Phosphoproteomics of cardiac Nav1.5 channels

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Céline Marionneau

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

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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

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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

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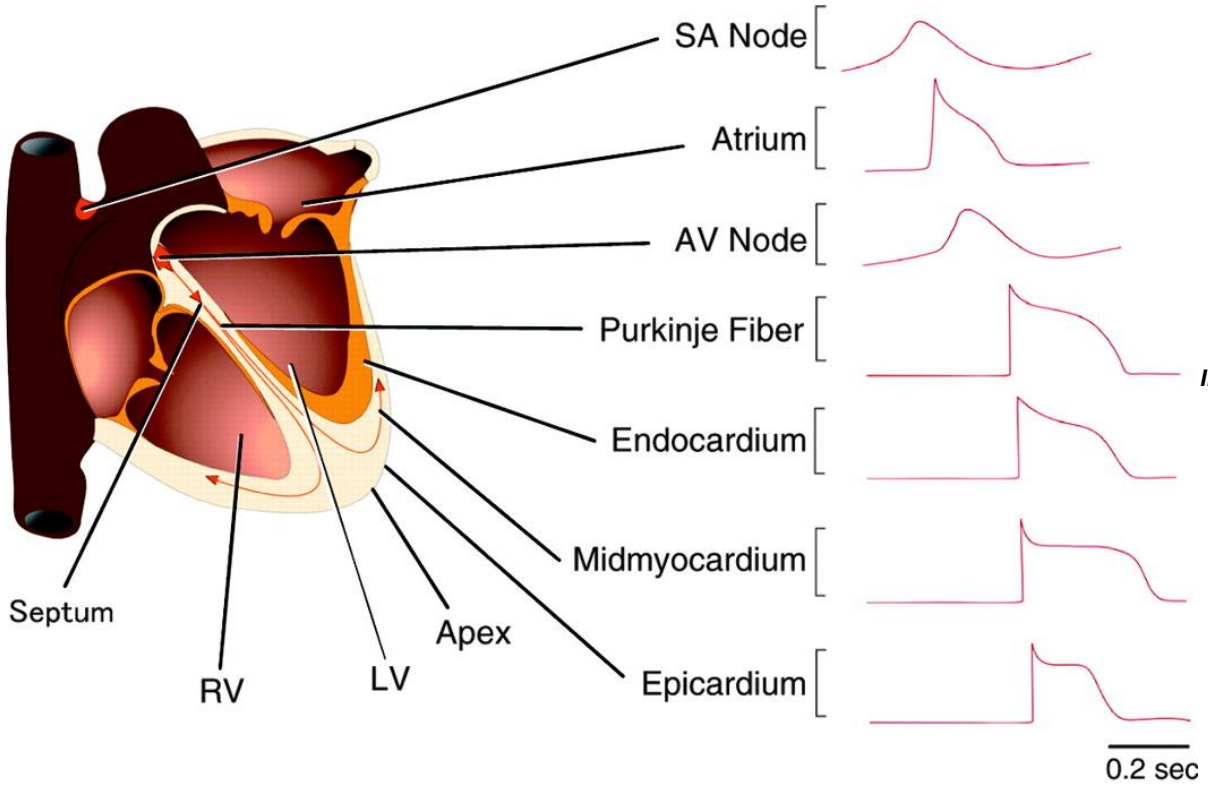
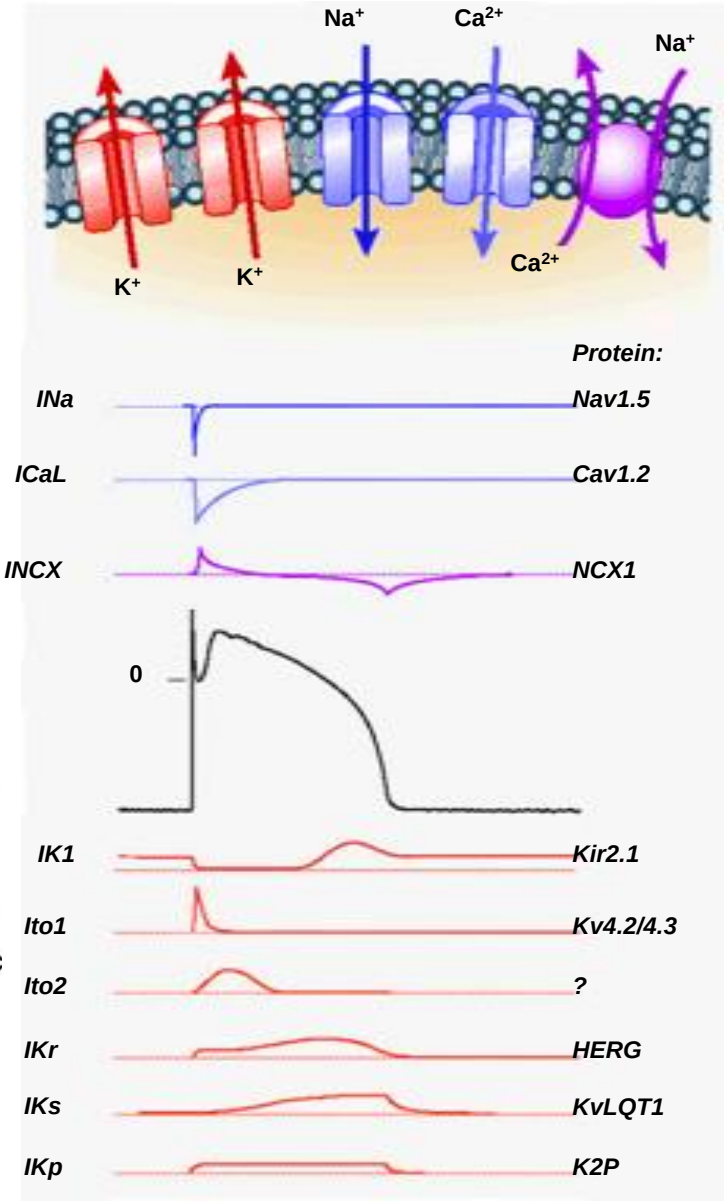
Cardiac excitability

