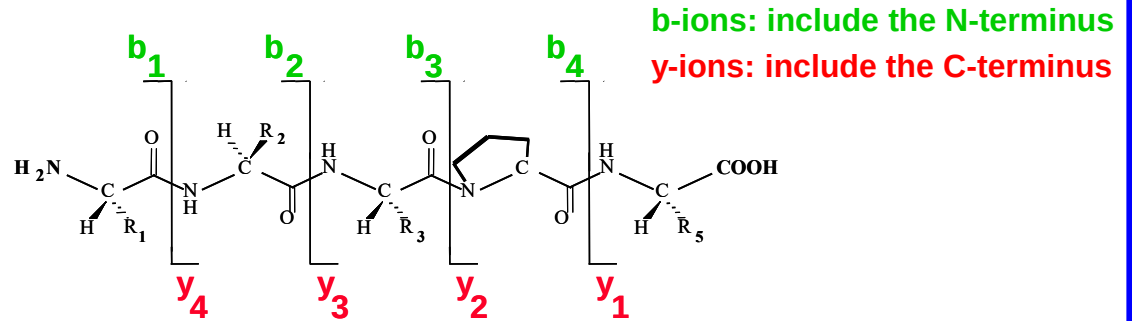
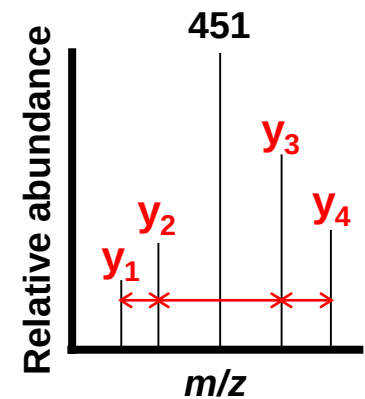


Isolation of the most abundant precursor ions



CID: Collision Induced Dissociation

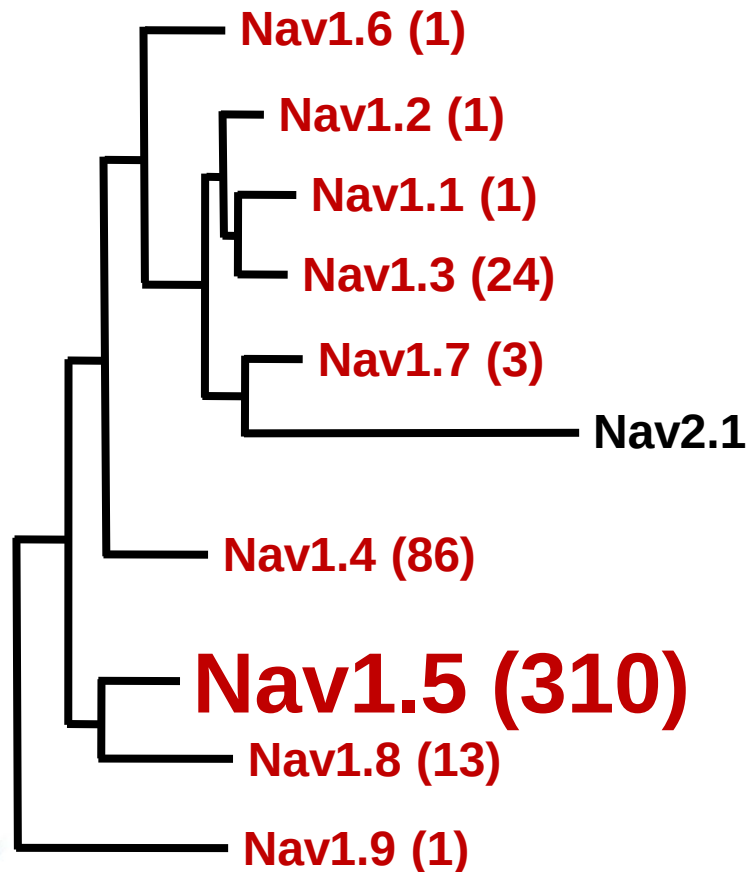
MS2 (MS/MS or tandem MS): fragmentation spectrum



