Title

Structural Modeling of Voltage-Gated Ion Channel Interactions with Drugs Using Rosetta

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Structural Modeling of Voltage-Gated Ion Channel Interactions with Drugs Using Rosetta

By

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DISSERTATION

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Committee in Charge

2022

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List of Scientific Abbreviations

Å ..... angstrom

Ca^{2+} ..... calcium ion

CaM ..... calmodulin

Ca_V ..... voltage-gated calcium channels

Ca_VAb ..... bacterial calcium channel

CiPA ..... Comprehensive in vitro Proarrhythmia Assay

Cryo-EM ..... cryogenic electron microscopy

EC ..... excitation-contraction

ECG ..... electrocardiogram

EEEE ..... selectivity filter residues of $Ca_V1.2$: Glu-393, Glu-763, Glu-1145, and Glu-1446

FR ..... fenestration region

hERG ..... human ether-a-go-go related gene channel, $K_V11.1$

HP ..... hydrophobic pockets

K^+ ..... potassium ion

K_V ..... voltage-gated potassium channels

LQTS ..... long QT syndrome

NAMD ..... Nanoscale Molecular Dynamics software

Na_V ..... voltage-gated sodium channels

NMR ..... nuclear magnetic resonance

PD ..... pore domain

PDBID ..... Protein Data Bank identification number

PhD ..... Doctor of Philosophy

PLIP ..... Protein Ligand Interaction Profiler

REU ..... Rosetta energy unit

RMSD ..... root mean square deviation

SF ..... selectivity filter

Tcl ..... Tool Command Language

TdP ..... Torsades de Pointes

VGIC ..... voltage-gated ion channel

VSD ..... voltage sensing domain

WT ..... wild type

XML ..... extensible markup language

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Abstract

Structural Modeling of Voltage-Gated Ion Channel Interactions with Drugs Using Rosetta

Many different types of drugs–from antibiotics to blood pressure medication–tend to interfere with the body's ability to control heart rhythm by disrupting the proteins in heart cells that control the movement of charged atoms (ions) across the cell membrane known as voltage-gated ion channels (VGICs). These drugs can cause dangerous arrhythmias (abnormal heart rhythms) that can increase the risk for heart failure, stroke, or death. Early and efficient assessment of cardiotoxicity is essential to the drug development process and to reducing drug development costs. Current methods for assessing safety are sensitive but not specific and can often result in false identification of unsafe treatments and the failure of potentially life-saving treatments to reach the public. Structural characterization of VGICs and their modulating interactions are necessary for rational design of safe therapeutics.

Human Ether-a-go-go-Related Gene (hERG) encodes a potassium-selective voltage-gated ion channel ($K_V11.1$) essential for normal electrical activity in the heart. Genetic hERG mutations and drug blockage of the channel pore can cause long QT syndrome (LQTS). LQTS predisposes individuals to arrhythmia and puts them at risk for stroke or sudden cardiac arrest. A major problem in antiarrhythmic drug therapies is the proclivity for these drugs to promote fatal arrhythmias through hERG channel blockade. However, not all hERG channel blocking drugs are pro-arrhythmic, and their differential affinities to discrete channel conformational states and their state stability modulations have been suggested to contribute to arrhythmogenicity.

Voltage-gated calcium (Ca_V) channels play a key role in muscular contraction, neuronal excitation, gene expression regulation, and the release of hormones or neurotransmitters. Dysregulation of Ca_V channels and the associated intracellular calcium homeostasis have been associated with various cardiac and neurological disorders. Found throughout the body, often as part of large complexes and/or clusters, the L-type $Ca_V1.2$ channel mediates the influx of Ca^{2+} into the cell in response to membrane depolarization. Mutations or blockage of the channel by drug molecules leading to altered functions of human $Ca_V1.2$ have been linked to cardiac arrhythmias, autism, bipolar disorder, and immunodeficiency. Many Ca_V channel blockers targeting the $\alpha 1$-subunit of $Ca_V1.2$ are used to treat hypertension, coronary artery disease and other cardiovascular medical conditions. However, few drugs have been approved for clinical use due to severe side effects (including cardiotoxicity) or limited efficacy.

In this study, Rosetta electron density refinement and homology modeling protocols were used to build voltage sensing and pore domain structural models of wild-type hERG channels in open and closed states, open-state hERG mutant variants (Y652A, F656A, and Y652A/F656A double mutant) based on cryo-electron microscopy (cryo-EM) structures of hERG (PDB ID: 5VA2) and EAG1 (PDB ID: 5K7L) channels as well as open- and closed-state models of the wild-type $Ca_V1.2$ $\alpha 1$-subunit using cryo-EM $Ca_V1.1$ (PDB ID: 5GJV), and $Na_V1.4$ (PDB ID: 6AGF) structures, respectively. The hERG channel models were developed as protein targets for Rosetta-based molecular docking studies of charged and neutral forms of amiodarone, nifekalant, dofetilide, d- and l-sotalol, flecainide, and moxifloxacin–a diverse set of pharmaceuticals chosen based on their different arrhythmogenic potentials and abilities to facilitate hERG current. The $Ca_V1.2$ models were used as targets for Rosetta docking studies with verapamil and amlodipine– representatives

of two different calcium channel blocking classes: phenylalkylamines and dihydropyridines, respectively. We present the results of our docking studies that provide structural insights into the molecular and state-dependent drug interactions with hERG and $Ca_V1.2$ channels that play a key role in differentiating safe and harmful ion channel blockers.

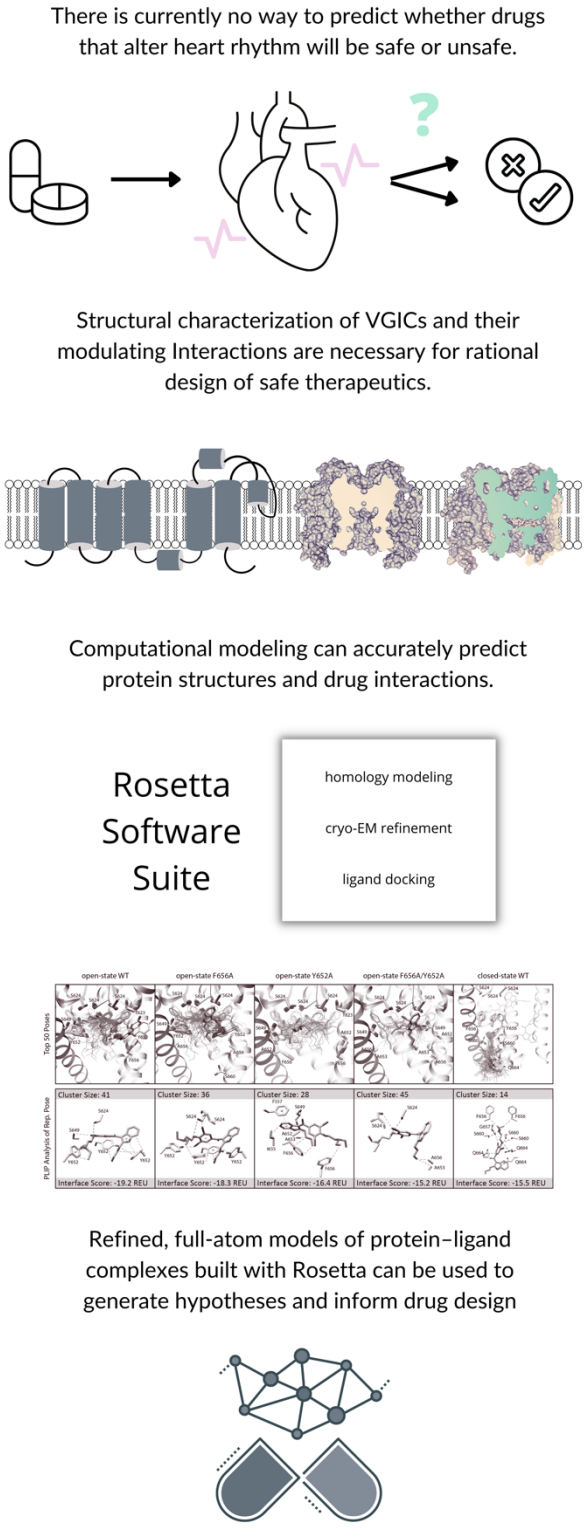
Key Findings:

- ◊ The pattern of hERG-drug interactions with the hydrophobic pocket is consistent with experimental data suggesting facilitating drugs may act as a wedge to bias hERG channel equilibrium towards the open state and increase hERG current amplitude in response to low-voltage depolarization.
- ◊ Open-state WT hERG interface scores are lower than, or similar to, Y652A mutants suggesting that these poses are relevant for amiodarone, nifekalant, flecainide, moxifloxacin, d-sotalol, and dofetilide, based on comparison to existing experimental data.
- ◊ Open-state WT hERG interface scores are not lower than the F656A mutants for nifekalant, neutral flecainide, neutral moxifloxacin, d-sotalol, l-sotalol, and dofetilide, suggesting limitations of our study using only two conformational states or limitations of Rosetta to model allosteric contributions of F656.
- ◊ The percentage of poses remaining within the closed-state hERG channels suggests that closed channels can accommodate known trapped drugs (nifekalant, flecainide, d/l-sotalol, and dofetilide), but not amiodarone or moxifloxacin (non-trapped drugs).
- ◊ Amlodipine and verapamil docked to $rCa_V1.2$ models in open and closed states recapitulated known binding orientations and similar positioning within pore but did not

reproduce known binding determinants necessitating revision of model development to include additional structural densities and increasing search radius in docking protocol in future studies.

Graphical Abstract

Figure 1 Infographic Visualization of Abstract



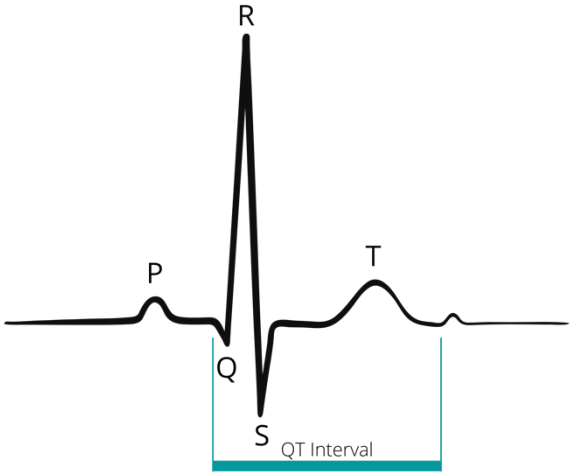
1. Introduction

"In few specialties of medicine are new promising drugs shown to be so much inferior to placebo, and even worse, to increase mortality."

–Sanderson, 1996[1]

There is often a fine line between therapeutic effect and dangerous cardiac rhythm disturbances. Voltage-gated ion channels (VGICs) are a class of transmembrane proteins that play a critical role in mediating the cardiac action potential and maintenance of normal heart excitation and contraction by selectively transporting ions across the cell membrane in response to changes in membrane potential. VGICs are critical to consider as both targets and off-targets during the drug development process[2]. Pharmaceutical treatments for arrhythmia, hypertension, coronary artery disease and other cardiovascular conditions often target various VGICs, while inherited and acquired channelopathies (a group of diseases caused by the dysfunction of ion channels) have been linked to many cardiac and neurological diseases such as cardiac arrhythmias, autism, bipolar disorder, and immunodeficiencies. Cardiotoxicity, especially due to drug-induced interference in channel function, is one of the most common causes of withdrawal or restriction of the use of marketed drugs[3]. Deviant cardiac electrical activity, often associated with the prolongation of the QT interval (long QT syndrome, LQTS) on an electrocardiogram (ECG), can increase the risk for fatal ventricular arrhythmias, such as Torsades de Pointes (TdP).

Figure 2 QT Interval of ECG Waveform



Few antiarrhythmic drugs have been approved for clinical use due to severe side effects (most significantly, cardiotoxicity) or limited efficacy[4]. Drug-induced arrhythmogenicity has been one of the most critical regulatory concerns for cardiovascular and other drugs in development. Historically, widely used predictors of drug proarrhythmic potential are the prolongation of the QT interval and the blockade of the hERG channel (a major cardiac voltage-gated K^+ channel that mediates repolarization current). However, these predictors are not selective enough since not all QT-prolonging and hERG-blocking drugs are arrhythmogenic. Currently, efforts are focused on improving these predictors through an understanding of interactions of various drugs with multiple cardiac ion channels, including hERG and $Ca_V1.2$. $Ca_V1.2$ is an L-type calcium channel essential to maintaining the intracellular calcium homeostasis and excitation-contraction (EC) coupling that can cluster into large oligomer complexes to amplify the resultant Ca^{2+} signal[5,6].

To better understand the interactions between various drugs and VGICs, atomic-scale prediction and refinement of protein structure and protein-ligand docking are necessary. The Rosetta suite of applications for computational modeling of protein structures and their interactions with ligands and other proteins is ideal for their fast computational speeds, sampling relevant conformational and sequence space, and validated energy evaluation[7]. Rosetta integrates largely knowledge-based energy functions derived from the Protein Data Bank (PDB) survey with knowledge-guided Monte Carlo sampling and gradient-based minimization–resulting in some of the most accurate structure and atomic interaction predictions available[7–13].

Loop modeling combined with comparative modeling (RosettaCM) methodology uses sequence alignments, fragment recombination, iterative fragment assembly and minimization, and high-resolution refinement to generate protein models with higher accuracy than other

methods[14–18]. Additional model accuracy can be derived through the automated use of cryo-electron microscopy (cryo-EM) maps and fragment-based enumerative sampling (RosettaES) to refine lower, near-atomic resolution (3-4.5Å) structures[19,20]. Combined with Monte Carlo minimization, ensembles of the ligand as well as protein backbone and side chain conformations can be used to sample conformational flexibility of protein-ligand complexes and accurately predict protein-ligand interfaces (RosettaLigand)[7,8,21–23].

The following chapters utilize these Rosetta methodologies to predict VGIC structures and small-molecule interactions that we know to play a pertinent role in normal and dysregulated cardiac electrical activity. These studies revealed key similarities and differences between various drug interactions with hERG and $Ca_V1.2$ in multiple states to provide useful structural insights into molecular mechanisms of drug action and their pro-arrhythmia proclivities and to inform future drug design.

2. Modeling of hERG Channel Interactions with Drugs Using Rosetta

2.1 Introduction

Human ether-à-go-go related gene (hERG) encodes a voltage-gated potassium channel Kv11.1 that mediates the rapid repolarization phase during a cardiac action potential[24]. The hERG channel cycles between closed, open and inactivated states in response to membrane voltage changes to tightly regulate the K^+ transport in the heart[25]. Genetic mutations of hERG or drug-induced interferences in channel function can result in long-QT syndrome (LQTS), potentially leading to fatal arrhythmias such as Torsade de pointes (TdP). Promiscuous block of the cardiac hERG channel by structurally varied drugs is a major research question and drug-design challenge.

A significant inhibitor to developing and approving new drugs is the low specificity of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) safety guidelines[5,26]. While early testing of QT prolongation and hERG channel block is extremely effective at eliminating the risk of approving potentially torsadogenic drugs, they are inadequate markers of true proarrhythmic risk[5,26,27] since several hERG blocking and QT-prolonging drugs demonstrate low pro-arrhythmia proclivities. Surrogate markers, such as hERG block in cell cultures or QT prolongation in animal models, often do not correlate with arrhythmogenicity in human subjects; however, multi-scale *in silico* models of drug cardiotoxicity assessment may provide better accuracy[28,29]. The Comprehensive *In Vitro* Proarrhythmia Assay (CiPA) Initiative seeks to establish a new paradigm for early safety assessment of drugs that differentiates effects on QT interval prolongation from TdP arrhythmia generation, removing the

current constraints on drug development[27]. Additionally, certain drugs not only block hERG but exhibit secondary effects on hERG current, such as “facilitation” that increases channel current potentials close to the threshold for channel activation[30–32] and has been postulated to lower the risk for arrhythmia, complicating cardiotoxicity assessment of the drugs. This study aims to elucidate the atomic-level interactions between open and closed states of the hERG channel and various drugs as a contribution to the multi-scale *in silico* models of proarrhythmic risk.

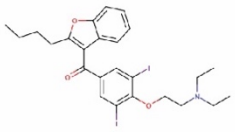
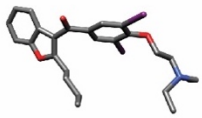
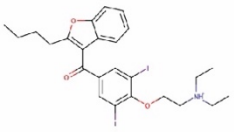
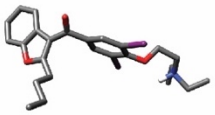
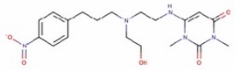
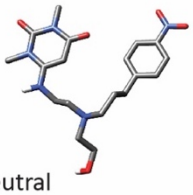
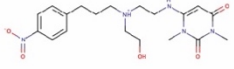
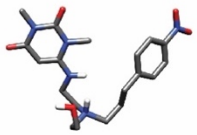
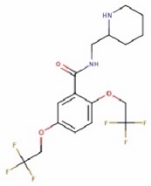
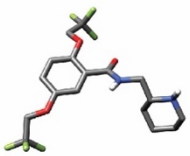
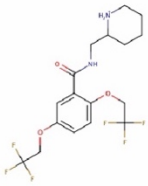
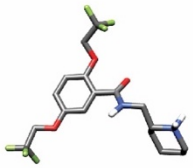
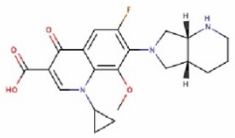
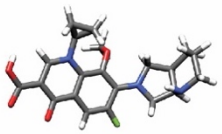
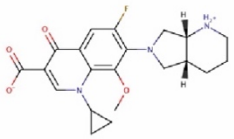
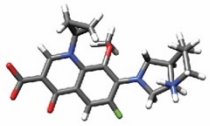
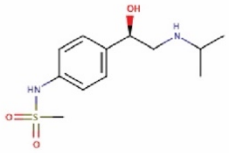
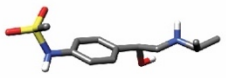
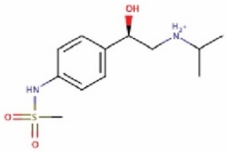
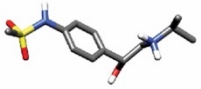
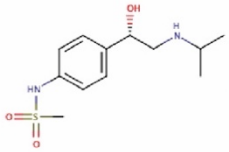
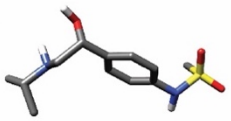
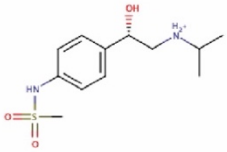
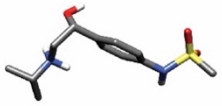
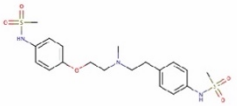
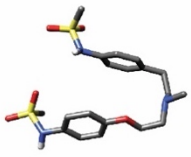
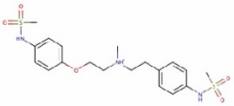
The hERG channel is homotetrameric, with each subunit composed of six transmembrane segments (S1-S6)[4]. The S1-S4 segments of each subunit form the voltage sensing domains; the S5 and S6 segments, along with intervening pore and turret helices and connecting loops, form the ion-conducting pore[33]. Several laboratories have identified molecular determinants for various drugs on the hERG channel and have shown that Y652 and F656 on the S6 segment form canonical drug binding residues[34–37], and their positioning may influence the sensitivity of hERG to certain drugs[38,39]. Additionally, the interactions between various drugs and the hERG channel are often shown to be protein conformational state dependent[40], typically showing preferential block for the open or inactivated states[41–50]. The Mackinnon laboratory solved the structure of a putatively open state of the hERG channel (PDB: 5VA2) using single particle cryo-electron microscopy at a 3.8 Å-resolution[51]. The homologous EAG1 channel’s structure in a closed state was also published by the Mackinnon laboratory (PDB: 5K7L)[52] and can be used to build a closed state model of hERG. Potentially inactivated state structures of channels homologous to hERG have not yet been resolved. Recently published hERG channel structures (PDB: 7CN0 and 7CN1) are inconclusive regarding which state they respresent[53]. Therefore, our study focused only on a putatively open and a closed state and deferred the inactivated state for later study.