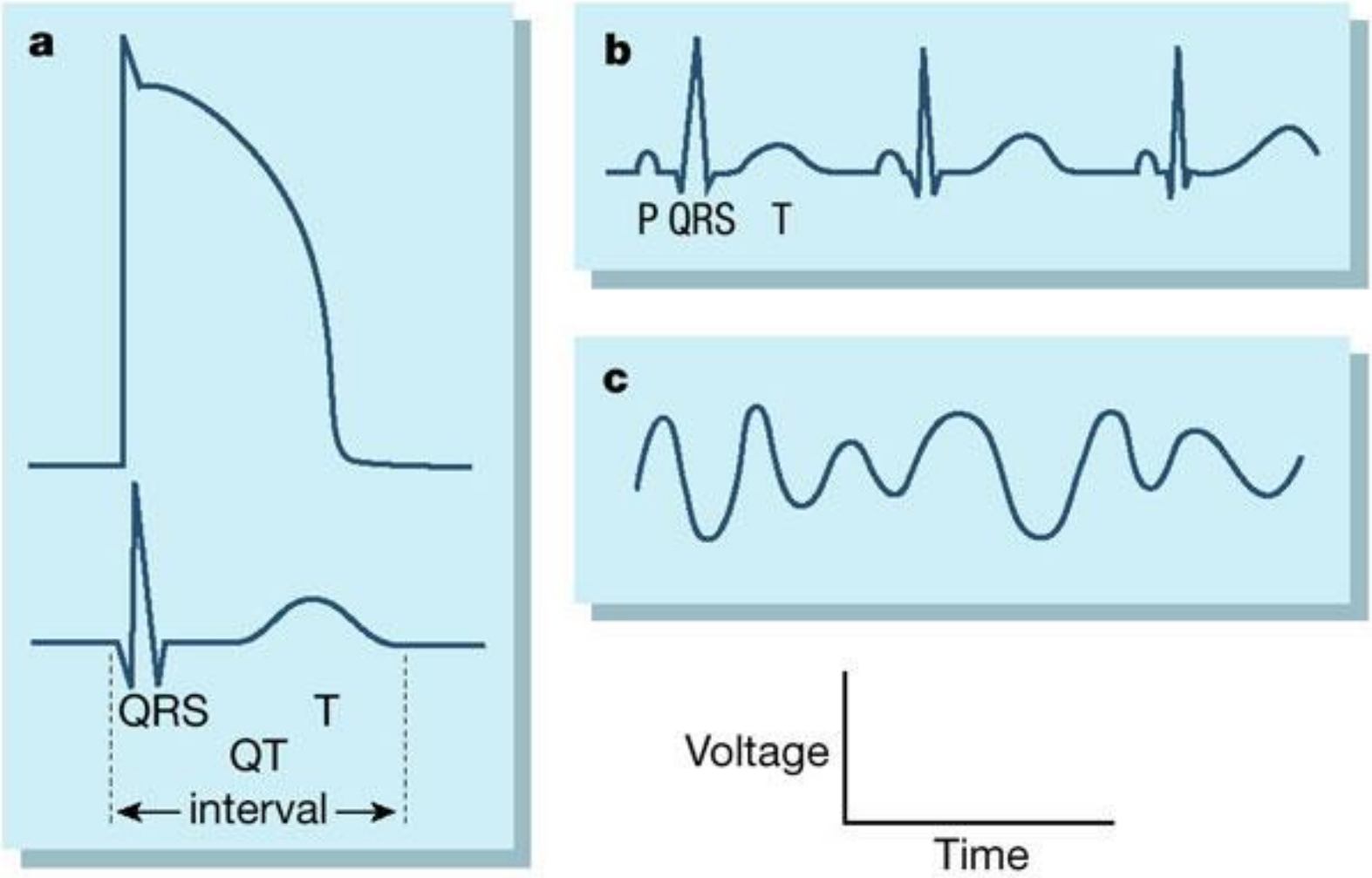


Nerbonne and Kass, *Physiol Rev 2005*



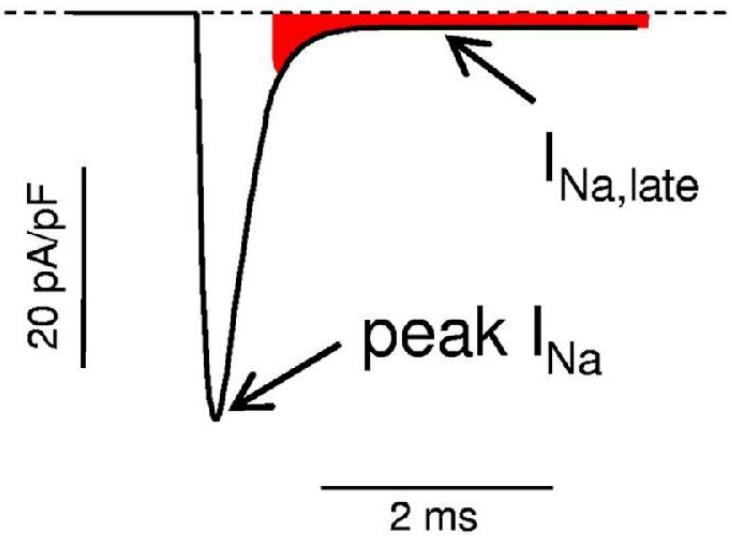
Marban, *Nature 2002*

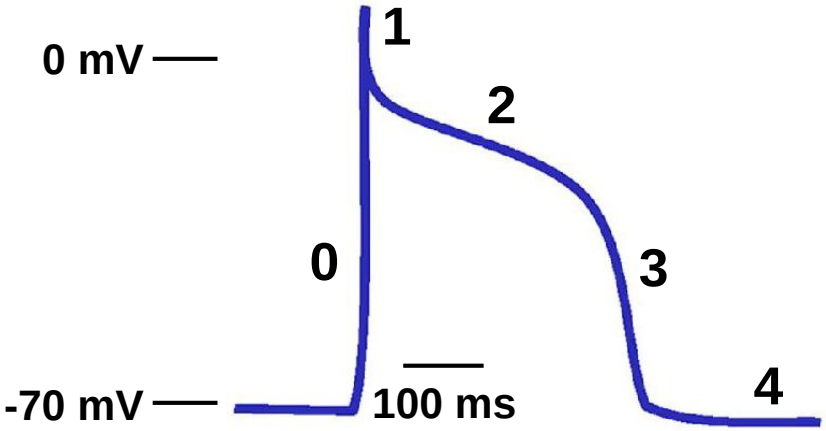
Electrocardiograms : ECG

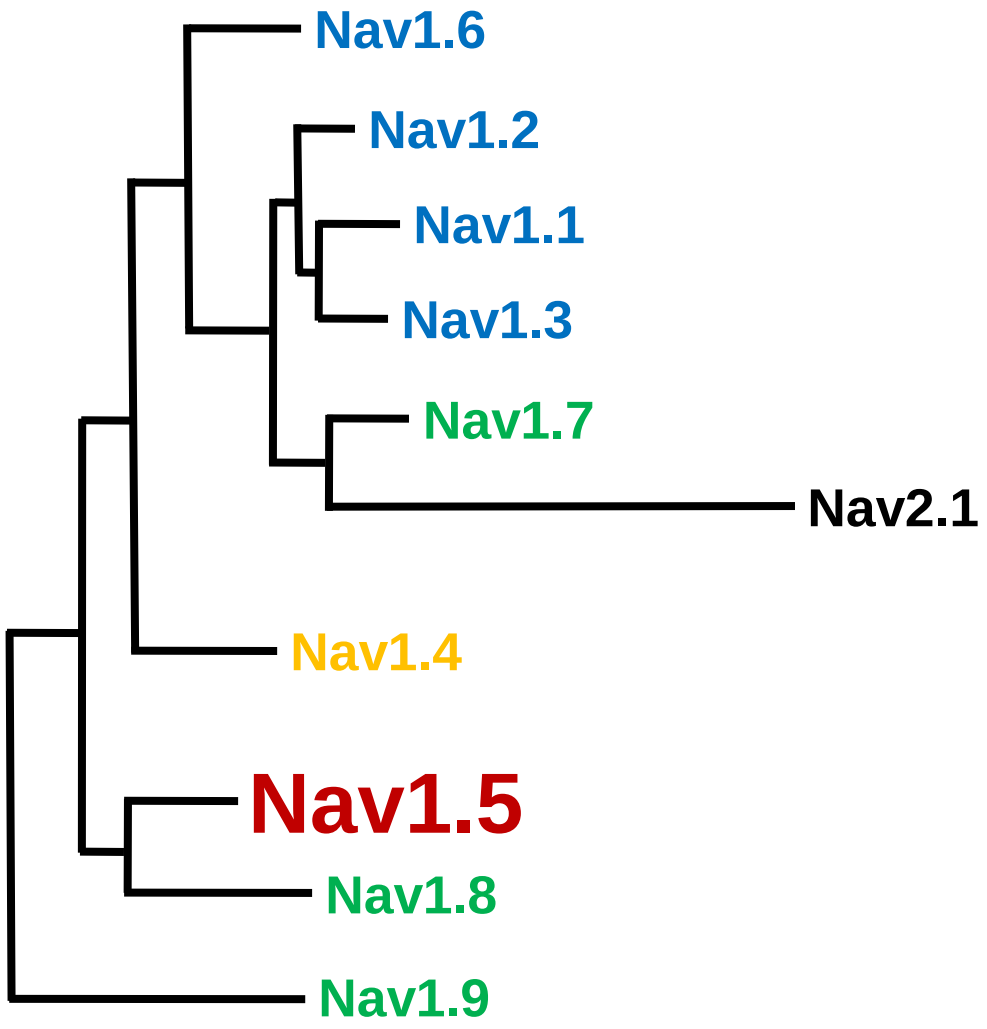


Marban, *Nature 2002*

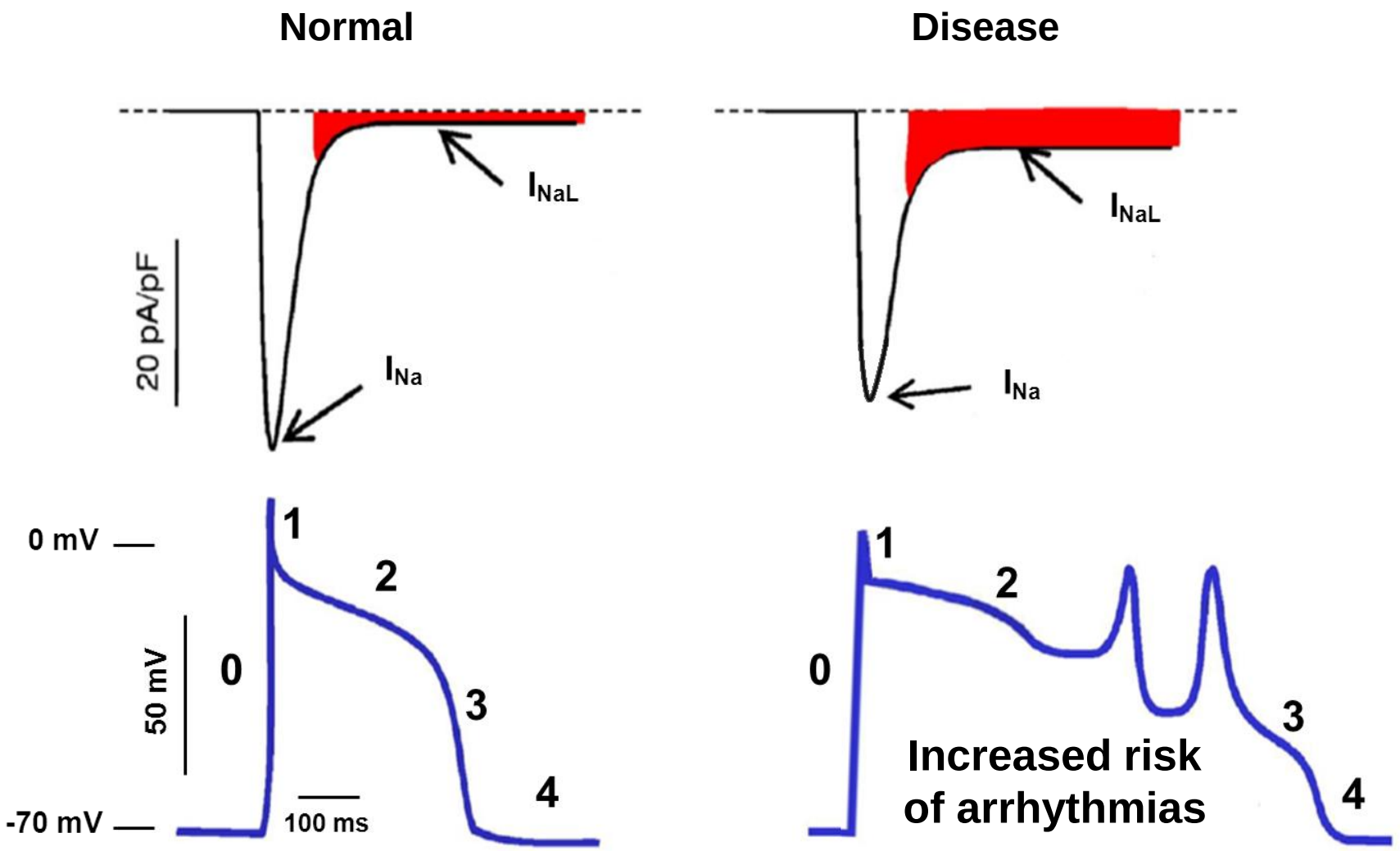
Cardiac voltage-gated Na^+ channels





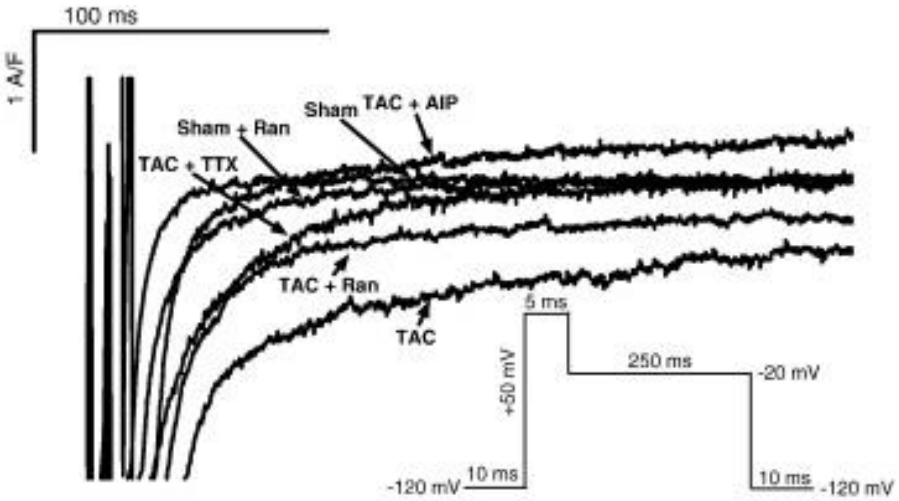


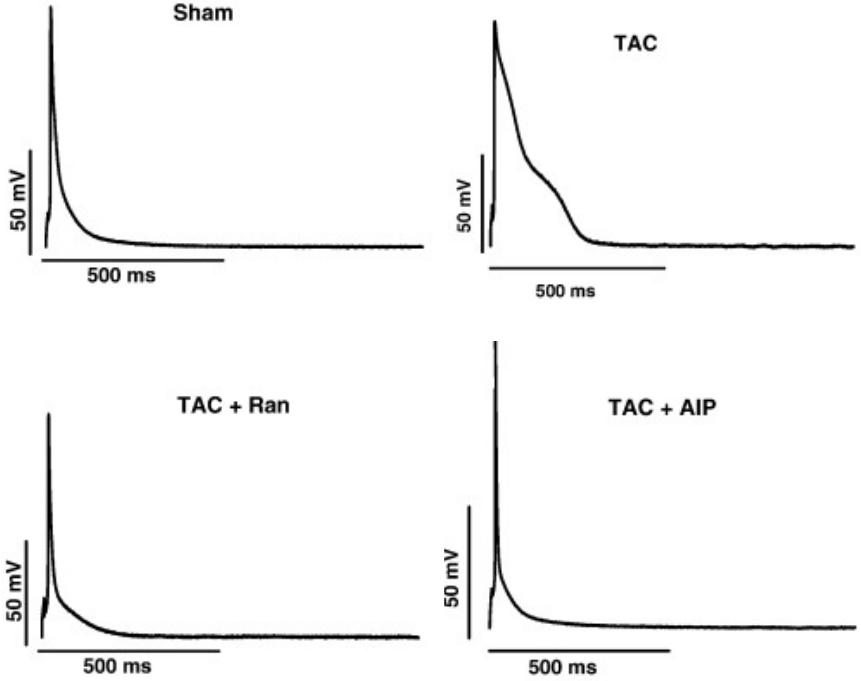
Nav1.5 channels in heart failure



Maier, 2011, modified

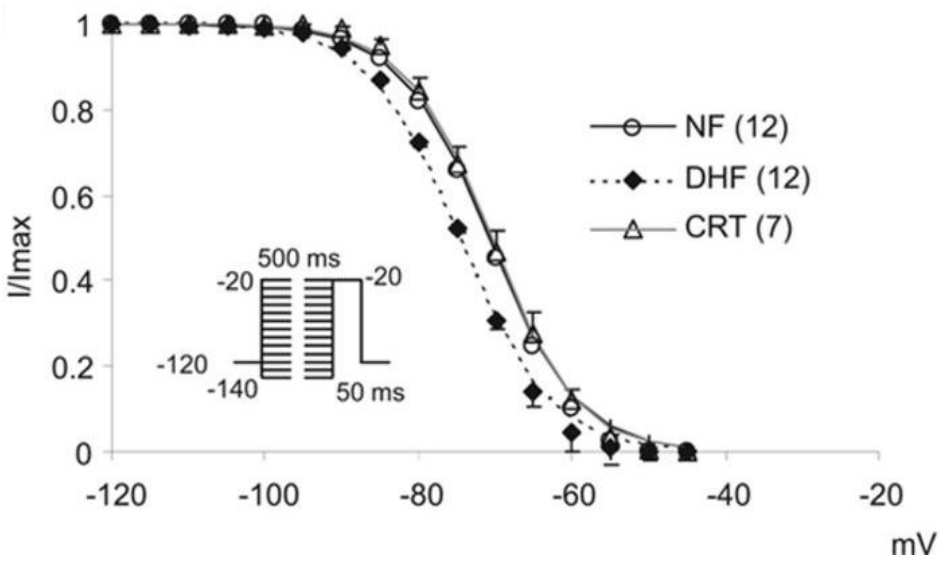
CaMKII increases I_{NaL} in heart failure