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 MAEKQARG SAT QE REGLP EEEA PRPQLDLQASKKLPDLYGNPPREL
N-TERM

76 IGEPLEDLDPFYSTQKTFIVLNKGT IFR FSATNALYVLSFHPVR RAAVKILVHSLF SMLIMCTILTN CVFMAQ
N-TERM IS1

151 HDPPFWTKYVEYTF TAIYTFESLVKILAR GFCLHAFTFLRDPWNWLD FSVIVMAYTTEFVDLGNV SALRTFVRVLR
IS2 IS3 IS4

226 ALK TISVISGLK TIVGALIQSVKK LADVMTLVF CLSVFALIGLQLFMGNLRHKCVRNFTELNGTNGSVEADGIV
IS5

301 WNSLDVYLNDPANYLLK NGTTDVLLCGN SSDAGTCPEGYR CLKAGENPDHGYTSFDSFAWAF LALFR LMTQDCWE
IS6

376 RLYQQT LRSAGKIY MIFFMLVIFLGSFYLVNLILAVVAMAYEEQNQATIAETEEKR FQEAEMMLKKEHEALT I
IS6

451 RGVDTVSRS SLEMSPLAPVTNHERRSKRRKRLSSGTEDGGDDLRLPKSD EDGPALNQL LTHGL RTSMRPRS
Loop I

526 RGSIFTFRRRDQG SEADFADDEN STAGESESHRT LLVFWPLRRP TQGPFGFT APGHVINGKRN STVDCNGV
Loop I

601 VSLLGAGDAEATSPGSHLLRPVLRDPDPTTTPSEEPGGPQMLTPQAPCADGFEPEGARQRAL SAVSVLTSALEE
Loop I

676 LEESHRCPPCWNRFQAHYLIWECCPLWMS IKQKV FVMDPFA DLTITMCIVLNTLFMALEHYNM TAEFEEMLO
IS1

751 VGNLVFTGIFTAETFK IIALDPYYFQCGWNIFDS IIVILS LIMEGLSR MGNLSVLR SFRLLRVFKLAKSWPTL
IS2 IS3 IS4

826 NTLIKI IGNSVGALGNL LTLVLAIIVFIFAVVGMQLFG NYSELRRHRISDSGLLP WHMMDFFHAF LIIFRILCGE
IS5

901 WIETMWDCEVSGQSLCLLVFLVMVIGNLVVLNLFALLLSFSADNL TAPDEGEMNNLQALARIQGR LRFV
IS6

976 KRTTWDFCCGLLR RP KPAALAT ESQLES CIAAPR PPPPEVEKAPPARKETRFEEDKRPQGTPGDTEPVCVF
Loop II