In this study, we used Rosetta modeling software to study atomic-level interactions between the hERG channel in open and closed states and various drugs with high, intermediate, and low risk for arrhythmia. The open state of the wild-type hERG channel, its mutants (Y652A, F656A, and Y652A/F656A double mutant), and the closed state of the wild-type hERG channel (Figure 1) were developed as protein targets for docking studies of cationic and neutral forms of antiarrhythmic medications amiodarone, dofetilide, flecainide, nifekalant, d- and l-sotalol and the fluoroquinolone antibiotic moxifloxacin (Table 1). We selected these drugs based on their different arrhythmogenic potentials[5,27,54] and abilities to facilitate hERG current[31,55].

Amiodarone is an iodine-rich, benzofuran-based class III antiarrhythmic drug targeting several K^+ channels with low arrhythmogenic risk[54]; it is used for treating supraventricular and ventricular arrhythmias but also exhibits blocking of Na^+ channels, beta-adrenoceptors, and Ca^{2+} channels[56–60]. It is a high-affinity hERG blocker with reported IC_{50} values of ~10μM[61–65]. Experimental data suggest that F656 and Y652 in the S6 segment of hERG channels and S624 at the base of the selectivity filter (SF) play key roles in amiodarone's ability to block hERG channel[61]. Nifekalant is a pyrimidinedione-based class III antiarrhythmic drug used to treat ventricular tachycardia with low arrhythmogenic risk[30]. It is a high-affinity hERG blocker with reported IC_{50} values of ~142nM[55,66–68]. Experimental data suggest that G648, Y652, and F656 residues in the S6 segment of hERG channels and T623 and V625 at the base of the SF play key roles in nifekalant's ability to block hERG channel[30]. Flecainide is a class Ic antiarrhythmic drug derived from trifluoroethoxy-benzamide used to treat paroxysmal ventricular tachycardia with moderate arrhythmogenic potential[69–71]. Its therapeutic mechanism of action is inhibition of cardiac voltage-gated sodium channel $Na_V1.5$[72]. Flecainide is a high-affinity hERG blocker with reported

IC_{50} values of ~3μM[73–75]. Experimental data suggest that F656 in the S6 segment of hERG channels and V625 at the base of the SF play key roles in flecainide's ability to block hERG channel[74]. T623, S624, G648, and Y652 are also known to affect flecainide inhibition of the hERG channel to a lesser degree[74]. Moxifloxacin is a fluoroquinolone antibiotic drug with reportedly mixed proarrhythmic potential[71,76]. Moxifloxacin blocks hERG with reported IC_{50} values of ~36μM[77–80]. Experimental data suggest that Y652 and F656 in the S6 segment of hERG channels and S624 at the base of the SF play key roles in moxifloxacin's ability to block hERG channel[77,81–83]. Sotalol is a class II (beta-adrenergic receptor blocking) and class III (K^+ channel blocking) antiarrhythmic sulfonamide drug comprising d- and l-enantiomers[84]. Sotalol is a low-affinity binder to hERG with reported IC_{50} values of ~290μM[85–87] and associated with a high risk of arrhythmia[1,85,88,89]. Experimental data suggest that F656 and Y652 in the S6 segment of hERG channels play key roles in sotalol's ability to block hERG channel[29,84,85,88,90,91]. Dofetilide is a sulfonamide class III antiarrhythmic drug used in the treatment of ventricular arrhythmia and considered to have high proarrhythmic risk[27,54]. Dofetilide binds hERG channel in a state-dependent manner with a 70-fold affinity for an inactivated state and reported IC_{50} values for an open-state block of 3.5-11 μM[41,43,92]. Experimental data suggest that F656 is a molecular determinant of high affinity binding (IC_{50} is ~75nM)[42,86,93] and plays a key role in dofetilide's ability to block hERG channel[41,42,44]. Additionally, the pore helix (T623, S624, and V625) and S6 domain (G648, Y652, and V659) residues are known from alanine-scanning mutagenesis studies to reduce block of methanesulfonanilide drugs such as dofetilide[88,94–96].

Table 1 Drug structures and ionization states

Name	pKa	Chemical Structure	3D Structure	%	Chemical Structure	3D Structure	%
Amiodarone	6.56		 Neutral	87		 Cationic	13
Nifekalant	9.8		 Neutral	24		 Cationic	76
Flecainide	6.3, 9.3		 Neutral	1		 Cationic	99
Moxifloxacin	9.3		 Neutral	1		 Zwitterionic	92
d-Sotalol	9.8		 Neutral	<1		 Cationic	>99
l-Sotalol	9.8		 Neutral	<1		 Cationic	>99
Dofetilide	7		 Neutral	72		 Cationic	28

Our results reveal key similarities and differences between various drugs interactions with wild-type and mutant hERG channel in an open and closed states and provide useful structural insights into molecular mechanisms of drug action and their pro-arrhythmia proclivities.

2.2 Materials and Methods

2.2.1 Rosetta modeling of hERG in open and closed states.

We used the Rosetta structural modeling software[97,98] and the cryo-EM structures of a putatively open-state hERG (PDBID: 5VA2)[51] and a closed-state EAG1 (PDBID: 5K7L)[52] as templates (Figure 1). Each structure was passed through the cryo-EM refinement protocol in Rosetta (Appendix I.A)[19]. The lowest scoring density-refitted models were then used in RosettaCM[14,99] to model the channel (XML protocol in Appendix I.B). We generated 10,000 structural models of both open and closed state and selected the top 1000 from each for clustering analysis (described below). The lowest energy structures were visually inspected before being selected for the docking study. UCSF Chimera's rotamer tool was used to make the F656A, Y652A, and Y652A/F656A mutations based on the final wild-type open-state model.

2.2.2 RosettaLigand modeling of hERG interaction with drugs.

We obtained the molecular structures of each drug from the ZINC[100] and PubChem[101] databases. OpenEye OMEGA (OpenEye Scientific Software) was used to generate conformers for the drugs[102]. At a physiological pH 7.4, each drug exists in a dominant ionized (cationic or zwitterionic) form. However, because the drug receptor site in the pore lumen region is hydrophobic, this may shift the ionization equilibrium. This indicates that we need to study both ionized and neutral forms of each drug when analyzing interactions with the hERG channel (Table 1)[56,103–109].

To uniformly and efficiently sample the pore region, drugs were placed at 10 different initial locations spanning the top and bottom of the pore lumen region and the four fenestration regions. As part of the standard Rosetta docking protocol, we set the initial random perturbation to a translation distance less than 5Å and the sampling radius to 5Å (XML protocol in Appendix I.C). The details of the RosettaLigand docking algorithm have been described previously[21,99,110]. A total of 100,000 docking models were generated for each drug and each protein. The top 10,000 were selected based on the total_score of the protein-ligand complex and then ranked by ligand binding energy represented by the Rosetta interface_delta_X score term. The top 50 most favorable interface score models were visually analyzed using Chimera[111]. The representative poses were further analyzed using the Protein-Ligand Interaction Profiler (PLIP)[112] web service. We performed T-tests on our docking results to determine if there is a significant difference between the means of the top 50 poses of open-state WT hERG model and the open-state models of Y652A and F656A mutants and the closed-state model.

2.2.3 Clustering analysis of ligand docking.

Clustering analysis of docking results was done in R Studio by calculating a similarity matrix between all top 50 poses clustered based on a cutoff parameter and minimum cluster size parameter (Table 2) using equation (1) where *z* is drug center of mass (COM) position with respect to hERG SF C_α COM along the *z*-axis, *l* is length of the vector between end-point atoms of a drug molecule, and Φ is the polar angle away from the z-axis (Appendix I.D).

$$Similarity = \sqrt{\left(\frac{z-z_{ref}}{z_{max}-z_{min}}\right)^2 + \left(\frac{l-l_{ref}}{l_{max}-l_{min}}\right)^2 + \left(\frac{\Phi-\Phi_{ref}}{\Phi_{max}-\Phi_{min}}\right)^2} \quad (1)$$

This ensures invariance to the rotation around the z-axis and, therefore, can account for the 4-fold symmetry of the hERG channel. Unique clusters were then identified using K-means optimization initialized using the lowest interface score structures from each cluster as the cluster centers (Appendix I.E).

Table 2 Clustering Parameters

Drug	Similarity Cutoff	Minimum Cluster Size	Endpoint Atoms
Amiodarone	0.1	3	C2, C22
Nifekalant	0.1	3	O2, O3
Flecainide	0.05	3*	C9, C16
Moxifloxacin	0.01	3	C11, O3
d-Sotalol	0.05	3	C8, C9
l-Sotalol	0.05	3	C8, C9
Dofetilide	0.1	3	S1, S2
* Neutral flecainide docked to closed-state hERG channel used a minimum cluster size of 2.			

Percentage within hydrophobic pocket calculated by proportion of poses of the top 50 models of each docking simulation with at least one atom positioned at or in the hydrophobic pocket of the hERG channel as visualized for each pose in Chimera (Figure 2). Percentage within closed pore of hERG channel calculated by counting number of poses of top 50 models for each docking simulation that are fully encapsulated within hERG channel pore or fenestration region (Figure 8).

2.3 Results and Discussion

2.3.1 Rosetta modeling of hERG in open and closed states

We used the Rosetta structural modeling software[97,98] and the cryo-EM structures of a putatively open-state hERG[51] and a closed-state EAG1[52] channels (Figure 1 Panel A) as templates for generating open and closed state hERG models, respectively, as described in Methods. Comparison of the selectivity filters (SF) of the open and closed state models to those of the original cryo-EM structures (Figure 1 Panel B) show 0.6Å and 0.7Å root-mean-square deviations (RMSD), respectively. There is a 0.4Å RMSD between the open and closed state SFs. HOLE (Figure 1 Panel C) estimates the maximal pore radius of the open and closed states to be ~4.2Å and ~2.8Å, respectively, with greatest constriction of the pore due to positioning of Y652

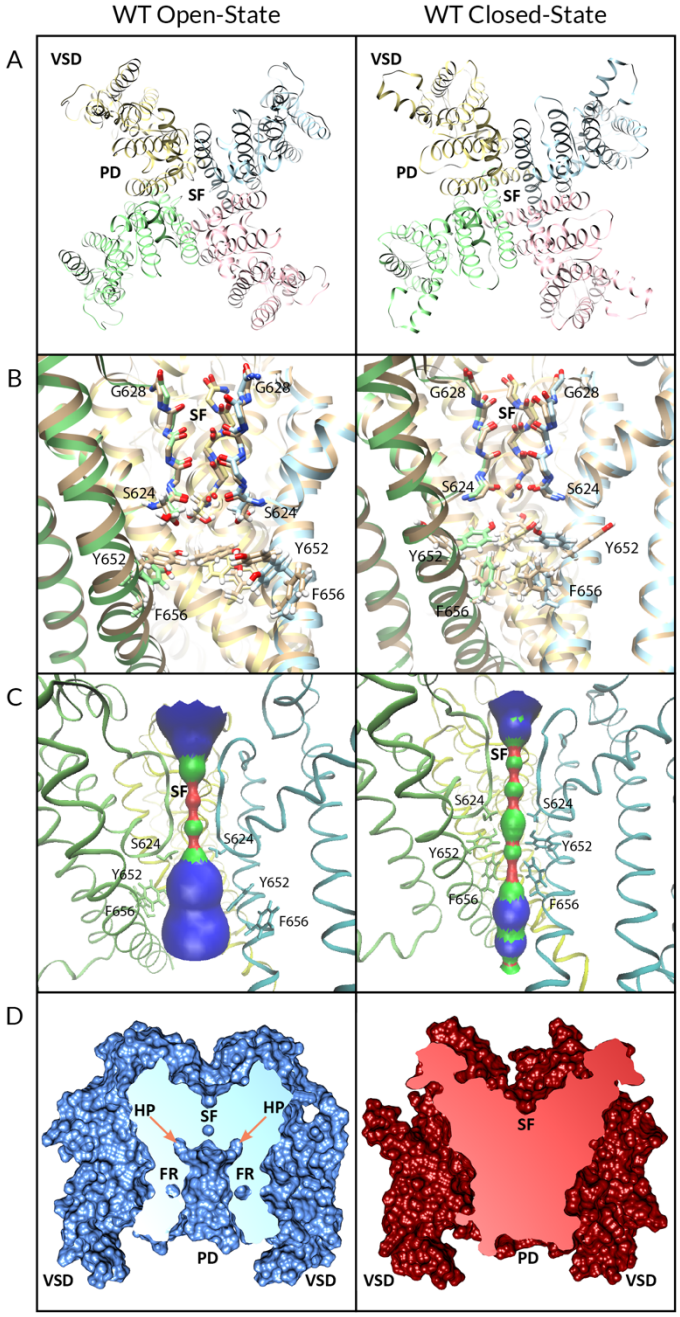


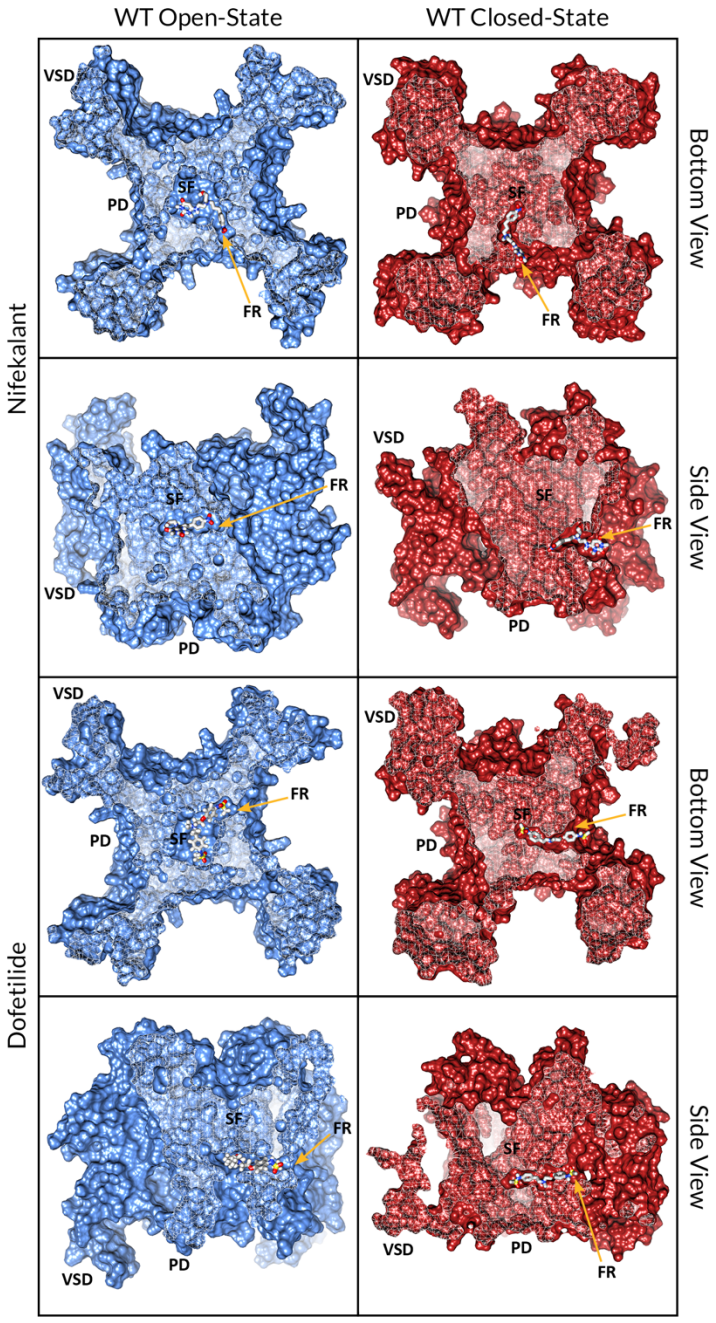
Figure 3 Open- and Closed-State hERG Models

Models of wild-type open state (Panel A) and closed state (panel B) hERG channel. Row A is top-down view channel. Row B compares pore domains of cryo-EM refined models (multi-colored) to (left) published hERG structure (gold, PDB: 5VA2) and (right) closed state hERG homology model (gold, PDB: 5K7L). Row C is HOLE profile of pore volume. Chain A is pink, Chain B is blue, Chain C is yellow, and chain D is green. Row D is a depiction of the protein surface cross-section in Chimera.

and F656. Cross sections of the protein surface (Figure 1 Panel D) by Chimera were unable to detect any accessible pore in the closed state of the hERG channel–although modeling indicates it can still accommodate certain drugs (See Figure 8 and Supplement Figure 13)–but revealed the wide pore and deep hydrophobic pockets that extend up from the pore at the base of the open-state hERG SF.

Figure 4 Bottom and Side Views of hERG Channel with Drug-Induced Fenestrations

Surface representations of open (blue) and closed (red) state WT hERG structures with fenestration regions (FR) available because of drug (white) docking interactions.



To study binding of various drugs with high (d/l-sotalol, dofetilide), intermediate (flecainide, moxifloxacin), and low (amiodarone, nifekalant) risks for arrhythmia (Table 2) to the hERG channel pore in the open and closed states, we used RosettaLigand as described in Methods. Protein-Ligand Interaction Profiler (PLIP) analysis was used to identify the hydrophobic interactions, the hydrogen and halogen bonds, and the π-π and cation-π interactions in top Rosetta Models.

2.3.2 Open-State WT hERG–Drug Interactions

We first studied open-state WT hERG interactions with neutral (Figure 2) and cationic or zwitterionic (Supplement Figure 1) drugs. Every drug pose was positioned in the center of the pore, below the selectivity filter (SF) adjacent to Y652. Variabilities between drug poses are characterized by the frequency and depth of positioning within the hydrophobic pocket (Figure 2 and Supplement Figure 1 Panels A-B, Table 2), and the variation in key binding residues of top clusters (Figure 2 and Supplement Figure 1 Panels C-D, Table 2). Rosetta-predicted interface scores cannot be compared between different drugs. Notably, there are four deep hydrophobic pockets extending from the central pore cavity up behind the selectivity filter between the S6 helix and the pore helix and formed by residues T623, S624, V625, G648, and Y652 as identified in the original hERG structure by Wang and MacKinnon[51]. These deep hydrophobic pockets are open and available to drugs in this putatively open-state WT hERG model and disappear in the closed-state WT hERG model (Figure 1D).

