Phosphoproteomics of cardiac Nav1.5 channels

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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

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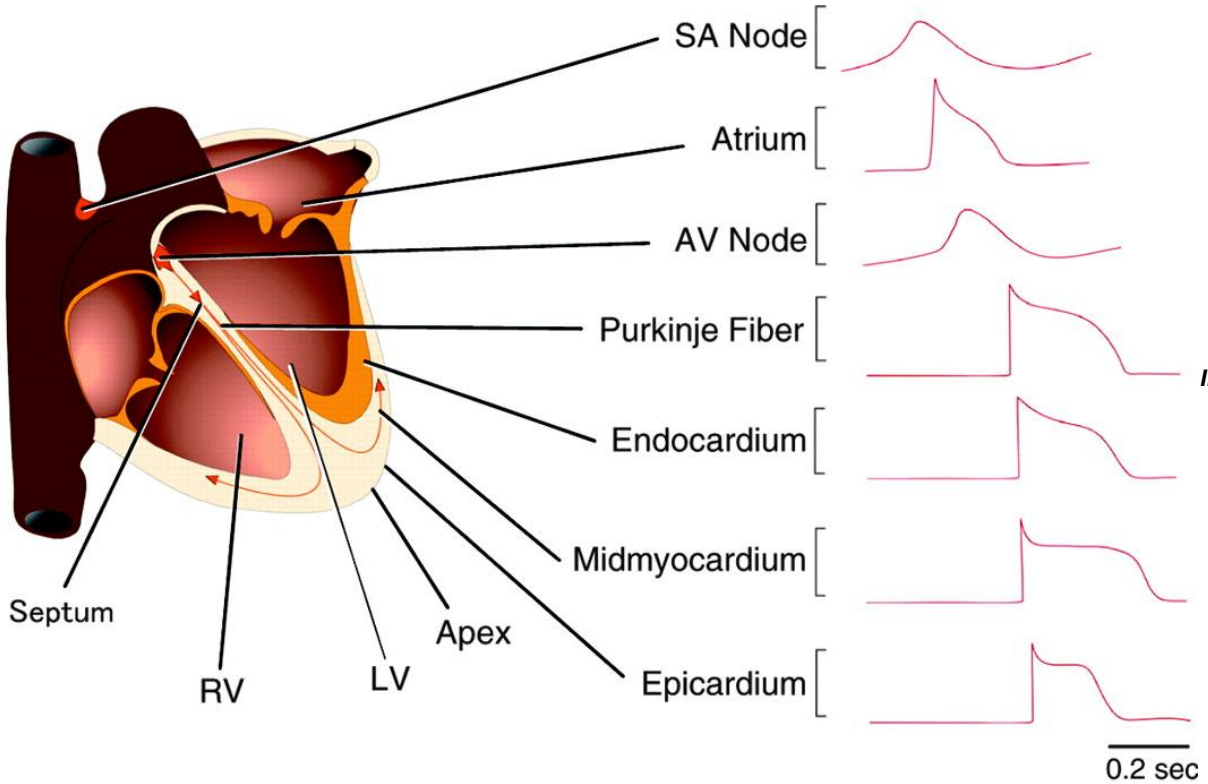
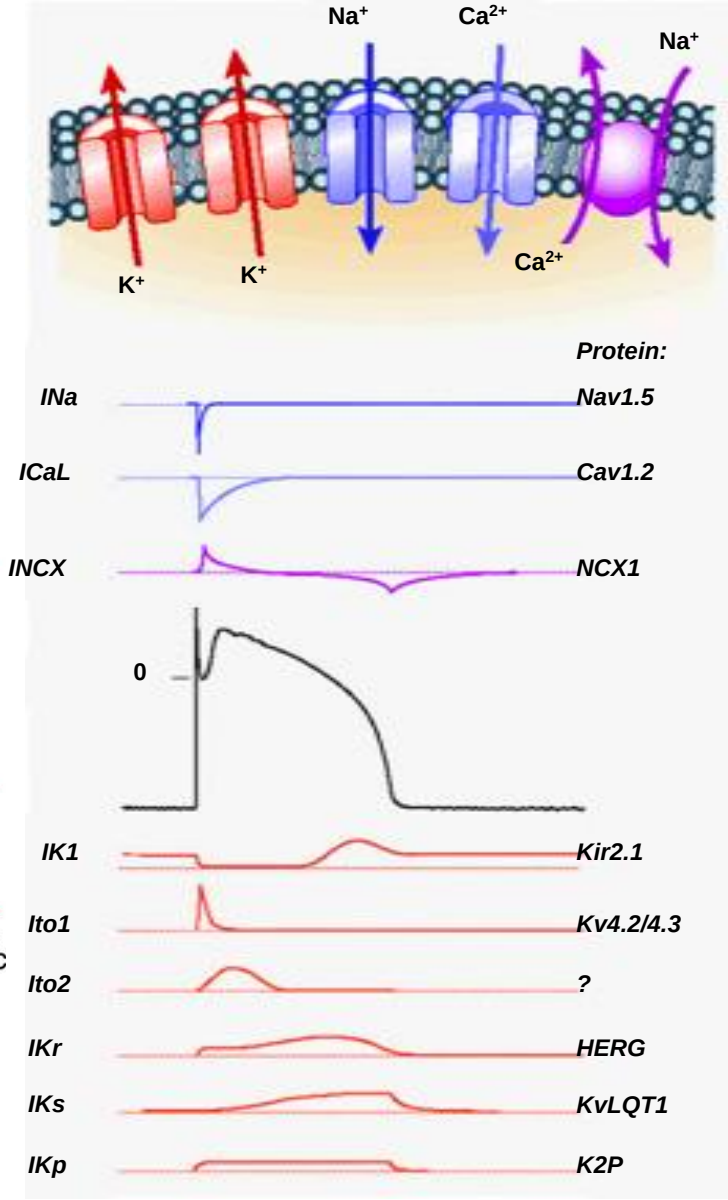
Cardiac excitability