1051 IAVAES DDDQEEDEENSLGTEEESSK [Q] QESQVVS GGHEPPQE PRAWSQVSET TS EAEASTSQADWQQERE
Loop II

1124 AEPRAPGCGETPED SYSEGSTADMTNTADLLEQIPDLGEDVDKPEDCFTEGCVRRCPCCMVDTTQAPGV VWWR LR
Loop II

1199 KTCYRIVEHWSWFETFI IFMILLSSGALAFEDIYLEER KTIK VILEYADKMFTYV FVLEMLLK WVA YGFKKYFTNA
IIIS1 IIIS2

1274 WCWLD FLIVDVS LVSLVANTLGFAEMGP IKSRLTRLRALRPLRALS RFE GMRVVVNALVGAI PSIMNVLLVCLIFW
IIIS3 IIIS4

1349 LIFSIMGVNL FAGK FGRCINQTEGDLPLNYTIVNNKSECESFNVTGELYTKVKVNFNDVNGAGYLALLQVATFKG
IIIS5

1424 WMDIMYAAVDSRGYEEQPQWEDNLYMYIYFVVFI IFGSFETLNLFIGVII DNFNQKKLGGQDI FMTEEQKKYY
IIIS6

1499 NAMKKLGSK KPQKPIPRPLNKYQGFIFDI VTRQAFDVTIMFLICLN MV TMMVETDDQSPEKVN ILAKINLLFVAI
Loop III IVS1 IVS2

1574 FTGECIVKMAALRHYYFTNSWNIFDFVVVLSIVGTVLSDI IQYFFSPTL FVRILARIGRI LR LIRGAKGIRT
IVS3 IVS4

1649 LLFALMMSLPALFN IGLLLFLVMFIYSIFGMANFAYVKWEAGIDDMFNQTFAN SMLCLF QITTSAGWDGLLSPI
IVS5

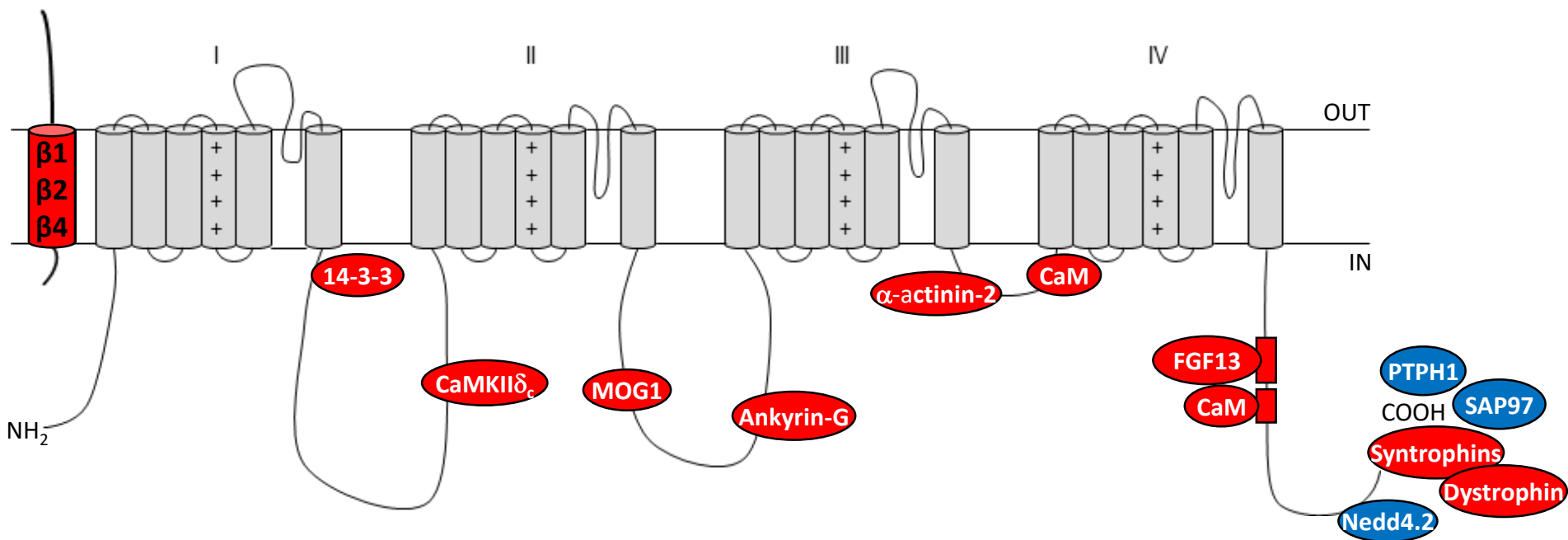
1724 LNTGPYPYCDPNLPNSNGSRGNCSPAVGILFETTYIIISFLIVNM YIAI ILENF SVATEESTEPLSEDDFDMFY
IVS6

1799 EIWEKFDPEA QFIEYLALSDFADALSEPLRIAKPNQISL INMDLPMVSGDR IHCDILFAF KRVLGESGEMDA
C-TERM

1874 LKIQMEEK FMAANPSK ISYEPITTTLRKHEEVSATVIQRAFRRHLLQRSVK HASFLFRQQAC SGLSDEDA PER
C-TERM

1949 EGLIAYMMNENFSRSGPLS SSISS TSFPSPSYDSVTRATSNL PVRASDYSRSED LADFP PDRDRESIV
C-TERM

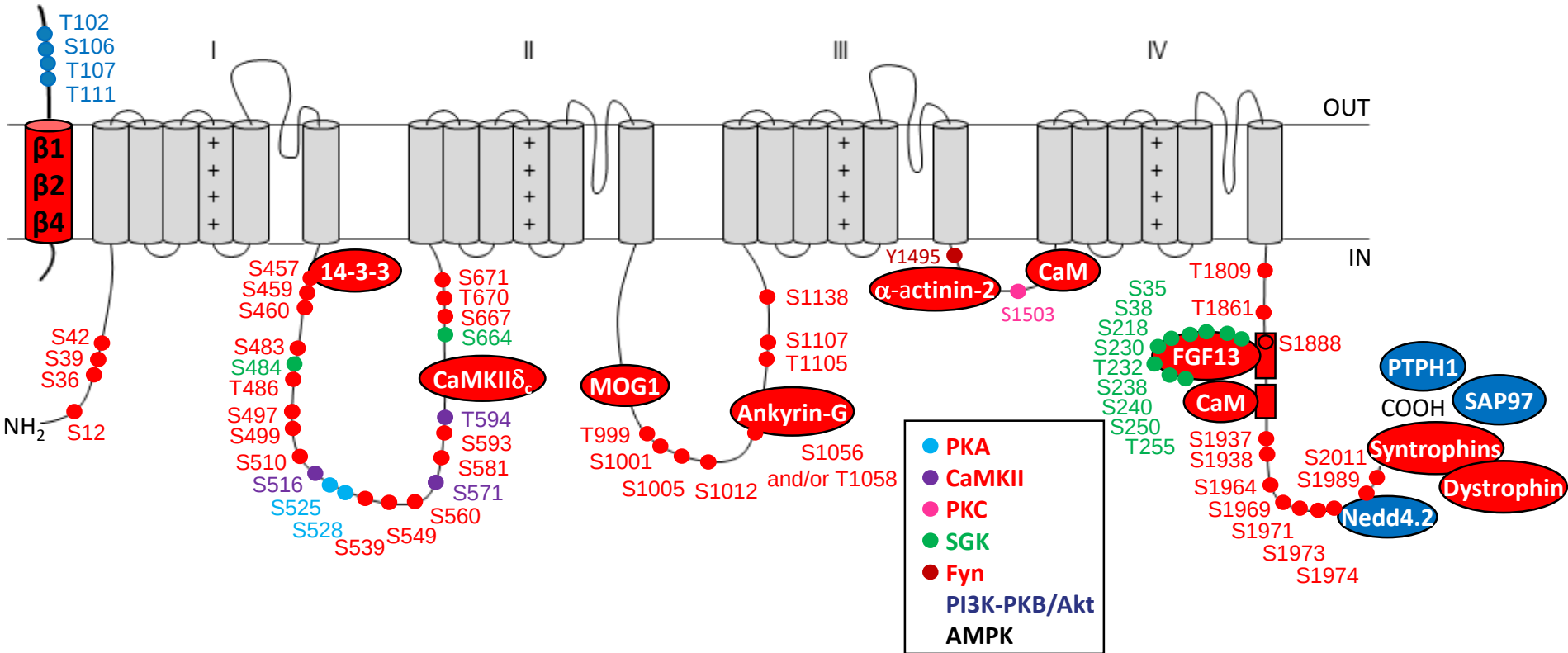
Identification of Nav interacting proteins



