A. COVER PAGE

Project Title: Calmodulin Regulation Na Channels: From Function and Structure to Disease	
Grant Number: 5R01HL128743-04	**Project/Grant Period:** 04/01/2016 - 03/31/2021
Reporting Period: 04/01/2019 - 03/31/2021	**Requested Budget Period:** 04/01/2019 - 03/31/2021
Report Term Frequency: Annual	**Date Submitted:**
Program Director/Principal Investigator Information: L MARIO AMZEL , PHD **Phone Number:** (410) 955-3955 **Email:** mamzel@jhmi.edu	**Recipient Organization:** JOHNS HOPKINS UNIVERSITY 3400 N. Charles Street BALTIMORE, MD 212182680 **DUNS:** 001910777 **EIN:** 1520595110A5 **RECIPIENT ID:**
Change of Contact PD/PI: NA	
Administrative Official: MARISA BAILEY 733 N. Broadway, Suite 117 Baltimore, MD 21205 **Phone number:** 443-287-0982 **Email:** mabailey@jhu.edu	**Signing Official:** TERESA LYNN PENNINGTON School of Medicine Biophysics & Biophysical Chem. 725 N. Wolfe Street Baltimore, MD 21205 **Phone number:** (410) 955-5032 **Email:** tpennin2@jhmi.edu
Human Subjects: Yes **HS Exempt:** NA **Exemption Number:** **Phase III Clinical Trial:** NA	**Vertebrate Animals:** NA
hESC: No	**Inventions/Patents:** Yes **If yes, previously reported:** Yes

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Mutations in Nav1.5 (cardiac) and Nav1.4 (skeletal) sodium channels, particularly in their carboxy tails (CTs), give rise to a number of arrhythmias and myotonias. Leveraging our recent discoveries the goals are to define how mutation-induced changes in channel structure and function produce disease, facilitating enhanced physiological understanding, and development of mechanistically-coherent therapies. Our work demonstrates that CaM regulation of Nav channels bears eerie similarity to Cav channels. Binding of Ca2+-free calmodulin (apoCaM) to a Nav channels strongly boosts peak channel open probability. ApoCaM binds the CTs of Cav and Nav channels and it is notable that many channelopathic mutations (Nav1.5 Brugada (BS); long QT syndromes (LQTS)) reside on CTs. Could such mutations simply reduce apoCaM affinity, dislodge this binding partner, and thus modulate channels in ways that directly rationalize disease? This potentially seminal hypothesis is explored by 3 aims.
Aim 1: Does apoCaM binding to Na channels modulate their peak opening and/or persistent opening? The subaims will explore the effects of CT channelopathic mutations in Nav1.5/1.4 with decreased apoCaM on peak open probability Ppeak and/or increased late persistent opening Ppersist using single channel recording. Systematic alanine substitutions into Nav1.5/1.4 will be introduced at predicted apoCaM/CT interfaces with assessment of CaM binding to the CT and functional assessment of CaM regulation of the holochannel. Design/test custom compensatory CaMs (ccCaMs) that preferentially bind channelopathic channels, as an orthogonal pair. This approach promises treatments of enormous molecular precision.
Aim 2: How does apoCaM binding to the carboxy tails of Nav1.5/Nav1.4 determine channel function? Our apoCaM/CT-Nav1.5 structure is a platform for new structure-function advance. We will determine structures of apoCaM bound to mutant Nav1.5 CTs, chosen based on the functional studies in Aim 1 to focus on variants with altered apoCaM binding. These structures will critically inform mechanism, and prove essential for ccCaM design. We will determine the atomic structure of the apoCaM/CT-Nav1.4 complex. An analogous interplay with Aim 1 may generalize the apoCaM-binding mechanism, and motivate further Nav1.4 channelopathic structures. We will perform Langmuir analysis to stringently test whether apoCaM/CT binding increases Ppeak and/or attenuates Ppersist.
Aim 3: Test for the underlying role of apoCaM/Nav mechanisms in Nav1.5 channelopathic disease. This aim will explore in disease models the role of apoCaM/Nav1.5 interaction in mediating channelopathies. We will express key channelopathic NaV1.5 channels in neonatal rat ventricular myocytes (NRVMs). Single-channel methods will assess altered Ppeak and Ppersist; optical maps will explore modified conduction and action potential morphology. For more integrative assessment, we will create/obtain transgenic mice with knock-in of channelopathic NaV1.5 (e.g., Nav1.5 IQ1909AA, S1904L). Single-channel methods will probe adult mouse ventricular myocytes; EKGs will be scrutinized for broadened QRS and QT prolongation and will allow tests proof-of-principle strategies to normalize function, culminating in inducible expression of ccCaMs in mice. To assess the relevance in humans, we will generate/obtain Nav1.5 channelopathic human iPSC-CMs. Single-channel and optical mapping methods will test for altered Ppeak and/or Ppersist, as well as perturbed AP waveforms. Evaluating action potential (AP) prolongation will be important here, where APs show a distinct plateau phase at baseline compared to mouse/rat models.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