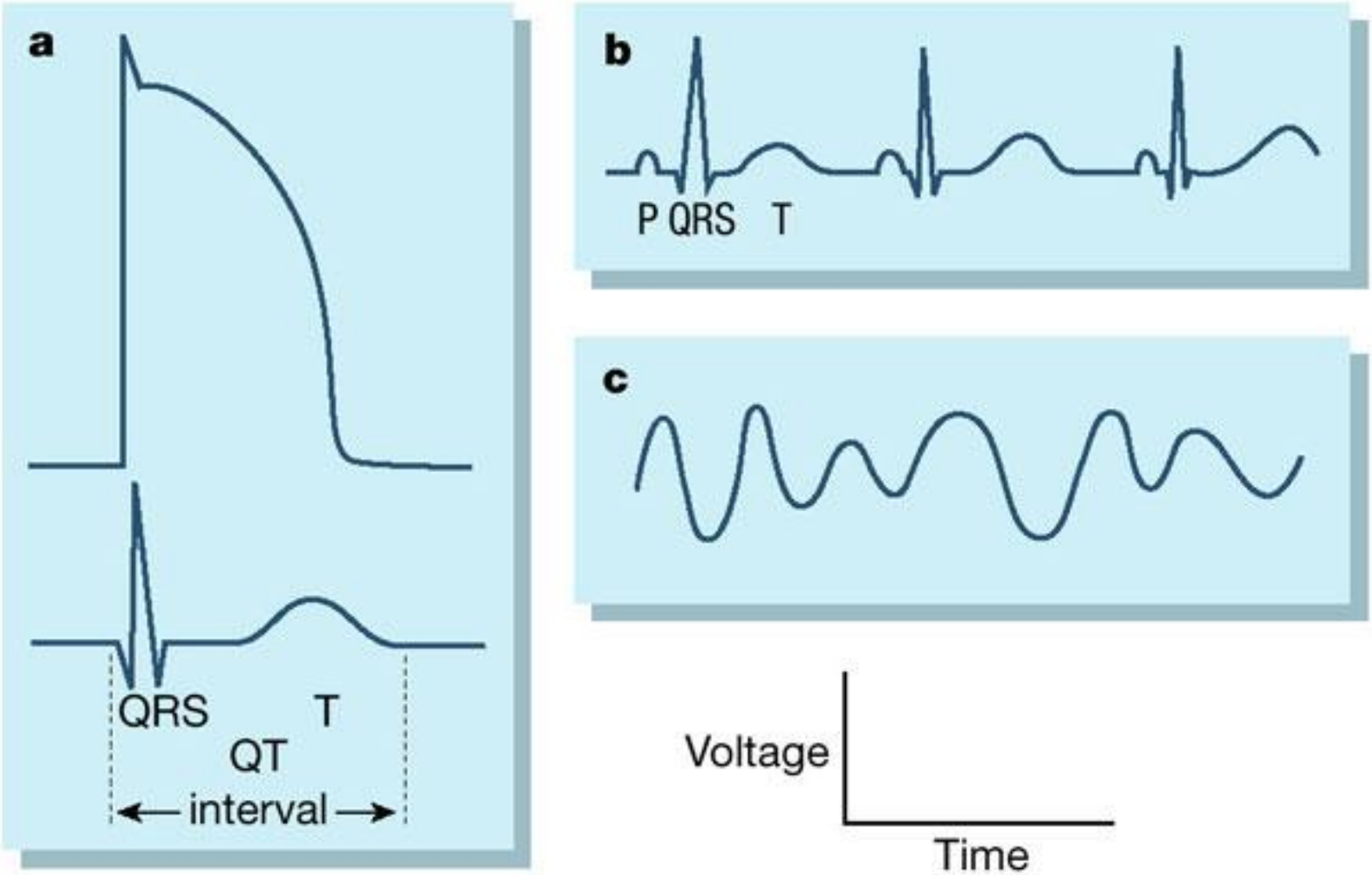


Nerbonne and Kass, *Physiol Rev 2005*



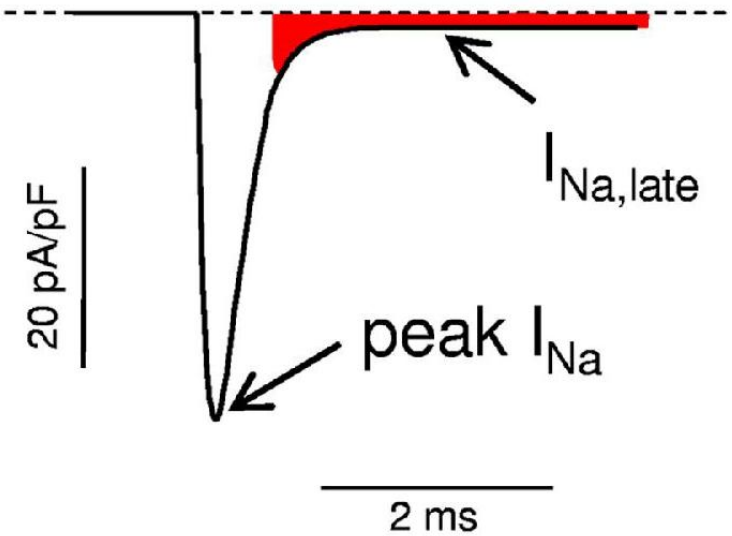
Marban, *Nature 2002*

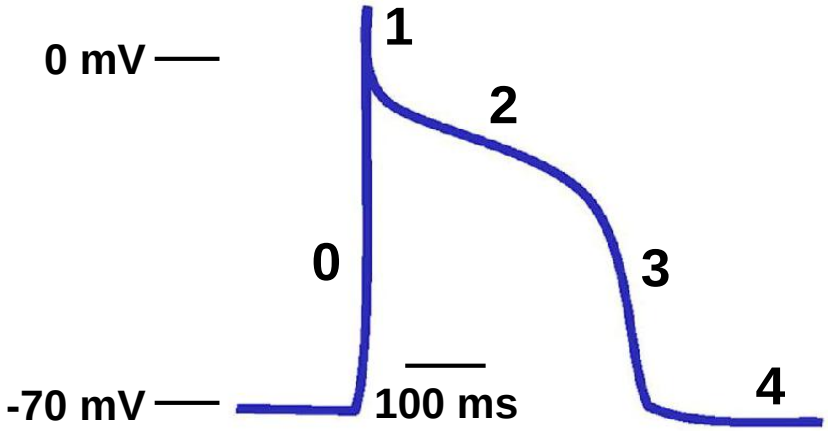
Electrocardiograms : ECG

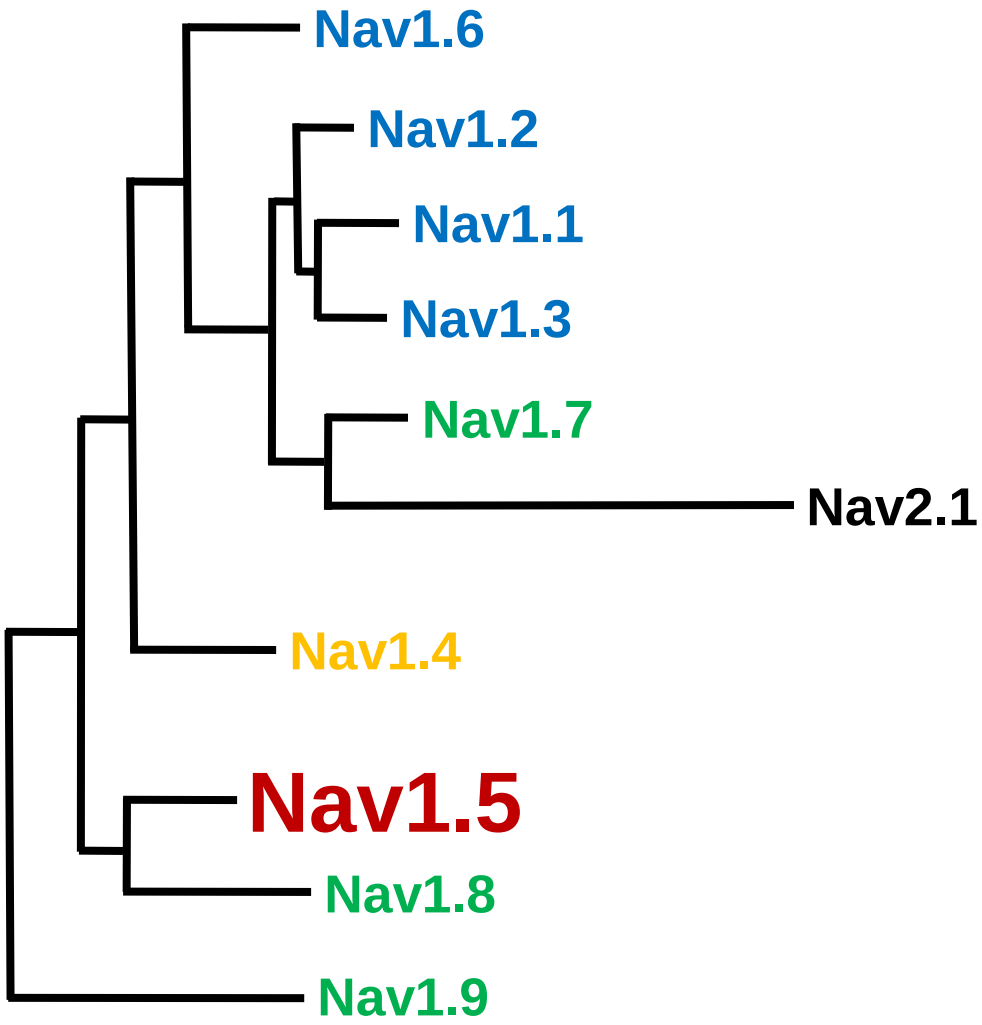


Marban, *Nature 2002*

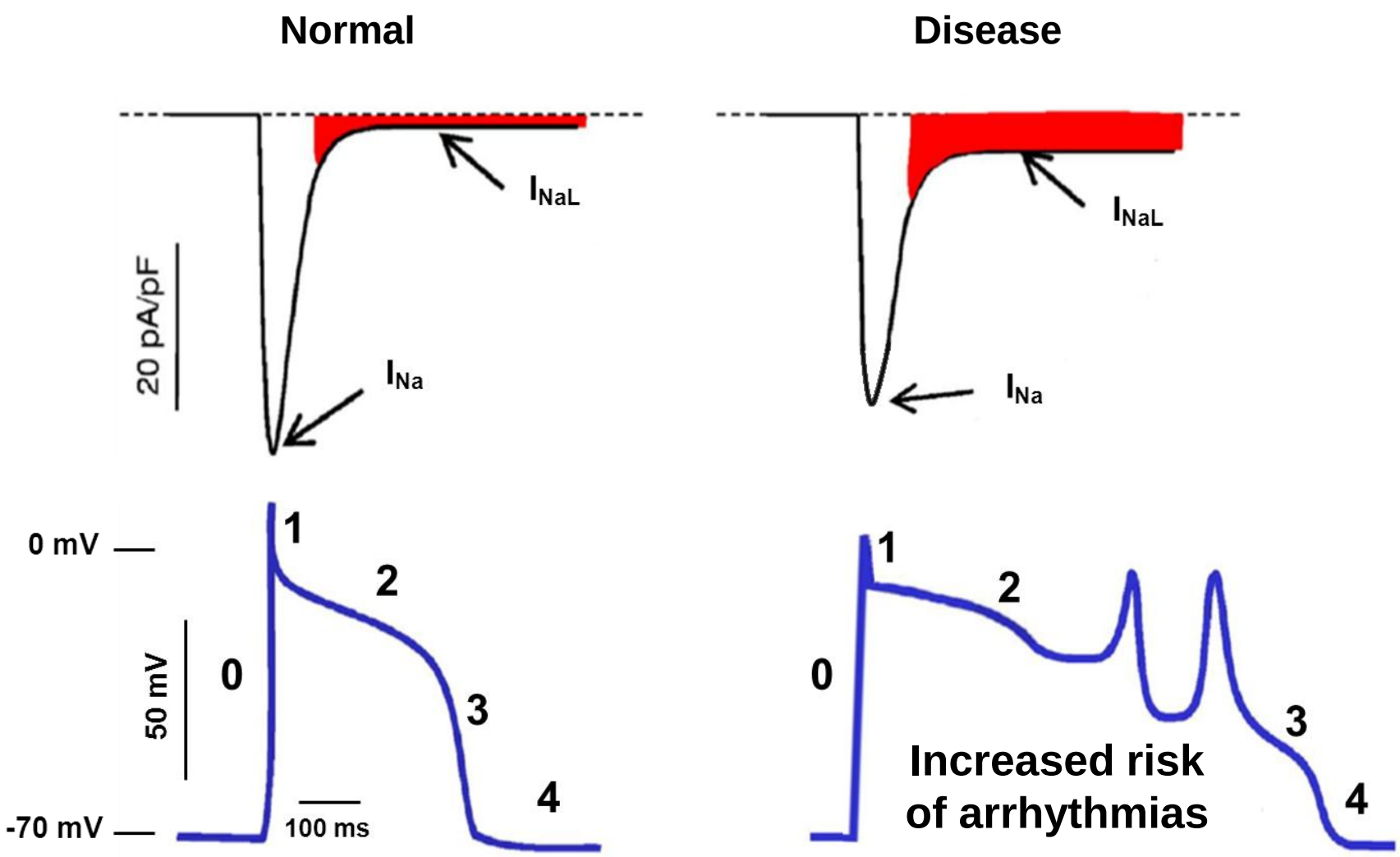
Cardiac voltage-gated Na^+ channels





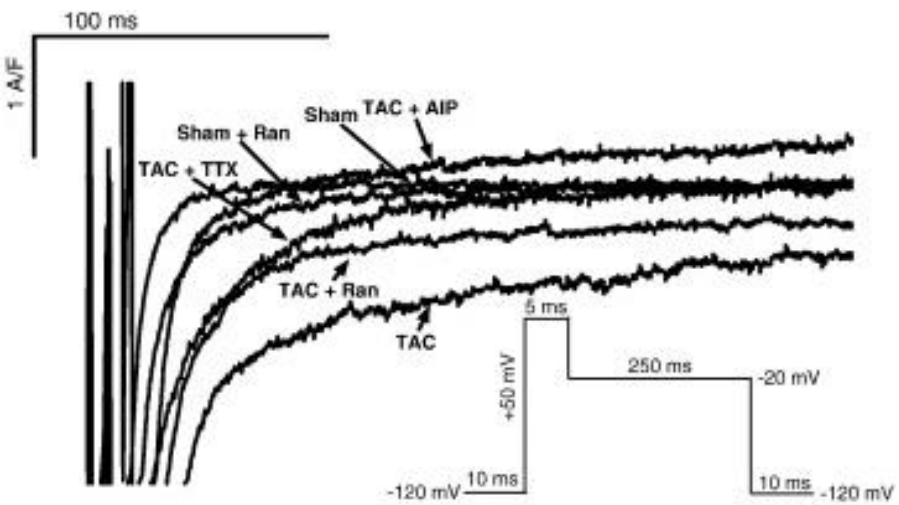


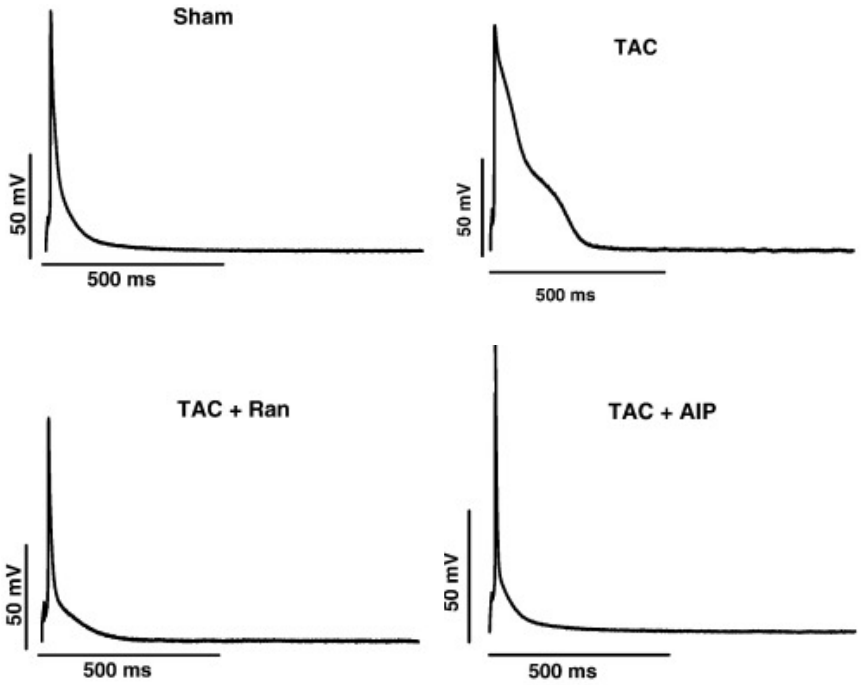
Nav1.5 channels in heart failure



Maier, 2011, modified

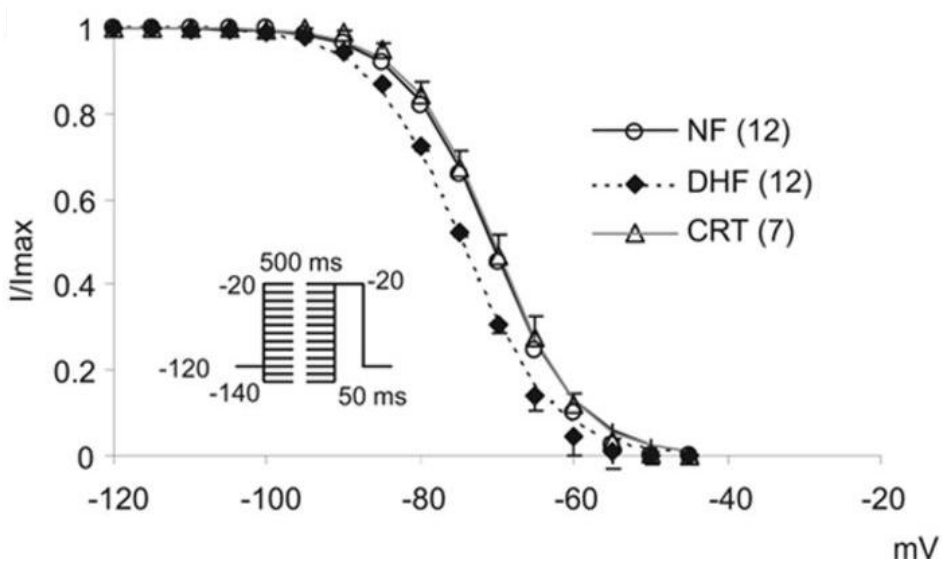
CaMKII increases I_{NaL} in heart failure