Caveolin-3, GPD1L, Cx43, Desmoglein-2, N-cadherin, CAR, Plakophilin 2, Dynamitin, 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

Identification of 62 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

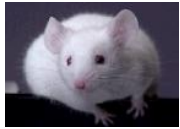
Kir2.1



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

TMT9 quantitative analysis from Sham/TAC mice

Sham / TAC mice



mαNa_vPAN-IPs

Labelling

Sham LV 1



Sham LV 2



Sham LV 3



Sham LV 4



TAC LV 1



TAC LV 2



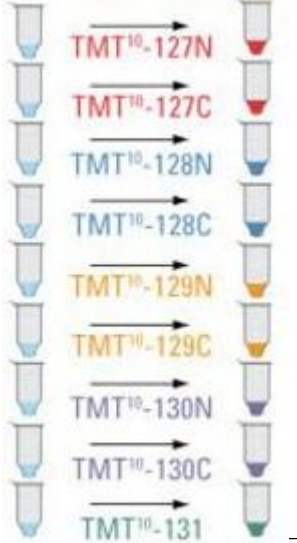
TAC LV 3



TAC LV 4



TAC LV 5



Combine

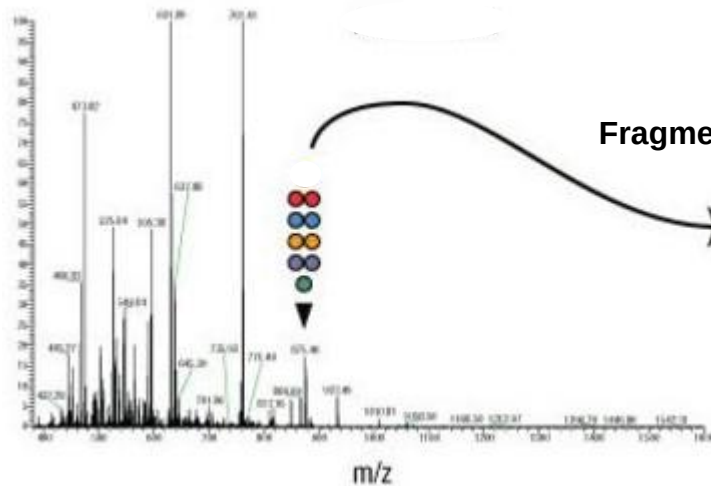


TMT methods

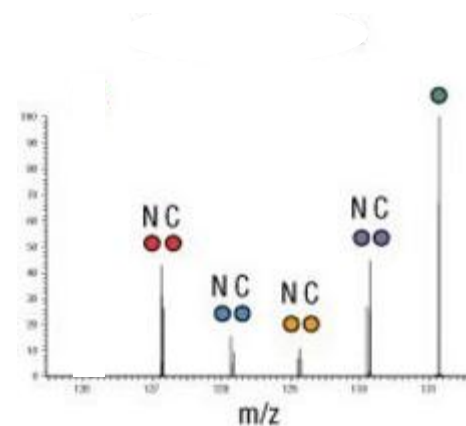
1 LC-MS/MS experiment



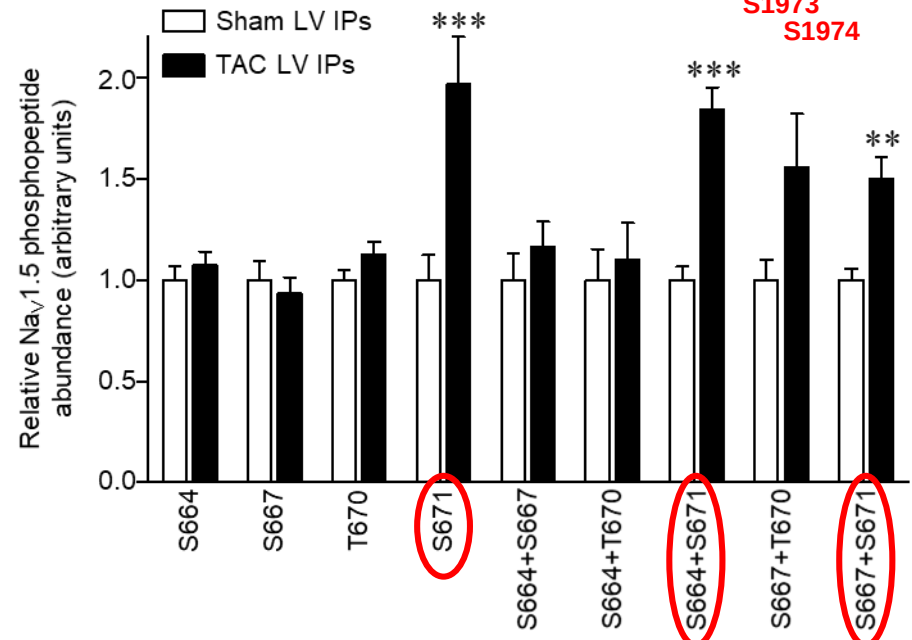
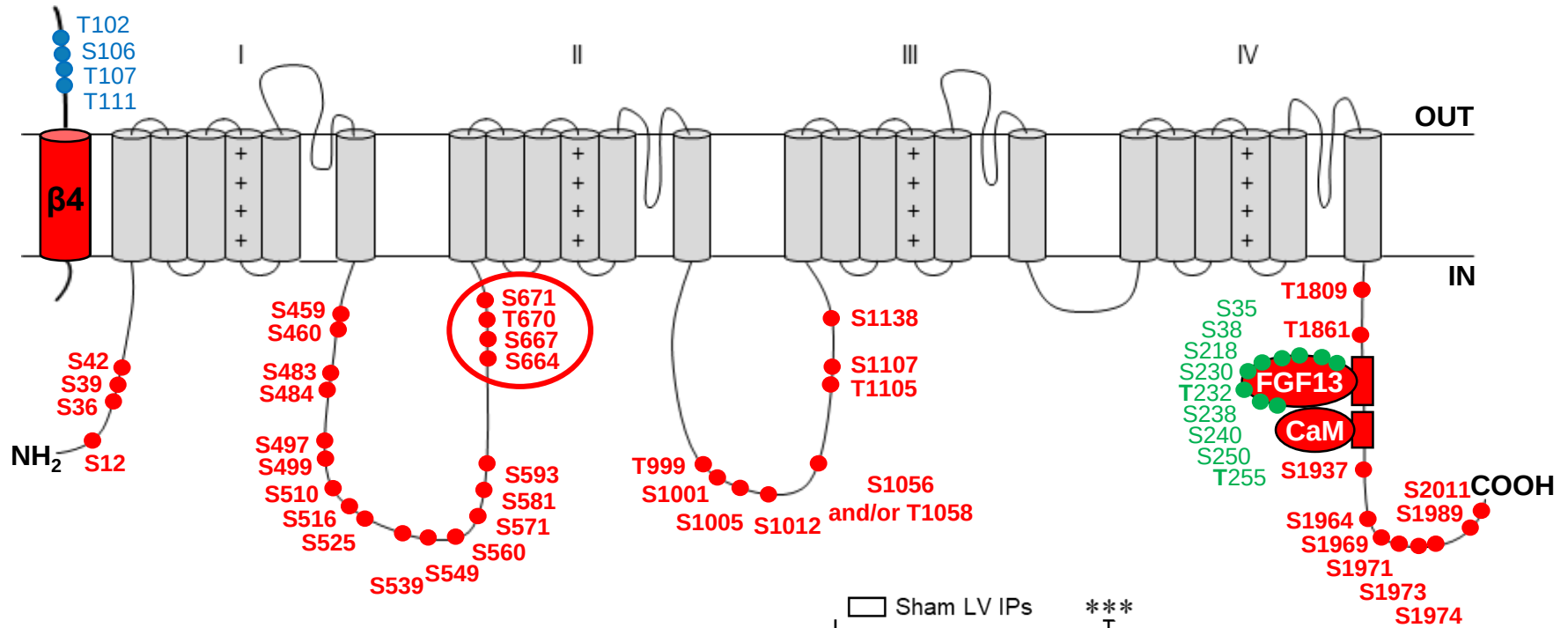
MS1 spectra: selection