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B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

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B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

Aspects of this work have been presented at national scientific meetings including the Biophysical Society, the American Heart Association (see publications), the American crystallography Association and the Gordon Research Conference on Ion channels.
The structure determined have been deposited at the PDB.
Seven papers have been published.

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Not Applicable

The award ‘Calmodulin regulation of Na channels: from function and structure to disease’ proposed an impressive multi-disciplinary exploration of a remarkable apoCaM-binding mechanism, hosted by a seasoned and synergistic team. Our team has been very successful and productive: We have published 7 major papers, deposited 3 structures in the PDB, and presented more than 5 posters in national meetings.

apoCaM binds the carboxy tail (CT) of Cav and Nav channels, and numerous mutations underlying channelopathies Nav1.5 Brugada (BS) and long QT syndromes (LQTS) as well as Nav1.4 myotonias are arrayed along the CT (2018, PRPL13). We have postulated that these mutations simply reduce channel affinity for apoCaM and dislodge this binding partner, resulting in a loss of modulation of the channel in a way that directly accounts for the disease phenotypes. We generated and characterized the functional properties of induced pluripotent stem cell-derived cardiomyocytes from a patient with D130G-CALM2-mediated LQTS, thus creating a platform with which to devise and test novel therapeutic strategies. We devised a strategy using CRISPR interference to selectively suppress the mutant gene while sparing the wild-type counterparts, making it a generalizable therapeutic strategy for any calmodulinopathy. This therapeutic strategy holds great promise for calmodulinopathy patients as it represents a generalizable intervention capable of specifically altering CaM expression and potentially attenuating LQTS-triggered cardiac events, thus initiating a path toward precision medicine. We published this work in Circ Research (2017, PRPL6). We also identified a patient with the $Nav1.5_{S1904L.}$ mutation displaying a mixed Brugada Syndrome phenotype. We have generated iPSCs from peripheral blood mononuclear cells to electrophysiologically characterize the mutation at a single cell level in comparison with the CRISPR corrected isogenic control hiPSC-CM. This sedimented the feasibility of investigating apoCaM/channel mechanisms in the hiPSC-CM model. We complemented the studies analyzing the binding affinity of CTNav1.5-CaM to $CTNav1.5_{S1904L}$-CaM (2019, PRPL7). We attempted to restore the binding affinity by designing custom compensatory mutations of CaM based on our lab’s crystal structures of the CTNav1.5-CaM complex(2014,PRPL4).

