**Generally, the model credibility reporting and update process will entail several pieces of information generally formatted to communicate to the uninformed reviewer/user an assessment of steps taken toward model credibility with respect to the intended use of the model, as well as communicate where the more interested reviewer/user can go to find more information.  This data includes:**

**1. Project title:** PREDICTIVE MULTISCALE IN SILICO CARDIO-PHARMACOLOGY

**2. A one/two sentence summary of the project topic with a hyperlink to more detailed information via the** [**IMAG wiki**](https://www.imagwiki.nibib.nih.gov/content/msm-participants)**.**

Disorders of excitability kill more Americans than any other cause and are difficult to treat with drugs. There is an urgent need to develop new approaches for predicting how drugs will affect cardiac rhythms. Our team will construct a novel computational multiscale model framework for predicting drug effects on emergent electrical activity in the heart.

**3. Details regarding Model Credibility plan following the**[**CPMS Ten Simple Rules (TSR)**](https://simtk.org/plugins/moinmoin/cpms/Ten%20Simple%20Rules%20of%20Credible%20Practice)**format.**

**Rule 1 – Define context clearly**:

* *Context of use: - to establish a multiscale model for predictive for predictive cardiopharmacology; The initial context of use is suggested as a computational screening framework for preclinical screening of drugs in early drug development to identify potential lead compounds and eliminate compounds with clear proarrhythmic effects. This will be done through combination of atomistic structural and protein, cell, tissue and organ scale functional modeling studies. For the latter in-house mathematical model code written in C and/or MatLab will be used, whereas atomistic modeling and simulations will be done using standard commercial software packages, freely available for academic users:*
* *Rosetta structural modeling software (https://www.rosettacommons.org/software and https://github.com/RosettaCommons ) is used to predict structure of human cardiac voltage-gated sodium channel and antiarrhythmic drugs interactions with the channel at atomic scale. Rosetta software is freely available to academic users with over 10,000 academic licenses already in use -* [*https://www.rosettacommons.org/software/license-and-download*](https://www.rosettacommons.org/software/license-and-download)
* *Nanoscale Molecular Dynamics (NAMD) (https://www.ks.uiuc.edu/Research/namd/) and DE Shaw Anton 2 (https://www.psc.edu/anton2) molecular modeling software is used to compute dynamical interactions of cardiac voltage gated sodium channel with local anesthetic and anti-arrhythmic drugs at multi-microsecond time scale with atomic resolution to predict their structural, energetic and kinetic determinants to be used for informing functional level models. NAMD is freely available to academic users and is one of the most widely used biomolecular simulation packages. Access to DE Shaw Anton 2, hosted by Pittsburgh Supercomputing Center, is through an NSF-sponsored grant and annual competitive computer allocation process ( https://www.psc.edu/anton-rfp).*

**Rule 2 – Use appropriate data:**

* *Appropriate data for functional scale modeling: V-clamp data from hiPSC derived myocytes, (2) V-clamp and AP-clamp data from adult ventricular myocytes/off-target effects, (3) Ca2+ measurements in cardiac myocytes, (4) whole heart optical mapping data.*
* *We used Rosetta software to predict structure of human cardiac voltage-gated sodium channel in multiple states based on cryo electron microscopy structures of eukaryotic voltage-gated sodium channels.*
* *Channel models developed by ROSETTA are used in Molecular dynamics (MD) simulations to validate their stabilities. Drug models are developed by CGENFF (generalized CHARMM force field) program web server (http://beowulf.engl.uky.edu/) and optimized using quantum mechanical reference data and established protocols (http://mackerell.umaryland.edu/~kenno/cgenff/download.php#tutor).*