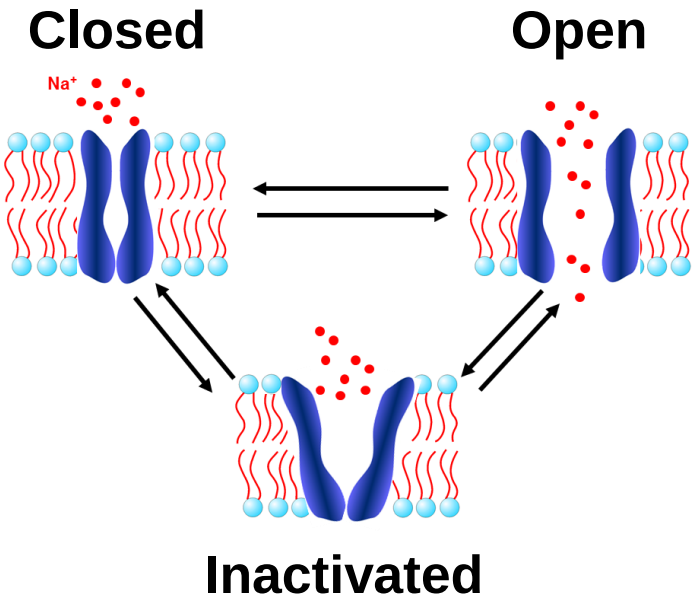


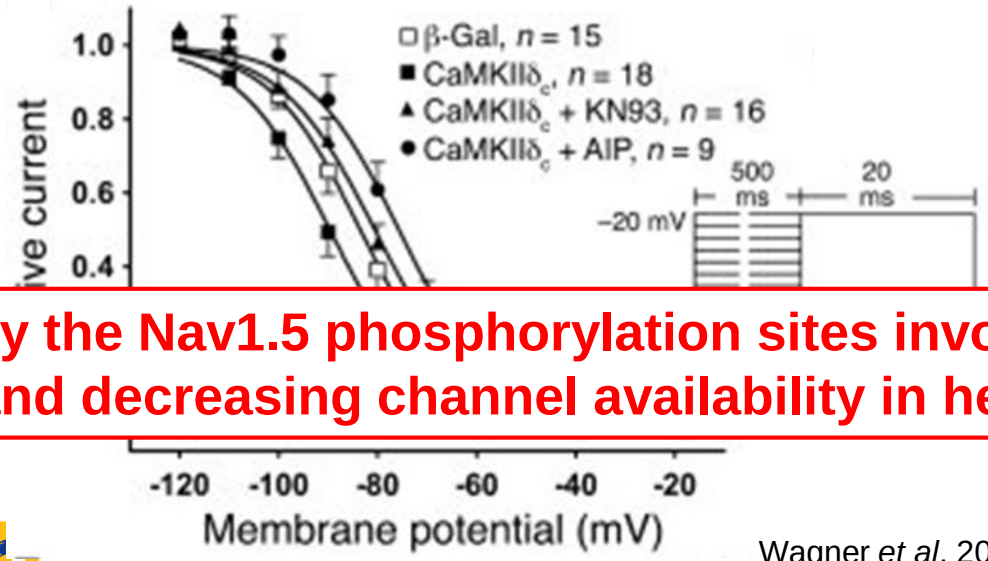
Toischer *et al*, 2013

CaMKII decreases Nav1.5 channel availability



Aiba *et al*, 2013





Wagner *et al*, 2006

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

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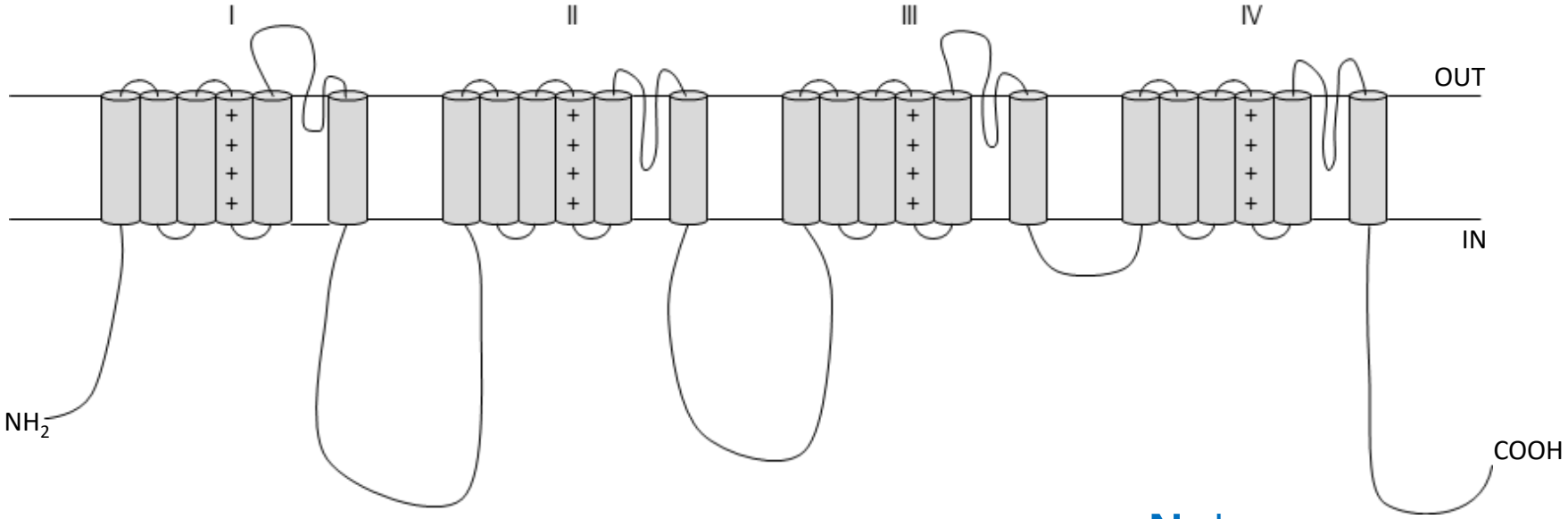
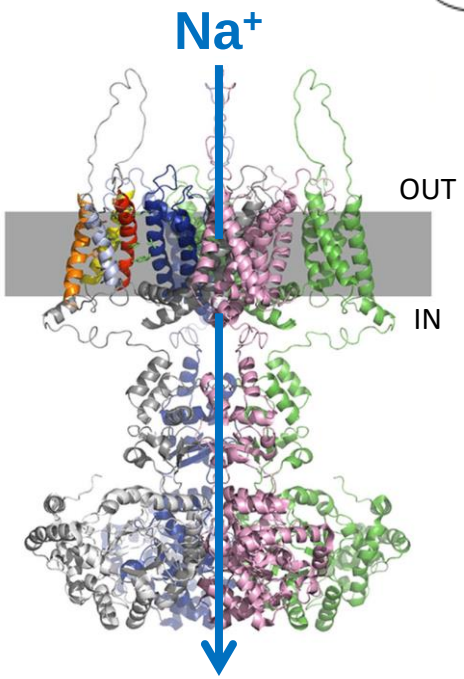
Cardiac Nav1.5 channel complexes





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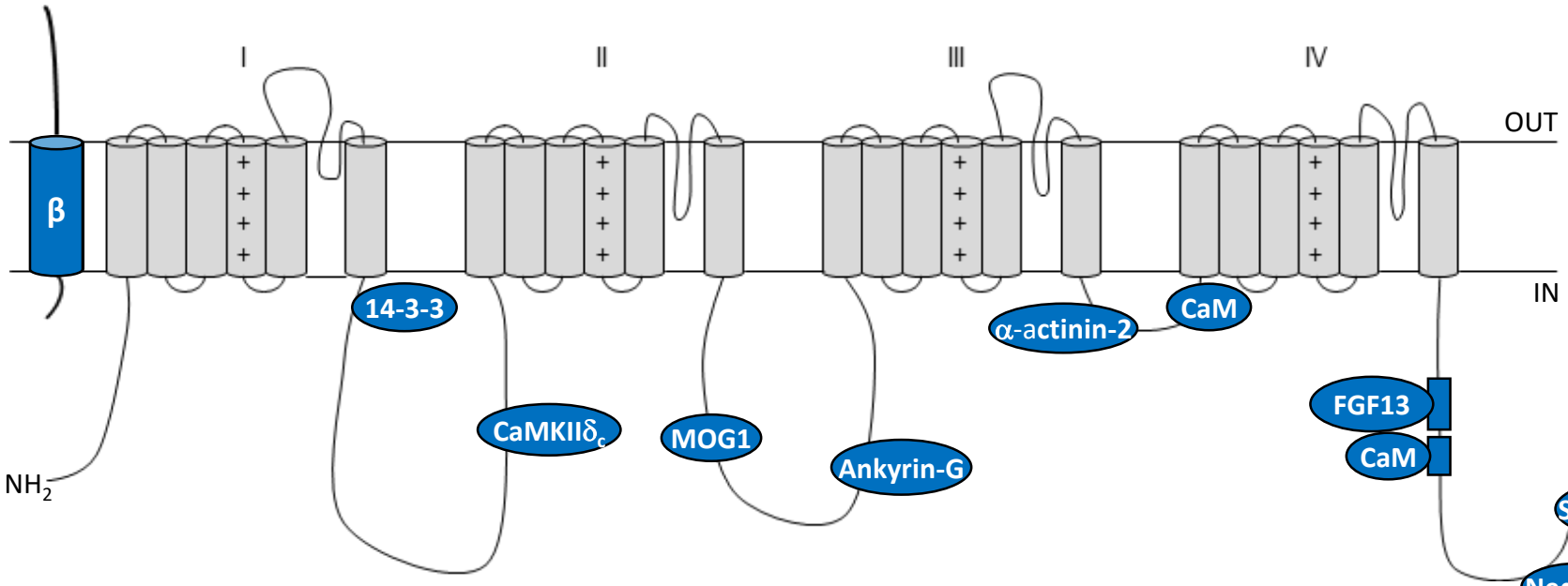

Caveolin-3 · GPD1L · Cx43 · Desmoglein-2 · N-cadherin · CAR · Plakophilin 2

Dynamitin · Telethonin · Slmap · CASK · Sirt1 · Vinculin · Kir2.1

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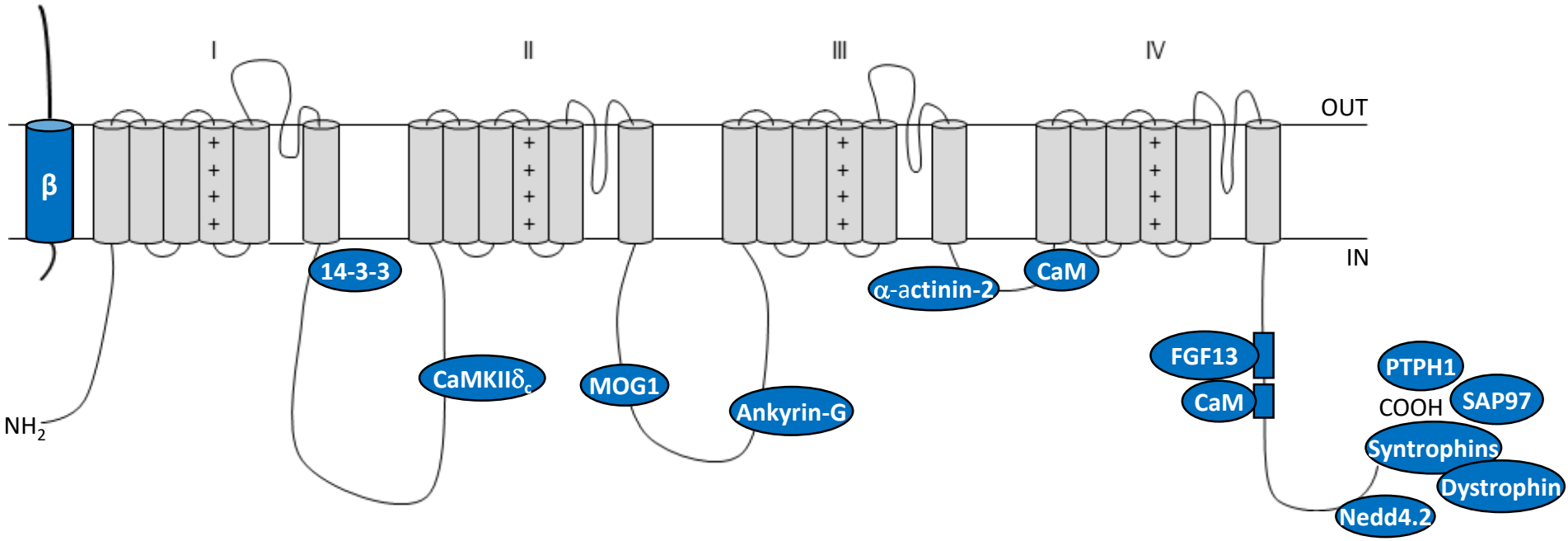
Cardiac Nav1.5 channel complexes



O-GlcNAcylation **Glycosylation** **Phosphorylation** **Methylation** **Acetylation**

Oxidation **Nitrosylation** **Lipoxidation** **Ubiquitylation** **Palmitoylation** **SUMOylation**

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

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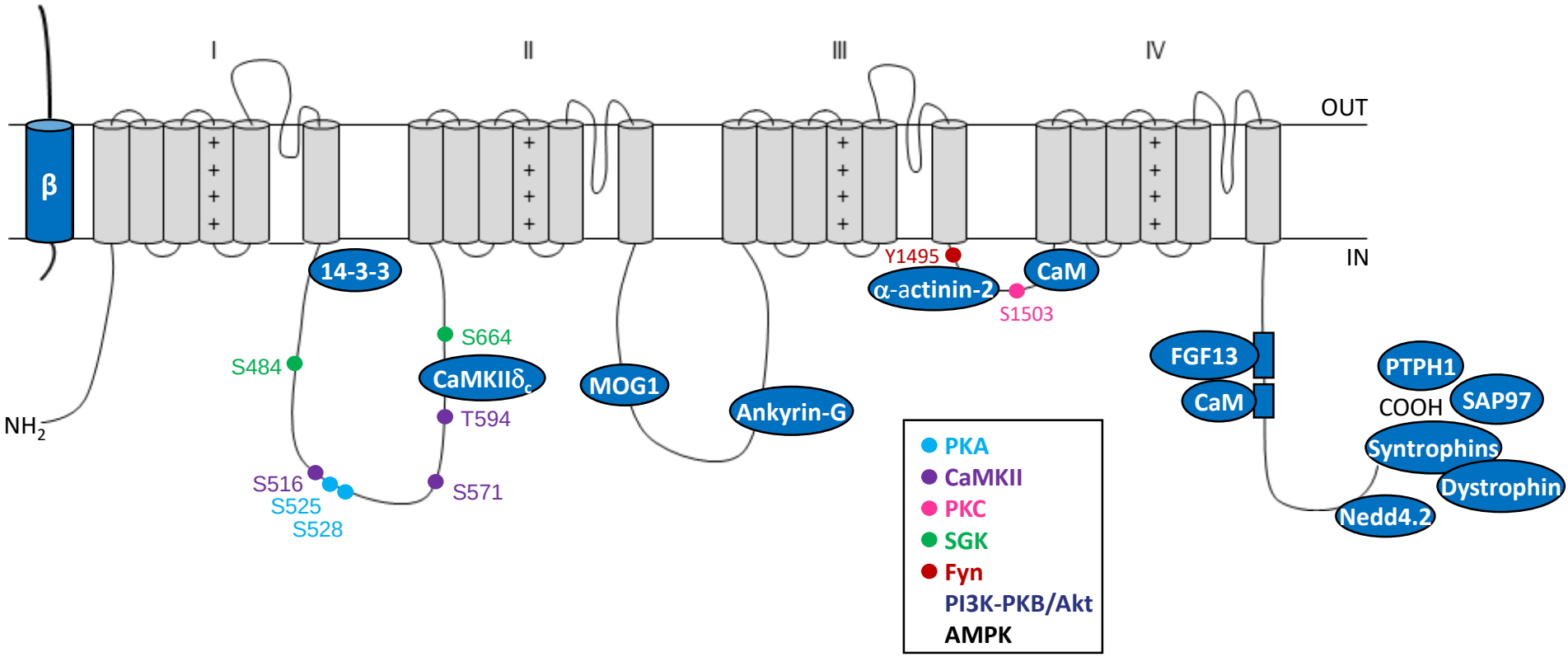
Marionneau and Abriel, ***JMCC 2015***