We analyzed the conformations of the cytoplasmic C-terminal fragments of Nav channels and CaM lobes in Nav-CaM and Nav-CaM-FHF complexes in the presence and absence of Ca^{2+} that have been published. We hypothesized that the structures are associated with specific functional states of the channels (2016,PRPL5). Moreover, as a team, we wrote a comprehensive review analyzing the structure of eukaryotic Navs determined by single-particle cryo-Electron Microscopy (cryo-EM) that emphasizes the new perspective that these structures have brought to the study of the channels. Alignment of the cryo-EM structures of the transmembrane channel pore with x-ray crystallographic structures of the cytoplasmic domains illustrates the complementary nature of the techniques and highlights the intricate cellular mechanisms that modulate the channels. We reviewed structural insights into the cytoplasmic C-terminal regulation of Nav1.4 and Nav1.5 with special attention to Ca^{2+} sensing by calmodulin, implications for disease, and putative channel dimerization(2020,PRPL7).

We described the allosteric regulators that selectively prevent Ca^{2+}-feedback of Cav and Nav channels in a paper published in eLife(2018,PRPL9). The work shows that Ca^{2+}/CaM-regulation of Cav1 and Nav1 families operates on allosteric sites within upstream portions of the respective channel CTs, distinct from the CaM-binding interface(2016, PRPL6). Our findings identify a general class of auxiliary proteins that modify Ca^{2+}/CaM signaling to individual targets allowing spatial and temporal orchestration of feedback, and outline strategies for engineering Ca^{2+}/CaM

signaling to individual targets. We further unified the scheme of Cav channel regulation by CaM by tethering CaM lobes to demonstrate that the bilobal architecture is obligatory for signaling Cav channels(2016, PRPL1). Specifically, with one CaM lobe bound, CT Cav rearranges itself. On the other hand, with two lobes it relieves inhibition and restores Ca^{2+} feedback (2018, PRPL2). The thermodynamic analysis of Ca^{2+} binding to CTNav1.4 together with the crystal structures of the CTNav1.4 in the presence and absence of Ca^{2+} provides a rational for the Calmodulin mediated Ca^{2+} dependent inactivation (CDI). Such inactivation is not observed in Nav1.5. Since the N-lobe binding motif of Nav1.5 is a mutational hotspot for inherited arrhythmias, we postulated that the contribution of mutation-induced changes in CDI to arrhythmia generation is a strong possibility(Yoder, Ben-Johny et al. 2019,PRPL14).

We have successfully raised a library of single domain VHH against Nav1.4 and Nav1.5. The phage display panning has resulted in >80 nanobodies specific for Nav1.4/5 that bind with nanomolar affinities (2019; PRPL12, 2020;PRPL11). As a difference with other antibodies against Navs, they are not pan-Nav(. 2020;PRPL10). Moreover they are key molecular tools for detecting Nav1.5 in skeletal muscle, cardiac muscle, hiPSC-CM and transiently expressed HEK293(2020;PRPL11). These data are seminal to this new presentation of the grant.

During the previous grant period the junior participants of the team were able to land outstanding positions for the next stage of their scientific careers. Dr. Ben-Johny was recruited as an Assistant Professor in Columbia University. Dr. Banerjee moved on professionally and now has a position at NIH and Jesse Yoder completed his PhD and is now a beam line scientist at NECAT.

B4. What opportunities for training and professional development has the project provided?

The doctoral thesis work of two graduate students, Jesse Yoder and Rebeca Joca, was supported by the proposal. Rahul Banerjee attended the 7th annual SIBYLS BioSAXS workshop at Lawrence Berkeley National Laboratory. Dr. Joca, is now a post doctoral fellow, attended the 2018 Biophysical Society meeting,
Dr. Federica Farinelli attended multiple Biophysical Society meetings presenting results. She got training on electrophysiology of iPSC cells.
Sara Nathan a graduate student that joined the laboratory as a rotation student working on this project got training on iPSC differentiation. She has presented at the Biophysical Society meeting on 2018,1019
Sofie Shoemacker, a JHU undergraduate, had learned to purify Nav, CaM and ITC.
Lakshmi Srinivasan has attended multiple Biophysical Society Meetings and American Crystallography meetings where she presented the nanobody data. She has got Cytiva training on SPR and on BLI to measure the binding of the nanobodies to the C Terminal region of the channel.