**Rule 3 – Evaluate within context:** *Verification and Validation:*

* *We will verify the reliability models as model components. Solvers will be benchmarked to dummy data set with known solutions to define numerical error tolerance. Models will be tested on two or more compilers, test compiled executables on various hardware, equations converge, steady-state derivatives sum to zero, outputs compare to benchmarks defined as the model outputs from deployment run. We will perform quantitative analyses of propagation of input uncertainties for model parameters. The benchmark is defined as a predefined tolerance of impact of input uncertainties on model outputs.*
* *We will generate an assessment report on assumptions and simplifications in the model components. The benchmark defined as a “go” if model assumptions and simplifications yield outputs that can be benchmarked to experimental validation within predefined error tolerances.*
* *We will generate a validation report on workflow that demonstrates the linkage between modules. The benchmark defined as a “go” if end user can utilize workflow components and framework to link models.*
* *We will generate novel models of parameterized using simulated and in vitro electrophysiology data. Model frameworks will be benchmarked to data above and must fall within a predefined error tolerance based on size of deviation of average distributions for acceptance.*
* *We will perform quantitative analyses of the propagation of input uncertainties. The benchmark is defined as a predefined tolerance of impact of input uncertainties on model outputs.*
* *Our team will generate an assessment report on assumptions and idealizations in reduced models of drug channel interactions. The benchmark defined as a “go” if model assumptions and simplifications yield outputs that can be benchmarked to experimental validation within predefined error tolerances.*
* *We will produce a public source code for models. The benchmark is defined as successful external end user execution of tools.*
* *We will integrate and verify the workflow into a Kepler Workflow and Jupyter Notebook. The benchmark defined as when workflow components are successfully linked and executed by end users accessing the workflow via GitHub.*
* *Rosetta software applications are being benchmarked and tested using Rosetta Benchmark server - http://benchmark.graylab.jhu.edu/*
* *NAMD local installation from source is tested upon installation by our admin using standard tools (make check). NAMD installation on computer clusters is tested by supercomputing center staff. In both cases suggested best practices are being followed (http://www.hpcadvisorycouncil.com/pdf/NAMD\_Best\_Practices.pdf). DE Shaw Anton 2 software is being rigorously tested by DE Shaw research and PSU personnel before being released, and user input is taken into account as well.*

**Rule 4 – List limitations explicitly.** Model Limitations:

* *In building models, data are compiled from various sources, obtained under varying experimental conditions and exhibit notable variability and heterogeneity. The complexity of channel dynamics and drug-channel interactions are by definition idealization in the model representations. Model representations are not unique. We will perform quantitative analyses of the propagation of input uncertainties and sensitivity analysis to determine the reliability range of the models.*
* *Rosetta structural modeling software is based on random sampling of conformational space using thousands or millions of independent trajectories and unless exactly the same seed number is used, distribution of different convergent clusters of models may vary.*
* *Molecular dynamics simulations running using NAMD or Anton 2 software are deterministic methods, but they are subject to numerical precision limitations and thus may provide different numerical results like protein conformations, drug distributions etc. even using the same starting point. This typically suggests that convergence was not achieved. Longer simulations and/or usage of advanced sampling methods such as umbrella sampling and metadynamics is employed in these cases.*

**Rule 5 – Use version control.** Version Control Plan:

* *All models will utilize version control systems in Kepler Workflows, Jupyter Notebooks and/or Github.*
* *Rosetta software public releases are historically numbered and we record specific Rosetta software version for each atomic scale modeling project.*
* *At the start of the project we use latest stable releases of the MD simulation software and adhere to their usage for the whole project, unless problems with the code accuracy or performance are detected. Currently we are using NAMD version 2.12 and Anton 2 software version 1.31.0.*

**Rule 6 – Document adequately.** Documentation and Provenance Plan:

* *All models will utilize documentation control systems in Kepler Workflows, Jupyter Notebooks and/or Github.*
* *We keep records of Rosetta software command lines and scripts used for specific modeling projects, which we will provide in the Supplementary Material of published papers.*
* *The detailed methodology including force field used and all the important simulation parameters are always provided as part of the supporting information. If a novel molecule was parameterized, its force field topology and parameters are included as well.*
* *For our MD simulations we keep input and stream files, molecular topology and parameter files, coordinate and restart files, all in text format, as well as binary molecular trajectory files. All these files are kept in multiple locations including remote and local data storages. They are freely available upon request upon paper publication.*

**Rule 7 – Disseminate broadly.** Dissemination Plan:

* *All models will be made available in multiple formats on Github.*
* *Rosetta software is freely available to academic users with over 10,000 academic licenses already in use - https://www.rosettacommons.org/software/license-and-download . Rosetta software command lines and scripts used for specific modeling projects will be provided in the Supplementary Material of published papers.*
* *NAMD is used by thousands of researchers all over the world. Anton 2 software is proprietary but is a modified version of DE Shaw Desmond software, freely available for academic users. It also uses the same file format and many of the simulation options (excluding Anton 2 architecture specific). NAMD and Anton 2 input files, structures and resultant molecular coordinate and trajectory files will be publicly disseminated upon request.*

**Rule 8 – Get independent reviews.** Independent *third-party users and developers*:

* *Have the M&S activity reviewed by independent third-party users and developers, essentially by any interested member of the community.*
* *We will produce a public source code via GitHub in a variety of formats for models. The benchmark for independent reviewing is defined as successful external end user execution of tools.*
* *Results of Rosetta structural modeling will be independently reviewed and tested by other M&S community members.*
* *Results of NAMD and Anton 2 MD simulations can be also independently analyzed and re-run by other researchers including M&S community members.*