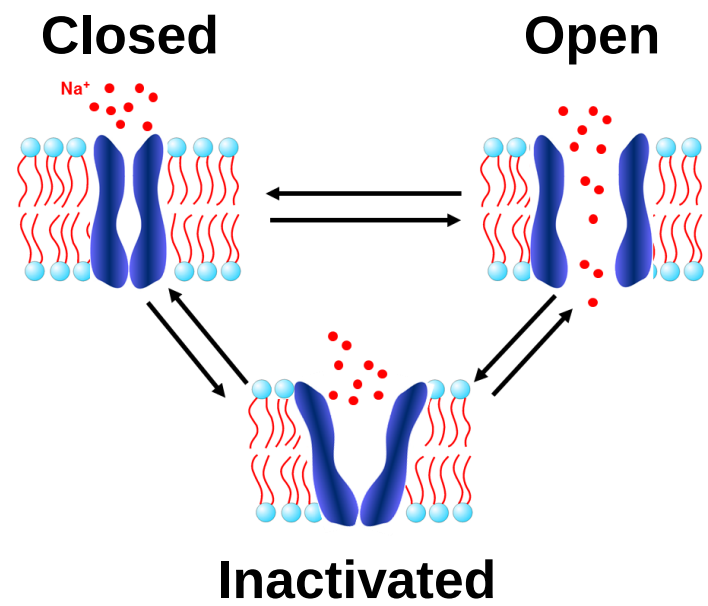


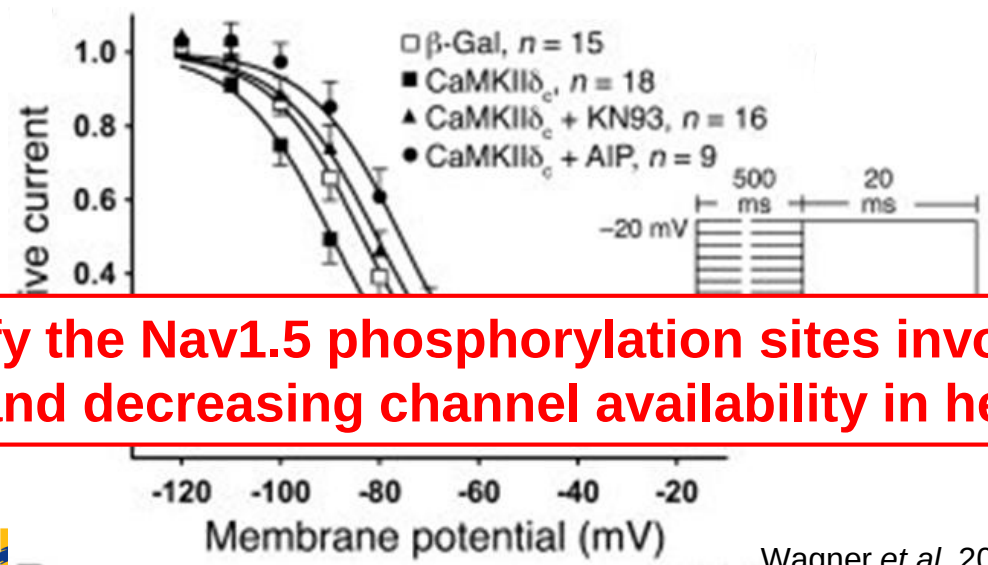
Toischer *et al*, 2013

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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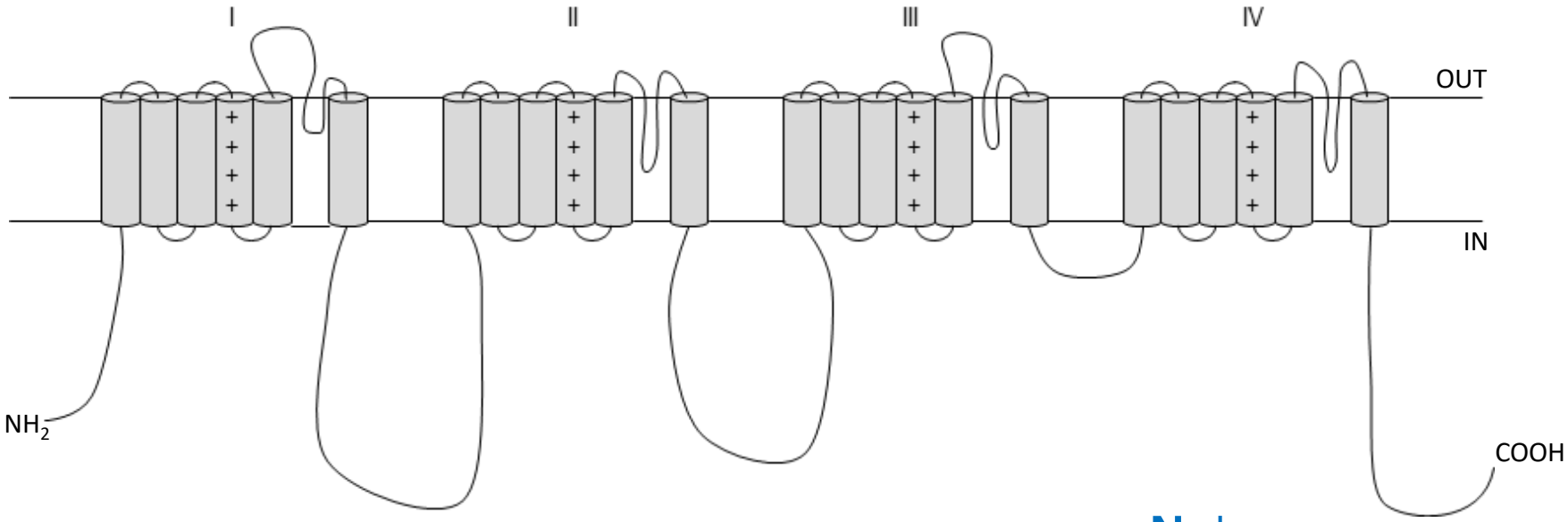
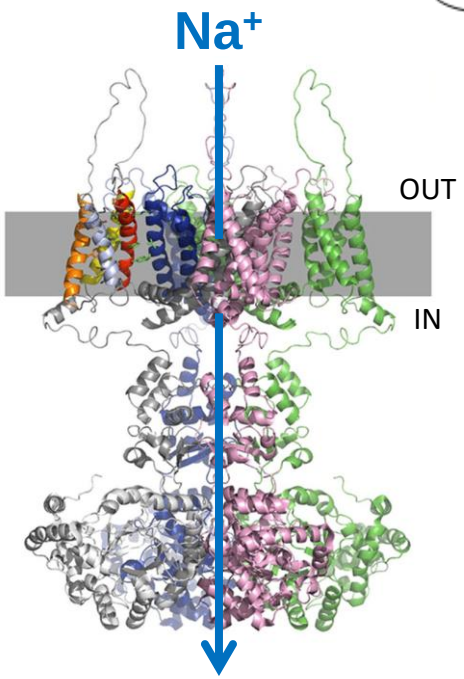
Cardiac Nav1.5 channel complexes





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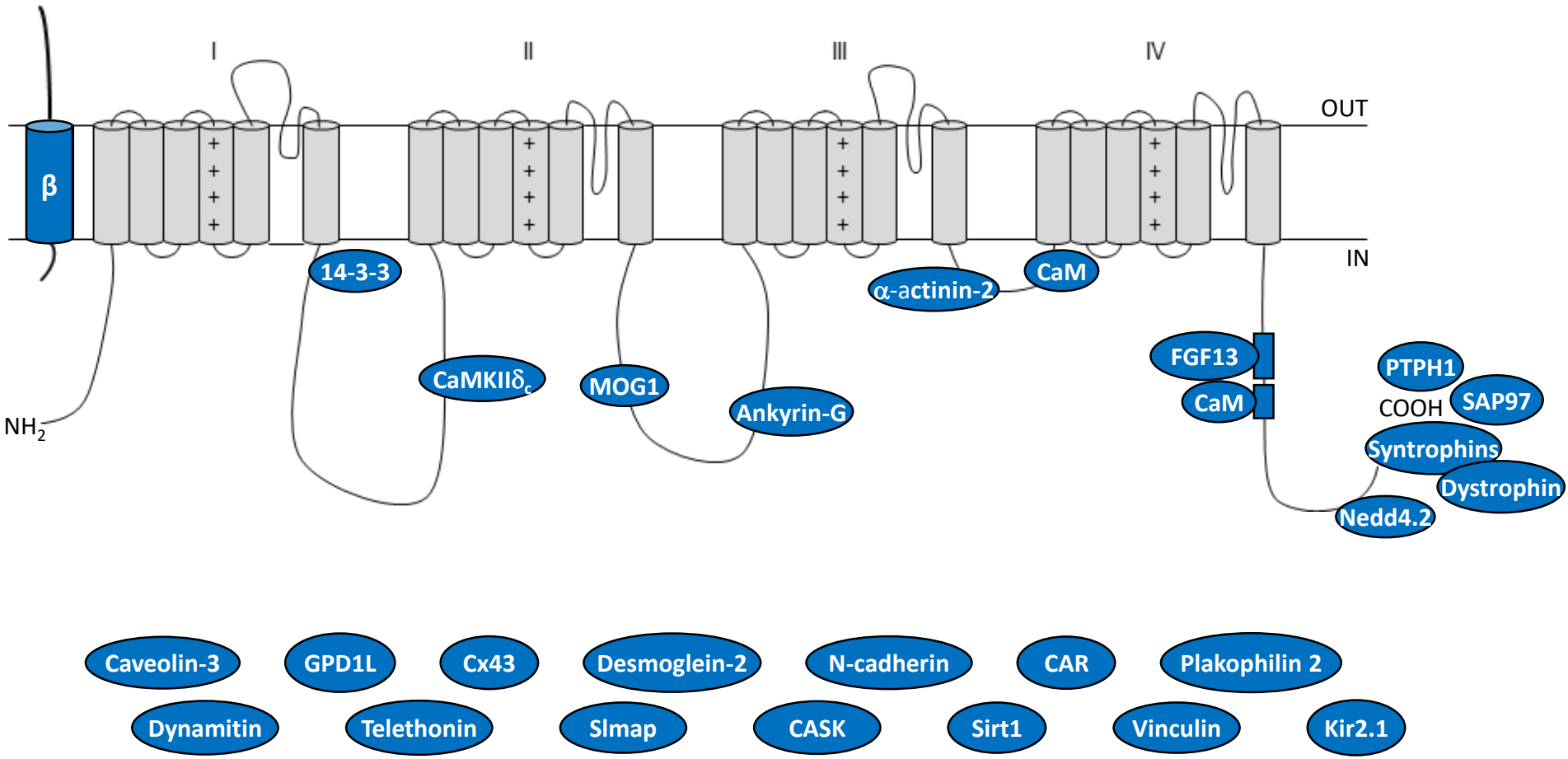
Cardiac Nav1.5 channel complexes