Under Dr. Gabelli mentoring, Dr. Yoder, Dr. Srinivasan and Ms. Nathan, got specific training on collecting x-ray diffraction data at NSLS-II, best in class high-flux mini beams of 4 μm size and low divergence.

Ms. Tihitina Aytenfisu, an urm undergraduate at JHU, learned to expressed and purify proteins. Her preliminary data was used to get her PURA (provost undergraduate research award). Tihitina grada

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Non-Compliant	Tomaselli GF, Farinelli F. Na_V Channels: Assaying Biosynthesis, Trafficking, Function. Methods in molecular biology (Clifton, N.J.). 2018;1722:167-184. PubMed PMID: 29264805; DOI: 10.1007/978-1-4939-7553-2_11.
Non-Compliant	Tomaselli GF, Farinelli F. Na_V Channels: Assaying Biosynthesis, Trafficking, Function. Methods in molecular biology (Clifton, N.J.). 2018;1722:167-184. PubMed PMID: 29264805; DOI: 10.1007/978-1-4939-7553-2_11.
Non-Compliant	Tomaselli GF, Farinelli F. Na_V Channels: Assaying Biosynthesis, Trafficking, Function. Methods in molecular biology (Clifton, N.J.). 2018;1722:167-184. PubMed PMID: 29264805; DOI: 10.1007/978-1-4939-7553-2_11.
Complete	Maheshwari S, Kim YS, Aripirala S, Murphy M, Amzel LM, Gabelli SB. Identifying Structural Determinants of Product Specificity in *Leishmania major* Farnesyl Diphosphate Synthase. Biochemistry. 2020 July 28;59(29):2751-2759. PubMed PMID: 32584028; PubMed Central PMCID: PMC8049779; DOI: 10.1021/acs.biochem.0c00432.
Complete	Maheshwari S, Kim YS, Aripirala S, Murphy M, Amzel LM, Gabelli SB. Identifying Structural Determinants of Product Specificity in *Leishmania major* Farnesyl Diphosphate Synthase. Biochemistry. 2020 July 28;59(29):2751-2759. PubMed PMID: 32584028; PubMed Central PMCID: PMC8049779; DOI: 10.1021/acs.biochem.0c00432.
Complete	Maheshwari S, Kim YS, Aripirala S, Murphy M, Amzel LM, Gabelli SB. Identifying Structural Determinants of Product Specificity in *Leishmania major* Farnesyl Diphosphate Synthase. Biochemistry. 2020 July 28;59(29):2751-2759. PubMed PMID: 32584028; PubMed Central PMCID: PMC8049779; DOI: 10.1021/acs.biochem.0c00432.
Complete	Nathan S, Gabelli SB, Yoder JB, Srinivasan L, Aldrich RW, Tomaselli GF, Ben-Johny M, Amzel LM. Structural basis of cytoplasmic NaV1.5 and NaV1.4 regulation. The Journal of general physiology. 2021 January 4;153(1). PubMed PMID: 33306788; PubMed Central PMCID: PMC7953540; DOI: 10.1085/jgp.202012722.
Complete	Nathan S, Gabelli SB, Yoder JB, Srinivasan L, Aldrich RW, Tomaselli GF, Ben-Johny M, Amzel LM. Structural basis of cytoplasmic NaV1.5 and NaV1.4 regulation. The Journal of general physiology. 2021 January 4;153(1). PubMed PMID: 33306788; PubMed Central PMCID: PMC7953540; DOI: 10.1085/jgp.202012722.
Complete	Nathan S, Gabelli SB, Yoder JB, Srinivasan L, Aldrich RW, Tomaselli GF, Ben-Johny M, Amzel LM. Structural basis of cytoplasmic NaV1.5 and NaV1.4 regulation. The Journal of general physiology. 2021 January 4;153(1). PubMed PMID: 33306788; PubMed Central PMCID: PMC7953540; DOI: 10.1085/jgp.202012722.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