**Rule 9 – Test competing implementations.**

* *Multiple solvers will be benchmarked to dummy data set with known solutions to define numerical error tolerance. Models will be tested on two or more compilers, test compiled executables on various hardware, equations converge, steady-state derivatives sum to zero, outputs compare to benchmarks defined as the model outputs from deployment run.*
* *I-TASSER, MODELER, and Schrodinger software will be used to test competing atomic scale protein structural modeling approaches.*
* *In addition to NAMD and Anton 2 software, MD simulations will be also test run using CHARMM, GROMACS, OpenMM to test their performance and force field and MD methodology implementation accuracy.*

**Rule 10 – Conform to standards.** Best practices will be followed:

***Models and Software sharing plan:*** *All tools developed as part of this project will be made immediately freely available and transferable upon publication to biomedical researchers and educators in the non-profit sector, such as institutions of education, research institutions, and government laboratories. The terms of software availability will make source code freely available to researchers for modification and sharing. We will take full responsibility for creating the original and subsequent versions of the computational tools outlined in this proposal.*

***Sharing and reproducibility of published simulation works:*** *Following publication of all work supported through this grant, specific standards for reproducibility of results will be imposed by source code sharing through shared resources (described below) as follows: For each published figure, a package of model components (fully documented source code), modeling parameters (and initial conditions) and associated datasets used for comparison or optimization will be will be bundled and contain all necessary information to allow any user to reproduce the published figure. This is a critical component of the model and software sharing plans as it will ensure reproducibility of published model predictions. These packages will be made available in C source code and to ensure dissemination of a standard format with cross compatibility, the packages will also be made available in GitHub. All packages will be tested prior to public dissemination.*

***Plans to link proposed models with other relevant models:*** *All software will be developed as stand alone modules that comply with current C11 standards in the C programming language: ISO/IEC 9899:2011 the current standard for the C programming language. These will be “plug and play” components that can be added to existing codes.*

***A software dissemination and distribution plan:*** *To further enhance the potential impact of the software that we will develop and to manage and disseminate the improvements or customizations of our tools and resources by others, we will make all tools available via GitHUb and SenseLab that include model parameters and equations, metadata, scripts and supporting data including images.  Once models are published, they will be publicly shared via GitHub (1-5).*

***Dissemination and distribution through other shared resources:*** *Tools will also be uploaded to other shared resources including GitHUb and where appropriate to SenseLab (*[*http://senselab.med.yale.edu/*](http://senselab.med.yale.edu/)*). Included are tools for parameter optimization, source code for simulations and routines used in sensitivity analysis.*

***Other terms of use:*** *The terms of software will allow dissemination and commercialization of enhanced or customized versions of the software, or incorporation of the software or pieces of it into other software packages. To ensure continued utility to the wider scientific and academic community, all software developed under this contract will transferable such that another individual or team can continue development. Code will be compatible with existing C compilers available on Unix, PC and Mac platforms.*

**D. Description of how the planned activities *will lead to* a credible model**

*The following elements will be included in model construction and model linking across scales: Verification methods will be applied via utilizing reduced models to compare analytical derivatives and solutions to numerical derivatives and solutions in complex models under comparable conditions to ensure that the algorithms used to solve the computational model are accurate within predefined bounds on numerical error. Upon model validation (see benchmark below), we will also assess the impact of uncertainty in our model formulation and parameters, due to natural variability, experimental error or lack of knowledge in experimental data, on model outputs. We will fit a probability distribution of model outputs (based on data) with probability distributions of model inputs (i.e., uncertain parameters). Perturbations in model parameters within their distribution and calibration with validation data distributions will allow us to assess the non-linear sensitivity of the system and serve as a mode of uncertainty quantificationto determine:* ***(1)*** *reliability of developed models, including an assessment of how model parameters were fitted to data, and the effect of assumptions and simplifications in model components; and* ***(2)*** *propagation of uncertainties in tissue scale models, which might be sensitive to cell scale behavior (APD restitution, Ca2+ handling) combined with tissue scale properties (tissue conductivities, passive mechanical properties, distribution of cell types).*