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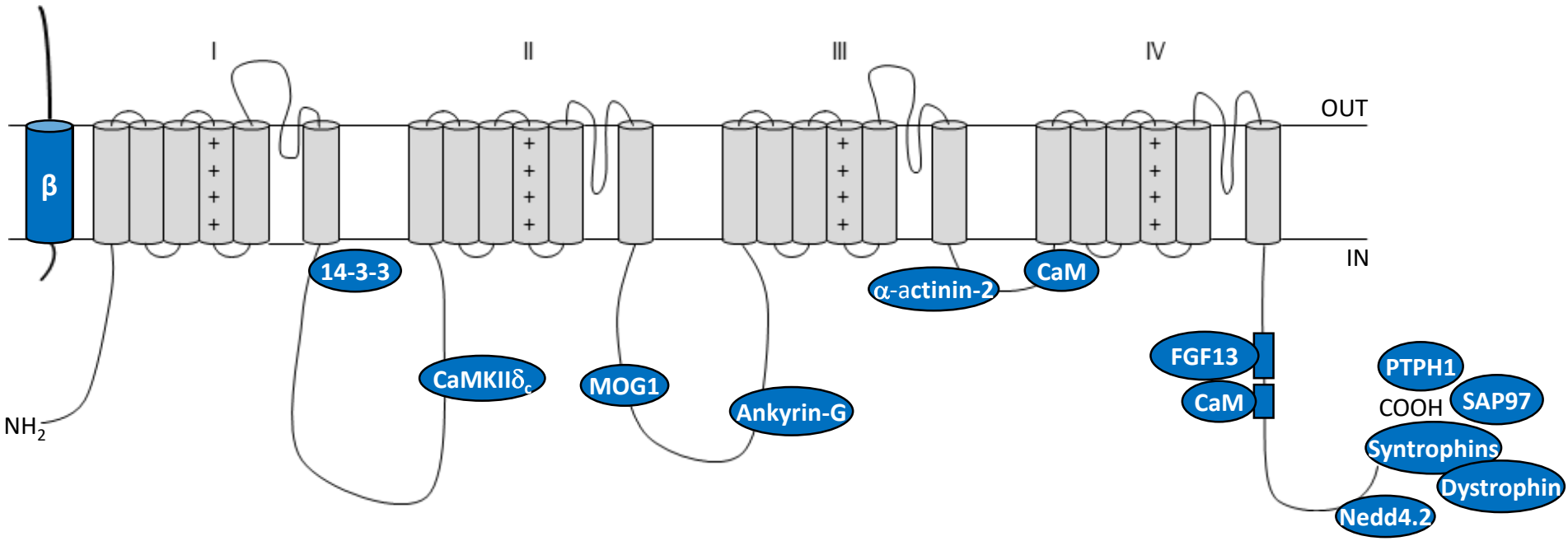
Cardiac Nav1.5 channel complexes



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

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

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

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

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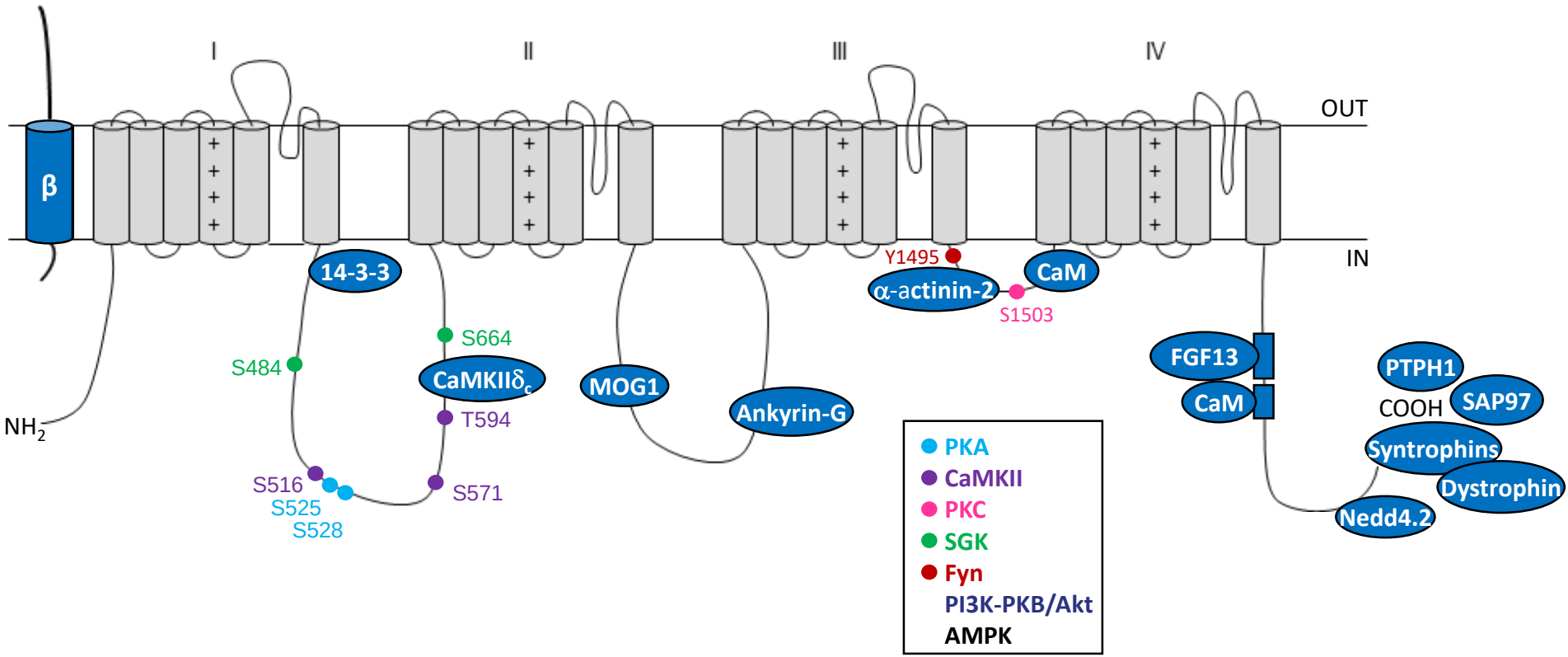
Marionneau and Abriel, ***JMCC 2015***

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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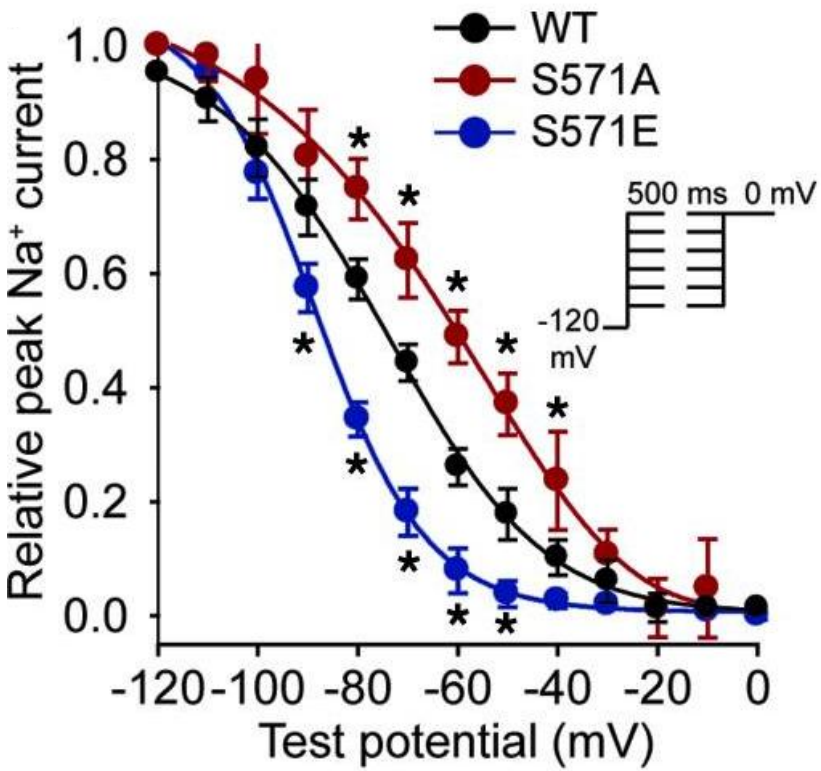
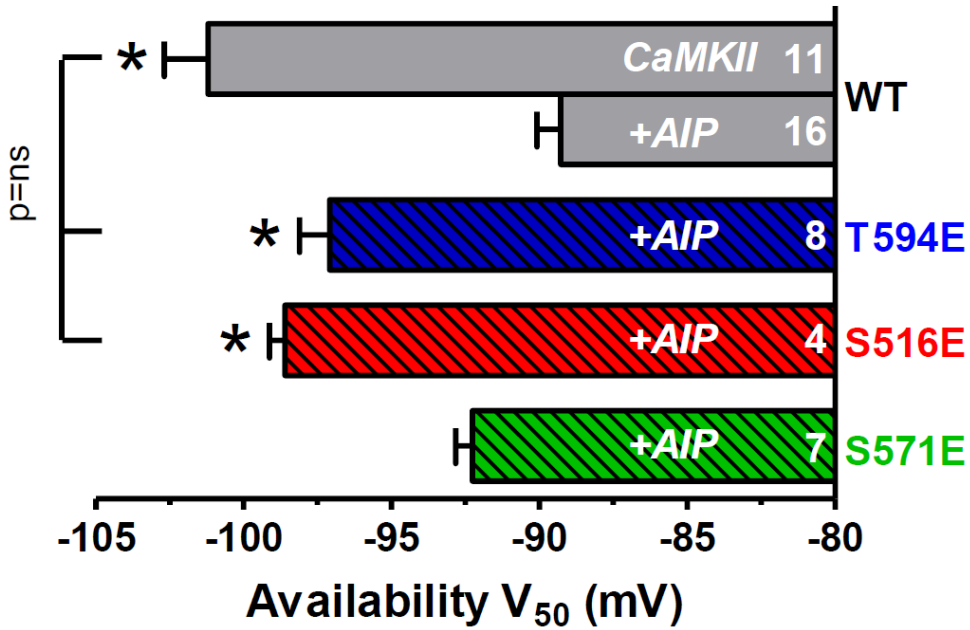
CaMKII phosphorylation sites