NOTHING TO REPORT

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period? Yes

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization? Yes

C.5 OTHER PRODUCTS AND RESOURCE SHARING

NOTHING TO REPORT

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
MAMZEL1	Y	Amzel, L. Mario	PHD	PD/PI	2.4	0.0	0.0			NA
RALDRICH	Y	Aldrich, Richard	BS,PHD	PD/PI	1.8	0.0	0.0			NA
GTOMASE1	Y	Tomaselli, Gordon Frank	BS,MD	PD/PI	1.8	0.0	0.0			NA
	N	Farinelli, Federica		Technician	6.0	0.0	0.0			NA
RJOCA1	N	Joca, Rebeca	PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position	12.0	0.0	0.0			NA
SNATHA10	N	Nathan, Sara	BS,PHD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position	12.0	0.0	0.0			NA
JESSEYODER	N	Yoder, Jesse	BA,PHD	Graduate Student (research assistant)	12.0	0.0	0.0			NA
RJOCA1	N	Joca, Rebeca Peres Moreno Maia	PHD,MS	Graduate Student (research assistant)	12.0	0.0	0.0			NA
	N	DiSilvestre, Deborah		Technician	3.0	0.0	0.0			NA
NOURDINE	N	Chakouri, Nourdine	PHD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position	9.0	0.0	0.0			NA
SGABELL1	N	Gabelli, Sandra	Ph.D.	Co-Investigator	2.4	0.0	0.0			NA
MJOHNY1	N	Johny, Manu Ben	Ph.D.	Co-Investigator	4.0	0.0	0.0			NA
R-BANERJEE	N	Banerjee, Rahul	PHD,MS	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position	6.0	0.0	0.0			NA

Glossary of acronyms:
S/K - Senior/Key
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support

DOB - Date of Birth	RE - Reentry Supplement
Cal - Person Months (Calendar)	DI - Diversity Supplement
Aca - Person Months (Academic)	OT - Other
Sum - Person Months (Summer)	NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Not Applicable

D.2.b New Senior/Key Personnel

Not Applicable

D.2.c Changes in Other Support

Not Applicable

D.2.d New Other Significant Contributors

Not Applicable

D.2.e Multi-PI (MPI) Leadership Plan

Not Applicable

E. IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

NOTHING TO REPORT

G. SPECIAL REPORTING REQUIREMENTS SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

Sub-Project ID	Study ID	Study Title	Delayed Onset	Clinical Trial	NCT	NIH-Defined Phase 3	ACT
	119744	Calmodulin Regulation Na Channels: From Function and Structure to Disease	NO	NO		NO	

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

NOT APPLICABLE

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE Not Applicable
G.11 PROGRAM INCOME Not Applicable
G.12 F&A COSTS Not Applicable

Section 1 - Basic Information (Study 119744)

OMB Number: 0925-0001 and 0925-0002

Expiration Date: 03/31/2020

1.1. Study Title *

Calmodulin Regulation Na Channels: From Function and Structure to Disease

1.2. Is this study exempt from Federal Regulations * ❍ Yes ● No

1.3. Exemption Number ❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8

1.4. Clinical Trial Questionnaire *

1.4.a. Does the study involve human participants?	● Yes	❍ No
1.4.b. Are the participants prospectively assigned to an intervention?	❍ Yes	● No
1.4.c. Is the study designed to evaluate the effect of the intervention on the participants?	❍ Yes	● No
1.4.d. Is the effect that will be evaluated a health-related biomedical or behavioral outcome?	❍ Yes	● No

1.5. Provide the ClinicalTrials.gov Identifier (e.g. NCT87654321) for this trial, if applicable

Section 2 - Study Population Characteristics (Study 119744)