Before 2012 : 9 phosphorylation sites



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Marionneau and Abriel, ***JMCC 2015***

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)

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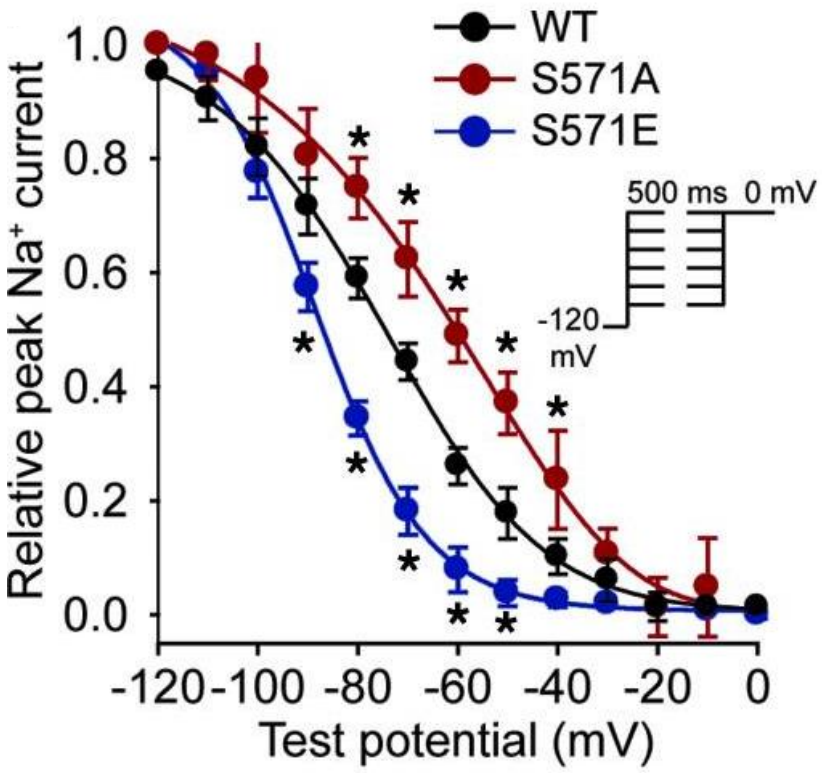
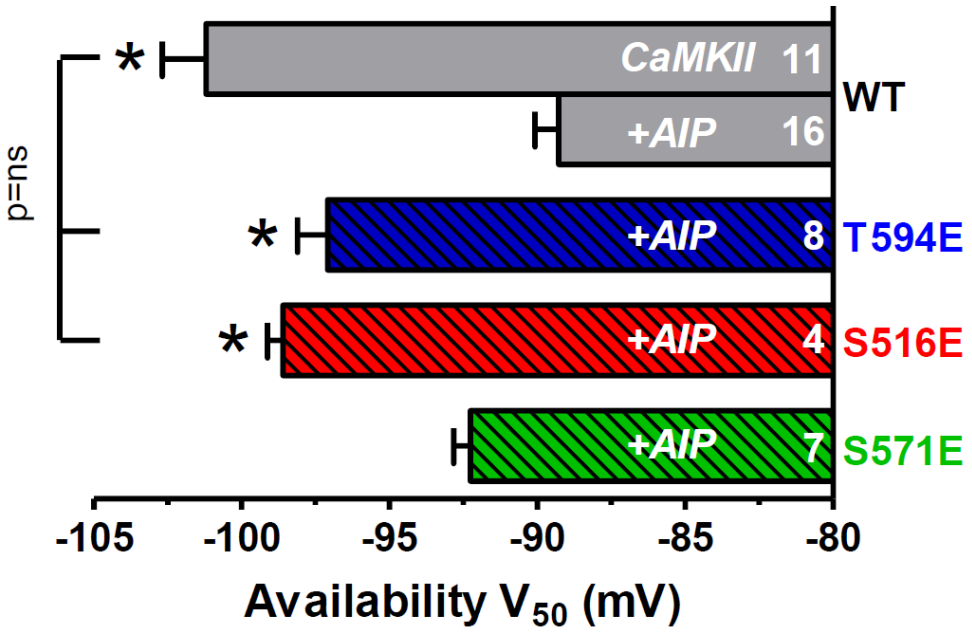
CaMKII phosphorylation sites