In silico



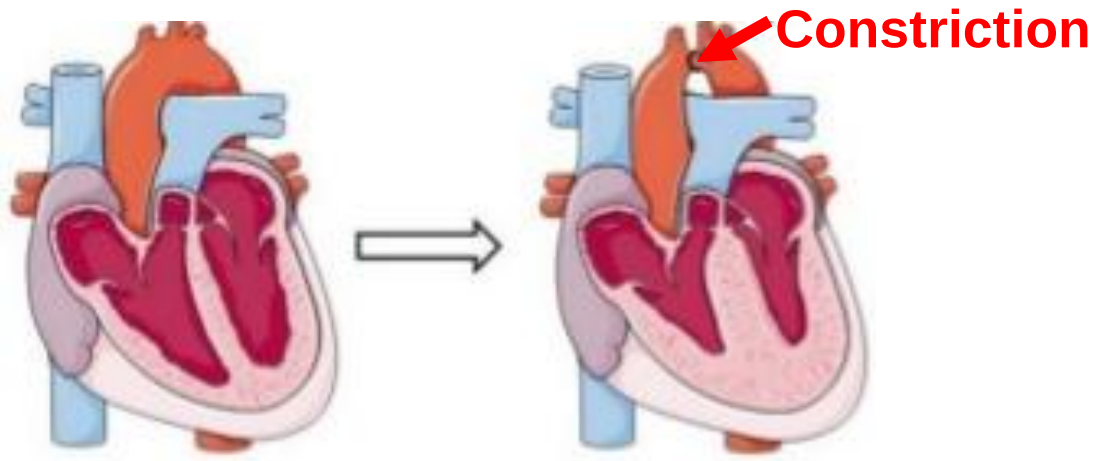
Hund *et al*, 2010

In vitro



Ashpole *et al*, 2012

Phosphoproteomics of cardiac Nav1.5 channels

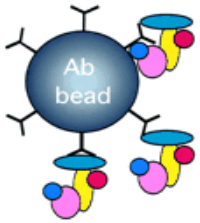


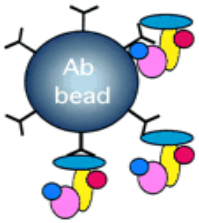
Sham Ventricles

TAC Ventricles

mαNavPAN-IPs

mαNavPAN-IPs

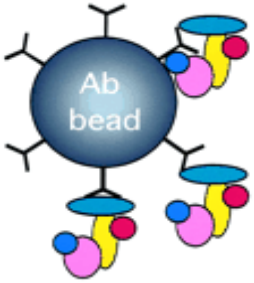




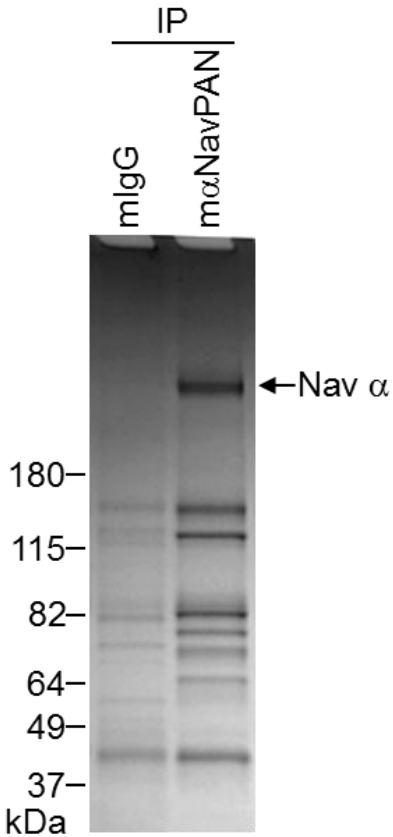
Quantitative phosphoproteomics

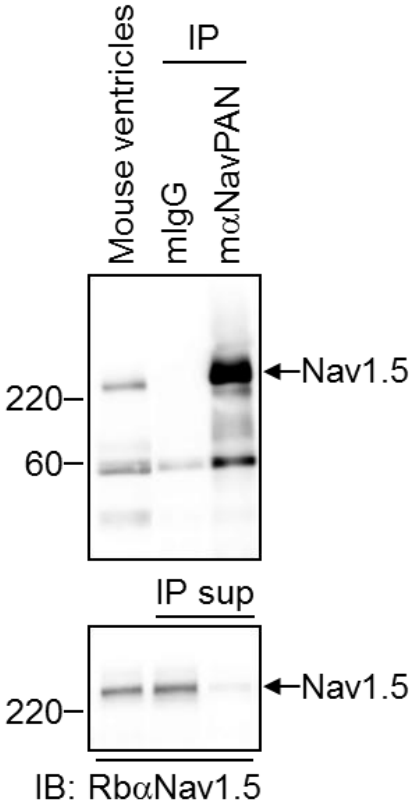
Purification of cardiac Nav1.5 channels

mαNa$_V$PAN IP



Digestion → **LC-MS/MS**





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

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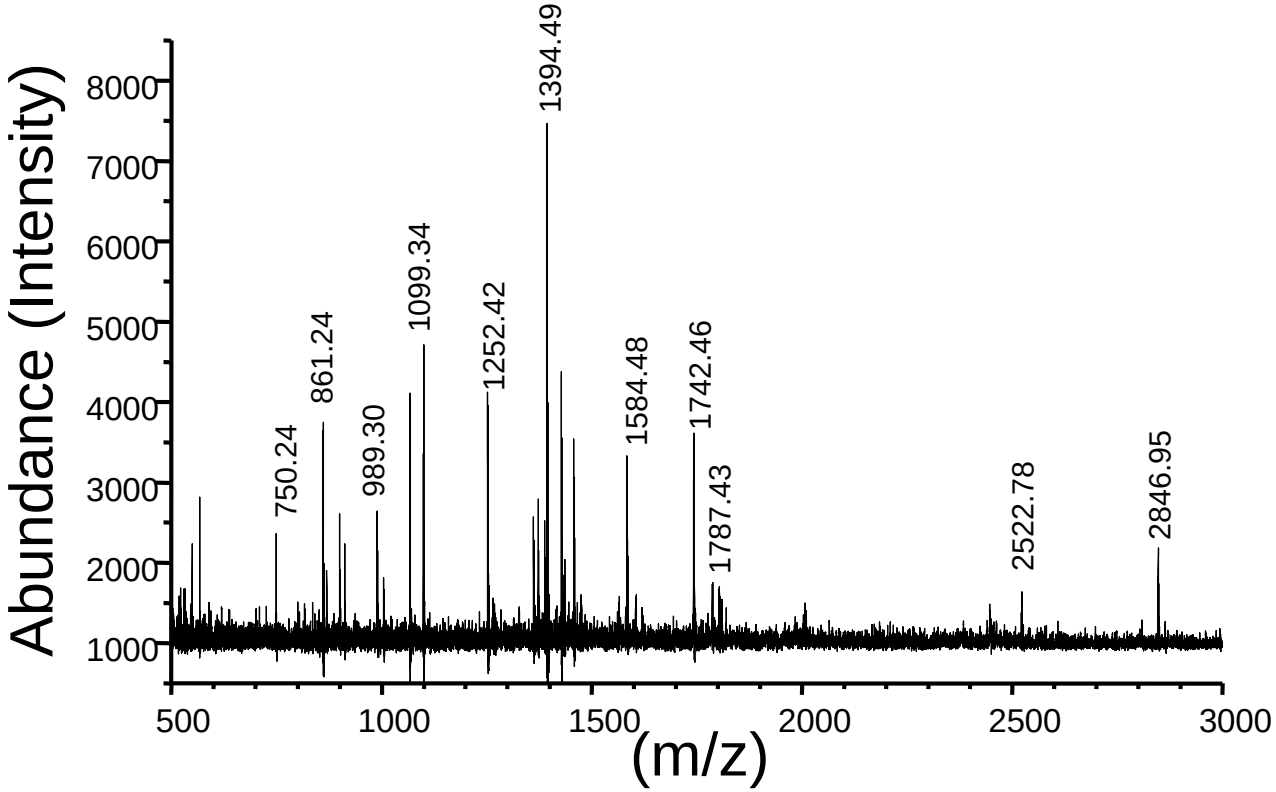
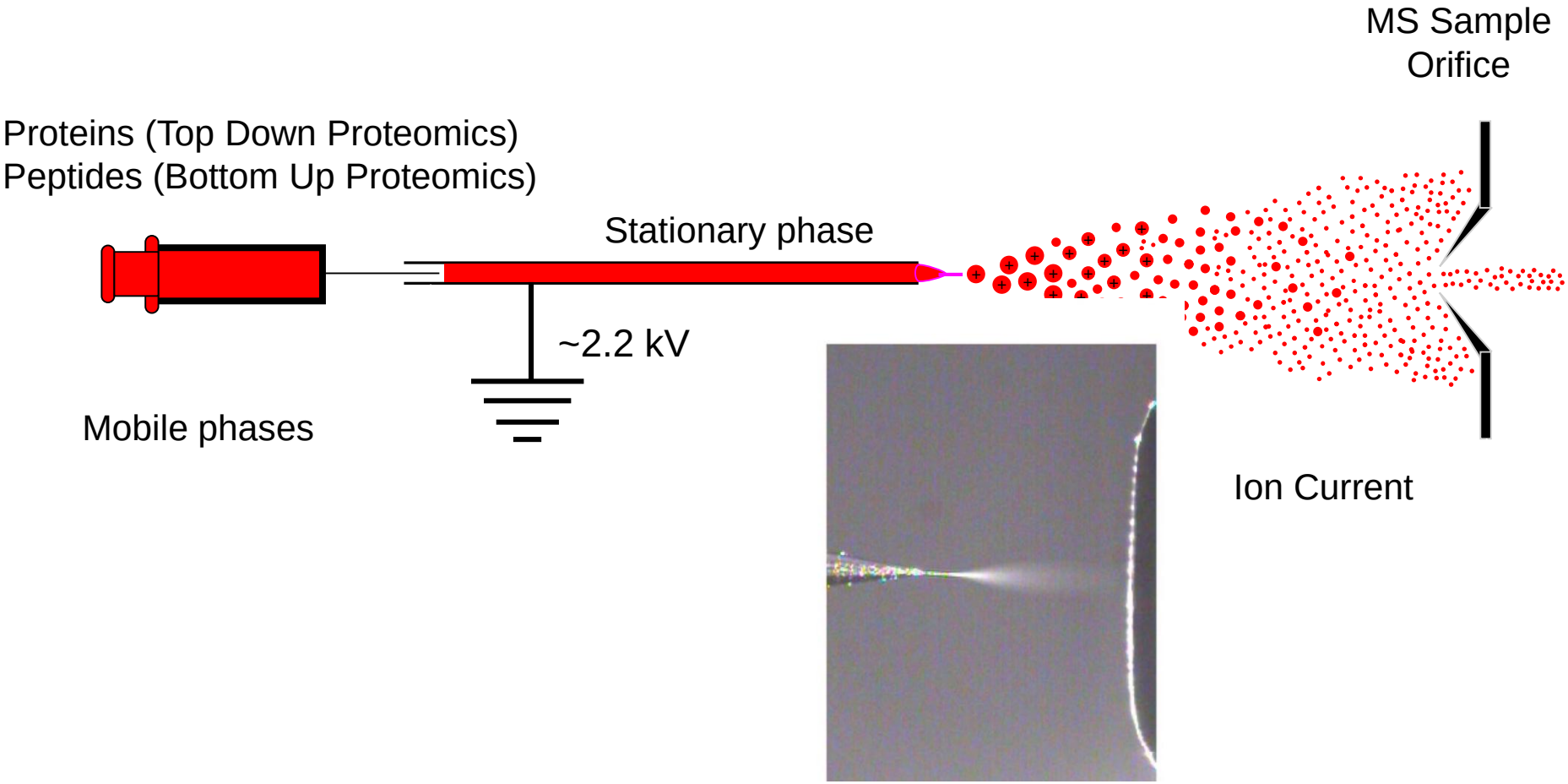
Mass Spectrometry – A Brief Definition

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

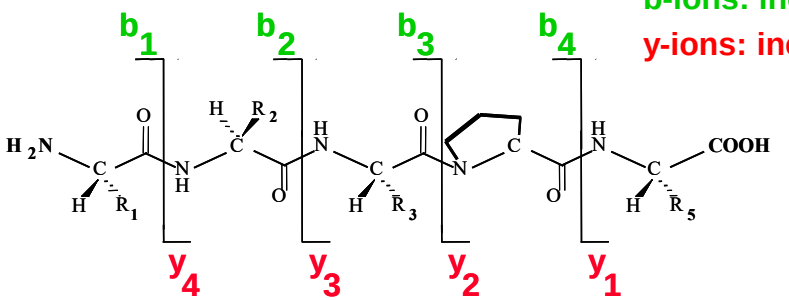
- Couples liquid chromatography to mass spectrometry
- Reversed-phase chromatography