Amiodarone. The most frequently sampled, lowest binding energy RosettaLigand poses of neutral (Figure 2) and cationic amiodarone (supplementary Figure 1)both interact with the WT and mutant open state hERG channel model pores in the region above F656 in the S6 segment and below the SF, with cationic amiodarone in an inverted orientation when compared to neutral amiodarone. For neutral amiodarone, 41 of the top 50 poses converged on a similar ligand orientation characterized by (1) the benzofuranyl group protruding up into the hydrophobic pocket, (2) an iodine on the benzene ring forming a halogen bond with S649 on the S6 segment, and (3) the butyl and ethyl groups at each end forming hydrophobic interactions with Y652 on opposing chains (Figure 2). For cationic amiodarone, 27 of the top 50 poses converged to a single

Table 3 Summary of Docking Results

Drug	Ion. St.	WT - Open State						Y652A			F656A			Y652A/F656A		
		Top Cluster Size and I.E.	Top Cluster Key Residues	Top Cluster Pore Regions	Second Cluster Size and I.E.	Second Cluster Key Residues	Second Cluster Pore Regions	Top Cluster Size and I.E.	Top Cluster Key Residues	Top Cluster Pore Regions	Top Cluster Size and I.E.	Top Cluster Key Residues	Top Cluster Pore Regions	Top Cluster Size and I.E.	Top Cluster Key Residues	Top Cluster Pore Regions
Amiodarone	0	41 -19.2 R.E.U.	S624 S649 Y652	SF base hydrophobic pocket	n/a	n/a	n/a	28 -15.9 R.E.U.	F557, S649 A652, A653 I655, F656	fenestration	36 -18.3 R.E.U.	S624 Y652	SF base hydrophobic pocket	45 -15.2 R.E.U	S624 A653 A656	SF base hydrophobic pocket
	+1	27 -17.7 R.E.U.	L622, S624 V625, Y652	SF base hydrophobic pocket	16 -16.5 R.E.U.	S624 Y652	SF base hydrophobic pocket	31 -15.6 R.E.U.	S624 A652 F656	SF base hydrophobic pocket	23 -17.7 R.E.U.	S624 Y652	SF base hydrophobic pocket	30 -13.6 R.E.U	S624 A652	SF base hydrophobic pocket
Nifekalant	0	13 -18.1 R.E.U.	S624 S649 Y652	SF base	6 -16.5 R.E.U.	S624 Y652	SF base hydrophobic pocket	8 -15.0 R.E.U.	F557, L622 S624, A652 I655, F656	SF base fenestration	17 -21.5 R.E.U.	L622.S624 S649, Y652	SF base	11 -15.5 R.E.U	F557, S624 S649, A652	SF base fenestration
	+1	14 -16.9 R.E.U.	S649 Y652	SF base hydrophobic pocket	12 -16.7 R.E.U.	S624, S649 Y652, A653	SF base	24 -15.2 R.E.U.	S624 S649	SF base	26 -18.5 R.E.U.	S649 Y652	SF base hydrophobic pocket	11 -14.8 R.E.U	F557 S624 A652	SF base fenestration
Flecainide	0	10 -9.6 R.E.U.	T623 Y652	SF base hydrophobic pocket	8 -10.2 R.E.U.	S649 Y652	hydrophobic pcoket	22 -9.9 R.E.U.	S624, S649 A652, F656	SF base	9 -11.5 R.E.U.	Y652 A653	SF base hydrophobic pocket	28 -9.1 R.E.U	S624 S649 A656	SF base
	+1	9 -11.1 R.E.U.	S624 S649 Y652	SF base hydrophobic pocket	6 -10.8 R.E.U.	L622 S649 Y652	hydrophobic pocket	25 -12.1 R.E.U.	S624, S649 A653, F656	SF base	13 -9.9 R.E.U.	T623, S624 S649, Y652	SF base hydrophobic pocket	37 -9.8 R.E.U	S624 A652 A656	SF base
Moxifloxacin	0	16 -12.7 R.E.U.	L622, S624 S649, Y652	SF base hydrophobic pocket	10 -13.2 R.E.U.	S624 S649 Y652	SF base hydrophobic pocket	21 -10.8 R.E.U.	S624, S649 A652, F656	SF base	14 -13.0 R.E.U.	L622, S624 S649, Y652	SF base hydrophobic pocket	12 -10.2 R.E.U	S624 S649 A654	SF base
	Z	27 -13.3 R.E.U.	S624 S649 Y652	SF base hydrophobic pocket	8 -12.5 R.E.U.	S624, S649 Y652, A653	SF base hydrophobic pocket	26 -11.9 R.E.U.	S649, A653 Y652, S660	central cavity only	21 -13.2 R.E.U.	S624 S649 Y652	SF base	37 -10.5 R.E.U	T623, S624 G648, S649 A652	SF base hydrophobic pocket
d-Sotalol	0	14 -12.6 R.E.U.	S624 Y652	SF base	8 -11.0 R.E.U.	S624 S649 Y652	SF base	13 -9.9 R.E.U.	S624, S649 A652, F656	SF base	11 -13.3 R.E.U.	S624 Y652	SF base	10 -9.8 R.E.U	T623, S624 S649, A652	SF base hydrophobic pocket
	+1	12 -13.0 R.E.U.	T623 S624 Y652	SF base	8 -13.0 R.E.U.	T623, S624 S649, Y652	SF base hydrophobic pocket	8 -10.1 R.E.U.	S624 S649	SF base	13 -13.3 R.E.U.	S624, V625 M645, S649 Y652	SF base hydrophobic pocket	14 -9.9 R.E.U	S624 S649	SF base
l-Sotalol	0	9 -11.5 R.E.U.	S624 S649 Y652	SF base	5 -11.2 R.E.U.	S624 S649 Y652	SF base	13 -10.1 R.E.U.	T623, S624 A653, F656	SF base	14 -12.2 R.E.U.	T623 S624 Y652	Sf base	27 -9.5 R.E.U	S649 A652	central cavity only
	+1	8 -12.0 R.E.U.	T623, S624 V625, Y652	SF base hydrophobic pocket	4 -12.3 R.E.U.	S624, S649 Y652, A653	SF base	7 -11.2 R.E.U.	S624, V625 A652, F656	Sf base hydrophobic pocket	15 -13.9 R.E.U.	T623, S624 V625, Y652	SF base hydrophobic pocket	37 -10.2 R.E.U	S649 A652 A653	central cavity only
Dofetilide	0	15 -14.1 R.E.U.	T623 S624 Y652	SF base	11 -13.8 R.E.U.	S624 S649 Y652	SF base	14 -12.9 R.E.U.	S624 A654 F656	SF base	9 -14.7 R.E.U.	S624 Y652	SF base	10 -10.7 R.E.U	T623, S624 S649, A652	SF base
	+1	11 -13.4 R.E.U.	S624 Y652	SF base	11 -13.1 R.E.U.	S624 S649 Y652	SF base	19 -11.3 R.E.U.	T623, S624 S649, A652	SF base	11 -13.9 R.E.U.	T623 S624 Y652	SF base	11 -10.5 R.E.U	L622 A652	SF base

Figure 5 Open-State WT hERG Interactions with Neutral Drugs

Rosetta docking results of wild-type (WT) hERG in open state with neutral forms of each drug. Chain A is colored pink, chain B is blue, chain C is yellow, chain D is green, and ligand C atoms are gray. Top pose from largest and second largest clusters are in stick form and are orange and dark green, respectively. O atoms – red, N – blue, S – yellow, I – violet. H atoms not shown for clarity. Panel A shows the surface of pore-lining residues colored by hydrophobicity. Panels A and B show top 50 poses in gray sorted by lowest interface score. Panels C and D highlight representative poses identified as the lowest energy pose from the largest and second largest cluster, respectively. PLIP-identified interactions are indicated by dashed lines. Halogen bonds are colored green, hydrogen bonds in blue, cation-pi in pink, pi-stacking in yellow, hydrophobic interactions in pale purple.

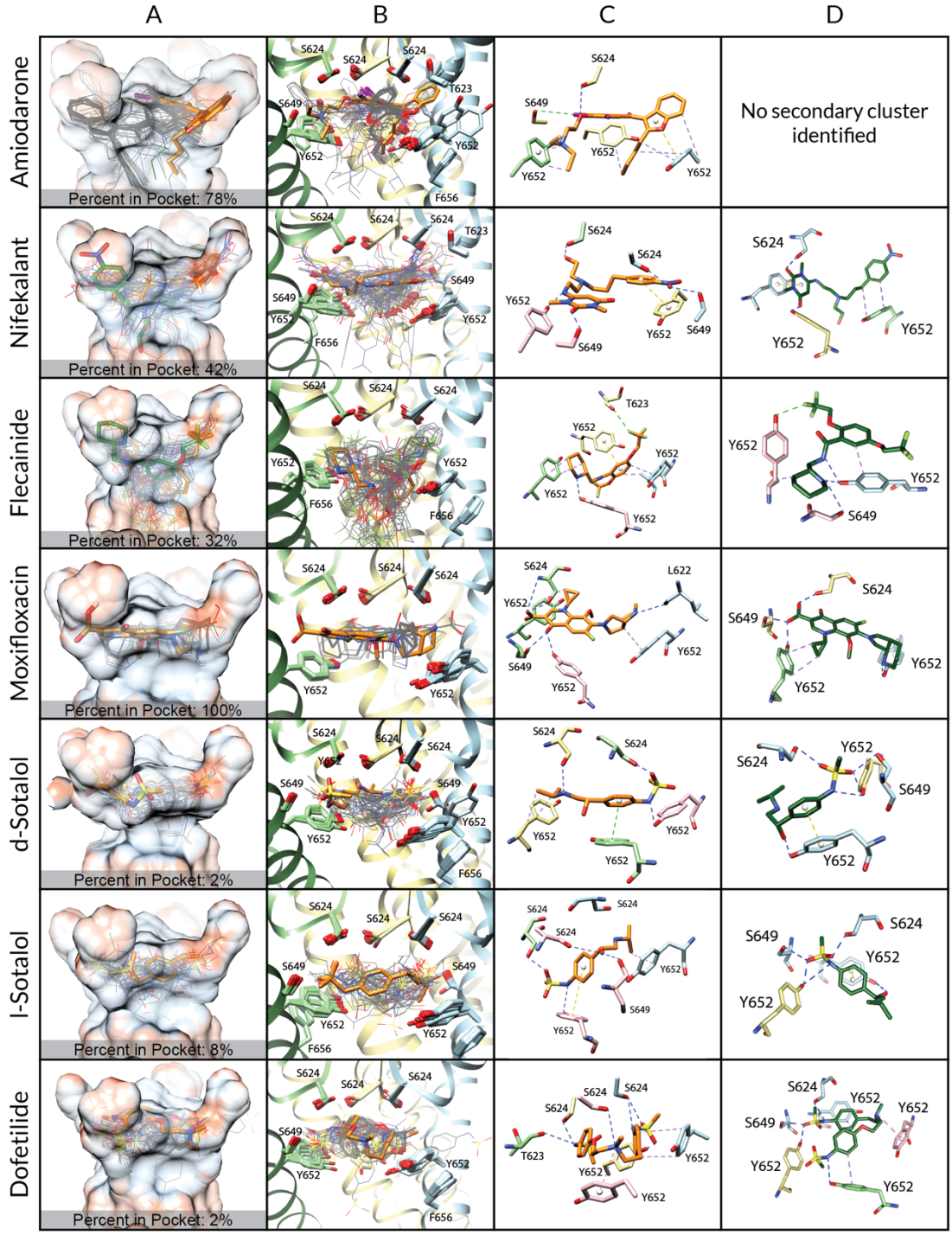
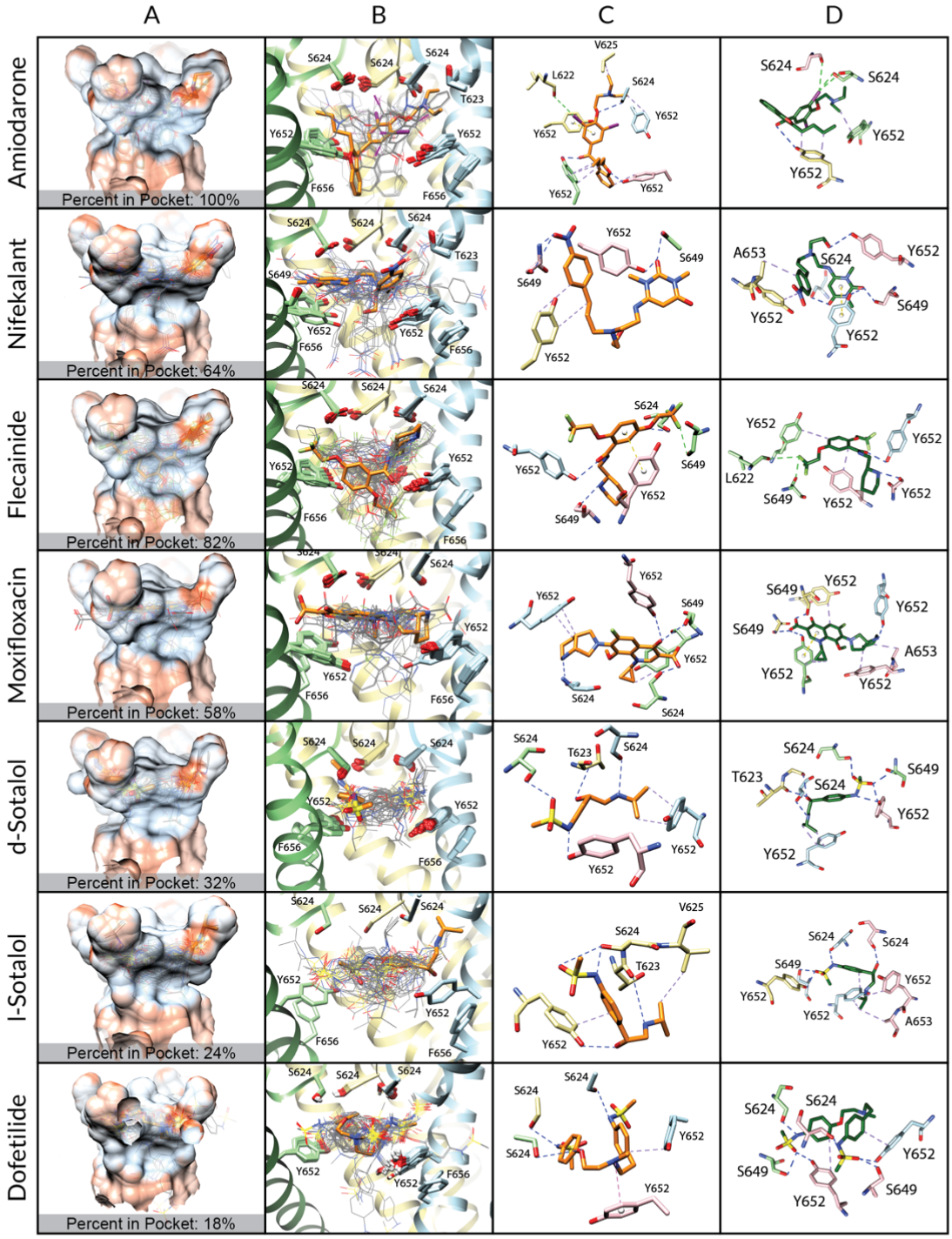


Figure 6 Open-State WT hERG Interactions with Cationic and Zwitterionic Drugs

Rosetta docking results of wild-type (WT) hERG in open state with cationic or zwitterionic forms of each drug. Chain A is colored pink, chain B is blue, chain C is yellow, chain D is green, and ligand C atoms are gray. Top pose from largest and second largest clusters are in stick form and are orange and dark green, respectively. O atoms – red, N – blue, S – yellow, I – violet. H atoms not shown for clarity. Panel A shows the surface of pore-lining residues colored by hydrophobicity. Panels A and B show top 50 poses in gray sorted by lowest interface score. Panels C and D highlight representative poses identified as the lowest energy pose from the largest and second largest cluster, respectively. PLIP-identified interactions are indicated by dashed lines. Halogen bonds are colored green, hydrogen bonds in blue, cation-pi in pink, pi-stacking in yellow, hydrophobic interactions in pale purple.



cluster characterized by (1) the diethylammonium group protruding into the hydrophobic pocket, (2) an iodine on the benzene ring forming a halogen bond with L622 on the pore helix near the base of the SF, (3) a π-stacking interaction between Y652 on one chain and the benzene ring of amiodarone, and (4) hydrophobic interactions between Y652 on multiple chains and the benzofuranyl and diethylammonium moieties of amiodarone (supplementary Figure 1). A second, smaller cluster was identified for cationic amiodarone that resembles the top cluster of neutral amiodarone.

Nifekalant. For neutral nifekalant, 13 of the top 50 RosettaLigand poses converged on a similar ligand orientation characterized by (1) π-stacking between the phenyl group and Y652, and (2) various hydrogen bonds between residues S624, S649, and Y652 and the pyrimidinedione, nitrophenyl, and hydroxyethylamino groups (Figure 2). A second, smaller cluster converged on a pose similar to the top cluster for cationic nifekalant where the nitrophenyl group protruded further up into the hydrophobic pocket (Figure 2). For cationic nifekalant, 14 of the top 50 poses converged on a similar ligand orientation characterized by (1) the nitrophenyl group protruding up into the hydrophobic pocket, (2) hydrogen bonds between S649 and Y652 sidechain residues on multiple chains and the oxygen atoms of the nitrophenyl and pyrimidinedione moieties, and (3) hydrophobic interactions between Y652 and the phenyl group (supplementary Figure 1). A second, smaller cluster converged on a pose where the pyrimidinedione moiety remained in the space between Y652 and S624 while the nitrophenyl group dipped down further into the pore rather than into the hydrophobic pocket.

Flecainide. The most frequently sampled, lowest binding energy RosettaLigand poses of neutral (Figure 2) and cationic (supplementary Figure 1) flecainide both interact with the WT and

mutant open state hERG channel model pores in the region below the SF and extending down towards F656A. For neutral flecainide, 10 of the top 50 poses converged on a similar ligand orientation characterized by (1) π-stacking between the benzene and Y652, (2) hydrogen bonding between Y652 and the piperidine group, (3) hydrophobic interactions between multiple Y652 residues and both the benzene and piperidine groups of flecainide, and (4) a halogen bond between T623 and a fluorine atom on one trifluoroethoxy group (Figure 2). A second, smaller cluster converged on a pose similar to the top cluster for cationic nifekalant where the piperidine group protruded further up into the hydrophobic pocket. For cationic flecainide, 9 of the top 50 poses also converged on a similar ligand orientation characterized by (1) the piperidine group protruding up into the hydrophobic pocket, (2) hydrogen bonds between S624, S649, and Y652 sidechain residues on multiple chains and the oxygen atoms of the trifluoroethoxy and amide moieties and the nitrogen of the piperidine group, (3) π-π stacking between Y652 and the benzamide group, and (4) a halogen bond between S649 and a fluorine atom on one trifluoroethoxy group (Supplemental Figure 1).