In silico



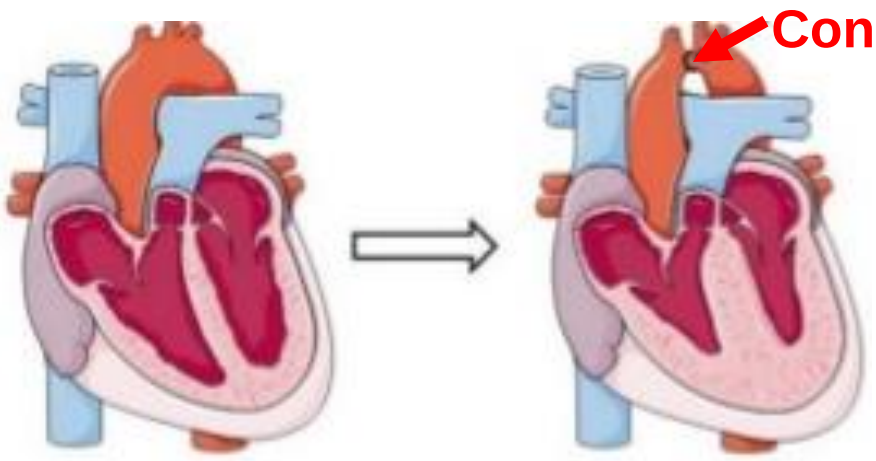
Hund *et al*, 2010

In vitro



Ashpole *et al*, 2012

Phosphoproteomics of cardiac Nav1.5 channels



Sham Ventricles

TAC Ventricles

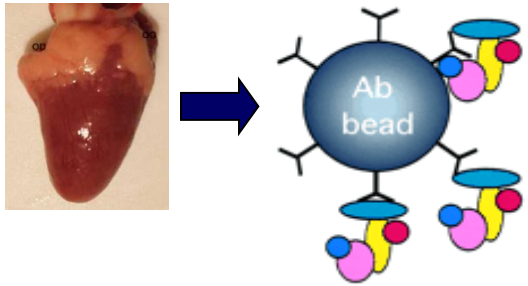
mαNavPAN-IPs

mαNavPAN-IPs

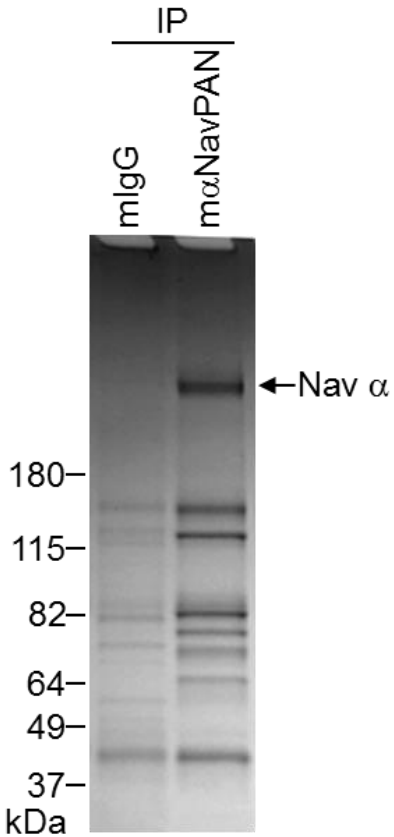
Quantitative phosphoproteomics

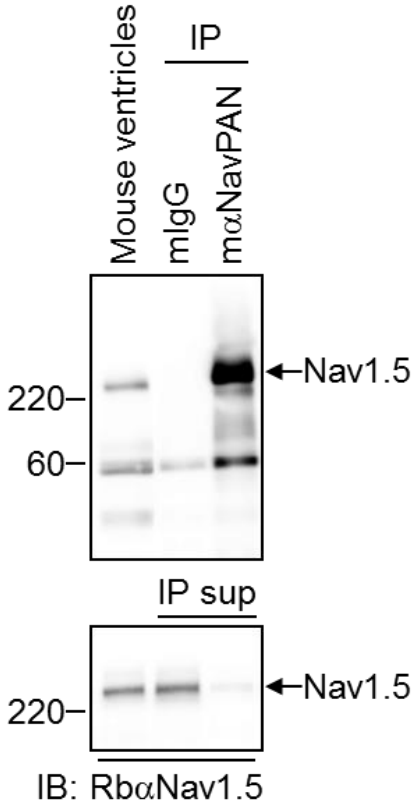
Purification of cardiac Nav1.5 channels

mαNa$_V$PAN IP



Digestion **LC-MS/MS**

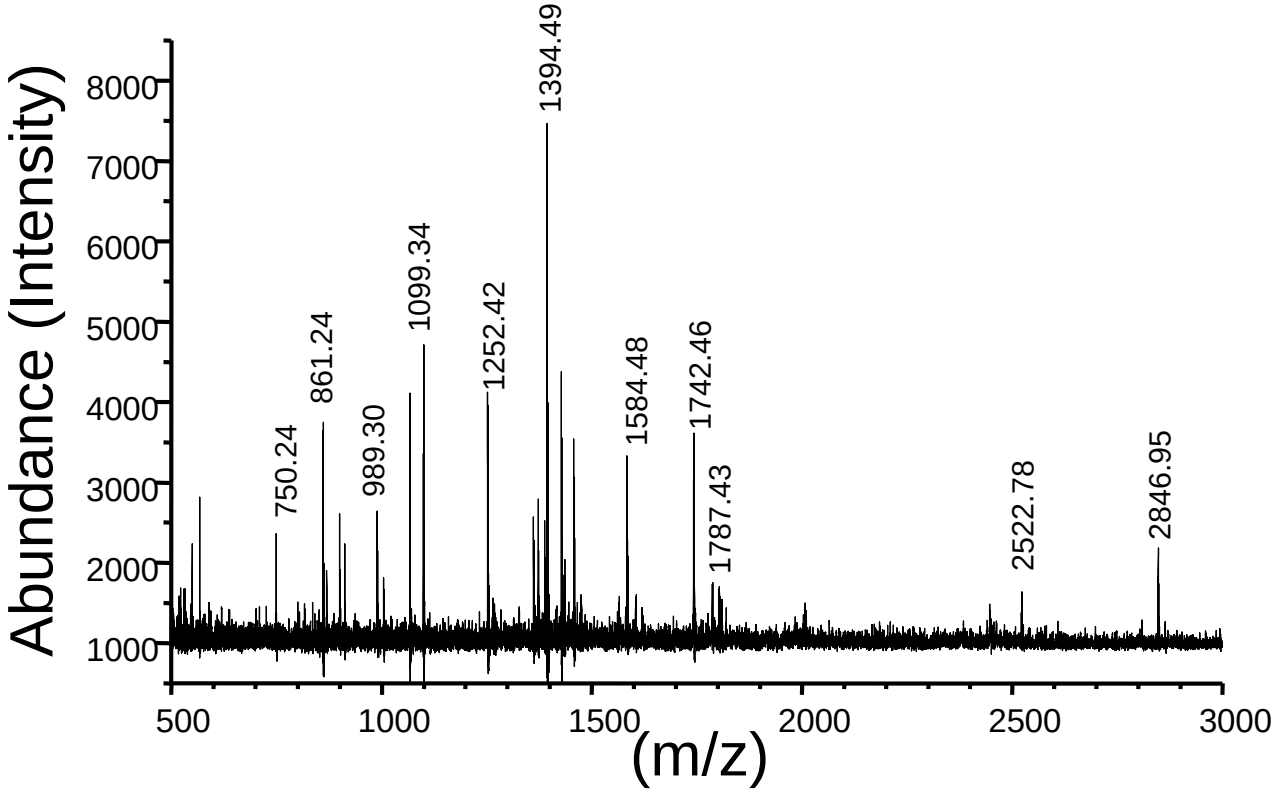




Marionneau et al, ***JPR 2012***

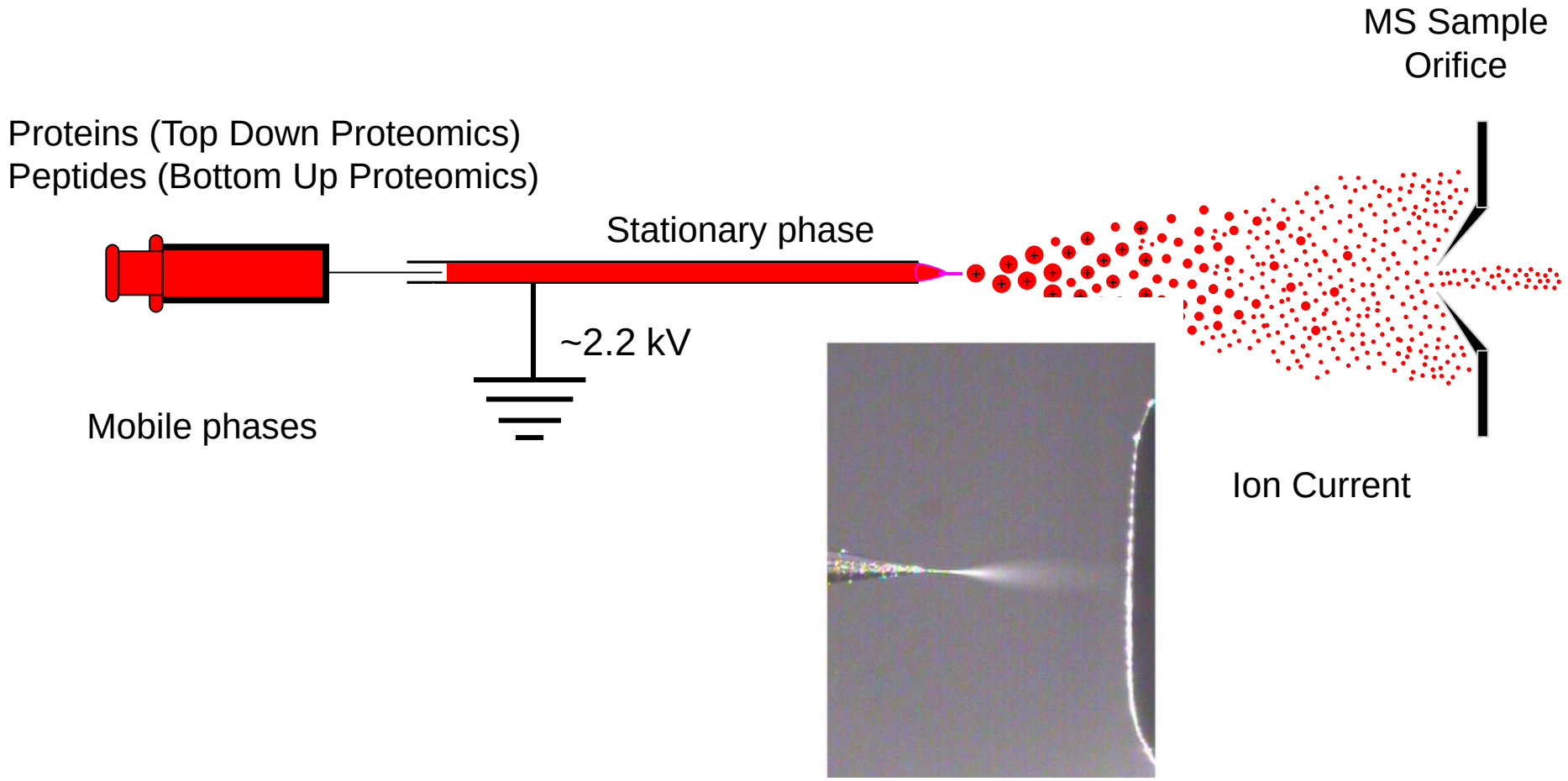
Mass Spectrometry – A Brief Definition

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).

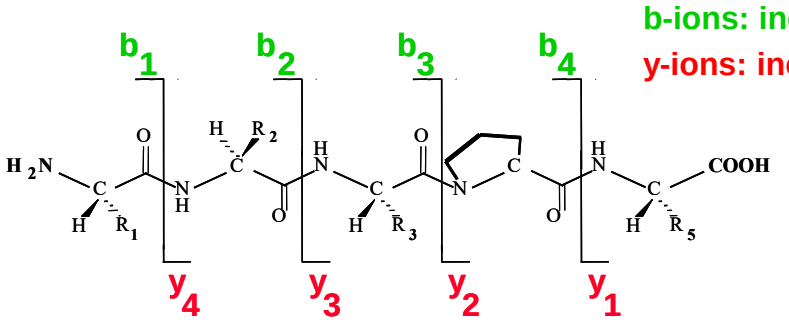


Electrospray ionization : ESI-MS

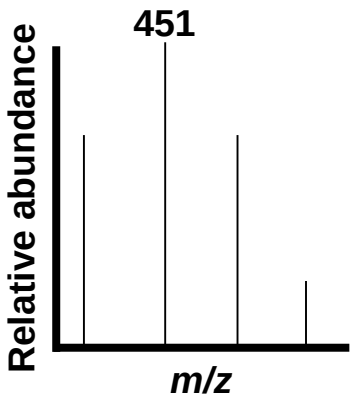
- Couples liquid chromatography to mass spectrometry
- Reversed-phase chromatography



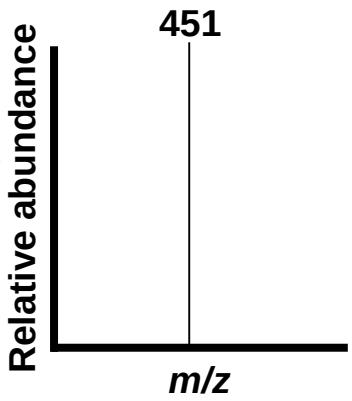
MS/MS analyses



MS1: **mass spectrum of precursor ions**

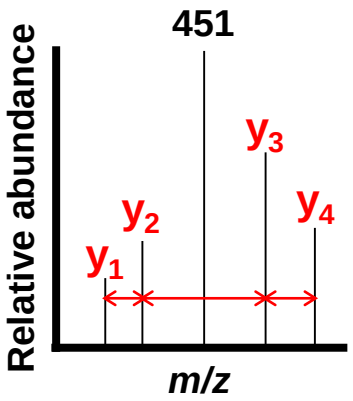


Isolation of the most abundant precursor ions



CID: Collision Induced Dissociation

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



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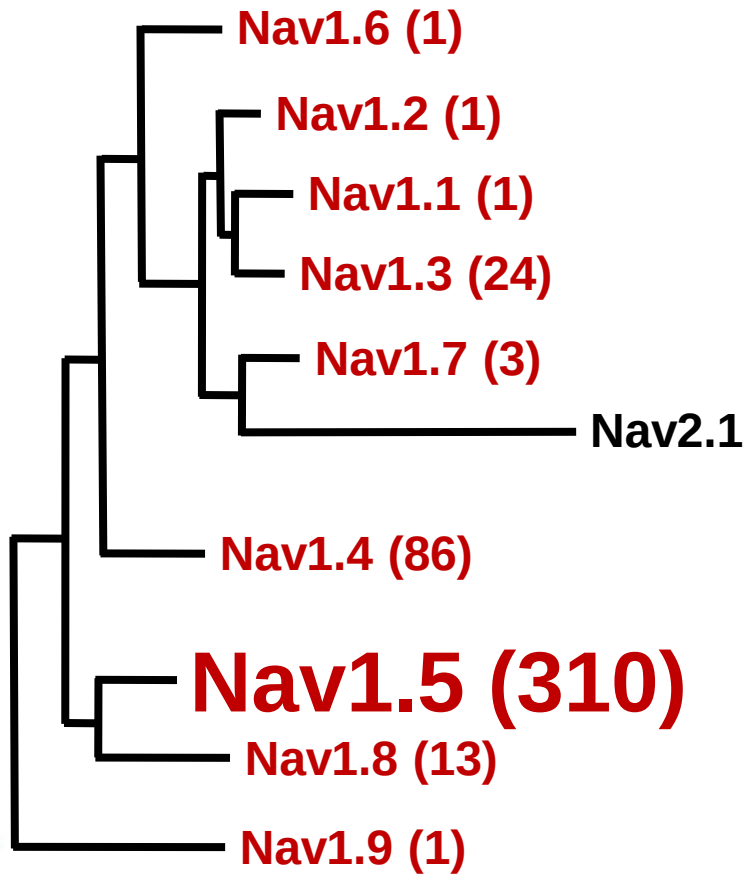
Identification of Nav pore-forming subunits