*Sensitivity analysis and variability: By introducing random variations in several model key parameters (e.g., conductances of ion channels, maximum transport rates, etc.), we will build populations of thousands model variants following the method described by the Sobie group and our group, where each model has different parameters (and outputs). This approach will allow investigation of the natural variability among cells in a tissue (intra-subject variability) or among different individuals (inter-subject variability). Moreover, the assessment of sensitivity of model predictions to parameter perturbations ensures enhanced robustness of the results of computational studies.* *We will also utilize population-based approaches and multivariable regression models to build a quantitative bridge between iPSC-CMs (iPSC-VSMs) and adult human cardiac (vascular) myocytes,**i.e., to map physiology from iPSCs to human adult myocytes. This method will allow to quantitatively predict patient-specific responses in adult human myocytes, hearts, and vessels from recordings and simulations in iPSCs.*

*Deliverables*

*We will utilize multiple modes of uncertainty quantification, including population-based approaches, throughout the project and in each task to determine: reliability of developed models; analysis and propagation of input uncertainties; effect of assumptions and simplifications in model components.*

*Benchmarks*

*We anticipate iteration between modeling and experiments to ensure the models are well constrained and robust. If a model fails to reproduce a given output, we will perform sensitivity analysis to determine the parameters (describing ion channels, Ca2+ handling, or signaling) to which given model output/process (AP or Ca2+ handling properties and their rate-dependency) is most sensitive. This will dictate new experiment(s) to be performed to further improve model parameterization. Where experimental data is sparse, we will (a) use experimental data from other species, or (b) utilize parameter values from previous models. Uncertainty quantification techniques will allow associating probability distributions of uncertain model parameters to a probability distribution of model outputs (to match experimental data).*

**E. Progress to-date and plans for the next reporting cycle (6 months). What has been achieved since last reporting?**

**MANUSCRIPTS:**

Docken, S.S., **Clancy, C.E.,**Lewis, T.J. 2018. “An idealized framework for rate-dependent effects of state-specific cardiac sodium channel blockers.”  To be submitted imminently.

Nguyen, P.T., DeMarco, K.R., Vorobyov, I., **Clancy, C.E.**, and Yarov-Yarovoy, V. **2018** “Structural Basis for Antiarrhythmic Drug Interactions with the Human Cardiac Sodium Channel” *Submitted to eLife. Preprint is available at BioRxiv:*

[*https://www.biorxiv.org/content/early/2018/09/29/430934*](https://www.biorxiv.org/content/early/2018/09/29/430934)

Yang, P.C, Purawat, S., Ieong, P.U., Jeng, M.T., DeMarco, K.R., Vorobyov, I., McCulloch, A.D., Altinas, I., Amaro, R.E. and **Clancy, C.E.** **2018** “A demonstration of modularity, reuse, reproducibility, portability and scalability for modeling and simulation of cardiac electrophysiology using Kepler Workflows” *Submitted to PLoS Computational Biology.*

DeMarco K. R., Bekker S., Vorobyov I. **2018** *“*Challenges and Advances in Atomistic Simulations of Potassium and Sodium Ion Channel Gating and Permeation” *Revised version submitted to Journal of Physiology.*

Agarwal SR, Gratwohl J, Cozad M, Yang PC, **Clancy CE**, Harvey RD. “Compartmentalized cAMP Signaling Associated With Lipid Raft and Non-raft Membrane Domains in Adult Ventricular Myocytes” ***Front Pharmacol.*** 2018 Apr 23;9:332. doi: 10.3389/fphar.2018.00332. eCollection 2018. PMID: 29740315

Perissinotti LL, De Biase PM, Guo J, Yang PC, Lee MC, **Clancy CE**, Duff HJ, Noskov SY. And “Determinants of Isoform-Specific Gating Kinetics of hERG1 Channel: Combined Experimental and Simulation Study.” ***Frontiers in Pharmacology.***  2018 Apr 12;9:207. doi: 10.3389/fphys.2018.00207

PMID: 29706893

Vorobyov, I. and **Clancy, C.E.** “Sex Drugs and Funky Rhythms”. ***Heart Rhythm.*** *Invited Editorial.* April 2018 Volume 15, Issue 4, Pages 485–486. PMID: 29605014

DeMarco, K.R., Bekker, S., **Clancy, C.E.**, Noskov, S.Y., and Vorobyov, I. “Digging into Lipid Membrane Permeation for Cardiac Ion Channel Blocker d-Sotalol with All-Atom Simulations”. ***Frontiers in Pharmacology.*** 01 February 2018 *|* <https://doi.org/10.3389/fphar.2018.00026> PMID: 29449809

**ABSTRACTS:**

• Kernik, D.C.,Morotti, S.,  Duff, H.J., Kurosawa, J., Jalife, J., Wu, J.C., Grandi, E., **Clancy, C.E.** 2018. “A computational analysis of inter-subject variability in induced pluripotent stem cell-derived cardiomyocytes.” 62nd Annual Biophysical Society Meeting. San Francisco, CA.