Moxifloxacin. The most frequently sampled, lowest binding energy RosettaLigand poses of neutral (Figure 1) and zwitterionic (Supplemental Figure 1) moxifloxacin interact with the WT and mutant open state hERG channel model pores in the region below the SF while only some poses of the zwitterionic form extend down towards F656A. For neutral moxifloxacin, 16 of the top 50 poses converged on a similar ligand orientation characterized by (1) hydrogen bonding between both sidechain atoms on L622, S624, S649, and Y652 and backbone atoms on S624 and various positions on moxifloxacin and (2) hydrophobic interactions between multiple Y652 residues and the cyclopropyl and pyrrolo-pyridine groups of moxifloxacin (Figure 2). Several

smaller clusters similar to the largest cluster are positioned in the same region of the pore between the SF and Y652. For zwitterionic moxifloxacin, 27 of the top 50 poses also converged on a very similar ligand orientation characterized by hydrogen bonding between both sidechain atoms on S624, S649, and Y652 and backbone atoms on S624 various positions on moxifloxacin and (2) hydrophobic interactions between multiple Y652 residues and the cyclopropyl and pyrrolo-pyridine groups of moxifloxacin (supplementary Figure 1). Thirteen of the top 50 poses of zwitterionic moxifloxacin extend into the fenestration region.

Sotalol. The most frequently sampled, lowest binding energy RosettaLigand poses of neutral (Figure 2) and cationic (supplementary Figure 1) d- and l-sotalol both interact with the WT open state hERG channel pore in the region below the SF and above Y652. There is little convergence of sotalol poses in the pore lumen for both WT and mutant poses, most likely due to sotalol being a low-affinity binder[85,88]. Neutral d- and l-sotalol (Figure 2) remained centered in the open WT pore forming hydrogen bonds and hydrophobic interactions with S624 and Y652 on multiple chains as well as a π-π stacking interaction between Y652 and the phenyl group of neutral sotalol. The 2-propylammonium group of the cationic l-sotalol top cluster and a secondary cluster of cationic d-sotalol (supplementary Figure 1) reach into the hydrophobic pocket interacting with T623 and V625. For neutral and cationic l-sotalol, the 2-propylamine group also interacted with the hydrophobic pocket of the Y652A mutant and formed hydrophobic interactions with T623 and V625, respectively. Two of the top 50 poses of cationic l-sotalol extend into the fenestration region.

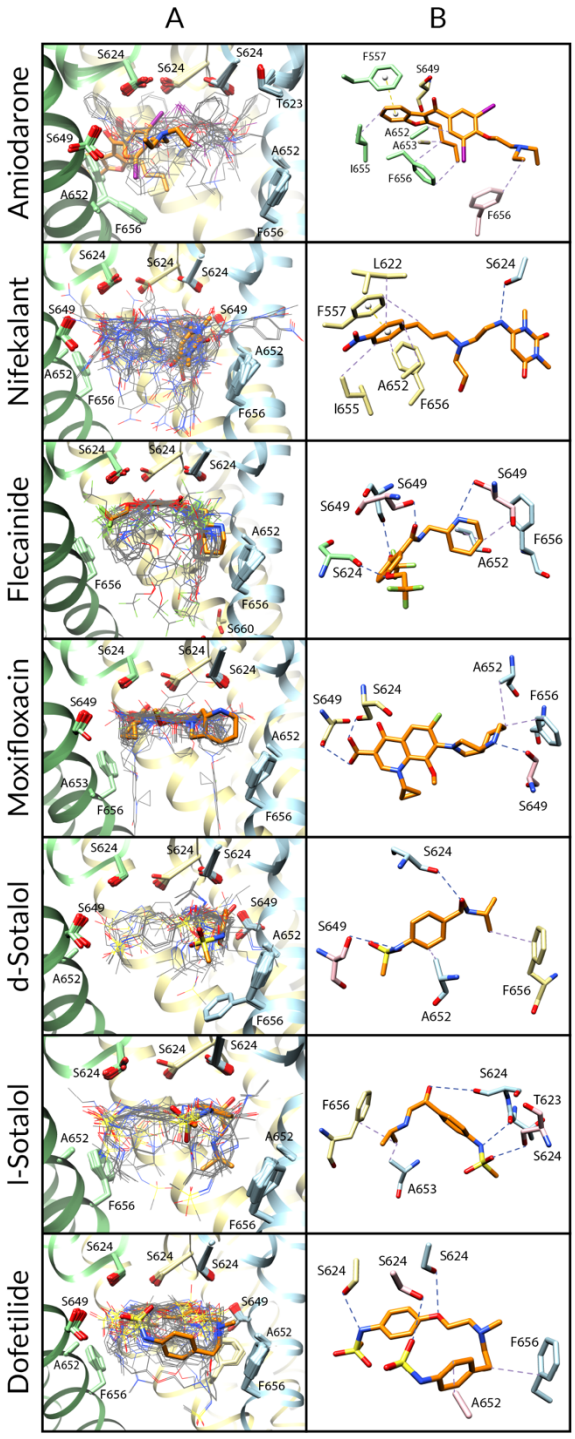
Dofetilide. The most frequently sampled, lowest binding energy RosettaLigand poses of neutral (Figure 2) or cationic (supplementary Figure 1) dofetilide both interact with the WT and

mutant open state hERG channel model pores in the region above Y652 and below the SF. For neutral dofetilide, 15 of the top 50 poses converged on a similar ligand orientation characterized by (1) cation-π interactions between the central methylamine of dofetilide and Y652, and (2) various hydrogen bonds between residues T623 and S624 of multiple chains and the oxygen of the phenoxy and the nitrogen of the sulfonamide moieties and the central methylamine (Figure 2). Residues T623 and S624 are demonstrated to affect high affinity binding of drugs[37,113]. An additional four clusters converged in the exact same region of the pore and in similar orientations. For cationic dofetilide, 11 of the top 50 poses converged on a very similar orientation as the top cluster for neutral dofetilide along with the several other identified clusters (Supplementary Figure 1). One cluster of cationic dofetilide did have one sulfonamide group protruding into the hydrophobic pocket while 2 of the top 50 poses extended into the fenestration region.

Summary. Top clusters of amiodarone, nifekalant, and flecainide are frequently (32-78% of poses) and deeply positioned within the hydrophobic pocket. All moxifloxacin poses are positioned at the opening of the hydrophobic pocket while very few poses (2-8%) of d/l-sotalol and dofetilide are positioned in the hydrophobic pocket. We hypothesize that drug binding within the hydrophobic pocket may allosterically affect channel gating by affecting the closure of the S6 helix bundle. This pattern of interaction with the hydrophobic pocket is consistent with experimental data suggesting facilitating drugs may act as a wedge to bias hERG channel equilibrium towards the open state and increase hERG current amplitude in response to low-voltage depolarization[30,32,55].

Figure 7 Open-State Y652A hERG Interactions with Neutral Drugs

Rosetta docking results of Y652A hERG channel mutant in open state with neutral forms of each drug. Chain A is colored pink, chain B is blue, chain C is yellow, chain D is green, and ligand C atoms are gray. Top pose from largest and second largest clusters are in stick form and are orange and dark green, respectively. O atoms – red, N – blue, S – yellow, I – violet. H atoms not shown for clarity. Panel A shows top 50 poses in gray sorted by lowest interface score. Panel B highlights representative poses identified as the lowest energy pose from the largest and second largest cluster, respectively. PLIP-identified interactions are indicated by dashed lines. Halogen bonds are colored green, hydrogen bonds in blue, cation-pi in pink, pi-stacking in yellow, hydrophobic interactions in pale purple.

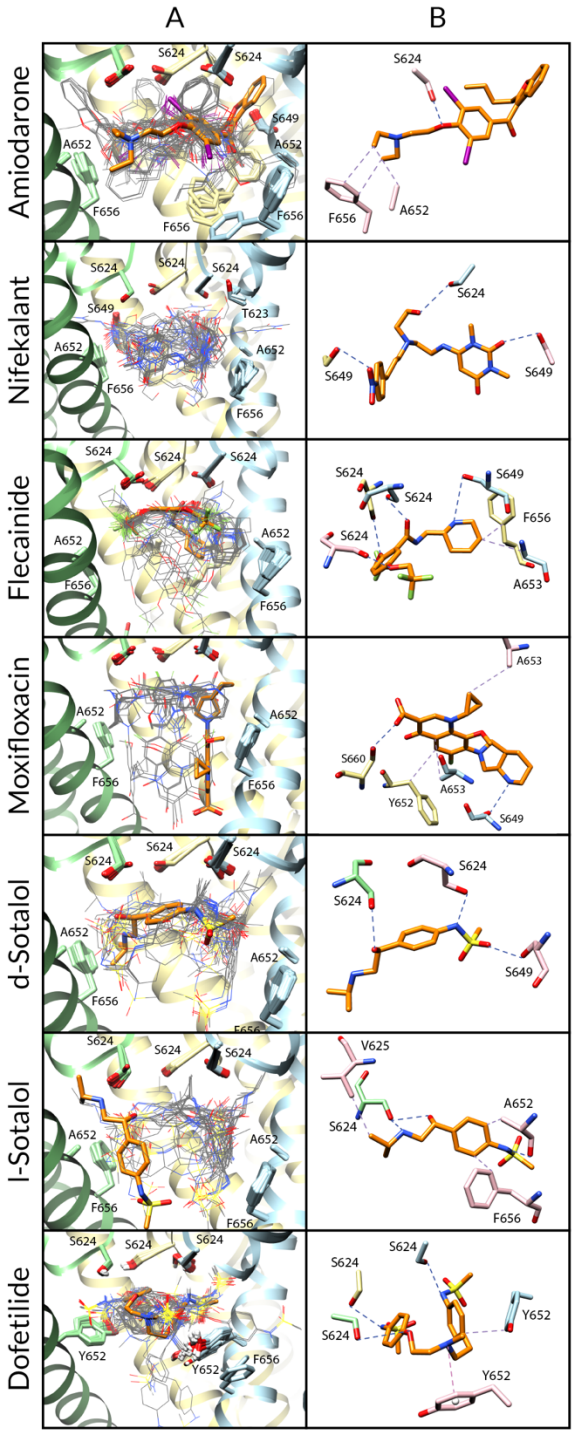


2.3.3 Open-State Mutant hERG-Drug Interactions

hERG residues Y652 and F656 residues on the S6 helix are known key determinants of drug binding in the hERG channel pore (Figure 1). Mutations of these residues would be expected to decrease binding affinity to hERG for each ligand. Rosetta interface scores are ligand-specific and cannot be compared across different drugs[8], but can be compared across various protein states. Interface scores (Table 3) show drug interactions with open state WT hERG to be lower (more favorable) than the Y652A mutants which could explain experimental data showing less potent IC50 values for Y652A hERG[25,30,35,37,37,40,41,43,44,49,66,85,90,95,114]. In disagreement with experimental data, interface scores for nifekalant, neutral flecainide, neutral

Figure 8 Open-State Y652A hERG Interactions with Cationic and Zwitterionic Drugs

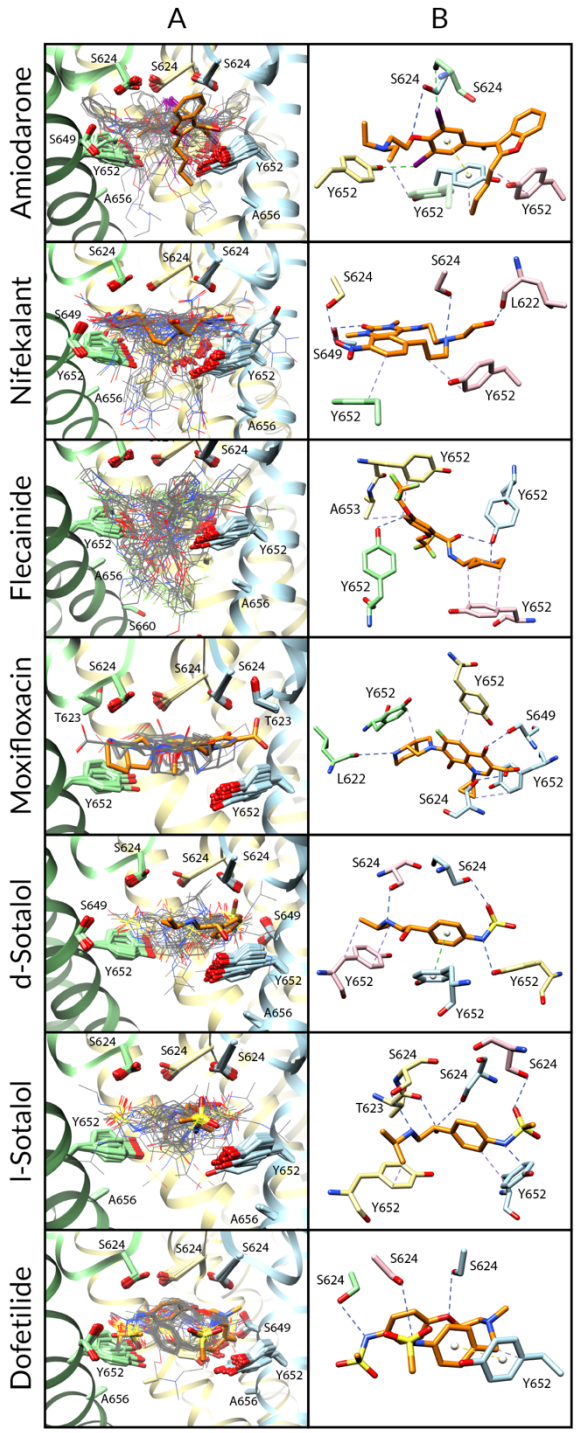
Rosetta docking results of Y652A hERG channel mutant in open state with cationic or zwitterionic forms of each drug. Chain A is colored pink, chain B is blue, chain C is yellow, chain D is green, and ligand C atoms are gray. Top pose from largest and second largest clusters are in stick form and are orange and dark green, respectively. O atoms – red, N – blue, S – yellow, I – violet. H atoms not shown for clarity. Panel A shows top 50 poses in gray sorted by lowest interface score. Panel B highlights representative poses identified as the lowest energy pose from the largest and second largest cluster, respectively. PLIP-identified interactions are indicated by dashed lines. Halogen bonds are colored green, hydrogen bonds in blue, cation-pi in pink, pi-stacking in yellow, hydrophobic interactions in pale purple.



moxifloxacin, d-sotalol, neutral l-sotalol, and dofetilide interacting with the F656A mutant hERG model tended to be more favorable than with the open-state WT hERG model. This may be due to the inability of Rosetta docking protocols to sample local and allosteric conformational changes upon drug binding within and near the receptor site formed by F656 and entropic contributions of F656 to ligand binding, as supported by molecular dynamic studies which reveal possible roles of F656 in ligand ingress to the binding pocket within the pore and indirect interactions through π-π stacking with Y652. Alternatively, high-affinity ligand binding is state-dependent[47] and this particular putatively-open substate of an F656A mutant hERG channel may

Figure 9 Open-State F656A hERG Interactions with Neutral Drugs

Rosetta docking results of F656A hERG channel mutant in open state with neutral forms of each drug. Chain A is colored pink, chain B is blue, chain C is yellow, chain D is green, and ligand C atoms are gray. Top pose from largest and second largest clusters are in stick form and are orange and dark green, respectively. O atoms – red, N – blue, S – yellow, I – violet. H atoms not shown for clarity. Panel A shows top 50 poses in gray sorted by lowest interface score. Panel B highlights representative poses identified as the lowest energy pose from the largest and second largest cluster, respectively. PLIP-identified interactions are indicated by dashed lines. Halogen bonds are colored green, hydrogen bonds in blue, cation-pi in pink, pi-stacking in yellow, hydrophobic interactions in pale purple.

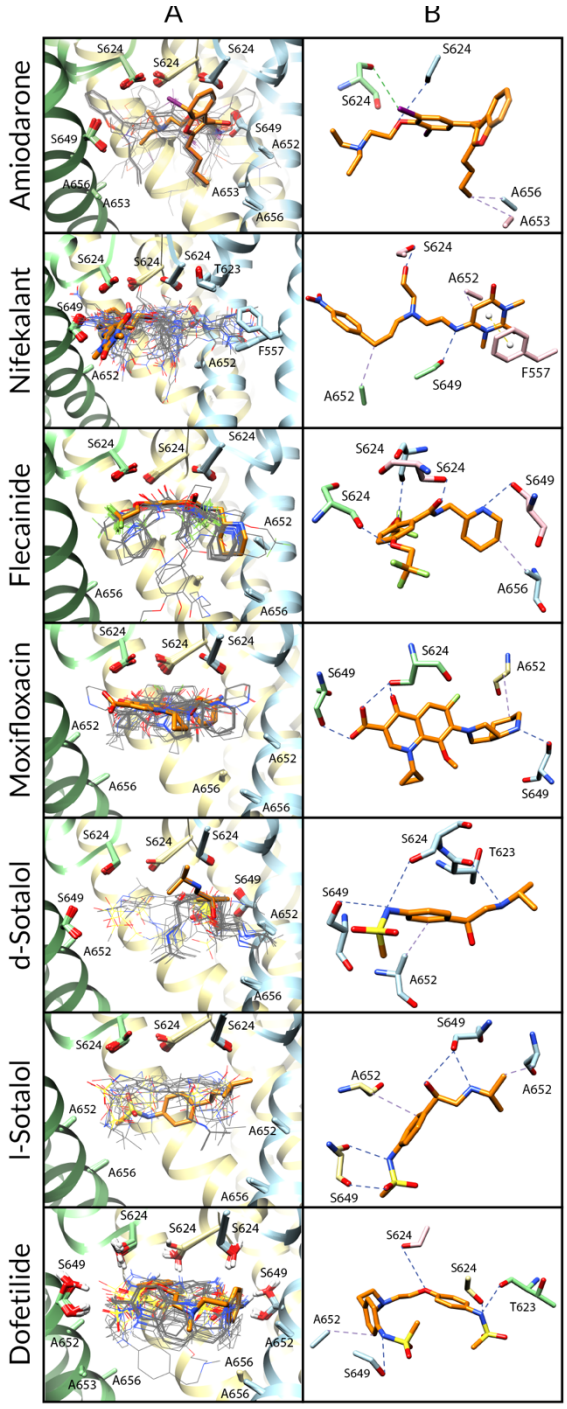


allow for more favorable binding than what the spectrum of hERG states and experimental evidence may reveal.