$Na_V1.5$:
- **Q1080 and Q1080 del isoforms**
- **310 unique peptides**
- **56% amino acid coverage**




```
1     MANFLLPRGTSSFRRFTRESLAAIEKRMAEKQARGSATLQESREGLPEEEAPRPQLDLQASKKLPDLYGNPPREL
                                                                N-TERM
76    IGEPLEDLDPFYSTQKTFIVLNKGKTIFRFSATNALYVLSPFHPVRAAVKILVHSLFSMLIMCTILTNCVFMAQ
                                                    N-TERM                  IS1
151   HDPPPWTKYVEYTFTAIYTFESLVKILARGFCLHAFTFLRDPWNWLDFSVIVMAYTTEFVDLGNVSALRTFRVLR
                          IS2                       IS3                       IS4
226   ALKTISVISGLKTIVGALIQSVKKLADVMVLTVFCLSVFALIGLQLFMGNLRHKCVRNFTELNGTNGSVEADGIV
                                                    IS5
301   WNSLDVYLNDPANYLLKNGTTDVLLCGNSSDAGTCPEGYRCLKAGENPDHGYTSFDSFAWAFLALFRLMTQDCWE
376   RLYQQTLRSAGKIYMIFFMLVIFLGSFYLVNLILAVVAMAYEEQNQATIAETEEKEKRFQEAMEMLKKEHEALTI
                                IS6
451   RGVDTVSRSSLEMSPLAPVTNHERRSKRRKRLSSGTEDGGDDRLPKSDSEDGPRALNQLSLTHGLSRTSMRPRSS
                                Loop I
526   RGSIFTFRRRDQGSEADFADDENSTAGESESHRTSLLVPWPLRRPSTQGQPGFGTSAPGHVLNGKRNSTVDCNGV
                                Loop I
601   VSLLGAGDAEATSPGSHLLRPIVLDRPPDTTTPSEEPGGPQMLTPQAPCADGFEEPGARQRALSAVSVLTSALEE
                                Loop I
676   LEESHRKCPPCWNRFAQHYLIWECCPLWMSIKQKVKFVVMDPFADLTITMCIVLNTLFMALEHYNMTAEFEEMLQ
                                                    IIS1
751   VGNLVFTGIFTAEMTFKIIALDPYYYFQQGWNIFDSIIVILSLMELGLSRMGNLSVLRSFRLLRVFKLAKSWPTL
                IIS2                    IIS3                    IIS4
826   NTLIKIIGNSVGALGNLTLVLAIIVFIFAVVGMQLFGKNYSELRHRISDSGLLPRWHMMDFFHAFLIIFRILCGE
                                IIS5
901   WIETMWDCMEVSGQSLCLLVFLLVMVIGNLVVLNLFLALLLSSFSADNLTAPDEDGEMNNLQLALARIQRGLRFV
                                IIS6
976   KRTTWDFCCGLLRRRPKKPAALAIHSQLPSCIAAPRSPPPPEVEKAPPARKETRFEEDKRPGQGTPGDTEPVCVP
                                Loop II
1051  IAVAESDIDDQEEDEENSLGTEEEESSK[Q]QESQVVSGGHEPPQEPRAWSQVSETTSSEAEASTSQADWQQERE