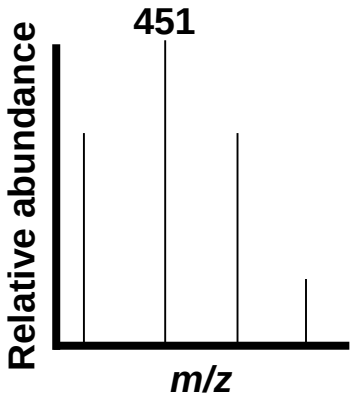
MS/MS analyses

b-ions: include the N-terminus

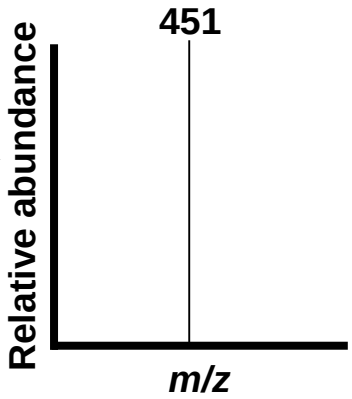
y-ions: include the C-terminus



MS1: mass spectrum of precursor ions

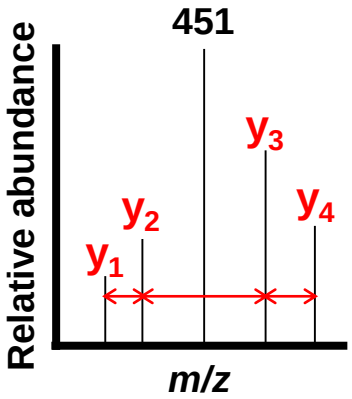


Isolation of the most abundant precursor ions



CID: Collision Induced Dissociation

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



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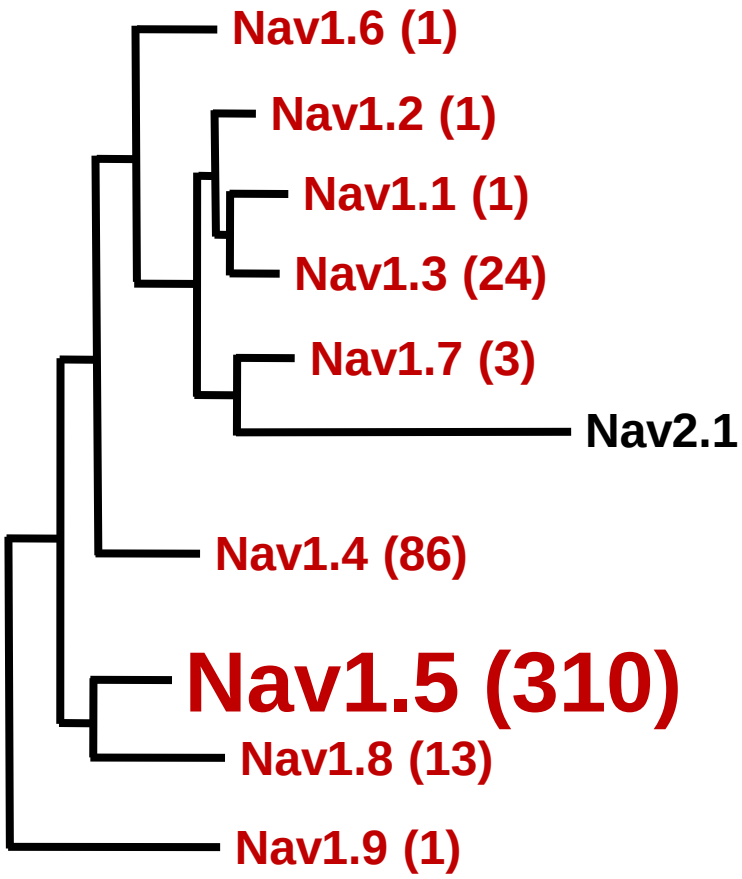
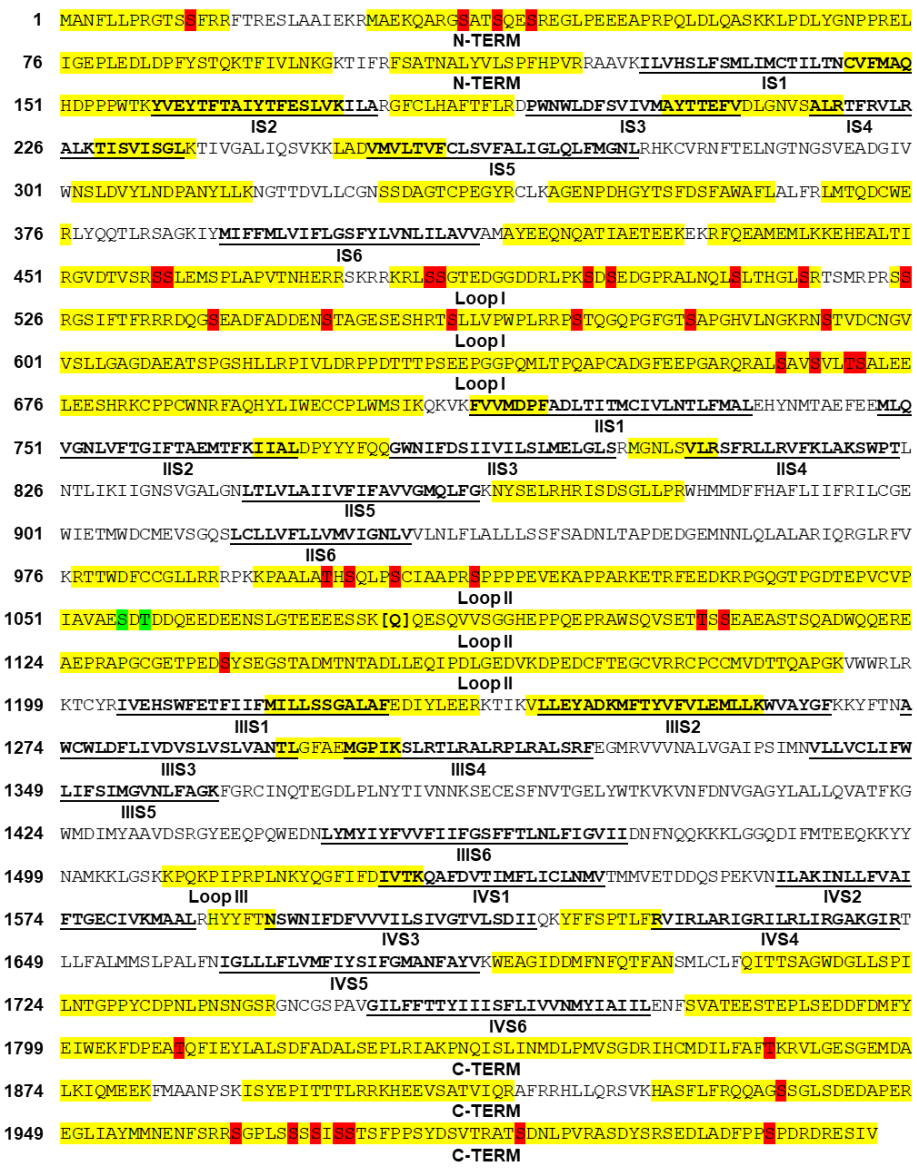
Identification of Nav pore-forming subunits

$Na_V1.5$:

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





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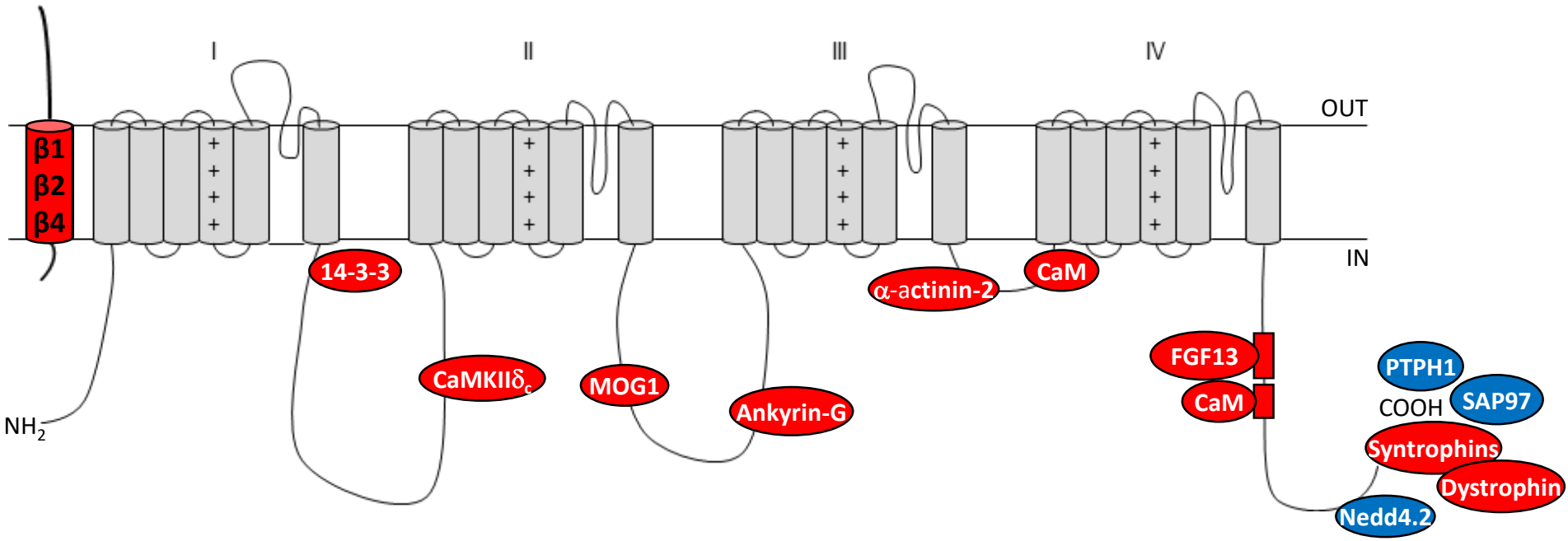
Identification of Nav interacting proteins



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

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

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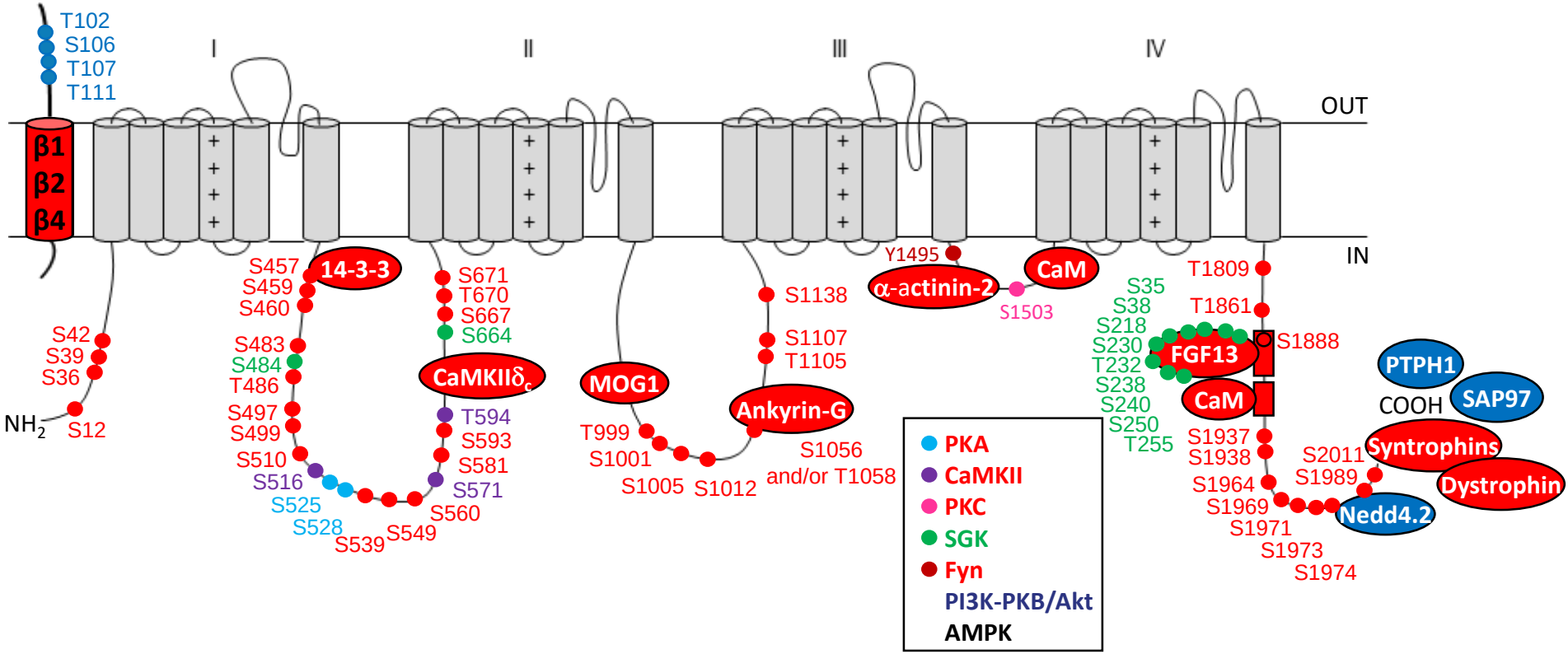
Marionneau and Abriel, ***JMCC 2015***
Marionneau et al, ***JPR 2012***
Burel et al, ***JBC 2017***
Lorenzini et al, ***JGP 2021***