Amiodarone. Neutral and cationic amiodarone remained in a similar region within the hERG pore located between F656 and the base of the SF for all the hERG channel mutants (Figure 3-5). For neutral amiodarone complexes with the hERG F656A and Y652A/F656A mutants, the top cluster poses revealed the benzofuranyl group protruding into the hydrophobic pocket with the butyl and diethylamino groups forming various hydrophobic contacts with Y652, F656, and A653 (Figures 4-5). The top cluster pose for neutral amiodarone docked to the hERG Y652A mutant showed the benzofuranyl group protruding into the fenestration region forming a π-stacking interaction with F557 on the S5 helix

Figure 10 Open-State Y652A/F656A hERG Interactions with Neutral Drugs

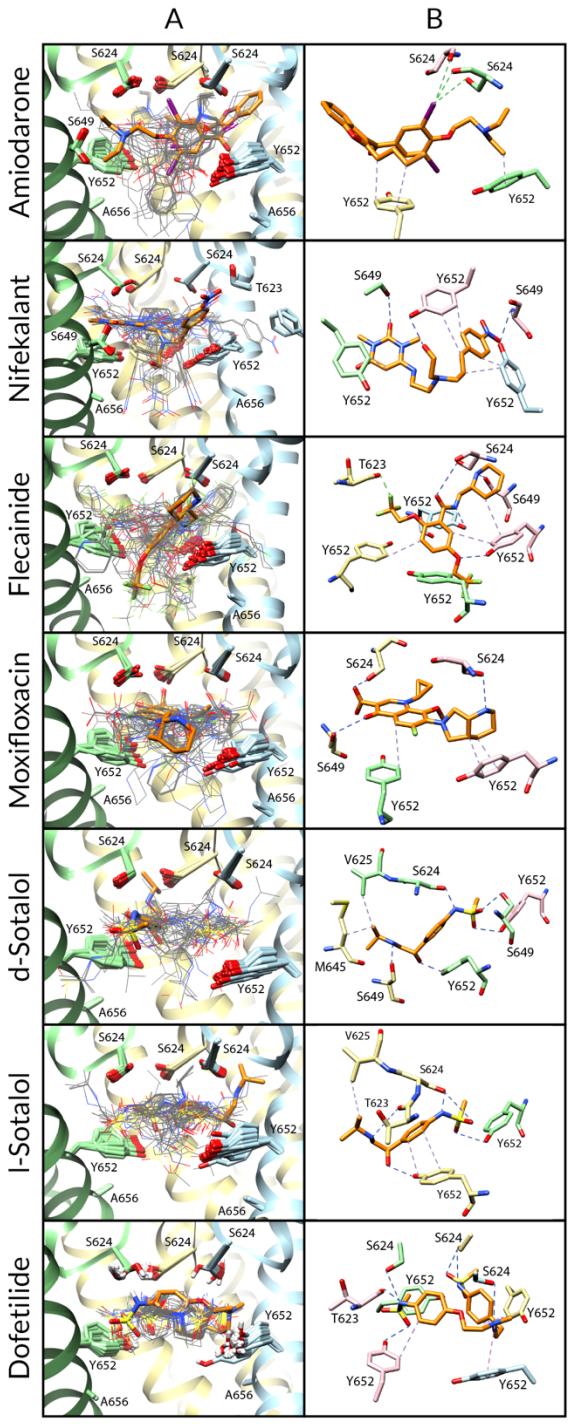
Rosetta docking results of Y652A/F656A hERG channel mutant in open state with neutral forms of each drug. Chain A is colored pink, chain B is blue, chain C is yellow, chain D is green, and ligand C atoms are gray. Top pose from largest and second largest clusters are in stick form and are orange and dark green, respectively. O atoms – red, N – blue, S – yellow, I – violet. H atoms not shown for clarity. Panel A shows top 50 poses in gray sorted by lowest interface score. Panel B highlights representative poses identified as the lowest energy pose from the largest and second largest cluster, respectively. PLIP-identified interactions are indicated by dashed lines. Halogen bonds are colored green, hydrogen bonds in blue, cation-pi in pink, pi-stacking in yellow, hydrophobic interactions in pale purple.



and a hydrophobic interaction with I655 on the S6 helix (Figure 3). While the representative neutral amiodarone binding pose for F656A hERG channel mutant is not located low enough in the pore to interact with the mutated residue A656, the top cluster of neutral amiodarone poses docked to Y652A and double-mutant hERG channel models both form hydrophobic contacts lower in the pore with F656. For cationic amiodarone complexes with the hERG Y652A, F656A and Y652A/F656A mutants, the top cluster poses revealed the benzofuranyl group protruding into the hydrophobic pocket but a reduced number of predicted nonbonded interactions with the other functional groups of cationic amiodarone forming hydrophobic interactions with residue 652 being either tyrosine or alanine

Figure 11 Open-State F656A hERG Interactions with Cationic and Zwitterionic Drugs

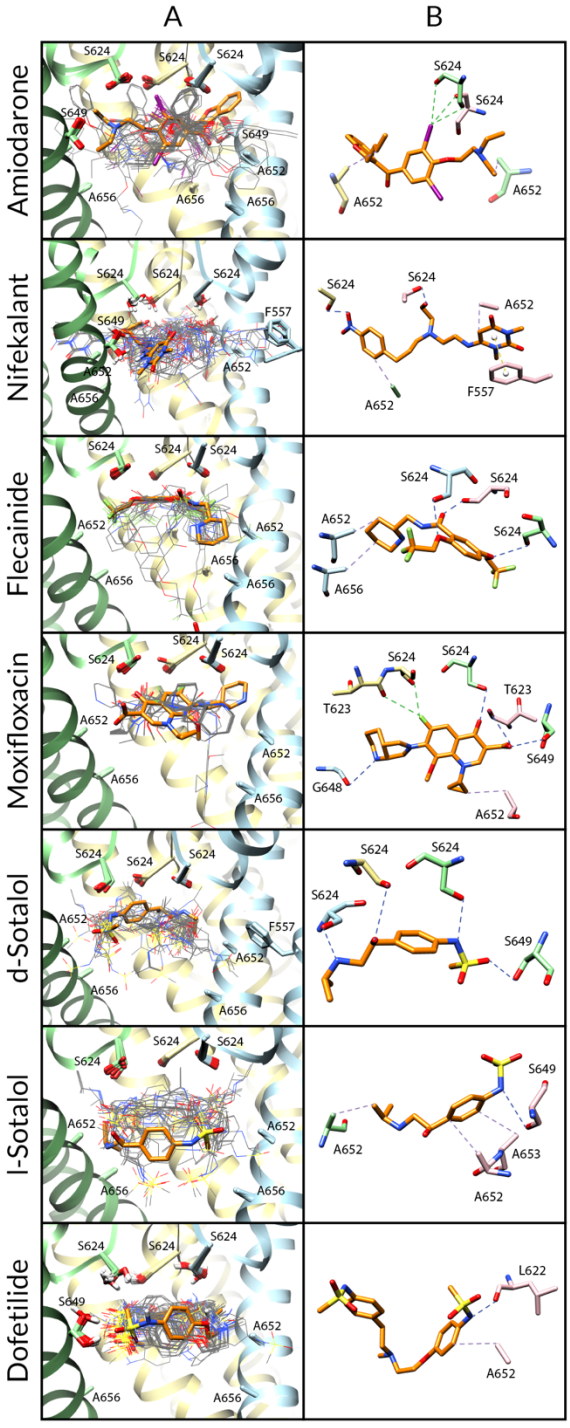
Rosetta docking results of F656A hERG channel mutant in open state with cationic or zwitterionic forms of each drug. Chain A is colored pink, chain B is blue, chain C is yellow, chain D is green, and ligand C atoms are gray. Top pose from largest and second largest clusters are in stick form and are orange and dark green, respectively. O atoms – red, N – blue, S – yellow, I – violet. H atoms not shown for clarity. Panel A shows top 50 poses in gray sorted by lowest interface score. Panel B highlights representative poses identified as the lowest energy pose from the largest and second largest cluster, respectively. PLIP-identified interactions are indicated by dashed lines. Halogen bonds are colored green, hydrogen bonds in blue, cation-pi in pink, pi-stacking in yellow, hydrophobic interactions in pale purple.



(supplementary Figures 2-4). The top cationic amiodarone pose of the top cluster only dips low enough into the pore to form hydrophobic interactions with F656 in the Y652A mutant hERG model. With the F656A and double mutant, an iodine on the benzene ring is available to form halogen bonds with multiple S624 residues at the base of the SF. For the Y652A hERG mutation, the iodinated benzyl ring is protruding lower in the pore, failing to find halogen bond contacts with any S624 residues. However, the oxygen in the ethoxy group connected to the benzene ring does make a hydrogen bond with the hydroxyl group of S624. For the F656A mutant hERG model interaction with both neutral and cationic amiodarone and the Y652A mutant with neutral amiodarone, the largest, low-energy clusters are smaller than those for the WT showing less

Figure 12 Open-State Y652A/F656A hERG Interactions with Cationic and Zwitterionic Drugs

Rosetta docking results of Y652A/F656A hERG channel mutant in open state with cationic or zwitterionic forms of each drug. Chain A is colored pink, chain B is blue, chain C is yellow, chain D is green, and ligand C atoms are gray. Top pose from largest and second largest clusters are in stick form and are orange and dark green, respectively. O atoms – red, N – blue, S – yellow, I – violet. H atoms not shown for clarity. Panel A shows top 50 poses in gray sorted by lowest interface score. Panel B highlights representative poses identified as the lowest energy pose from the largest and second largest cluster, respectively. PLIP-identified interactions are indicated by dashed lines. Halogen bonds are colored green, hydrogen bonds in blue, cation-pi in pink, pi-stacking in yellow, hydrophobic interactions in pale purple.



convergence to a similar binding pose. The largest, low-energy cluster of the Y652A hERG mutant interaction with cationic amiodarone and the double-mutant interaction with both neutral and cationic amiodarone are larger than those for the WT showing tighter convergence.

Nifekalant. Neutral (Figures 3-5) and cationic (supplementary Figures 2-4) nifekalant remained in the region within the hERG pore located between residue 656 and the base of the SF for all the hERG channel mutants. However, both neutral and cationic nifekalant showed a reduced number of poses interacting with the hydrophobic pocket for all hERG channel mutants. For neutral nifekalant complexes with the Y652A and F656A/Y652A mutants as well as cationic nifekalant with the F656A/Y652A mutant, the largest clusters protrude into the

lipid membrane facing fenestration region forming π-stacking interactions with F557 on the S5 helix.

Flecainide. Neutral (Figures 3-5) and cationic (supplementary Figures 2-4) flecainide clusters remained in the region within the hERG pore located between Y656 and the base of the SF for all the hERG channel mutants, although some poses extended further down to interact with S660. However, both neutral and cationic flecainide showed a reduced number of poses interacting with the hydrophobic pocket for Y652A and F656A/Y652A hERG channel mutants while F656A showed more similarity to WT. For neutral and cationic flecainide complexes with the Y652A and F656A/Y652A mutants, the largest clusters do not show any π-stacking and almost no poses extend into the hydrophobic pocket or fenestration regions. Interface scores of the lowest binding energy poses from each of the largest clusters (representative poses) for Y652A and F656A/Y652A mutants were less favorable than those for the WT poses for both neutral and cationic flecainide while F656A mutant showed more favorable interface scores.

Moxifloxacin. Neutral moxifloxacin (Figures 3-5) clusters remained tightly clustered in the region within the hERG pore located between residue 652 and the base of the SF for all the hERG channel mutants in a similar pose as the WT open state, although some poses extended further down to interact with residue 656 and S660 (Figure 3). Zwitterionic moxifloxacin (supplementary Figures 2-4), in contrast, did not remain as tightly clustered. For the Y652A mutant, 26 of the top 50 poses cluster with the dihydroquinoline group of zwitterionic moxifloxacin extending down into the pore towards A656 (Figure 3). For the F656A mutant, 40 of the top 50 poses of zwitterionic moxifloxacin remain in the region between the SF and Y652 while 10 poses have the pyrrolo-pyridine group tilted down below the Y652 towards A656 (Figure 4). For the

Y652A/F656A double mutant, zwitterionic moxifloxacin tightly clustered with 37 of the top 50 poses remaining in the pore region between the bottom of the SF and A652 (Figure 5). No pose interacts in the hydrophobic pocket except for zwitterionic moxifloxacin with the Y652A/F656A double mutant. However, several do extend towards the fenestration region in all open state models further than dofetilide but do not reach out of the pore like nifekalant or amiodarone.

Sotalol. The F656A mutation in the hERG channel model did not change the interaction profile for any form of sotalol (Figures 3-5) except that the cationic d-sotalol pose reaching into the hydrophobic pocket became the largest cluster rather than a secondary cluster. However, the Y652A and Y652A/F656A mutations distinctly change the interaction profiles for sotalol where the most frequently sampled, lowest binding energy RosettaLigand poses of neutral and cationic d- and l-sotalol either shift to form hydrogen bonds with S649 or down to form hydrophobic interactions with F656. For the Y652A/F656A double mutant, the 2-propylamine group of neutral d-sotalol in large secondary cluster extended out into the fenestration region. For neutral and cationic l-sotalol, the 2-propylamine group also interacted with the hydrophobic pocket of the Y62A mutant and formed hydrophobic interactions with T623 and V625, respectively.

Dofetilide. Studies of neutral (Figures 3-5) and cationic (supplementary Figures 2-4) dofetilide interactions with hERG Y652A, F656A, and Y652A/F656A mutants in an open state remained in the region within the hERG pore located between Y652 and the base of the SF for all the hERG channel mutants as cluster size remained similar and no poses extended into the hydrophobic pockets or fenestration regions.

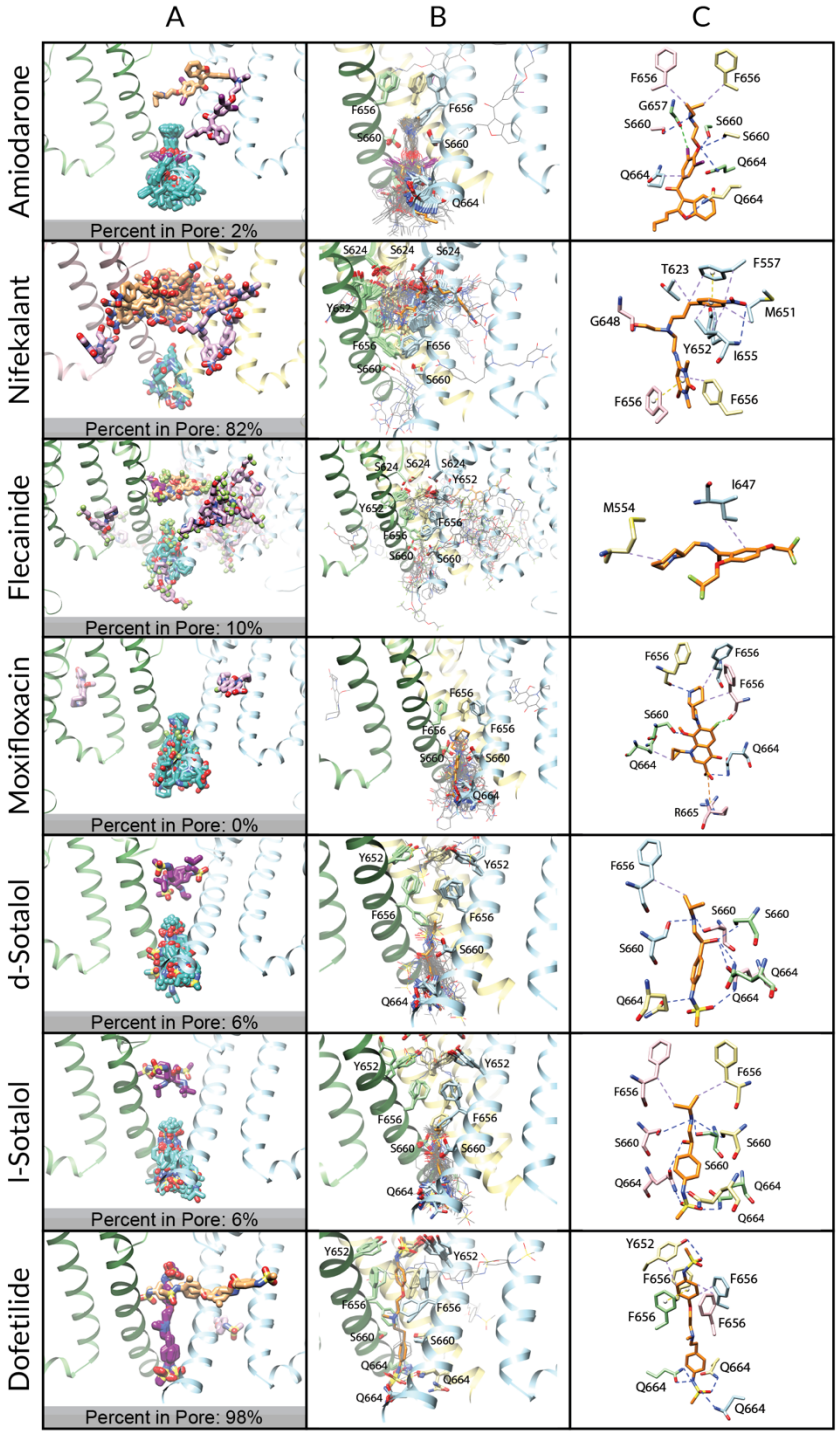
Summary. Open-state WT hERG interface scores are lower than, or similar to, Y652A mutants suggesting that these poses are relevant for amiodarone, nifekalant, flecainide, moxifloxacin, d-sotalol, and dofetilide, based on comparison to existing experimental data. However, a comparison of the largest cluster interface scores between WT hERG channel and F656A hERG mutants across each drug do not reflect experimental evidence of F656 as a key binding determinant for cationic amiodarone, nifekalant, neutral flecainide, neutral moxifloxacin, d-sotalol, l-sotalol, and dofetilide. This may be due to our study being limited to two conformational states, the inability of the RosettaDock approach to sample local and allosteric conformational changes upon drug binding within and near the receptor site formed by F656, and/or capture entropic contributions of F656 to ligand binding[115]. Furthermore, our putatively open state hERG channel model represents only one state out of potentially multiple other nearby open and inactivated states for which drugs may have a higher affinity, but which are only available upon drug binding.