                                Loop II
1124  AEPRAPGCGETPEDSYSEGSTADMTNTADLLEQIPDLGEDVKDPEDCFTEGCVRRCPCCMVDTTQAPGKVWWRLR
                                Loop II
1199  KTCYRIVEHSWFETFIIFMILLSSGALAFEDIYLEERKTIKVLLEYADKMFTYVFVLEMLLKWVAYGFKKYFTNA
                        IIIS1                   IIIS2
1274  WCWLDFLIVDVSLVSLVANTLGFAEMGPIKSLRTLRALRPLRALSRFEGMRVVVNALVGAIPSIMNVLLVCLIFW
                IIIS3                   IIIS4
1349  LIFSIMGVNLFAGKFGRCINQTEGDLPLNYTIVNNKSECESFNVTGELYWTKVKVNFDNVGAGYLALLQVATFKG
                IIIS5
1424  WMDIMYAAVDSRGYEEQPQWEDNLYMYIYFVVFIIFGSFFTLNLFIGVIIDNFNQQKKKLGGQDIFMTEEQKKYY
                                IIIS6
1499  NAMKKLGSKKPQKPIPRPLNKYQGFIFDIVTKQAFDVTIMFLICLNMVTMMVETDDQSPEKVNILAKINLLFVAI
                Loop III                IVS1                    IVS2
1574  FTGECIVKMAALRHYYFTNSWNIFDFVVVILSIVGTVLSDIIQKYFFSPTLFRVIRLARIGRILRLIRGAKGIRT
                        IVS3                            IVS4
1649  LLFALMMSLPALFNIGLLLFLVMFIYSIFGMANFAYVKWEAGIDDMFNFQTFANSMLCLFQITTSAGWDGLLSPI
                                IVS5
1724  LNTGPPYCDPNLPNSNGSRGNCGSPAVGILFFTTYIIISFLIVVNMYIAIILENFSVATEESTEPLSEDDFDMFY
                                IVS6
1799  EIWEKFDPEATQFIEYLALSDFADALSEPLRIAKPNQISLINMDLPMVSGDRIHCMDILFAFTKRVLGESGEMDA
                                C-TERM
1874  LKIQMEEKFMAANPSKISYEPITTTLRRKHEEVSATVIQRAFRRHLLQRSVKHASFLFRQQAGSSGLSDEDAPER
                                C-TERM
1949  EGLIAYMMNENFSRRSGPLSSSSISSTSFPPSYDSVTRATSDNLPVRASDYSRSEDLADFPPSPDRDRESIV
                                C-TERM
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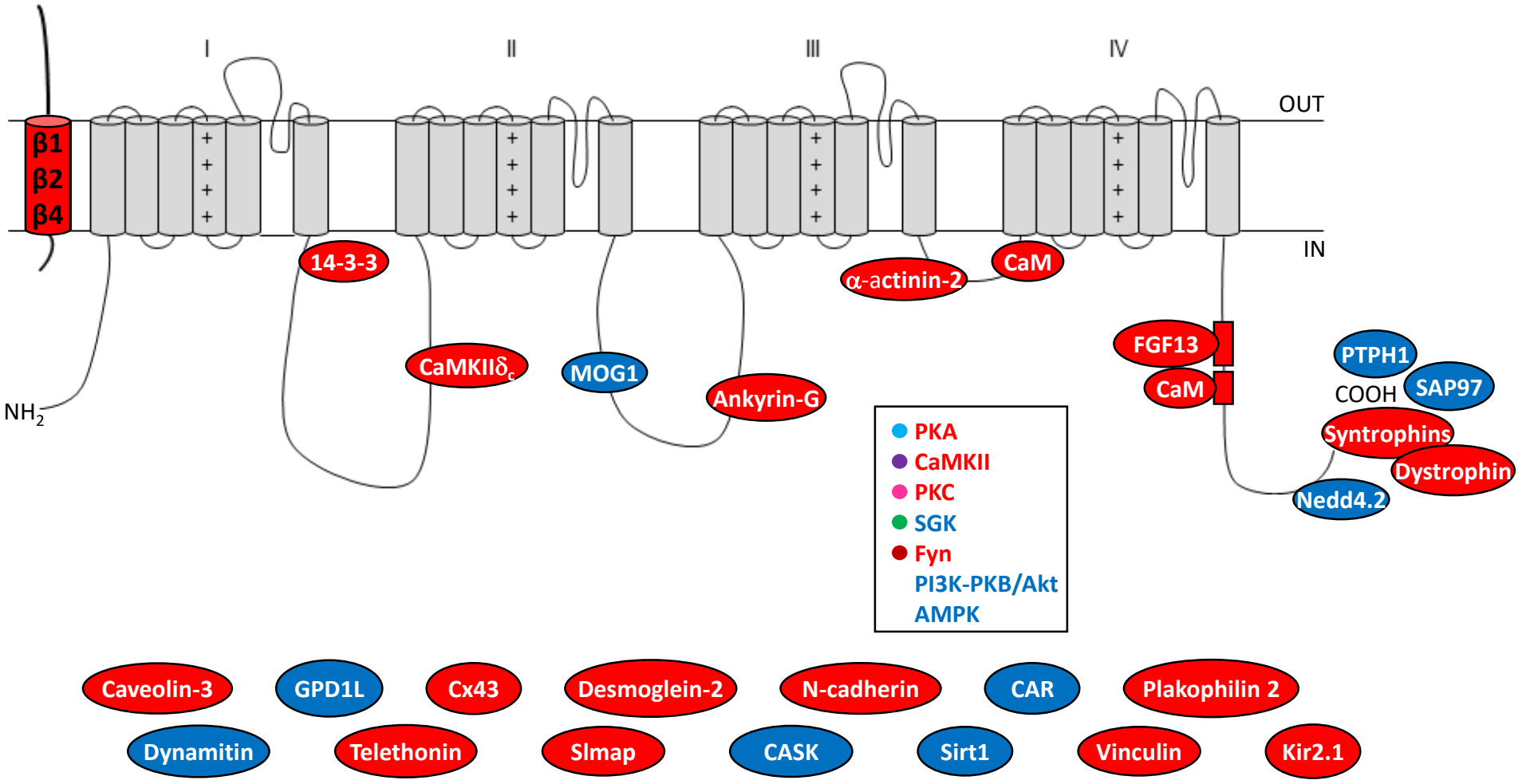
Characterization of cardiac Nav1.5 channel complexes



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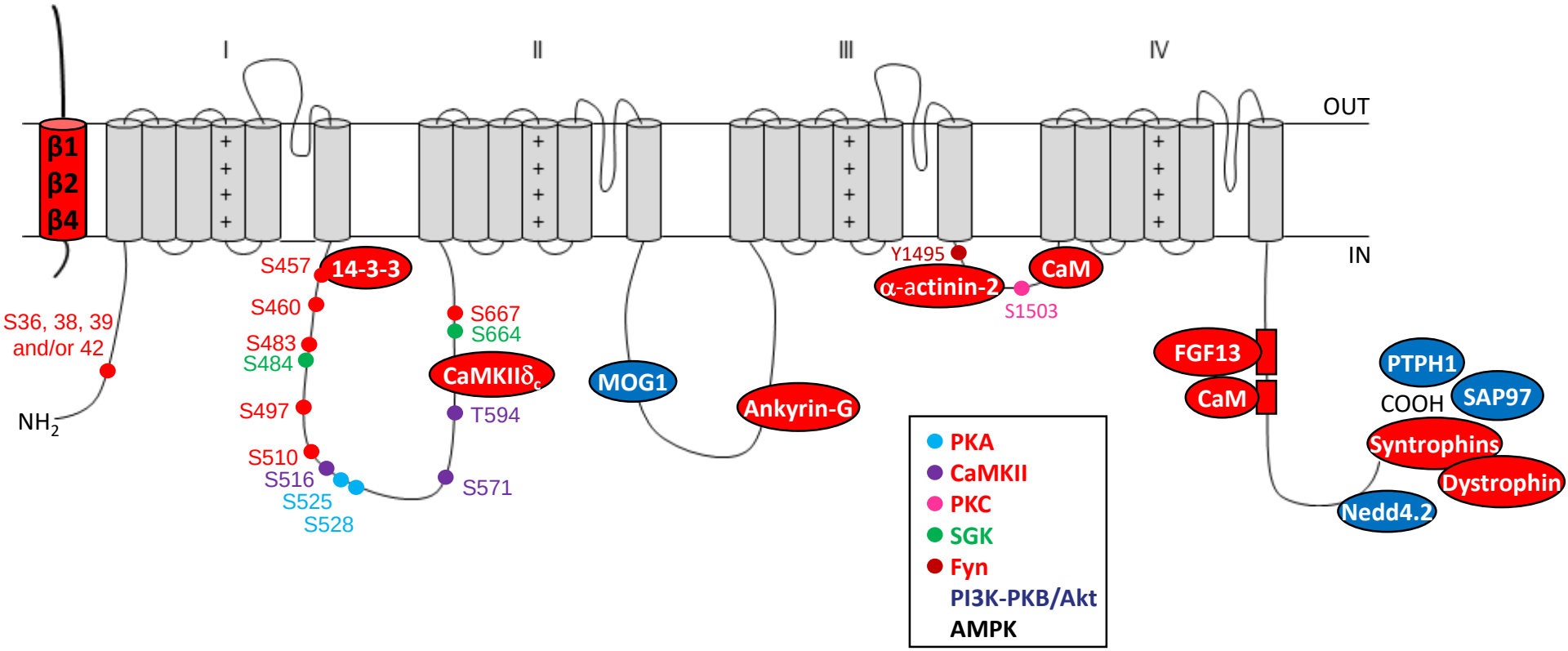
2012 : 9 + 7 = 16 phosphorylation sites



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

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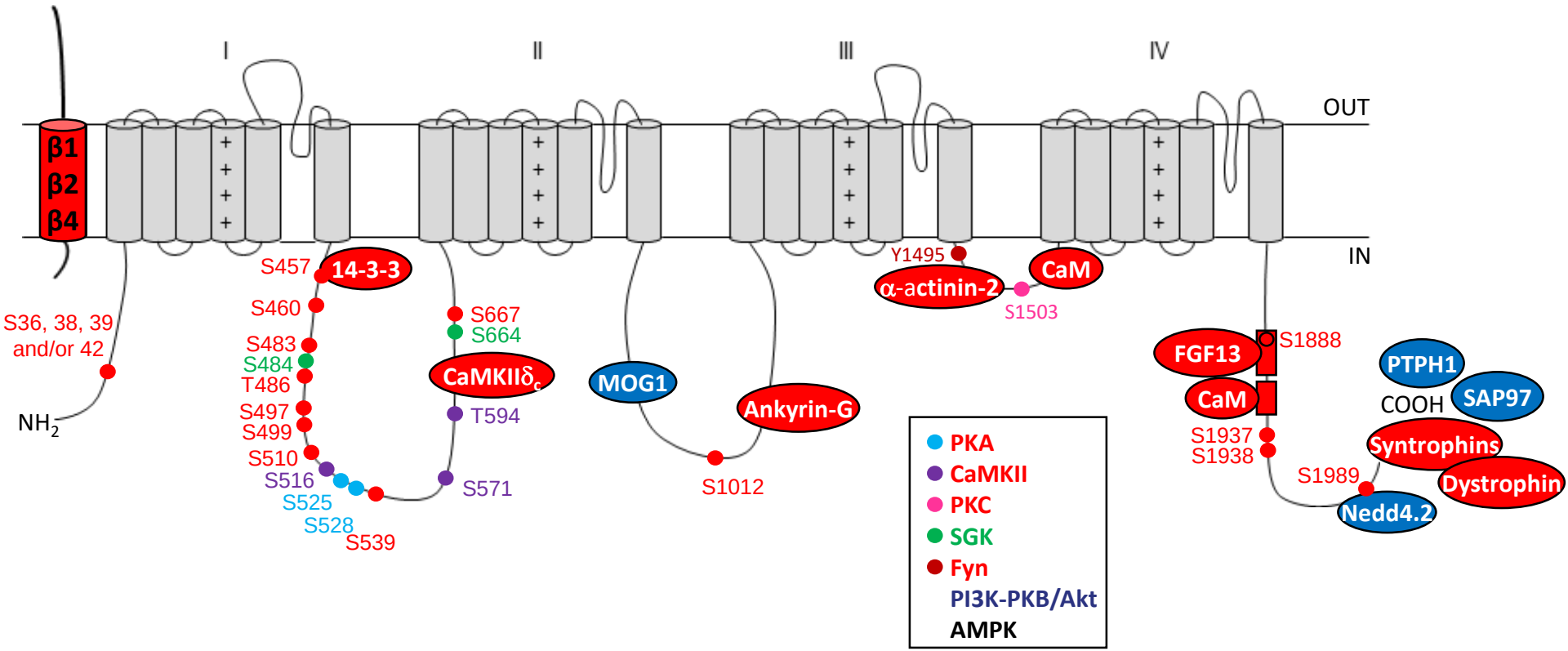
Marionneau and Abriel, ***JMCC 2015***
Marionneau et al, ***JPR 2012***