Identification of 62 phosphorylation sites



Glycosylation **Phosphorylation** **Méthylation** **Acétylation**

Oxidation **Nitrosylation** **Lipoxidation** **Ubiquitylation**

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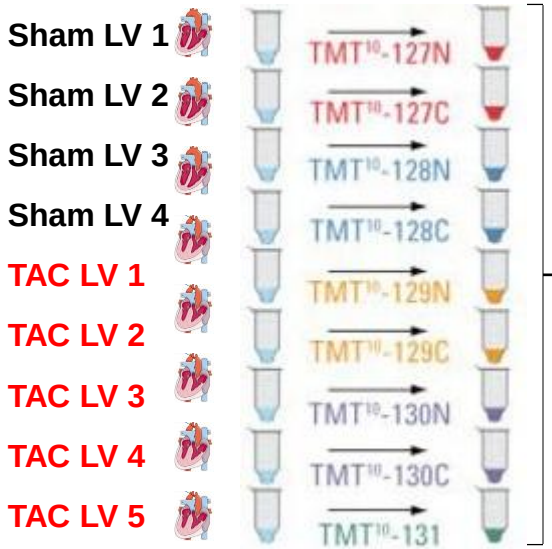
Burel et al, ***JBC 2017***
Lorenzini et al, ***JGP 2021***

TMT9 quantitative analysis from Sham/TAC mice

Sham / TAC mice

mαNa$_V$PAN-IPs **Labelling**

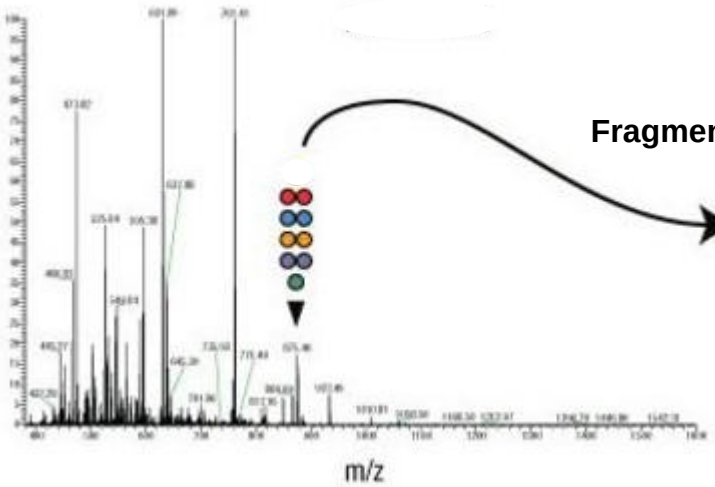


TMT methods

1 LC-MS/MS experiment

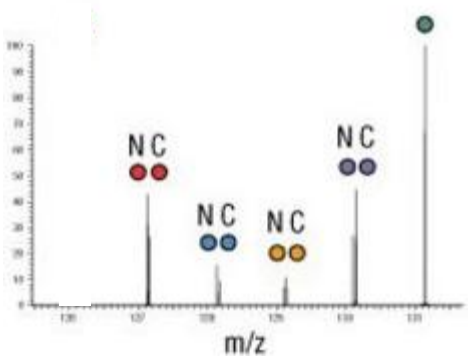
Combine

MS1 spectra: selection



Fragmentation

MS/MS spectra: Quantification



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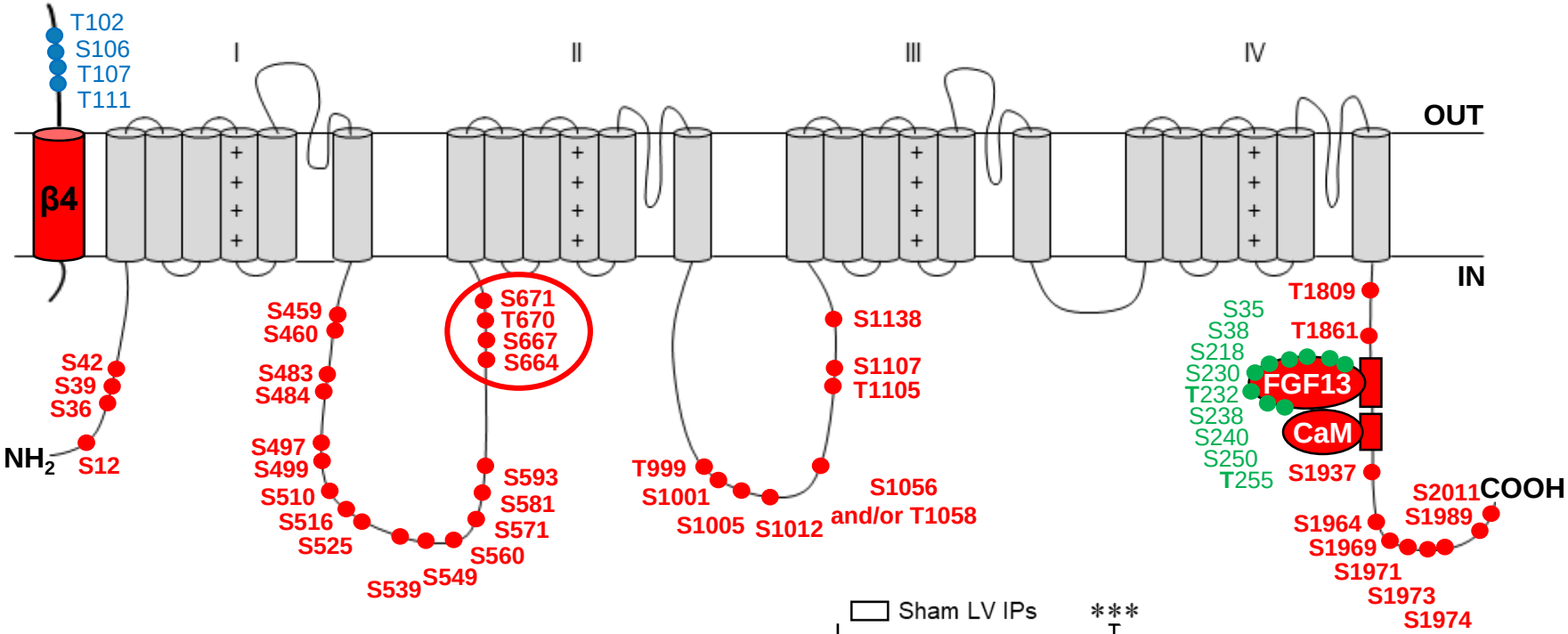
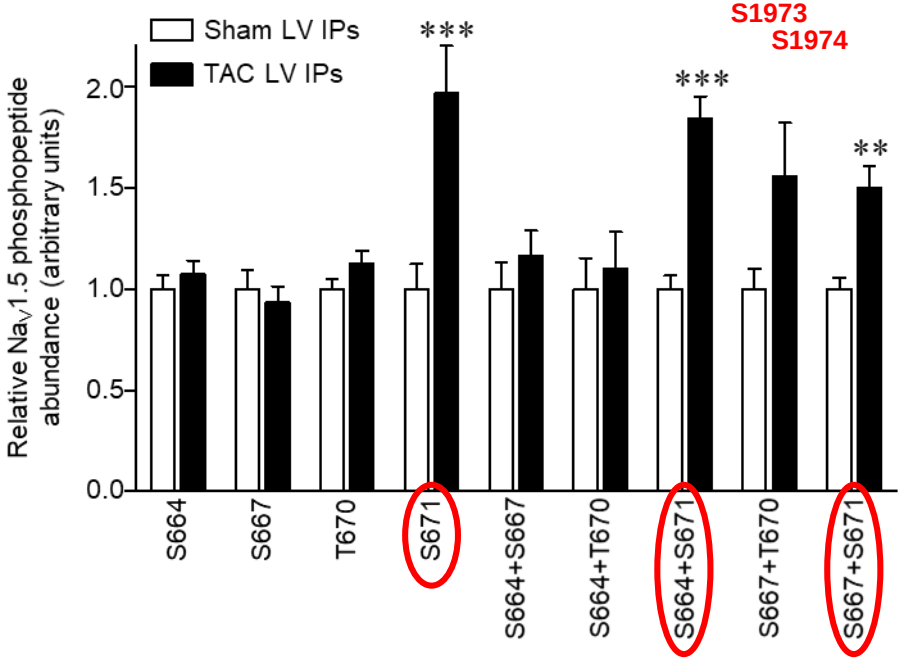
Quantitative phosphoproteomics in Sham/TAC mice





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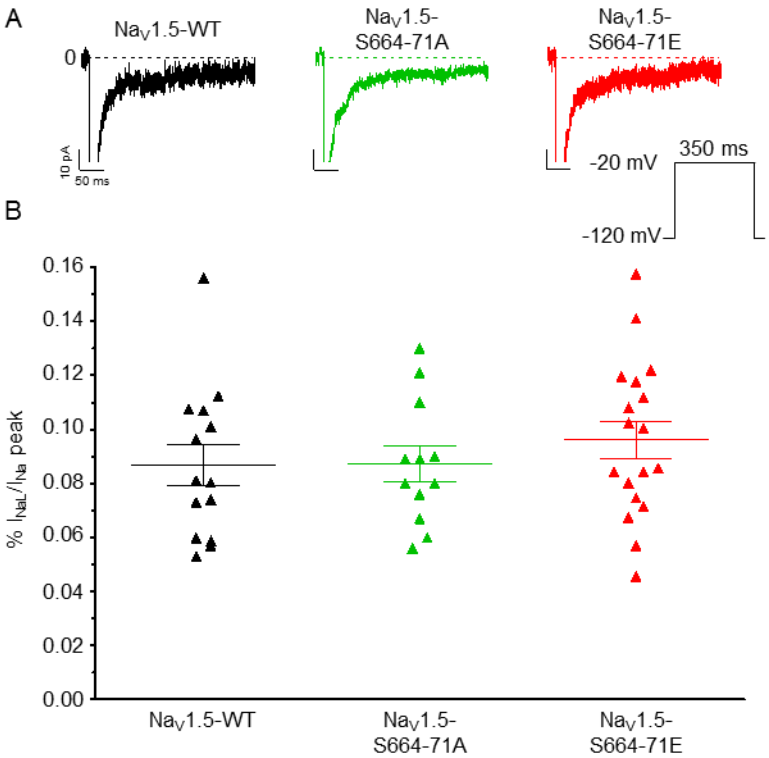
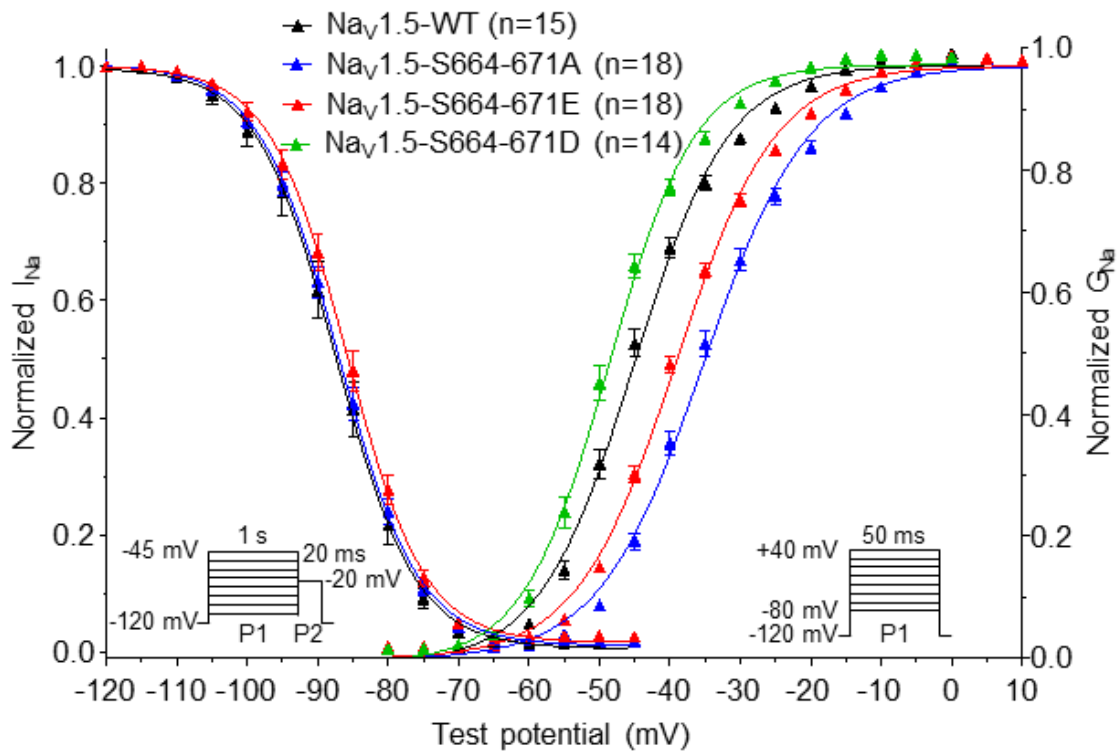
**p<0.01, ***p<0.001 in TAC *vs* Sham