2.3.4 Closed-State WT hERG–Drug Interactions

The closed hERG channel pore is known to be able to accommodate ligands of various sizes[116–119]. Gating-induced changes in the spatial location of F656 as well as open-state interactions with key residues in the pore (S624, Y652, F656) are thought to be particularly important in drug trapping phenomena[118,120–122]. Experimental data suggest that nifekalant, flecainide, sotalol, and dofetilide can be trapped within the hERG channel pore as the channel gate closes while amiodarone and moxifloxacin do not remain within the closed pore[73,116–125]. Either none or one of the top 50 docked poses remain in the pore for moxifloxacin and amiodarone, respectively, while multiple of the top 50 docked poses (6-98%) remain within the

Figure 13 Closed-State WT hERG Interactions with Neutral Drugs

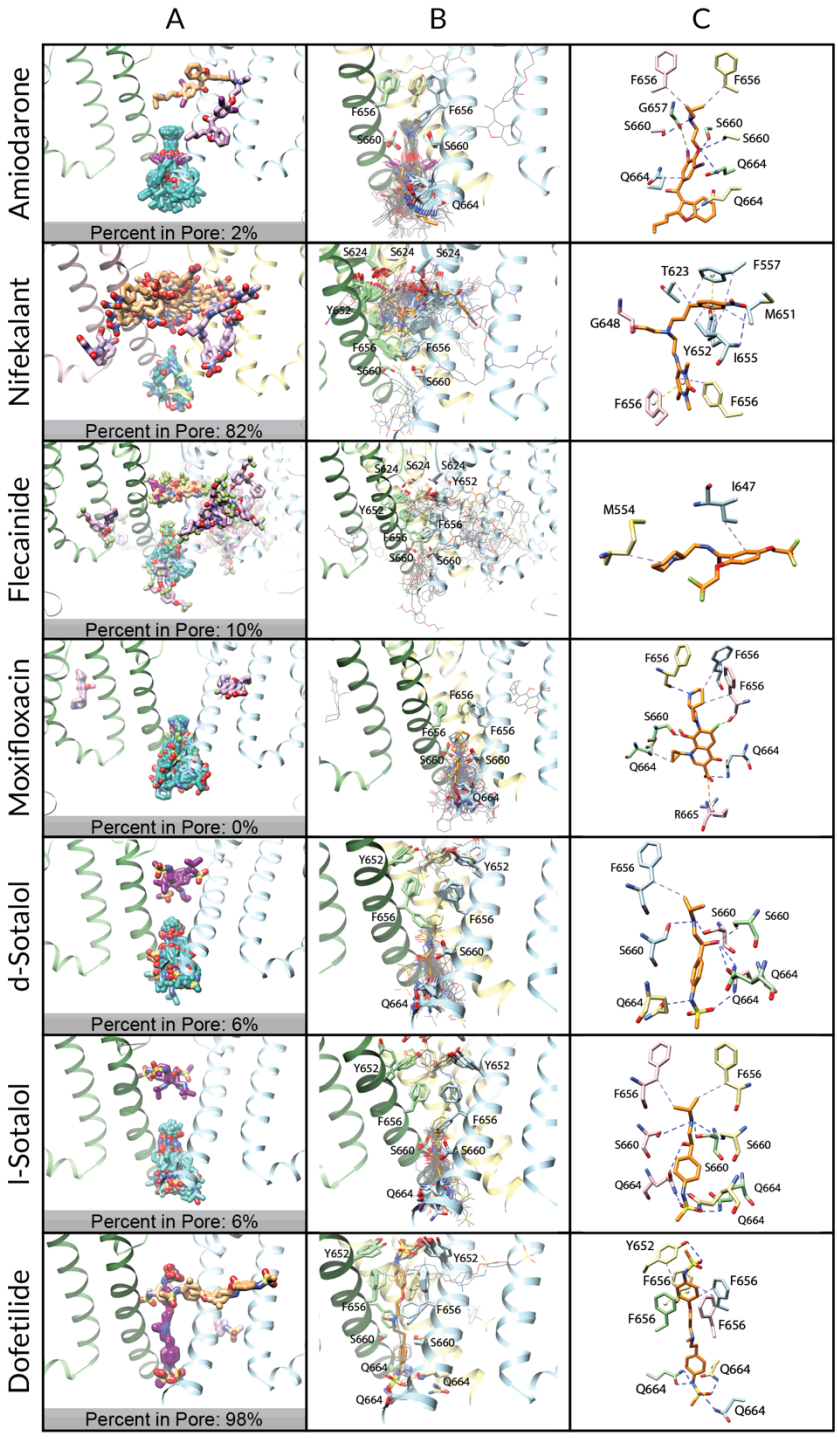
Rosetta docking results of closed-state WT (5) hERG channel interactions with neutral drugs. Chain A is colored pink, chain B is blue, chain C is yellow, chain D is green, and ligand C atoms are gray or orange (for top pose). O atoms – red, N – blue, S – yellow, I – violet. H atoms not shown for clarity. Panel A shows top 50 poses colored by position. Within pore is dark magenta, fenestration is orange, intracellular gate is cyan, and membrane is pale pink. Panel B shows top 50 poses in gray sorted by lowest interface score. Panel C highlights representative poses identified as the lowest energy pose from the largest cluster. PLIP-identified interactions are indicated by dashed lines. Halogen bonds are colored green, hydrogen bonds in blue, cation-pi in pink, pi-stacking in yellow, hydrophobic interactions in pale purple.



pore cavity for sotalol, flecainide, nifekalant, and dofetilide. These interactions with the closed state may reflect experimentally observed trapped behavior. While all sotalol and all but one top docked pose of dofetilide are entirely contained within the pore cavity, the docked poses remaining within the pore for nifekalant and flecainide prefer the fenestration region where one end of the ligand is within the pore

Figure 14 Closed-State WT hERG Interactions with Cationic and Zwitterionic Drugs

Rosetta docking results of closed-state WT (5) hERG channel interactions with cationic or zwitterionic drugs. Chain A is colored pink, chain B is blue, chain C is yellow, chain D is green, and ligand C atoms are gray or orange (for top pose). O atoms – red, N – blue, S – yellow, I – violet. H atoms not shown for clarity. Panel A shows top 50 poses colored by position. Within pore is dark magenta, fenestration is orange, intracellular gate is cyan, and membrane is pale pink. Panel B shows top 50 poses in gray sorted by lowest interface score. Panel C highlights representative poses identified as the lowest energy pose from the largest cluster. PLIP-identified interactions are indicated by dashed lines. Halogen bonds are colored green, hydrogen bonds in blue, cation-pi in pink, pi-stacking in yellow, hydrophobic interactions in pale purple.



cavity while the other end extends horizontally from the pore towards the cell membrane.

Amiodarone.

Modeling of neutral (Figure 6) and cationic (Supplement Figure 5) amiodarone to the closed hERG Channel pore revealed that almost all top 50 binding poses for both neutral and cationic amiodarone can be characterized by (1) hydrophobic interactions between F656 and the diethylamino tail of amiodarone, (2) several

hydrogen and halogen bonds between amiodarone oxygen and iodine atoms and G657, S660, and Q664 sidechains, and (3) the benzofuranyl group located at the base of the channel pore and extending into the intracellular region. For both neutral and cationic amiodarone binding to a closed-state model of the hERG channel, the interface scores of the representative poses were less favorable than for open-state WT but more favorable than for the double mutant hERG channel models.

Nifekalant. Modeling of neutral (Figure 6) nifekalant to the closed hERG channel pore revealed that 39 of the top 50 binding poses either extend into the fenestration region or remain in the pore with some protruding into the hydrophobic pocket. Of these, 11 of the top poses in a cluster converged to a similar ligand orientation characterized by (1) a π-stacking bond between the nitrophenyl group and F557 on the S5 helix within the fenestration region, (2) a π-stacking and hydrophobic interactions between the pyrimidinedione group and F656 within the pore, and (3) a hydrophobic interaction network between the nitrophenyl group and residues F557, T623, M651, and I655. In contrast, in the top 50 binding poses for cationic (Supplement Figure 5) nifekalant almost all of them escape from the pore laterally towards the membrane or, in the largest cluster, shift down towards the intracellular side. Characterizing 27 of the top 50 poses that compose this largest cluster and converge on a similar ligand orientation are (1) parallel and perpendicular π-stacking interactions between Y652 and the nitrophenyl group, (2) hydrogen bonds between the pyrimidinedione and hydroxyethyl groups and residues S660 and Q664 on multiple chains, and (3) hydrophobic interactions between the nitrophenyl group and residues Y652 and F656.

Flecainide. Modeling of neutral (Figure 6) flecainide to the closed hERG channel pore revealed that all top 50 binding poses for cationic flecainide either escape from the pore laterally towards the membrane or, shift down to the intracellular gate. Similarly, in the top 50 binding poses for cationic (Supplement Figure 5) flecainide all of them escape from the pore laterally towards the membrane or, in the second cluster, shift down towards the intracellular side. However, for cationic flecainide, two clear clusters can be identified in these two areas with the top pose outside but near the fenestration region characterized by (1) halogen bond between a trifluoroethoxy group and the backbone nitrogen of M645, and (2) a hydrophobic interaction network between the benzamide and piperidine groups and residues F551, A558, F619, I642, and L646.

Moxifloxacin. Modeling of neutral (Figure 6) and zwitterionic (Supplement Figure 5) moxifloxacin to the closed hERG channel pore revealed that nearly all top 50 binding poses shift down to the intracellular gate and the largest clusters are characterized by (1) halogen bond between the fluorine of moxifloxacin and the backbone oxygen of F656, (2) a hydrophobic interaction network between moxifloxacin and F656 and Q664 on multiple subunits, (3) a hydrogen bond network between moxifloxacin and S660, Q664, and the backbone of F656, and (4) a salt bridge between the carboxylic acid on moxifloxacin and R665.

Sotalol. Modeling of neutral (Figure 6) and cationic (Supplement Figure 5) d- and l-sotalol revealed that all the top 50 binding poses for cationic and 94% of the top 50 poses for neutral d- and l-sotalol shift down to the intracellular gate. For neutral d- and l-sotalol, however, a 6% of poses remain trapped in the pore interacting with Y652 and F656. All the top-cluster poses for neutral and cationic d- and l-sotalol can be characterized by large hydrogen bond networks

between sotalol and S660 and Q664 residues of all four chains simultaneously with some hydrophobic interactions with the β-carbon of F656.

Dofetilide. Modeling of neutral (Figure 6) dofetilide to the closed hERG channel pore revealed that nearly all (44) of the top 50 binding poses remained seemingly trapped in the pore region with one sulfonamide end near the top of the pore below the SF and the other at the base of the pore forming bonds with Y652 and Q664, respectively. This cluster converged to an orientation characterized by (1) a π-stacking bond between the phenoxy group and F656, (2) hydrophobic interactions between residues Y652 and F656 and the phenoxy groups, and (3) hydrogen bonds between residues Y652 and Q664 on multiple chains and both sulfonamide groups. Similarly, the top cluster for cationic (Supplement Figure 5) dofetilide also remains trapped within the closed state in an extended conformation within the pore. This cluster, however, is only composed of 9 models and is shifted to sit higher within the pore with one sulfonamide end protruding into the fenestration region while the other reaches down to S660. The remaining 41 of the top 50 binding poses for cationic dofetilide escape from the pore either laterally towards the membrane or shift down towards the intracellular gate.

Summary. The percentage of poses remaining within the closed-state hERG channels suggest that closed channels can accommodate known trapped drugs (nifekalant, flecainide, d/l-sotalol, and dofetilide), but not amiodarone or moxifloxacin (non-trapped drugs). Trapped drugs that do not demonstrate facilitation effects (sotalol and dofetilide) do not interact in the closed state pore regions expected to affect gating-induced closure of the S6 helix (hydrophobic pocket, fenestration region). Trapped drugs that demonstrate facilitation effects (nifekalant) interact with the closed state hydrophobic pockets and fenestration regions which further support the

hypothesis that facilitating drugs (nifekalant, amiodarone, moxifloxacin) may act as a wedge to bias hERG channel equilibrium towards the open state and increase hERG current amplitude in response to low-voltage depolarization[30,32,55]. This data would predict that moxifloxacin would be a non-trapped facilitator of hERG current

2.4 Conclusions

We have investigated similarities and differences between various drugs interactions with hERG channel in an open and closed states and highlighted key structural insights into molecular mechanisms of drug action. Rosetta docking simulations using models of both the putatively open- and closed-state hERG channel suggest that previously identified residues (S624 and Y652) are important in binding for each drug studied while other pore-lining residues (L622, T623, V625, and S649) are often also involved. This is supportive of previous mutational studies and pharmacophore models which, together, identify hydrophobic features of drugs that may interact in/near the hydrophobic pocket (L622, T623, V625) while ionizable functional groups may favor interactions on the S6 helix near S649 and Y652[30,32,66,94,114,122,126].

Notably, top clusters of amiodarone, nifekalant, flecainide, and moxifloxacin positioned within the hydrophobic pocket while only few poses of d/l-sotalol and dofetilide are positioned in the hydrophobic pocket. We predict that drug interactions within the hydrophobic pocket–a region absent from our closed-state hERG model–may impact the closure of the S6 helix bundle thereby affecting channel gating. Consistent with our results, experimental data suggests that facilitating drugs may act as a wedge to bias hERG channel equilibrium toward an open state conformation and can increase hERG current amplitude in response to low-voltage depolarization[30,32,55].

The more favorable interface energies in drug binding with the F656A hERG mutant when compared to WT hERG channel do not support experimental evidence of F656 as a key binding determinant for nifekalant, neutral flecainide, neutral moxifloxacin, d-sotalol, l-sotalol, and dofetilide. This may be due to the limitations of the RosettaDock approach to sample allosteric conformational changes upon drug binding near the receptor site formed by F656 and evaluate entropic contributions of F656 to ligand binding[115]. Furthermore, our putatively open state hERG channel model represents only one state out of potentially multiple other nearby open and inactivated states for which drugs may have a higher affinity, but which are only available upon drug binding.

Our results suggest a potential structural model for hERG channel facilitation through drug interactions with the hydrophobic pocket and fenestration region of the hERG pore domain. Models of facilitating drugs interact in the open state with the hydrophobic pocket more than non-facilitating drugs. Models of trapped, facilitating drugs interact with the fenestration region more than trapped, non-facilitating drugs. Experimental testing and molecular dynamics simulations are needed to test these hypotheses.

3. Modeling of $Ca_V1.2$ Channel Interactions with Drugs Using Rosetta

3.1 Introduction

Calcium (Ca^{2+}) is one of the fundamental biosignaling ions involved in complex interactions associated with membrane transport, as a second messenger in signaling and regulatory pathways, intracellular calcium repositories, cellular energy metabolism, and other physiological processes[127]. Cytosolic calcium concentration is tightly regulated and is altered as calcium either enters the cell or is released from the cellular stores through ion channels, transporters, and pumps[127]. Voltage-gated calcium channels (Ca_V) play a key role in muscular contraction, neuronal excitation, gene expression regulation, and release of hormones or neurotransmitters. Particularly, the L-type calcium channel $Ca_V1.2$ is essential to maintaining the intracellular calcium homeostasis and can cluster into large oligomer complexes to amplify the resultant Ca^{2+} signal[128–132]. Ca^{2+}-activated calmodulin (CaM) binding to the C-terminal pre-IQ domain of the channel plays a key role in this process, promoting channel–channel interaction and cooperative opening. CaM association with $Ca_V1.2$ can also enhance inactivation of this channel current upon increased Ca^{2+} influx[133], although its role in this process has been debated.

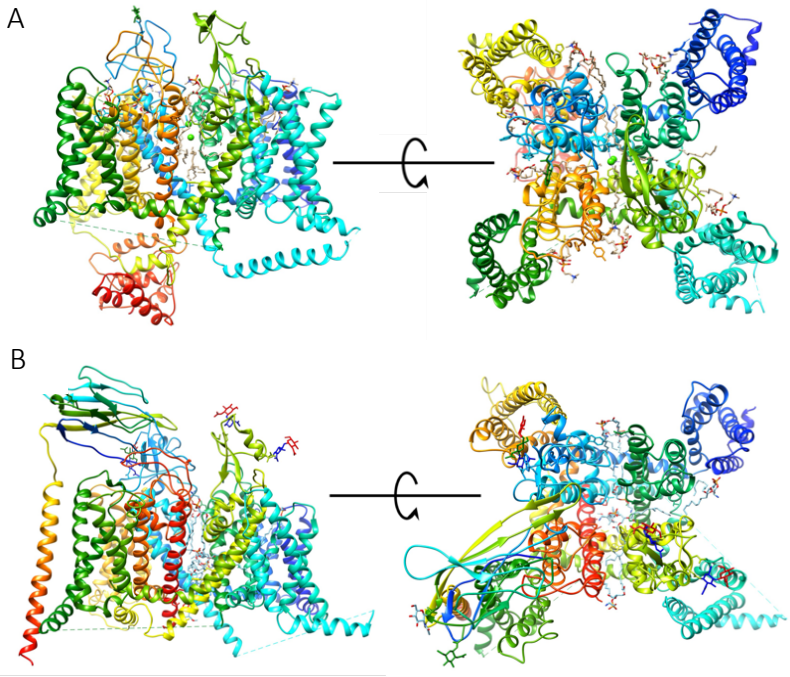
Mutations or blockage of $Ca_V1.2$ channels can lead to dysregulation of their gating and thus cell signaling pathways[6]. Inherited or acquired channelopathies due to disturbed functions of human $Ca_V1.2$ have been linked to many cardiac and neurological diseases such as cardiac arrhythmias, autism, bipolar disorder, and immunodeficiency[134]. Pharmaceutical treatments for hypertension, coronary artery disease and other cardiovascular conditions include dihydropyridines, phenylalkylamines and benzothiazepines, which function as Ca_V channel blockers targeting the α1-subunit of $Ca_V1.2$[135–137]. Ca_V channel blockers alter action potential

duration in cardiomyocytes, and there is often a fine line between their therapeutic effect and cardiac rhythm disturbances[5]. Few antiarrhythmic drugs have been approved for clinical use due to severe side effects (most significantly, cardiotoxicity) or limited efficacy[4]. Drug-induced arrhythmogenicity is one of the most critical regulatory concerns for cardiovascular and other drugs in development.

Historically, widely used predictors of drug proarrhythmic potential are the prolongation of the QT interval on the ECG and the blockade of the hERG (a major cardiac voltage-gated K^+ channel) current. However, these predictors are not selective enough, since not all QT-prolonging and hERG-blocking drugs are arrhythmogenic. Currently, efforts are focused on improving these predictors through understanding of interactions of various drugs with multiple cardiac ion channels, including $Ca_V1.2$[5]. The Comprehensive *In Vitro* Proarrhythmia Assay (CiPA) initiative is a novel preclinical drug cardiotoxicity screening approach through the development of computational models to predict drug effects on multiple cardiac ion channels and model validations by electrophysiological tests on stem cell-derived

Figure 15 $Ca_V1.1$ and $Na_V1.4$ published cryo-EM structures.

Top and side views of the published mammalian Ca_V and Na_V channel cryo-EM structures: (A) rabbit CaV1.1 (PDB ID: 5GJV); (B) NaV1.4 (PDB ID: 6AGF).



cardiomyocytes[138]. Amlodipine and verapamil are part of the CiPA initiative drug test list. Verapamil is a known hERG blocker but does not cause long QT syndrome (LQTS) or Torsades de Pointes (TdP) arrhythmia (often associated with hERG block)–potentially due to $Ca_V1.2$ block, which might reverse the action potential duration prolongation effect[139,140]. This study assesses $Ca_V1.2$-drug interactions at the atomic resolution.