2017 : 16 + 8 = 24 phosphoryaltion sites



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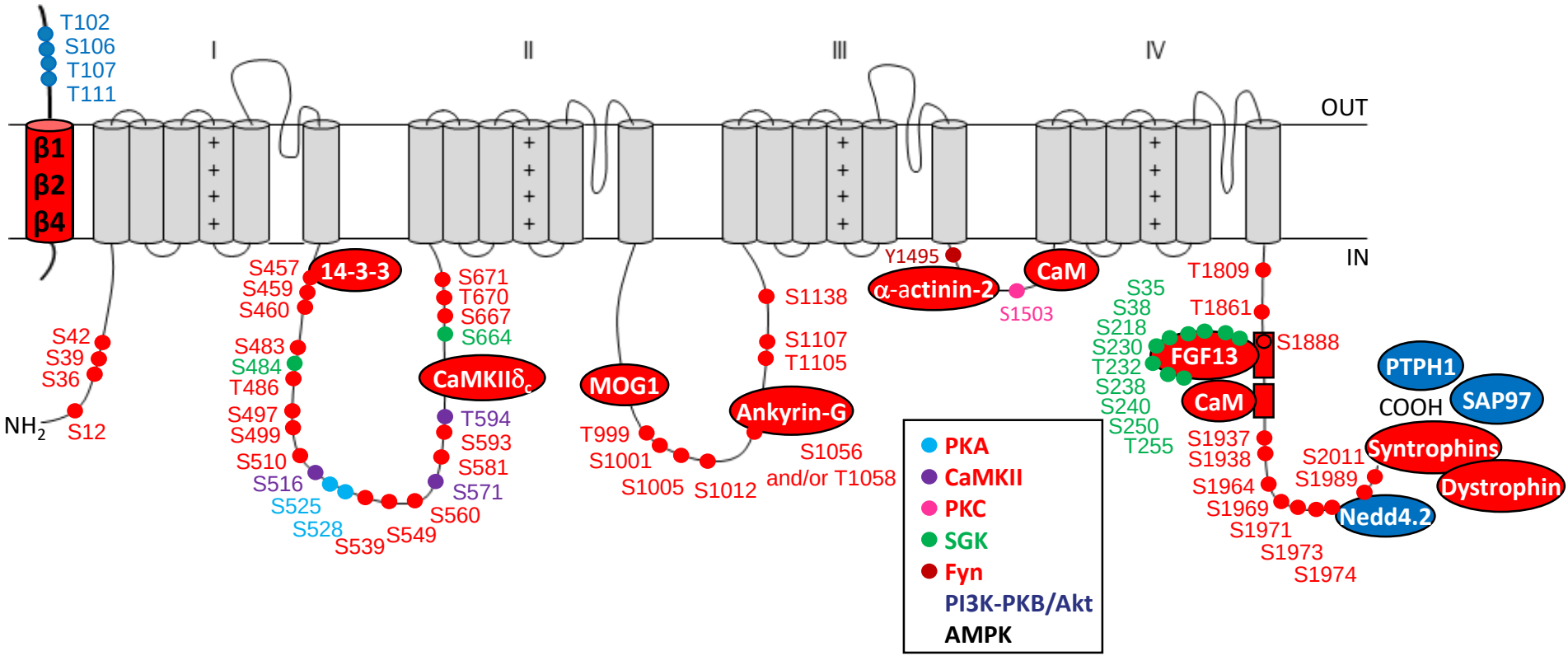
Marionneau and Abriel, ***JMCC 2015***
Marionneau et al, ***JPR 2012***
Burel et al, ***JBC 2017***

2018 : 49 + 4 + 9 = 62 phosphorylation sites



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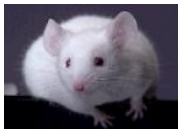
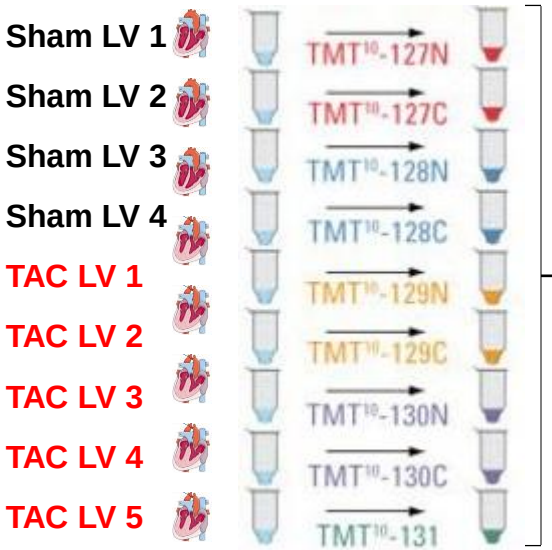
Marionneau and Abriel, ***JMCC 2015***
Marionneau et al, ***JPR 2012***
Burel et al, ***JBC 2017***
Lorenzini et al, ***JGP 2021***

TMT9 quantitative analysis from Sham/TAC mice

Sham / TAC mice



mαNa$_V$PAN-IPs **Labelling**

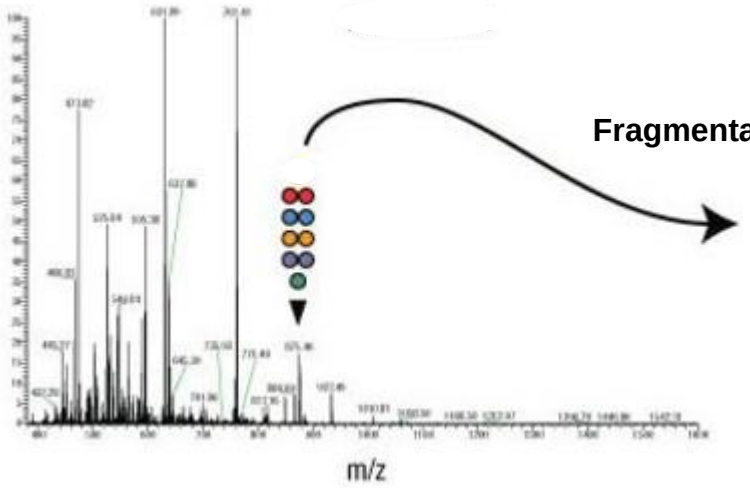


TMT methods

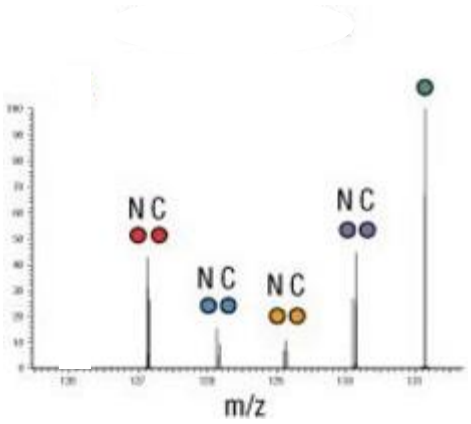
1 LC-MS/MS experiment

Combine

MS1 spectra: selection **MS/MS spectra: Quantification**



Fragmentation



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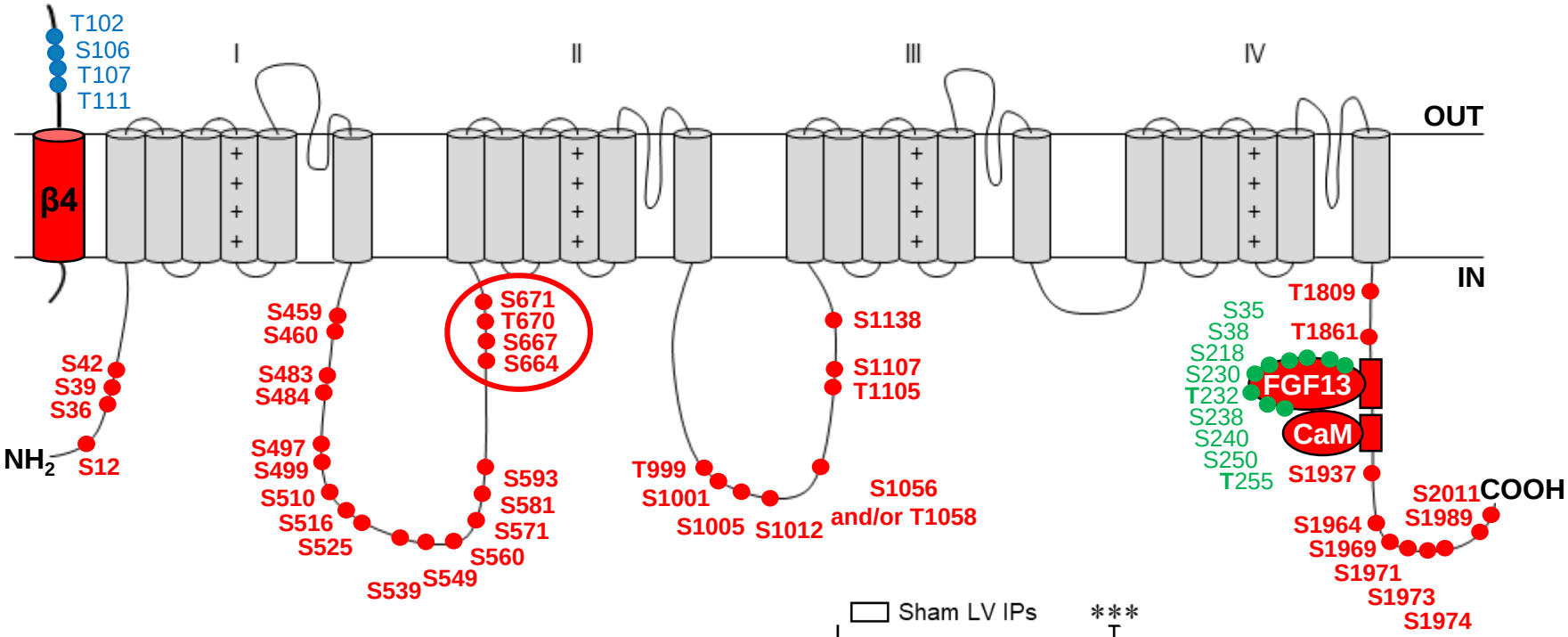
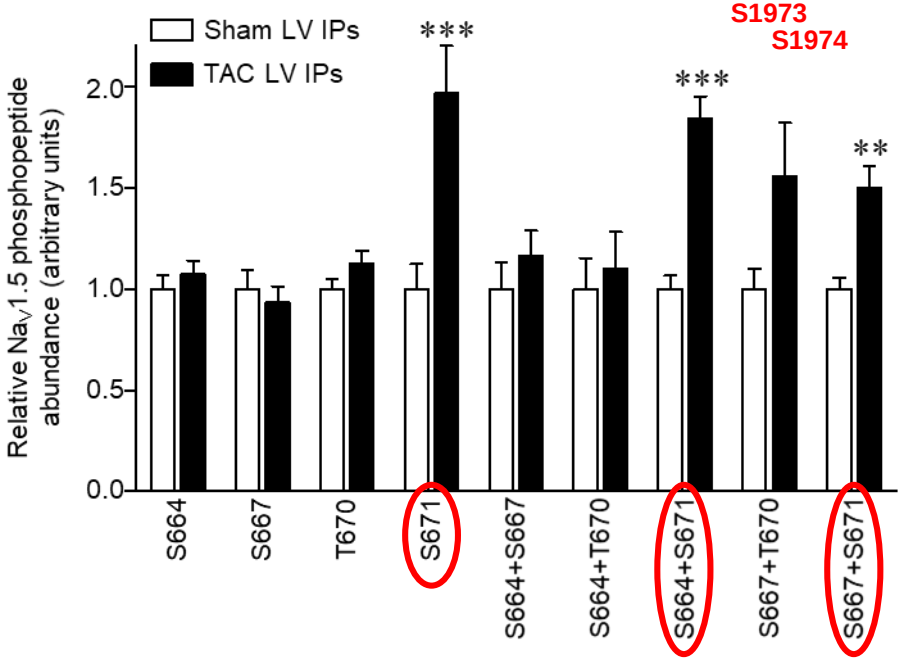
Quantitative phosphoproteomics in Sham/TAC mice





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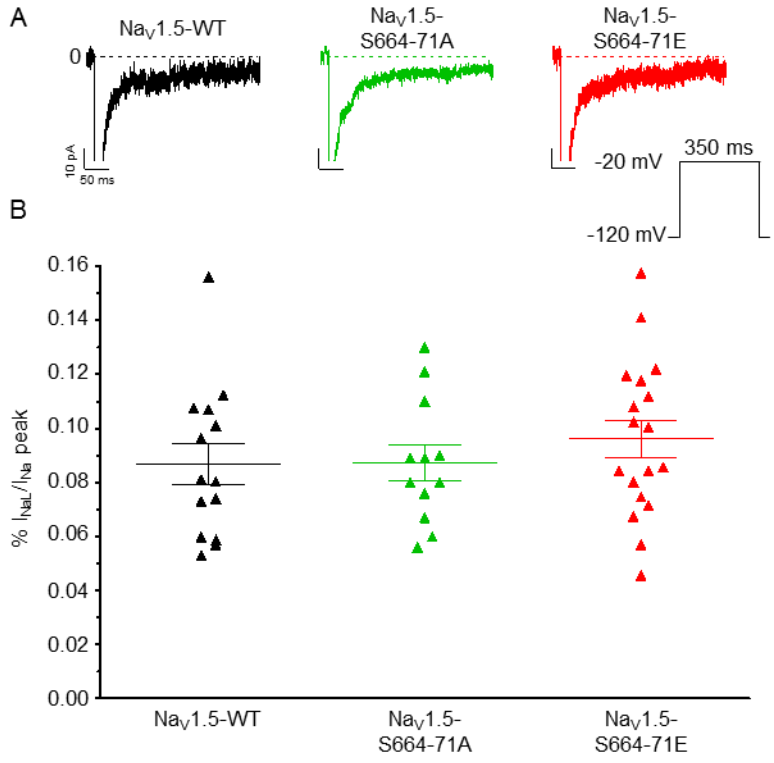
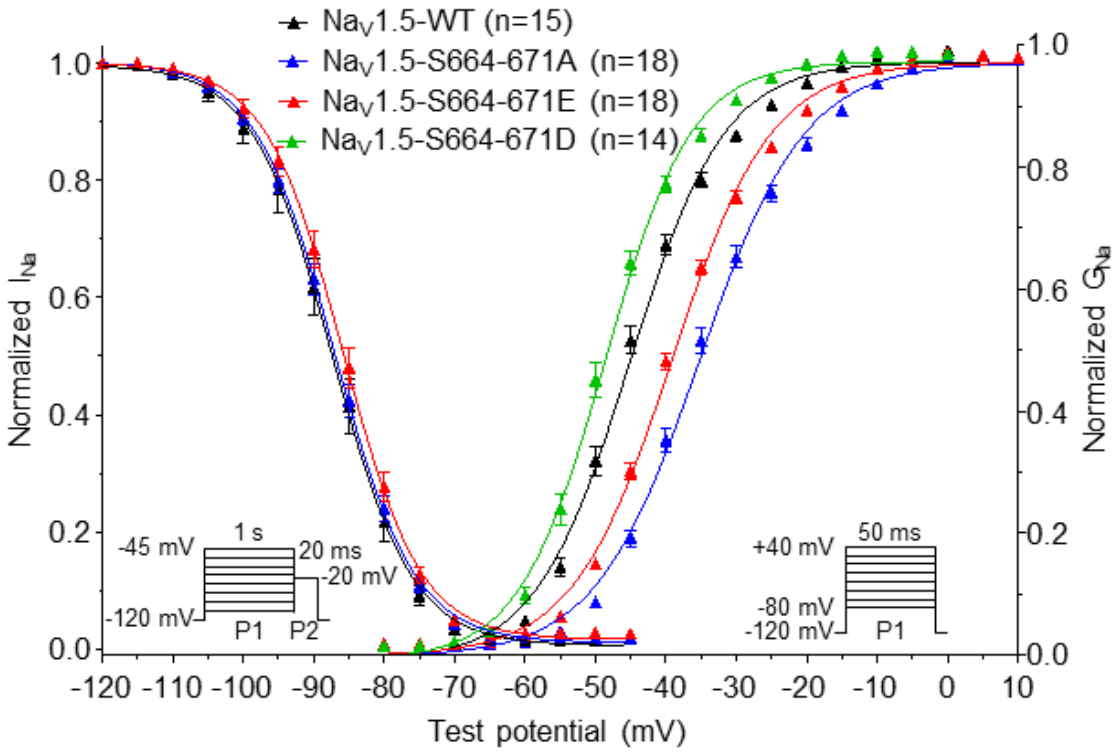
**p<0.01, ***p<0.001 in TAC *vs* Sham

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





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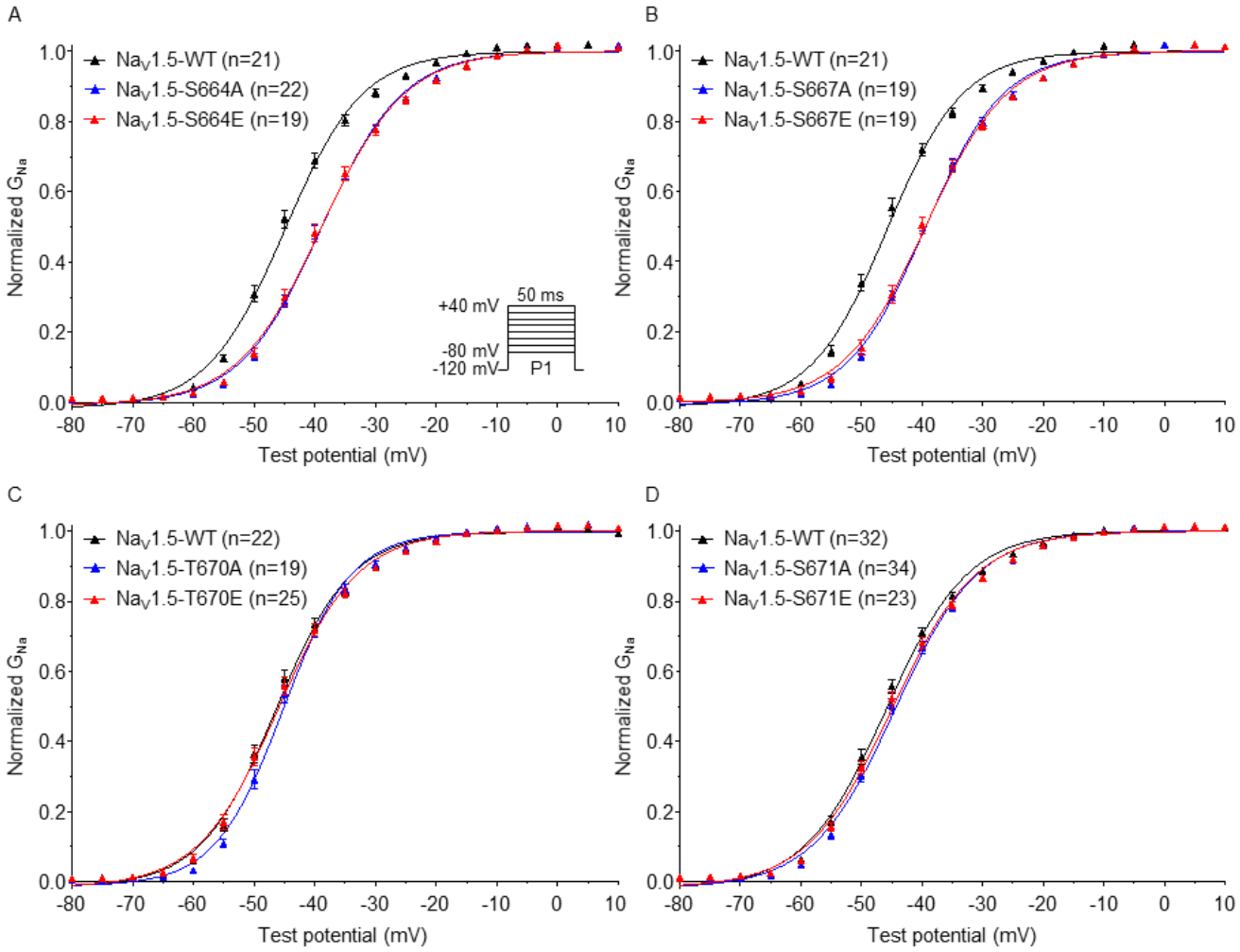
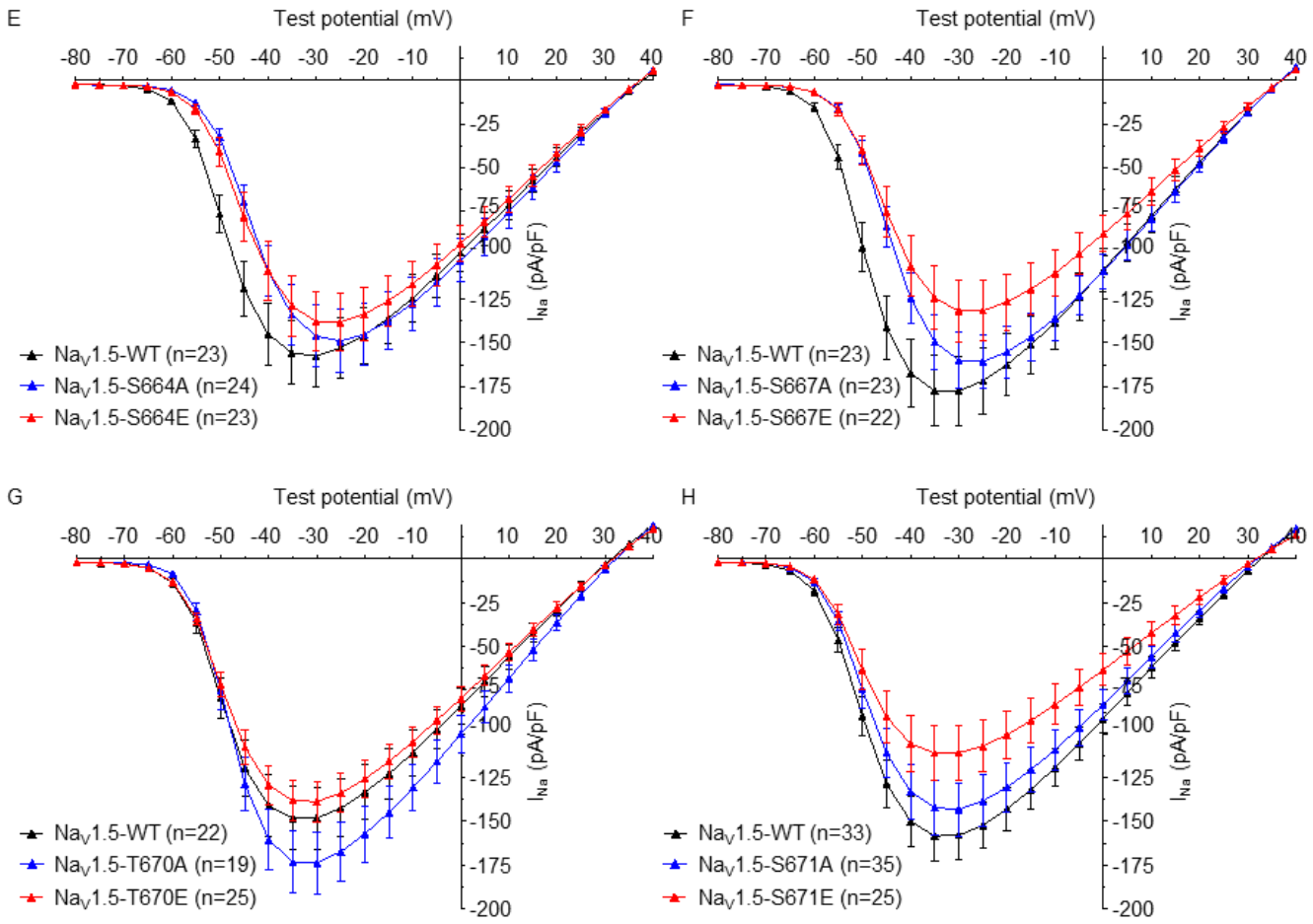
Electrophysiological analysis of simple Nav1.5 phosphomutants



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

Electrophysiological analysis of simple Nav1.5 phosphomutants



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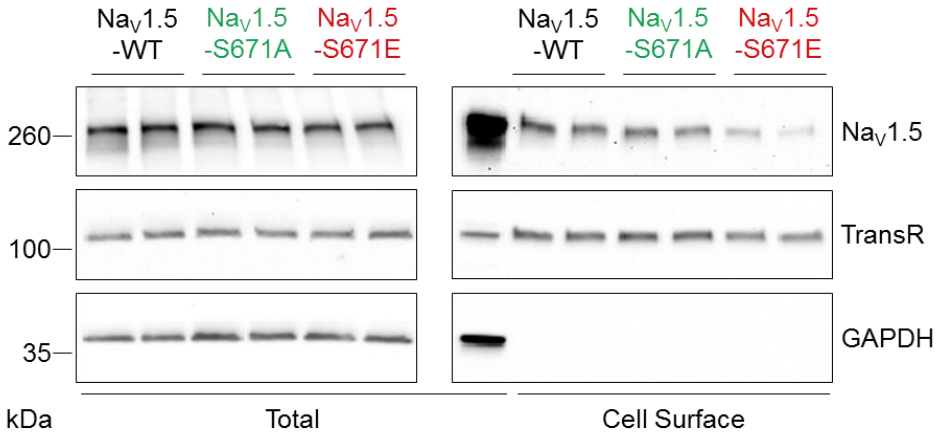
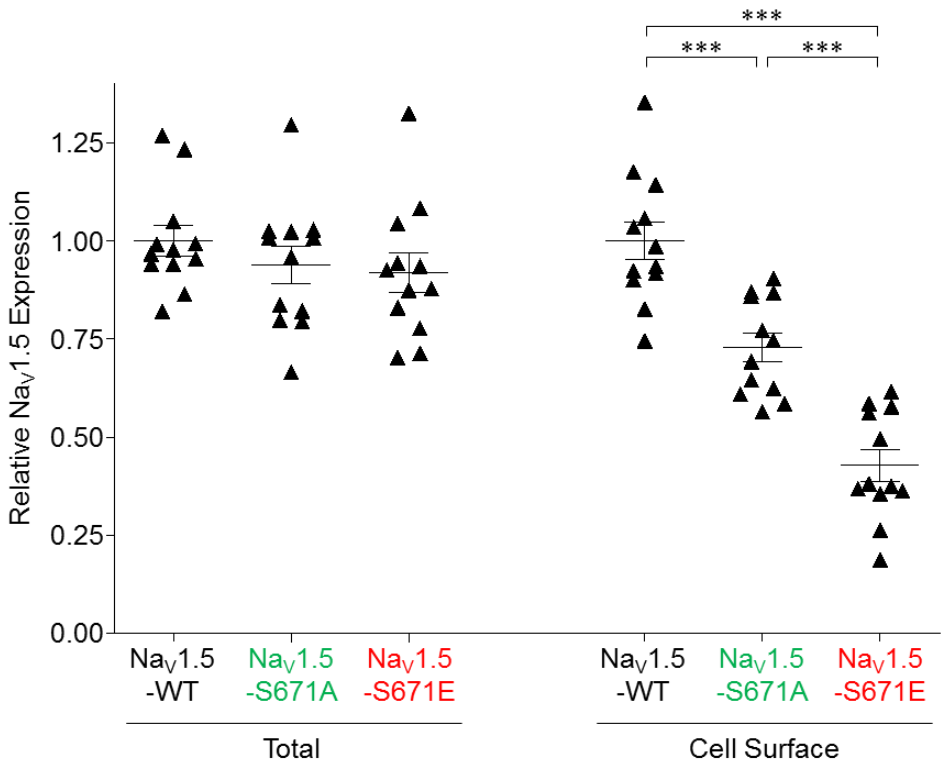
Main finding: Phosphorylation at S671 decreases I_{Na}.

Phosphorylation at S671 decreases Nav1.5 cell surface expression





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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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