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





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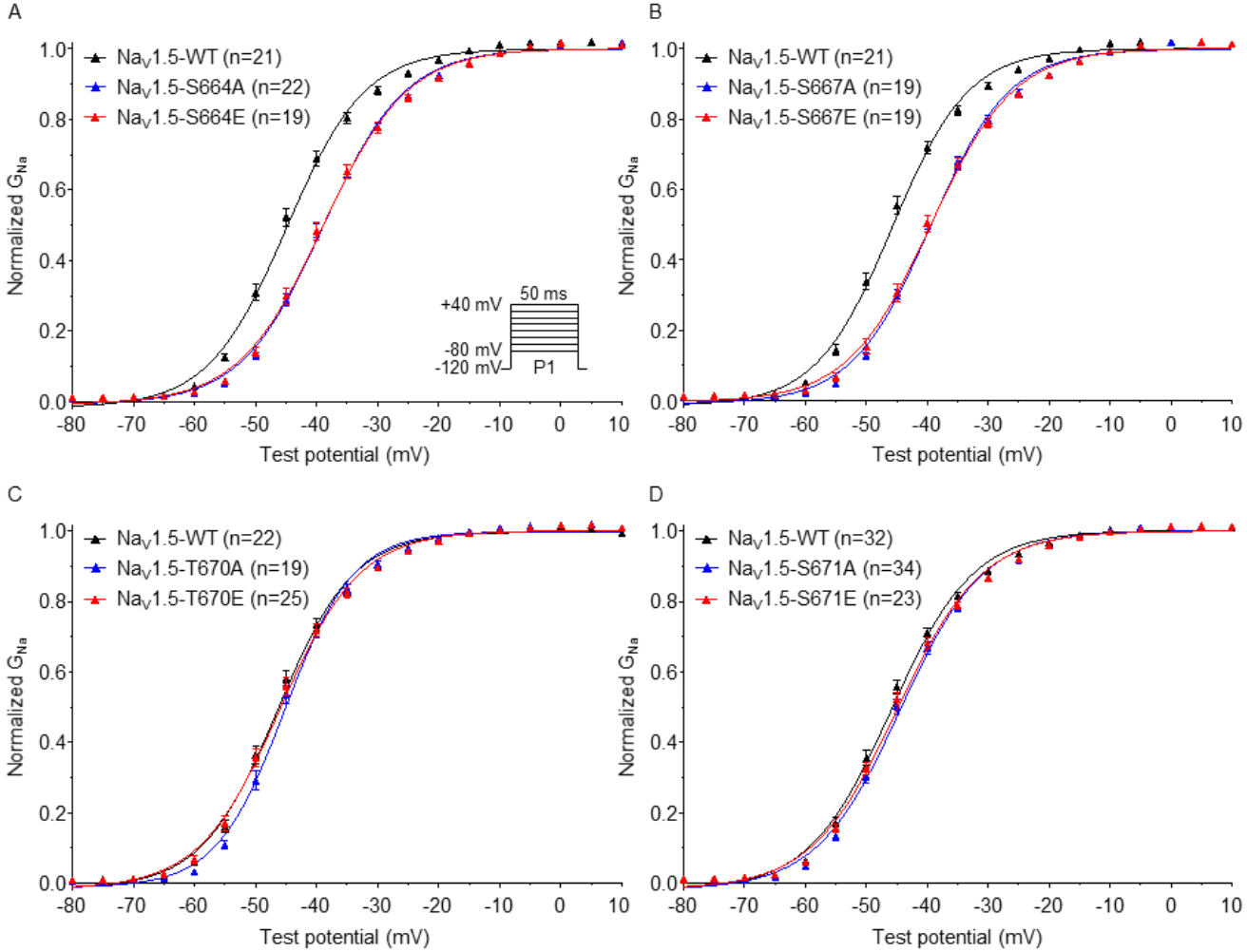
Electrophysiological analysis of simple Nav1.5 phosphomutants



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

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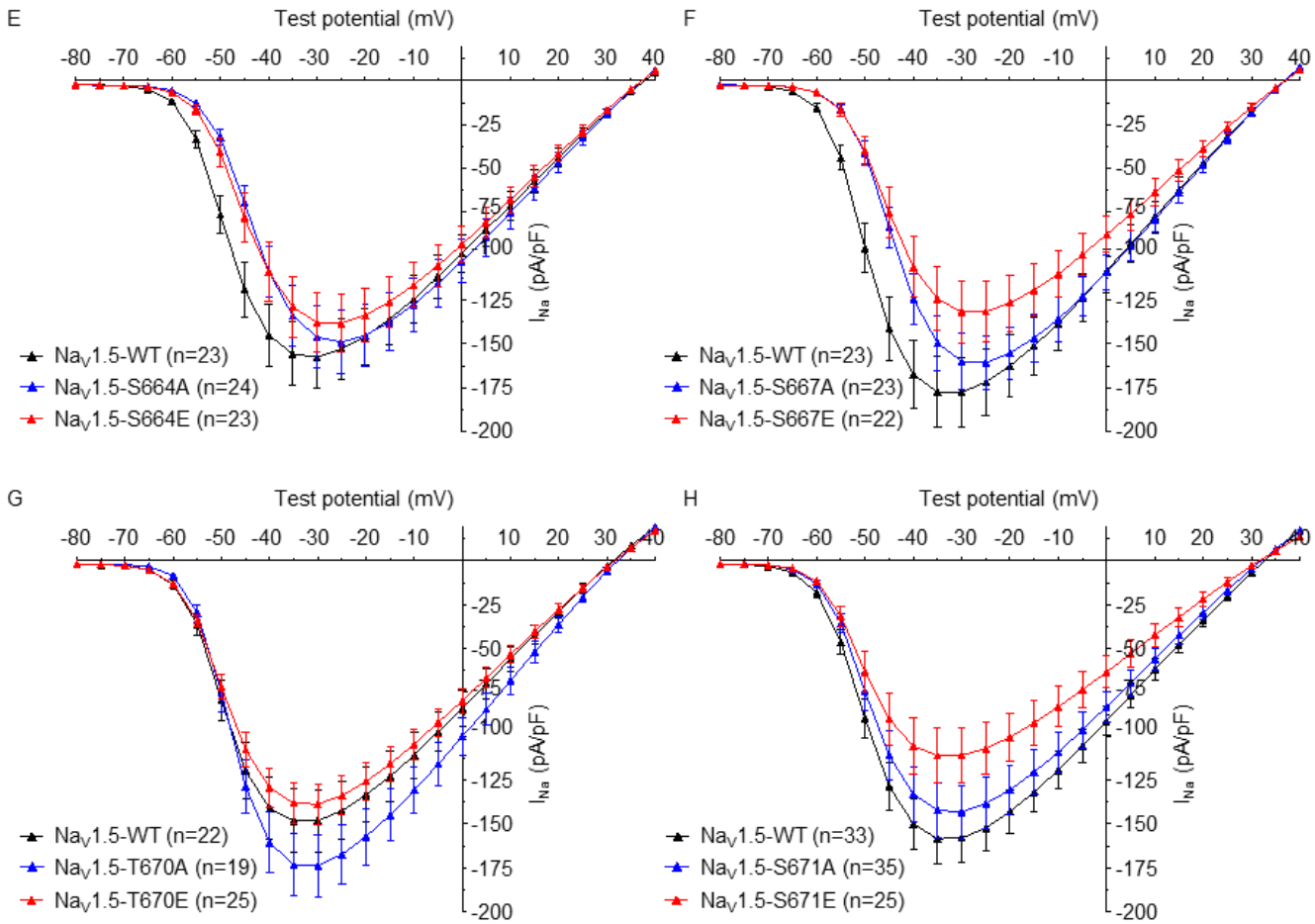
Electrophysiological analysis of simple Nav1.5 phosphomutants



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

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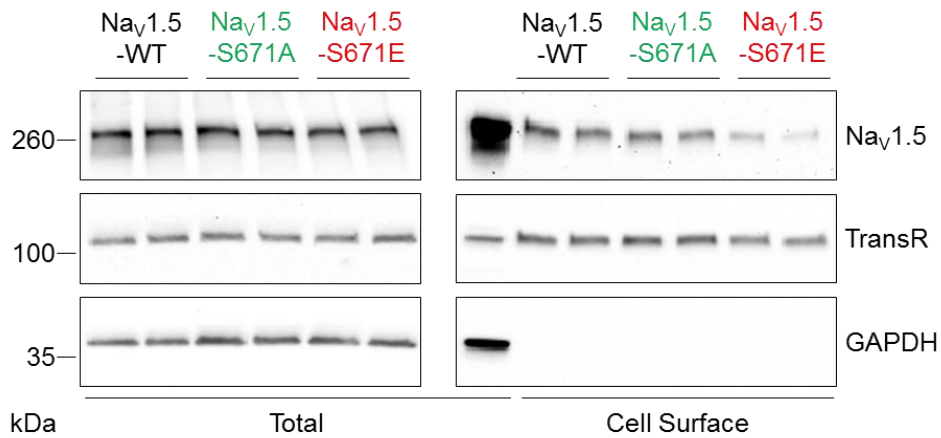
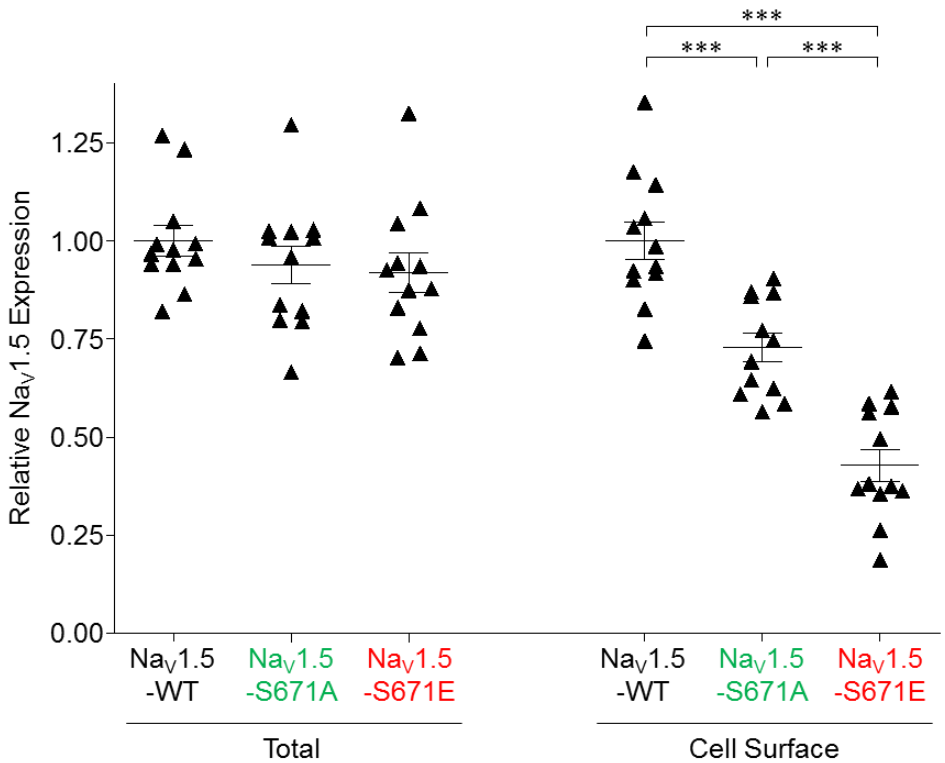
Phosphorylation at S671 decreases Nav1.5 cell surface expression





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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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