2.1. Conditions or Focus of Study

2.2. Eligibility Criteria

2.3. Age Limits Min Age: Max Age:

2.4. Inclusion of Women, Minorities, and Children

2.5. Recruitment and Retention Plan

2.6. Recruitment Status

2.7. Study Timeline

Inclusion Enrollment Reports

IER ID#	Enrollment Location Type	Enrollment Location
IER 119744	Domestic	

Inclusion Enrollment Report 119744

Using an Existing Dataset or Resource* : ● Yes ❍ No

Enrollment Location Type* : ● Domestic ❍ Foreign

Enrollment Country(ies): USA: UNITED STATES

Enrollment Location(s):

Comments:

Planned

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/ Alaska Native	0	0	0	0	0
Asian	1	1	0	0	2
Native Hawaiian or Other Pacific Islander	0	0	0	0	0
Black or African American	1	1	0	0	2
White	4	4	0	0	8
More than One Race	0	0	0	0	0
Total	6	6	0	0	12

Cumulative (Actual)

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/ Alaska Native	0	0	0	0	0	0	0	0	0	0
Asian	0	0	0	0	0	0	0	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0	0	0	0	0	0	0	0
Black or African American	0	0	0	0	0	0	0	0	0	0
White	0	0	0	0	0	0	0	0	0	0
More than One Race	0	0	0	0	0	0	0	0	0	0
Unknown or Not Reported	0	0	0	0	0	0	0	0	0	0
Total	0	0	0	0	0	0	0	0	0	0

Section 3 - Protection and Monitoring Plans (Study 119744)

3.1. Protection of Human Subjects

3.2. Is this a multi-site study that will use the same protocol to conduct non-exempt human subjects research at more than one domestic site? ❍ Yes ❍ No ❍ N/A

If yes, describe the single IRB plan

3.3. Data and Safety Monitoring Plan

3.4. Will a Data and Safety Monitoring Board be appointed for this study? ❍ Yes ❍ No

3.5. Overall structure of the study team

Section 4 - Protocol Synopsis (Study 119744)

4.1. Brief Summary

4.2. Study Design

4.2.a. Narrative Study Description

4.2.b. Primary Purpose

4.2.c. Interventions

Type	Name	Description

4.2.d. Study Phase

Is this an NIH-defined Phase III Clinical Trial? ❍ Yes ● No

4.2.e. Intervention Model

4.2.f. Masking ❍ Yes ❍ No

❑ Participant ❑ Care Provider ❑ Investigator ❑ Outcomes Assessor

4.2.g. Allocation

4.3. Outcome Measures

Type	Name	Time Frame	Brief Description

4.4. Statistical Design and Power

4.5. Subject Participation Duration

4.6. Will the study use an FDA-regulated intervention? ❍ Yes ❍ No

4.6.a. If yes, describe the availability of Investigational Product (IP) and Investigational New Drug (IND)/ Investigational Device Exemption (IDE) status

4.7. Dissemination Plan

I. OUTCOMES

I.1 What were the outcomes of the award?

Mutations in Nav1.5 (cardiac) and Nav1.4 (skeletal) sodium channels, particularly in their carboxy tails (CTs), give rise to a number of arrhythmias and myotonias. Leveraging our recent discoveries the goals are to define how mutation-induced changes in channel structure and function produce disease, facilitating enhanced physiological understanding, and development of mechanistically-coherent therapies. Our work demonstrates that CaM regulation of Nav channels bears eerie similarity to Cav channels. Binding of Ca2+-free calmodulin (apoCaM) to a Nav channels strongly boosts peak channel open probability. ApoCaM binds the CTs of Cav and Nav channels and it is notable that many channelopathic mutations (Nav1.5 Brugada (BS); long QT syndromes (LQTS)) reside on CTs. We have shown how such mutations affect apoCaM affinity, probably by dislodging this binding partner. To assess their relevance in humans, we differentiated Nav1.5 channelopathic human iPSC-CMs such as the 1904 Nav1.5 channelopathy. We determined the structures of apoCaM and CaCaM bound to Nav1.4 CT. These structures critically inform about the mechanism, and allowed generalization and differences between Nav1.4 and Nav1.5.