All Ca_V channels are composed of a pore-forming $\alpha 1$ subunit and several auxiliary subunits ($\alpha 2$, β and γ)[141]. High-resolution structures of a mammalian Ca_V channel, $Ca_V1.1$[142–145] (PDB IDs: 5GJV, 6JPA, 7JPX), and the homologous human sodium channel, $Na_V1.4$, structure[146] (PDB ID: 6AGF) stimulate a new age of structure-informed Ca_V research. Previously published $Ca_V1.1$ structures have a closed pore, whereas the $Na_V1.4$ structure is in a putatively open-inactivated state, allowing us to develop $Ca_V1.2$ models in multiple conformational states. Atomic scale studies of $Ca_V1.2$ structure, dynamics and drug interactions linked to the channel biological functions and their modulation are necessary to develop predictive kinetic models of drug binding and stimulate future rational design of pharmacotherapies with improved cardiac safety profiles and more efficient treatment of Ca_V-associated pathologies.

3.2 Materials and Methods

The Rosetta software suite offers several powerful tools for the prediction of protein structures[14,19,99]. Homology modeling in combination with cryo-EM density refinement can utilize the newly available $Na_V1.4$ and $Ca_V1.1$ structures (Figure 14) as templates for modeling of human $Ca_V1.2$ models in open-inactivated and closed states. Rosetta approach utilizes multiple sequence alignments, sequence-based fragments, and restraint functions to develop accurate, all-atom models with optimized sidechains, backbone refinements and energy minimizations. Once

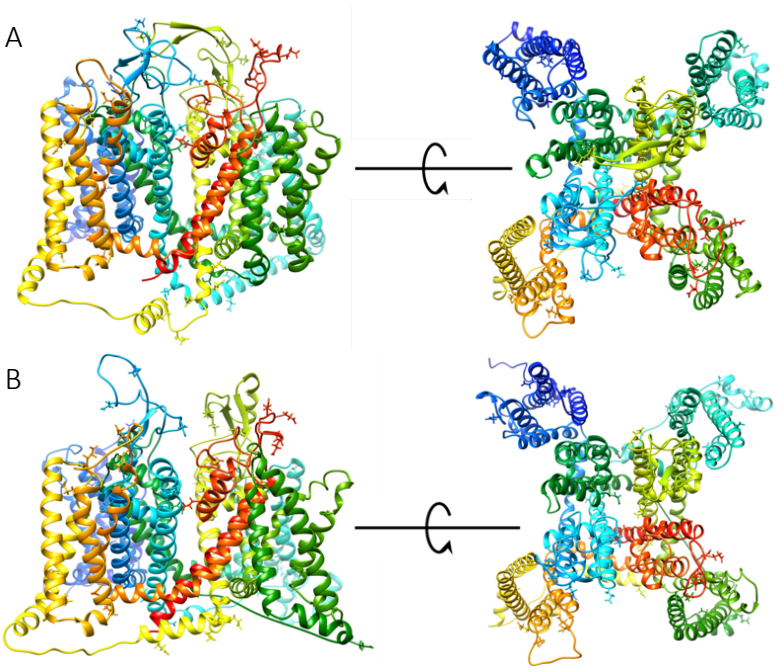
accurate models have been generated, they can be used as the basis for ligand and protein docking studies and all-atom and coarse-grained molecular dynamics simulations. Structural alignments were performed in Chimera using best-aligning chain pairing with the Needleman-Wunsch alignment algorithm.

3.2.1 Rosetta modeling of $Ca_V1.2$ in open-inactivated and closed states.

Homology modeling uses a known protein structure as a template to model the three-dimensional structure of a protein of interest[14,99]. Previously solved cryo-EM structures of rabbit $Ca_V1.1$[143] and human $Na_V1.4$[146] channels were used as templates to develop preliminary homology models of rabbit $Ca_V1.2$ ($rCa_V1.2$) in closed and open-inactivated states, respectively. Rabbit $Ca_V1.2$ was specifically selected because our collaborators Drs. Santana and Dixon study this isoform of Ca_V channel using experimental approaches[128–131]. To improve accuracy of the template structures, structural refinement was performed for $Ca_V1.1$ and $Na_V1.4$ structures into their respective cryo-EM density maps using Rosetta model-building and refinement[19]. This allows for more accurate $Ca_V1.2$ structure

Figure 16 Open-inactivated and closed-state homology models of $Ca_V1.2$

The homology models of rabbit $Ca_V1.2$ channel in different conformational states based on the cryo-EM density refined structures of: (A) closed rabbit $Ca_V1.1$ channel (PDB ID: 5GJV); (B) open-inactivated human $Na_V1.4$ (PDB ID: 6AGF) channels.



prediction using local iterative refinement and corrections to improve geometries and side-chain placements without overfitting. We generated 10,000 $Ca_V1.2$ models for each channel state and then selected the most frequently sampled lowest energy models for ligand docking studies.

3.2.2 RosettaLigand modeling of $Ca_V1.2$ interactions with drugs.

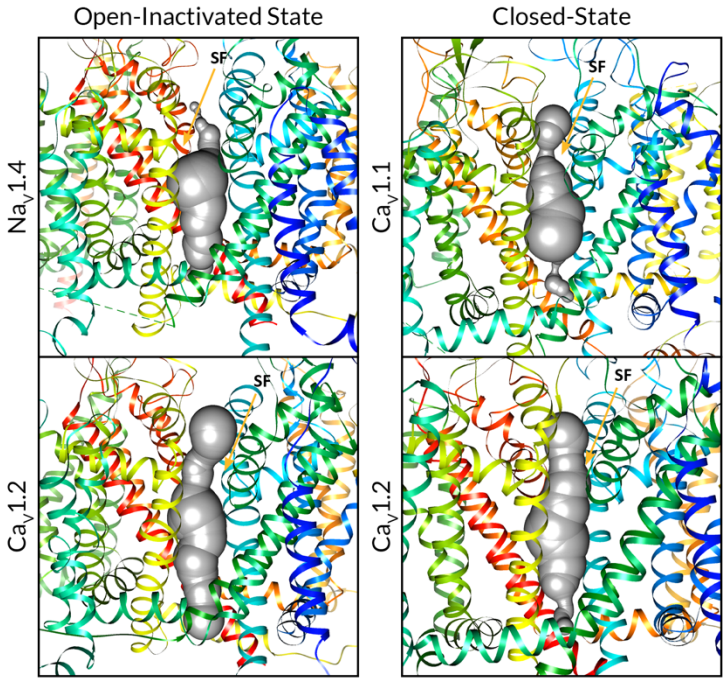
Computational ligand docking is a useful tool to study protein - drug interactions and is a key approach for rational drug design. RosettaLigand docking can explicitly model full flexibility of a ligand and protein side-chains and backbone atoms – contributing to its promising ability to accurately model conformational changes of the receptor site upon drug binding[21,22,99]. The $rCa_V1.2$ open-inactivated and closed state models were used as the receptor for docking studies of cationic verapamil and amlodipine, which are Ca_V channel blockers and hypertension therapeutics. Docking used 20 different starting points in and near the pore region of the channel to generate 20,000 different conformations using RosettaLigand. We selected the lowest 10% models by overall Rosetta energy score (total_score). From this set we then selected the 50 lowest energy poses by Rosetta interface energy score (interface_delta_X)[21,22,97].

3.3 Results and Discussion

3.3.1 Rosetta modeling of $Ca_V1.2$ in open-inactivated and closed states

Figure 17 Pore profiles of $Ca_V1.2$ models and refined $Ca_V1.1$ and $Na_V1.4$ structural templates

Channel pore is shown as a space-filling gray surface computed using MOLE software, whereas protein is depicted using ribbon representation color-coded by domain(?).



We used the Rosetta structural modeling software software[97,98] and the cryo-EM structures of closed-state $Ca_V1.1$ and open-inactivated state $Na_V1.4$ channels (Figure 17) as templates for generating open-inactivated and closed state $rCa_V1.2$ models, respectively, as described in Methods. While all four cryo-EM refined models share a similar maximal pore radius from 4.3-4.9Å, pore analyses (Figure 17) by MOLEonline[147] reveal two main constriction sites: the selectivity filter and intracellular gate. In the density-refined, closed-state $Ca_V1.1$ channel and corresponding closed-state $Ca_V1.2$ model, the radii at the intracellular gate (lined by residues V329, F1060, and F1376) are 0.6 Å and 1.1 Å, respectively, confirming that the pore is closed and inaccessible for ions to pass through. The radii at the intracellular gate for density-refined, open-inactivated state $Na_V1.4$ channel and corresponding open-inactivated state $Ca_V1.2$ model are 1.9 Å and 2.8 Å, respectively, suggesting a semi-closed pore phenotype likely preventing ion translocation. In the density-refined, closed-state $Ca_V1.1$ channel and corresponding closed-state

$Ca_V1.2$ model, the radii at the selectivity filter (EEEE locus) are 1.9 Å and 2.6 Å, respectively. The radii at the selectivity filter for density-refined, open-inactivated state $Na_V1.4$ channel and corresponding open-inactivated state $Ca_V1.2$ model are 2.3 Å and 2.1 Å, respectively. When a single domain of a hybrid bacterial calcium channel, Ca_VAb (PDBID: 5KLB)[139], is aligned to domain II of our open-inactivated and closed-state $Ca_V1.2$ models, the selectivity filter backbone atoms RMSD are 1.1 Å and 1.6 Å, respectively. This data suggests that there is a considerable, conformational shift in the $Ca_V1.2$ selectivity filter compared to the homologous $Ca_V1.1$, $Na_V1.4$, and Ca_VAb channel structures. While there is no available $Ca_V1.2$ structure to directly compare to, a recently published homology model[148] of $Ca_V1.2$ based on the same closed-state $Ca_V1.1$ channel structure constructed using the SWISS-MODEL server, resulted in a very similar structure with a whole-protein root-mean-square deviation (RMSD) of 1.1 Å.

3.3.2 Ligand docking of amlodipine and verapamil to $Ca_V1.2$

Amlodipine is a known calcium channel blocker belonging to the dihydropyridine subgroup with high affinity for Ca_V1 and Ca_VAb channels[149–154]. It is used to treat hypertension and angina pectoris and is thought to allosterically modulate $Ca_V1.2$ channel activation through interaction with the pore helix[145,149–152,155]. Verapamil is a high affinity, phenylalkylamine calcium channel blocker used to treat arrhythmia, angina pectoris, and hypertension[144,148,156,157]. Verapamil is thought to bind in the central pore, directly blocking the ion conducting pathway[93,148,158–161]. While high-resolution structures of $Ca_V1.2$ bound to ligands have not yet been resolved, X-ray crystallographic structures of various dihydropyridines and phenylalkylamines bound to pre-open and inactivated Ca_VAb and $Ca_V1.1$ have been characterized, including amlodipine and verapamil[139,144,145].

Figure 18 Drugs docked to $Ca_V1.2$ channel model in multiple conformational states

Top 50 poses for each docking colored in gray with lowest interface energy pose colored in orange. Protein colored by residue in rainbow (N-terminus in blue to C terminus in red).

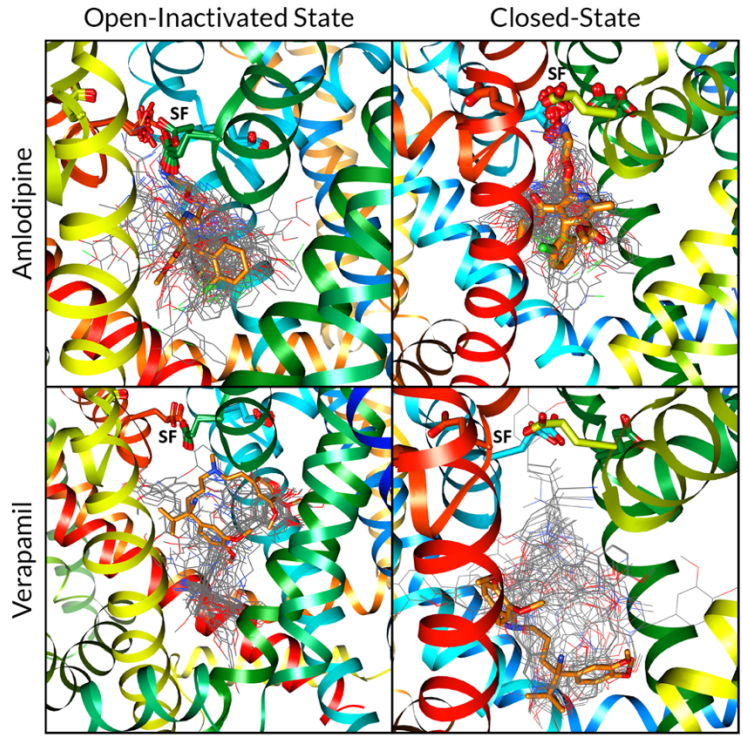


Figure 18 shows the top 50 poses selected out of 20,000 decoys first by total energy and then by interface energy (see Section 3.2.2 above) for the docking of amlodipine and verapamil to our open-inactivated and closed-state $Ca_V1.2$ channel models. All the top amlodipine poses interact with the pore helices and induce an asymmetry in the selectivity filter, in agreement with crystallographic data[139]. However, positioning of amlodipine relative to the membrane in our models is closer to the pore center than in published Ca_VAb–amlodipine and $Ca_V1.1$–amlodipine complex structures (Figure 19). This disagreement may be due to the differences in sequence and structure between Ca_VAb/$Ca_V1.1$ and $Ca_V1.2$ or limited sampling of ligand conformational space in a wide, asymmetric pore. The positioning of the 2-

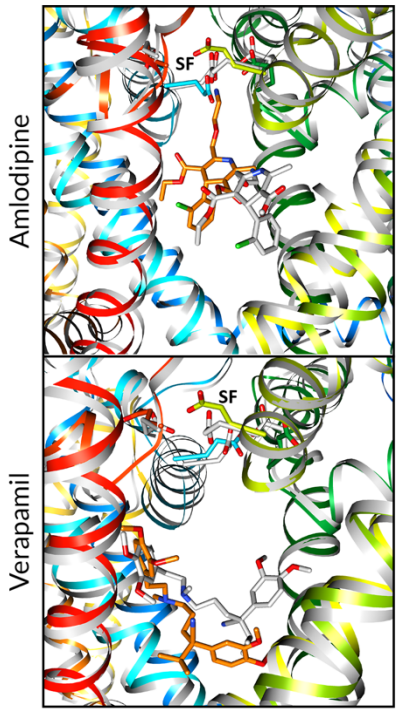


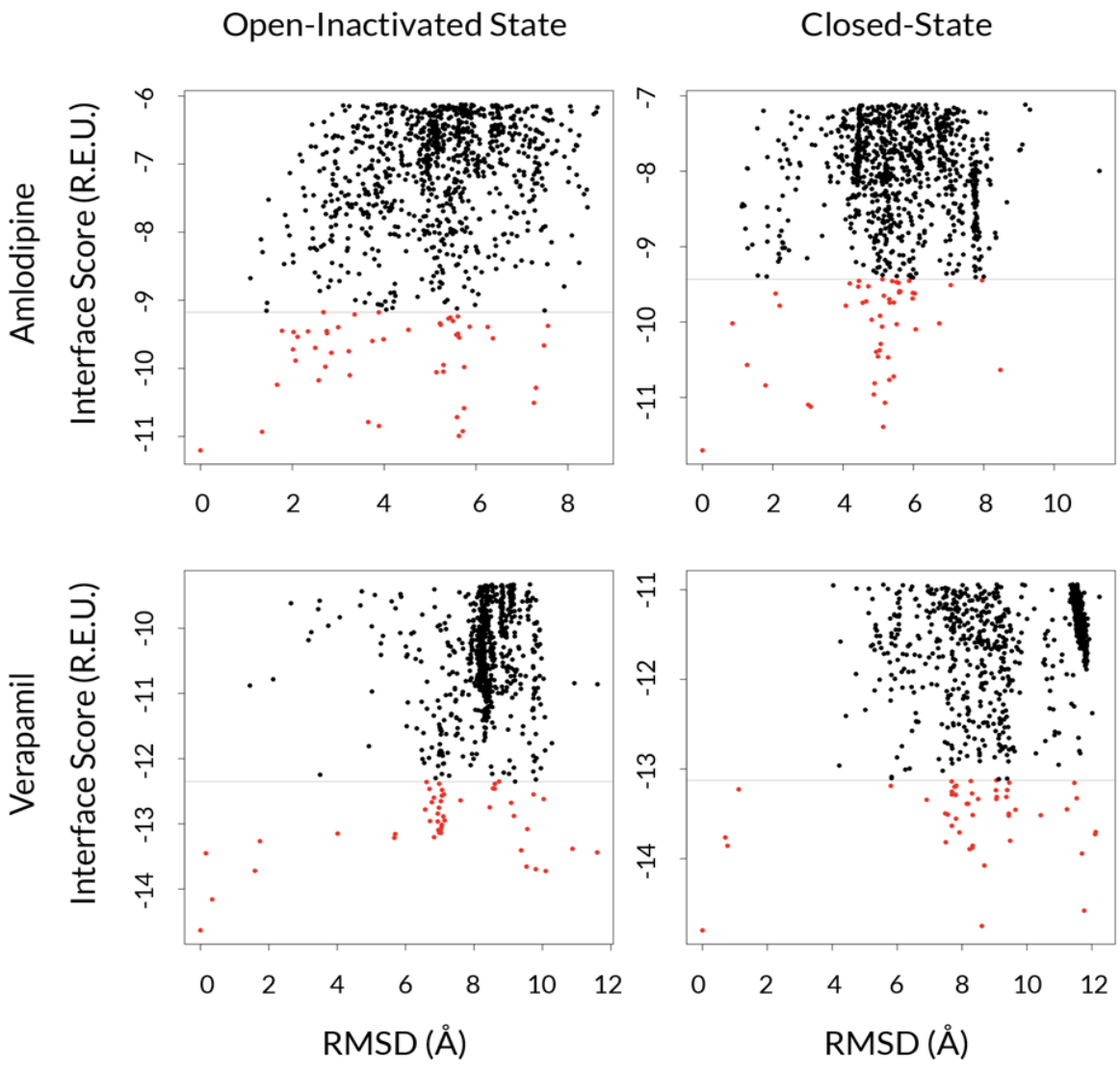
Figure 19 $Ca_V1.1$ and $Ca_V1.2$ in complex with amlodipine and verapamil

$Ca_V1.2$ is rainbow with lowest energy pose in orange. CaV1.1–amlodipine complex (PDB ID: 7JPX), top, and CaV1.1-verapamil complex (PDB ID:6JPA),bottom, overlayed and colored in light gray using Chimera-generated structural alignment.

aminoethoxymethyl moiety of amlodipine also extends up towards the center of the EEEE SF motif when docked to $Ca_V1.2$ but is turned down towards the pore center in the published $Ca_V1.1$-amlodipine complex structure (Figure 19)[145].

In agreement with crystallographic Ca_VAb data, the top pose of verapamil bound to open-inactivated state $rCa_V1.2$ occluded the ion conducting pathway with its tertiary amino group facing up, extracellularly toward the selectivity filter. In agreement with the published cryo-EM

Figure 20 Interface Score vs. RMSD plots for top 1000 docked $Ca_V1.2$–drug poses



$Ca_V1.1$-verapamil complex structure, verapamil is positioned between domain I and IV of the $Ca_V1.2$ channel, with one end of the ligand angled up towards the pore helix and the other extending into the central pore (Figure 19). However, verapamil binding was less converged, and positioning varied considerably within the pore cavity, more so than amlodipine. This can be visualized in the interface score vs. ligand RMSD plots (Figure 20) where there was greater variation in ligand RMSD of verapamil in both open-inactivated and closed states revealing a need for greater conformational sampling to identify a single, high-affinity binding site. Additional structure refinement and expansion of Rosetta search radius may be necessary to differentiate real and artificial differences in amlodipine and verapamil binding between various Ca_V structures.