• Kernik, D.C.,Morotti, S.,  Duff, H.J., Kurosawa, J., Jalife, J., Wu, J.C., Grandi, E., **Clancy, C.E.**2018. “A computational analysis of inter-subject variability in induced pluripotent stem cell-derived cardiomyocytes.” Centre Européen de Calcul Atomique et Moléculaire Meeting on Multiscale Modelling in Electrophysiology: From Atoms to Organs. Lugano, Switzerland.

• Docken, S.S., **Clancy, C.E.,**Lewis, T.J. 2018. “Mechanisms underlying rate-dependent effects of state-specific binding of sodium channel blockers in cardiovascular tissue: Insights from idealized models.” Biophysical Society 62nd Annual Meeting. San Francisco, CA.

• Vorobyov, I., Brown, B.M., DeMarco, K.R., Noskov, S.Y., Yarov-Yarovoy, V., Wulff, H., **Clancy, C.E.** 2018. “Molecular determinants of steroid hormone and drug induced arrhythmogenesis via hERG channel block.” 62nd Annual Biophysical Society Meeting. San Francisco, CA.

• DeMarco, K.R., Bekker, S., **Clancy, C.E.**, Noskov, S.Y., Vorobyov, I. 2018. “Atomistic simulation of lipid membrane permeation for cardiac ion channel blockers.” 62nd Annual Biophysical Society Meeting. San Francisco, CA.

• Emigh, A.M., DeMarco, K.R., Furutani, K., Bekker, S., Sack, J.T., **Clancy, C.E.**, Vorobyov, I., Yarov-Yarovoy, V. 2018. “Structural modeling of hERG channel interactions with drugs using ROSETTA.” 62nd Annual Biophysical Society Meeting. San Francisco, CA.

• Nguyen, P.T., DeMarco, K.R., Vorobyov, I., **Clancy, C.E.**, Yarov-Yarovoy, V. 2018. “Structural modeling of local anesthetic and antiarrhythmic drug binding to the human cardiac voltage gated sodium channel.”  62nd Annual Biophysical Society Meeting. San Francisco, CA.

• Kudaibergenova, M, Perissinotti, L.P., Duff, H.J., **Clancy, C.E**., Noskov, S.Y., 2018, “Multi-Scale Model for Evaluation of Drug-Induced QT Prolongation”, CECAM Workshop “Multiscale Modelling in Electrophysiology: From Atoms to Organs”, Lugano, Switzerland

• Maly J., Emigh A.M., DeMarco K.R., Vorobyov I., **Clancy C.E.**, and Yarov-Yarovoy V., 2018 Rosetta modeling and drug interactions of the hERG channel in an inactivated state. RosettaCon, Leavenworth, WA.

• Yang, P. C., Perissinotti, L.L., Wang, Y. , DeMarco, K.R., Jeng, M.-T., Vorobyov, I., Yarov-Yarovoy, V., Noskov, S. Y., Clancy. C. E. “Elucidating sex dependence of cardiac arrhythmias via multiscale modeling and simulations” 2018 IMAG Futures Meeting – Moving Forward with the MSM Consortium. National Institutes of Health, Bethesda, Maryland.

• DeMarco, K. R.; Vorobyov, I.; Emigh, A.; Bekker, S.; Dawson, J.; Yarov-Yarovoy, V.; Clancy C.E. 2018 “Using Atomistic Modeling and Simulation to Search for the Molecular Mechanisms of Drug-induced arrhythmogenesis via hERG blockade.*”* “The Heart by Numbers: Integrating Theory, Computation and Experiment to Advance Cardiology” Biophysical Society Thematic Meeting, Berlin, Germany**.**

•  Vorobyov, I.; DeMarco, K. R.; Bekker, S.; Dawson, J.; Emigh, A.; Furutani, K.;Sack, J.T.; Yarov-Yarovoy, V.; Clancy C.E. “Investigating Molecular Mechanisms of State-Dependent hERG Block via Atomistic Modeling and Simulations” Annual Meeting of the Society of General Physiologists, Woods Halls, Massachusetts**.**

**4. Issues/concerns identified as critical or problematic to achieve the standard of credibility set by MSM Consortium.**

None as yet identified.

**5. What other factors, if any, contribute to credibility but cannot be reported within the TSR structure? In requesting this information, we seek to identify credibility activities/issues and appropriate ways to report them at upcoming IMAG/MSM meetings.**

 None as yet identified.