MS/MS spectra: Quantification

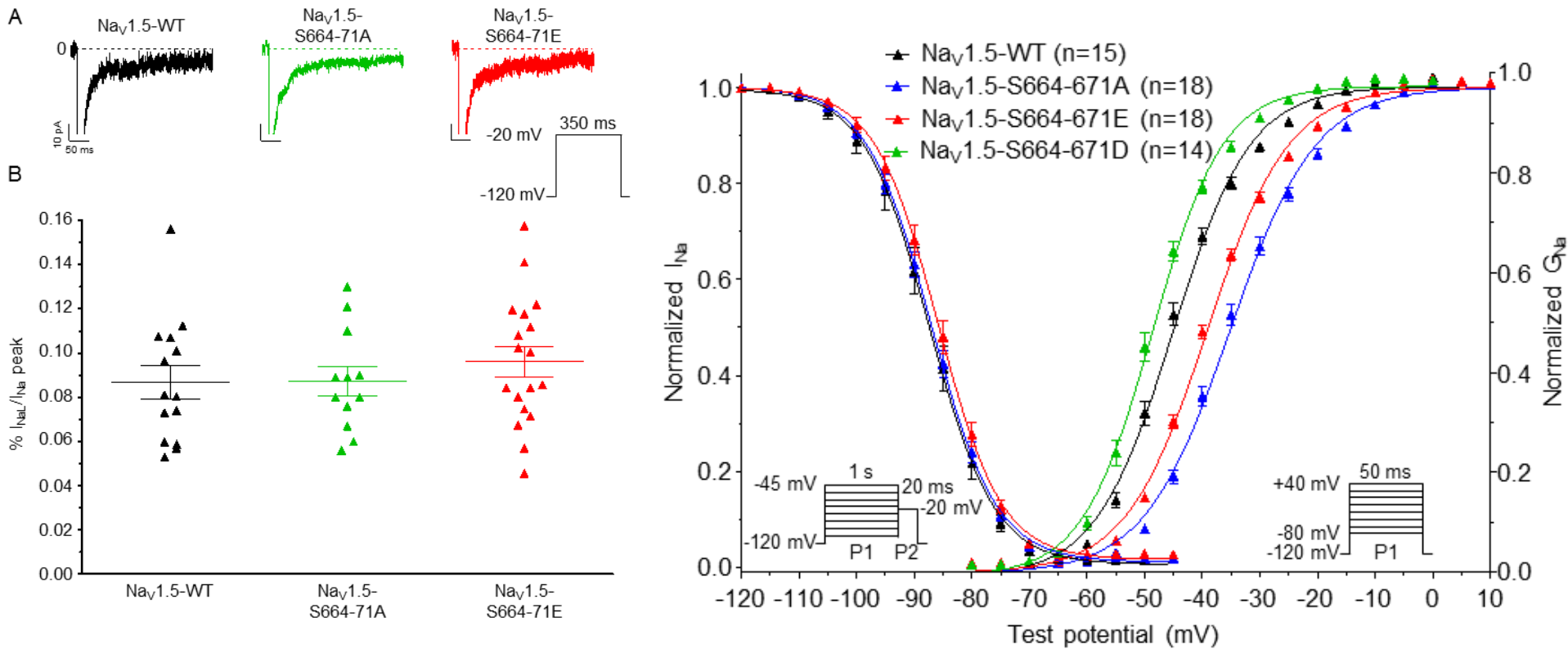


Quantitative phosphoproteomics in Sham/TAC mice

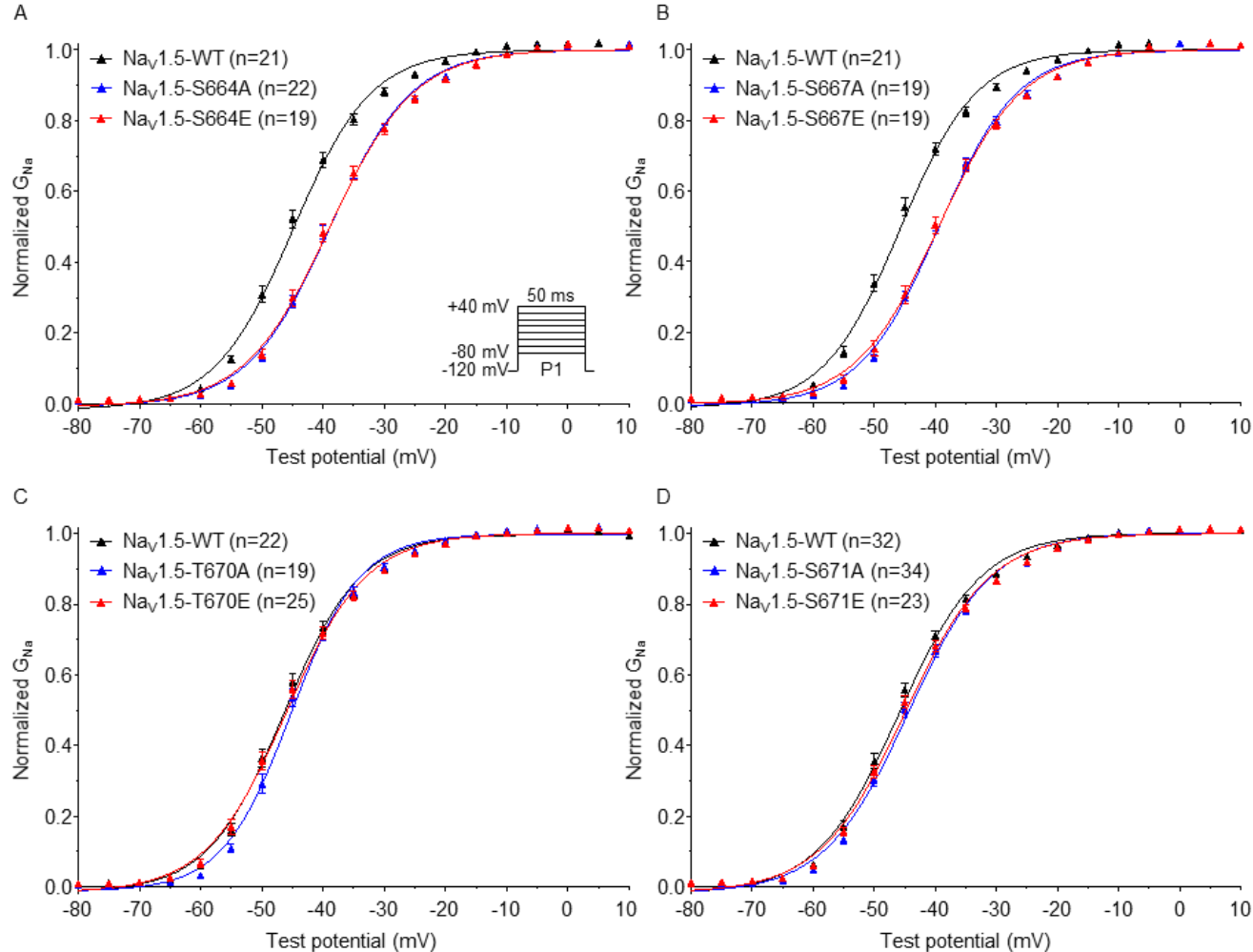


** $p < 0.01$, *** $p < 0.001$ in TAC vs Sham

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

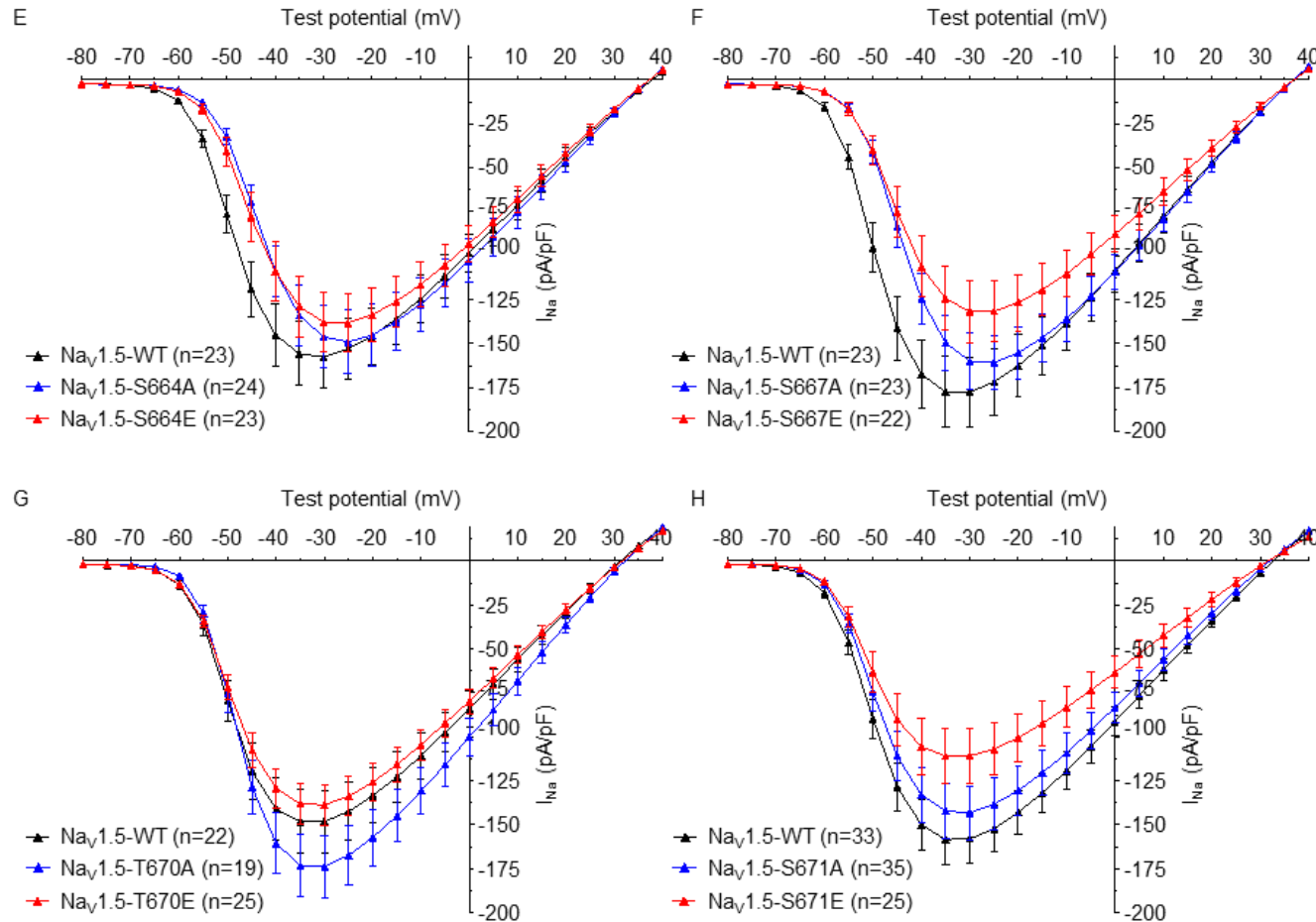


Electrophysiological analysis of simple Nav1.5 phosphomutants



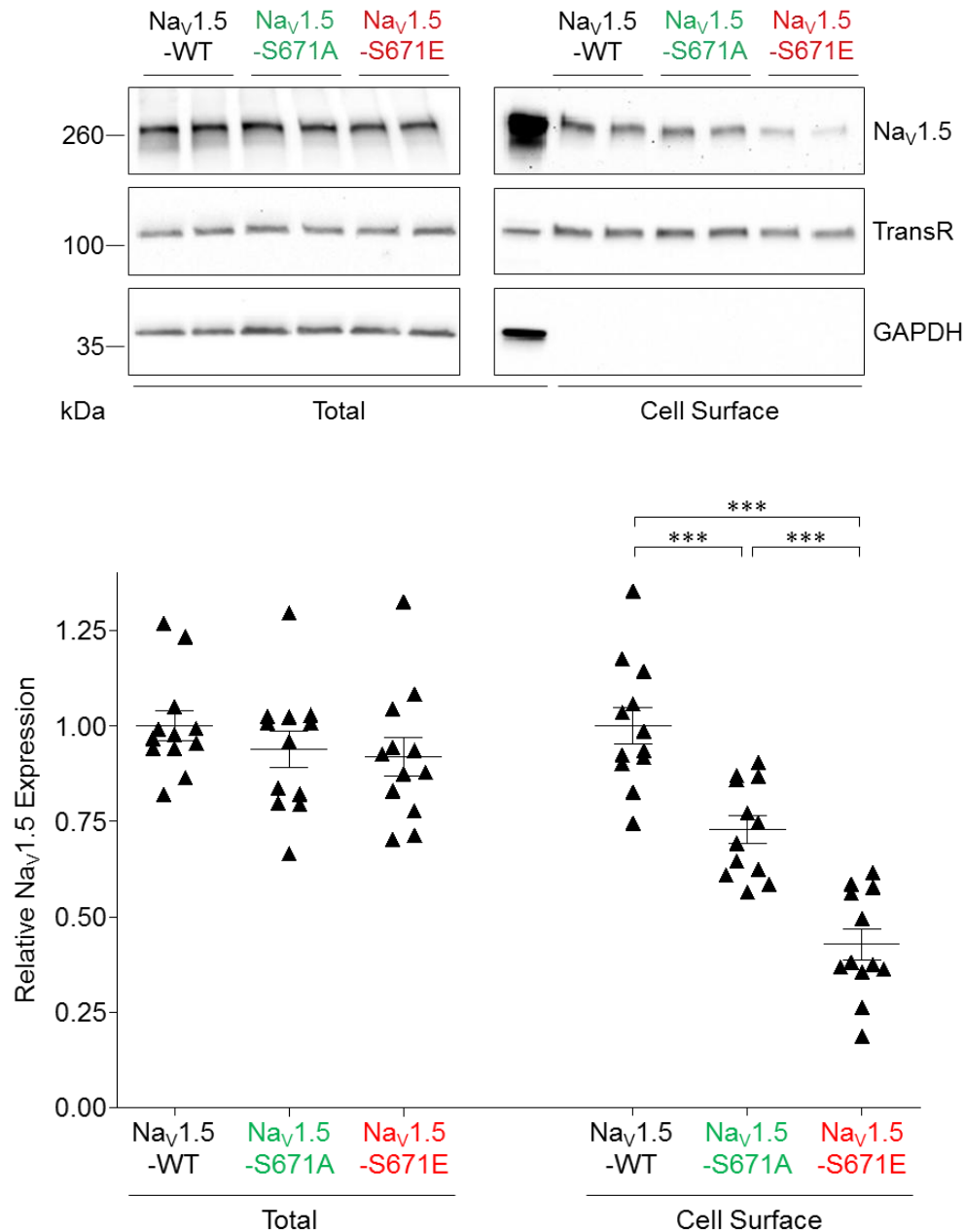
Main finding: Phosphorylation at S664 and S667 regulates Nav1.5 channel activation.

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

L'institut du thorax, Nantes, France

Agnès Tessier

Grégoire Rio

Gildas Loussouarn

Jérôme Montnach

Michel De Waard

Flavien Charpentier

CRCI²NA, Nantes, France

François Guillonneau

Benjamin Barre

Contact : celine.marionneau@univ-nantes.fr



**Marie Curie 7th Framework Program
of the European Commission**