3.4 Conclusions and Future Directions

Several similarities exist between our $Ca_V1.2$ docking and published Ca_V binding data for both amlodipine and verapamil, including drug binding domains and drug orientation within the pore. However, differences exist when comparing the published structural interactions between ligand and channel (Figure 19). Along with the limited verapamil pose convergence, our results necessitate further study. Expansion of the Rosetta search radius parameter to increase conformational search space and additional $Ca_V1.2$ model development using multiple structure densities and co-evolutionary analysis may help more accurately elucidate drug binding orientation to $Ca_V1.2$ and better inform future experimental drug design and extension of $Ca_V1.2$ channel modeling and structure/modulation studies.

3.4.1 Full Channel Modeling

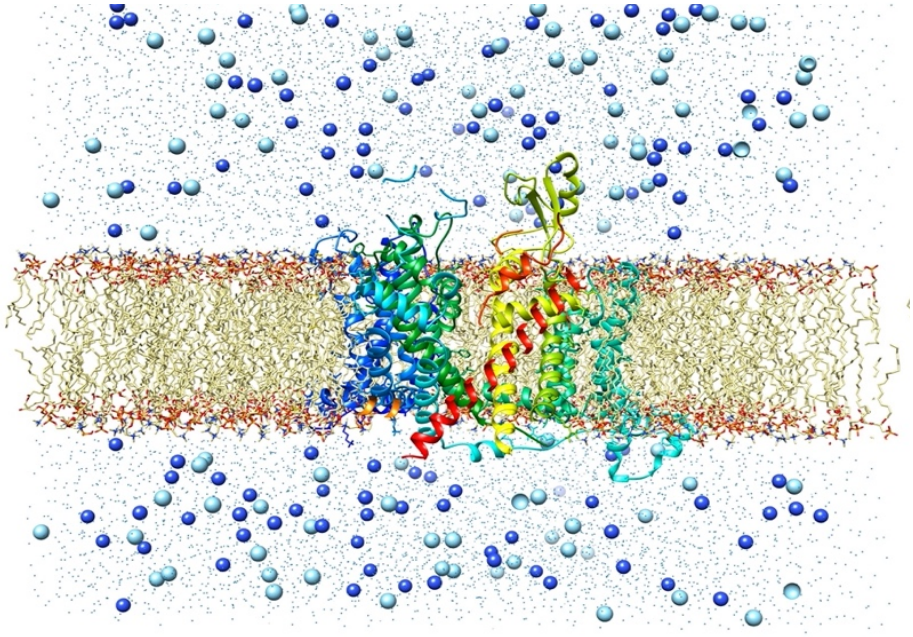
All the published $Ca_V1.2$-homologous structures lack C-terminal IQ and pre-IQ domains which are essential for CaM binding and channel oligomerization. There are several X-ray and NMR structures of CaM bound to C-terminal pre-IQ and/or IQ domain fragments[130,133,162,163], including one of a dimer. The developed open-inactivated- and closed-state $Ca_V1.2$ models can be combined with available X-ray and NMR structures of the missing C-terminal domains to construct the first complete model of $Ca_V1.2$. Because sequence identity between $Ca_V1.2$ and $Na_V1.4$ is only ~21% (the similarity is ~35%), a Rosetta homology modeling hybrid approach that combines the cryo-EM densities of the $Ca_V1.1$ structure in closed state and voltage-gated sodium channel structures in open and inactivated states may be needed to develop more accurate $Ca_V1.2$ channel structures in open-conducting and open-inactivated states to use as the basis to study the multi-state drug-channel interactions. Additionally, cryo-EM structures of the full length SK4 Ca^{2+}-activated potassium channel and the EAG1 voltage-gated potassium channel in complex with CaM[52,52] and the NMR structures[164] of a $Ca_V1.2$ IQ-domain derived peptide complexed with the EF3 and EF4 hands of α-actinin and with apo-CaM validated through mutagenesis and electrophysiological experiments may be useful to develop alternative models of the $Ca_V1.2$-CaM complex.

3.4.2 Further analysis of $Ca_V1.2$-drug interactions

$Ca_V1.2$ channel models should be developed with mutations at specific residues known to affect drug binding, such as S666 and A752 in the human $Ca_V1.2$ channel, which are critical for nifedipine block and also known to influence slow inactivation of the channel[149]. There are also other Ca_V residues known to be important for drug block[137,150,157,165] which can be used to further investigate drug-channel interactions. To compute drug affinities and binding/unbinding rates, Nanoscale Molecular Dynamics (NAMD) software can be used to perform molecular dynamics (MD) simulations of drug-$Ca_V1.2$ interactions in closed and open states[45]. An example of the system is depicted in Figure 21 which would use careful drug parameterization and advanced sampling and analysis techniques such as metadynamics and Markov state modeling. The molecular determinants that govern the interactions between $Ca_V1.2$ and various drugs will allow development of testable hypotheses about the mechanisms of action for each drug. MD simulations could provide kinetic parameters of drug–channel interactions to be utilized in functional models of cardiac cells

Figure 21 Cross-section of an open-state $rCa_V1.2$ embedded in membrane

A molecular system for all-atom MD simulations composed of an open-state $rCa_V1.2$ embedded in a POPC lipid bilayer solvated by 0.05 M aqueous $CaCl_2$.



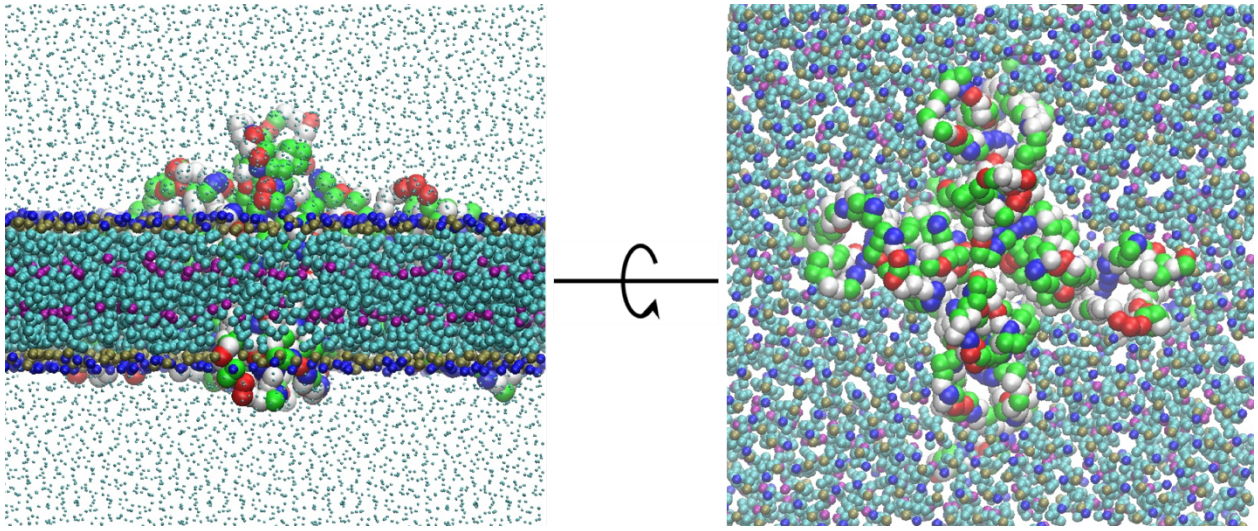
and tissues as part of a multiscale approach to predict cardiac safety[29].

3.4.3 Channel Clustering, Complexing, and Coarse-Grained Modeling

Understanding how $Ca_V1.2$ channel clustering and regulation by calmodulin can affect channel gating dynamics and drug interactions is essential to successful prediction of cardiac safety. It is known from biophysical experiments performed by Drs. Santana and Dixon and others that $Ca_V1.2$ channels cluster and may form functional dimers[166] and/or larger oligomers[128,129] and are co-localized with other proteins forming signaling cascades in cellular membranes. Those interactions will likely modulate $Ca_V1.2$ channel structure, dynamics and drug interactions[167].

Figure 22 Coarse-Grained representation of a $Ca_V1.2$ channel in a hydrated lipid bilayer

Top and side views of open-state $Ca_V1.2$ coarse-grained model in POPC lipid membrane, solvated by ions and water.



Co-evolving residues can be identified using our developed $Ca_V1.2$ models combined with Ca_V-CaM complex structures[162,163,166] and coevolutionary analysis of Ca_V sequences using the GREMLIN server[168–170] and used to constrain model development. Currently, simulating large, complexed proteins for physiologically relevant timescales is beyond the capacity of all-atom molecular dynamics. So, our all-atom model of the open state $Ca_V1.2$ will be used as the foundation for developing a coarse-grained MARTINI[171] model of the channel in a hydrated 1-

Palmitoyl-2-oleoylphosphatidylcholine (POPC) lipid membrane (Figure 22) for multi-μs molecular dynamics simulations of the channel complexes with calmodulin and their clusters. Model development of the large molecular complexes will lead to specific structural hypotheses about key protein-protein interactions necessary for complex formation that can be experimentally tested and validated. This may be able to answer the ongoing debate over the nature, extent, and determinants of $Ca_V1.2$ clustering into potentially functional dimers and oligomers and estimate how $Ca_V1.2$ channel complex formations modulate action of and are modulated by drugs. Detailed structural and dynamical information about $Ca_V1.2$ protein-protein and protein-drug interactions will be crucial in future rational drug design.

Summary

Our results identified a pattern of hERG-drug interactions with the hydrophobic pocket of hERG that is consistent with experimental data suggesting facilitating drugs may act as a wedge to bias hERG channel equilibrium towards the open state and increase hERG current amplitude in response to low-voltage depolarization. Open-state WT hERG interface scores are lower than, or similar to, Y652A mutants suggesting that our modeled poses are relevant for amiodarone, nifekalant, flecainide, moxifloxacin, d-sotalol, and dofetilide, based on comparison to existing experimental data. Open-state WT hERG interface scores are not lower than the F656A mutants for nifekalant, neutral flecainide, neutral moxifloxacin, d-sotalol, l-sotalol, and dofetilide, suggesting limitations of our study using only two conformational states or limitations of Rosetta to model allosteric contributions of F656. The percentage of poses remaining within the closed-state hERG channels suggest that closed channels can accommodate known trapped drugs (nifekalant, flecainide, d/l-sotalol, and dofetilide), but not amiodarone or moxifloxacin (non-trapped drugs). Amlodipine and verapamil docked to $rCa_V1.2$ models in open and closed states recapitulated known binding regions and similar positioning within pore but did not reproduce known binding residues, necessitating revision of model development to include additional structural densities and increasing search radius in docking protocol in future studies. Overall, this study demonstrated that atomistic modeling can be used to develop structural hypotheses about the mechanism of action of safe and unsafe drugs. Experimental testing and molecular dynamics simulations should be done to validate and test these hypotheses.

Appendix

I. Protocols and Scripts

A. XML Cryo-EM Refinement Protocol

```
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  <SCOREFXNS>
    <ScoreFunction name="dens" weights="beta_cart">
      <Reweight scoretype="elec_dens_fast" weight="35.0"/>
      <Set
scale_sc_dens_byres="R:0.76,K:0.76,E:0.76,D:0.76,M:0.76,C:0.81,Q:0.81,H:0.81,N:0.81,T:0.81,S:0.81,Y:0.
88,W:0.88,A:0.88,F:0.88,P:0.88,I:0.88,L:0.88,V:0.88"/>
    </ScoreFunction>
  </SCOREFXNS>

  <MOVERS>
     <SetupForDensityScoring name="setupdens"/>
     <LoadDensityMap name="loaddens" mapfile="/share/yarovlab/amemigh/projects/input/nav1.4-
fitting/emd_9617.map"/>
     <FastRelax name="relaxcart" scorefxn="dens" repeats="2" cartesian="1"/>
  </MOVERS>

  <PROTOCOLS>
    <Add mover="setupdens"/>
    <Add mover="loaddens"/>
    <Add mover="relaxcart"/>
  </PROTOCOLS>
  <OUTPUT scorefxn="dens"/>

</ROSETTASCRIPTS>
```

B. XML Comparative Modeling Protocol

```
<ROSETTASCRIPTS>
<SCOREFXNS>
   <ScoreFunction name="stage1" weights="score3" symmetric="1">
      <Reweight scoretype="atom_pair_constraint" weight="0.5"/>
   </ScoreFunction>
   <ScoreFunction name="stage2" weights="score4_smooth_cart" symmetric="1">
      <Reweight scoretype="atom_pair_constraint" weight="0.5"/>
   </ScoreFunction>
   <ScoreFunction name="fullatom" weights="ref2015_cart" symmetric="1">
      <Reweight scoretype="atom_pair_constraint" weight="0.5"/>
   </ScoreFunction>
   <ScoreFunction name="ref2015" weights="ref2015" symmetric="1">
   </ScoreFunction>
```

```
</SCOREFXNS>
<MOVERS>
  <Hybridize name="hybridize" stage1_scorefxn="stage1" stage2_scorefxn="stage2"
fa_scorefxn="fullatom" batch="1" stage1_increase_cycles="1.0" stage2_increase_cycles="1.0">
    <Template pdb="/share/yarovlab/amemigh/projects/input/hERG-EAG-hybrid/herg-template-
1D.pdb" cst_file="AUTO" weight="1.000" symmdef="/share/yarovlab/amemigh/projects/input/hERG-
EAG-hybrid/herg-template-1D.symm"/>
    Fragments three_mers="aaherg-1D_03_05.200_v1_3" nine_mers="aaherg-1D_09_05.200_v1_3" />
  </Hybridize>
  <FastRelax name="relax" scorefxn="ref2015"  />
</MOVERS>
<PROTOCOLS>
  <Add mover="hybridize"/>
</PROTOCOLS>
<OUTPUT scorefxn="ref2015" />
</ROSETTASCRIPTS>
```

C. XML Docking Protocol

```
<ROSETTASCRIPTS>
  <SCOREFXNS>
    <ligand_soft_rep weights="ligand_soft_rep">
      <Reweight scoretype="fa_elec" weight="0.42"/>
      <Reweight scoretype="hbond_bb_sc" weight="1.3"/>
      <Reweight scoretype="hbond_sc" weight="1.3"/>
      <Reweight scoretype="rama" weight="0.2"/>
    </ligand_soft_rep>

    <hard_rep weights=ligand>
      <Reweight scoretype="fa_intra_rep" weight="0.004"/>
      <Reweight scoretype="fa_elec" weight="0.42"/>
      <Reweight scoretype="hbond_bb_sc" weight="1.3"/>
      <Reweight scoretype="hbond_sc" weight="1.3"/>
      <Reweight scoretype="rama" weight="0.2"/>
    </hard_rep>
  </SCOREFXNS>
  <LIGAND_AREAS>
    <docking_sidechain chain="X" cutoff="7.0" add_nbr_radius="true" all_atom_mode="true"
minimize_ligand="10"/>
    <final_sidechain chain="X" cutoff="7.0" add_nbr_radius="true" all_atom_mode="true"/>
    <final_backbone chain="X" cutoff="7.0" add_nbr_radius="false" all_atom_mode="true"
Calpha_restraints="0.3"/>
  </LIGAND_AREAS>

  <INTERFACE_BUILDERS>
    <side_chain_for_docking ligand_areas="docking_sidechain"/>
    <side_chain_for_final ligand_areas="final_sidechain"/>
```

```
    <backbone ligand_areas="final_backbone" extension_window="3"/>
  </INTERFACE_BUILDERS>

  <MOVEMAP_BUILDERS>
    <docking sc_interface="side_chain_for_docking" minimize_water="true"/>
    <final sc_interface="side_chain_for_final" bb_interface="backbone" minimize_water="true"/>
  </MOVEMAP_BUILDERS>

  <SCORINGGRIDS ligand_chain="X" width="15">
    <vdw grid_type="ClassicGrid" weight="1.0"/>
  </SCORINGGRIDS>

  <MOVERS>
    <Transform name="transform" chain="X" box_size="5.0" move_distance="0.1" angle="5"
cycles="1000" repeats="1" temperature="5" initial_perturb="5.0"/>
    <HighResDocker name="high_res_docker" cycles="6" repack_every_Nth="3"
scorefxn="ligand_soft_rep" movemap_builder="docking"/>
    <FinalMinimizer name="final" scorefxn="hard_rep" movemap_builder="final"/>
    <InterfaceScoreCalculator name="add_scores" chains="X" scorefxn="hard_rep"
compute_grid_scores="0"/>
    AddJobPairData name="system_name" key="system_name" value_type="string"
value_from_ligand_chain="X"

    <ParsedProtocol name="low_res_dock">
      <Add mover_name="transform"/>
    </ParsedProtocol>

    <ParsedProtocol name="high_res_dock">
      <Add mover_name="high_res_docker"/>
      <Add mover_name="final"/>
    </ParsedProtocol>

    <ParsedProtocol name="reporting">
      <Add mover_name="add_scores"/>
      <Add
mover_name="system_name"/>
    </ParsedProtocol>
  </MOVERS>

  <PROTOCOLS>
    <Add mover_name="low_res_dock"/>
    <Add mover_name="high_res_dock"/>
    <Add mover_name="reporting"/>
  </PROTOCOLS>

</ROSETTASCRIPTS>
```

D. Clustering Inputs Extraction Tcl Script

```
proc getz {l d} {
   set com1 [measure center $l weight mass]
   set com2 [measure center $d weight mass]
   set dz [vecsub $com2 $com1]
   return [lindex $dz 2]
}

proc theta {s c} {
      #Get tilt angle between drug and z
      #special arctan
   return [expr atan2($s,$c)]
}

proc phi {v} {
      #Get azimuth angle between drug and x
   set x [lindex $v 0]
   set y [lindex $v 1]
   return [expr atan2($y,$x)]
}

proc rad2deg {a} {
      set PI 3.14159
   return [expr $a * (180.0/$PI)]
}

set moln 0

mol new top50_1.pdb type pdb waitfor all

for { set j 2 } { $j <= 50 } { incr j 1 } {
   animate read pdb top50_${j}.pdb beg 0 end -1 skip 1  waitfor all $moln
   }

set outfile [open ligand-tumble_closed_nif1.dat w]
set nf [molinfo top get numframes]

      puts "number of frames $nf"

set sf [atomselect top "sequence SVGFG"]
set drug [atomselect top "chain X"]
set PI 3.14159
   return [expr $a * (180.0/$PI)]
}

set moln 0
```

```
mol new top50_1.pdb type pdb waitfor all

for { set j 2 } { $j <= 50 } { incr j 1 } {
   animate read pdb top50_${j}.pdb beg 0 end -1 skip 1  waitfor all $moln
   }

set outfile [open ligand-tumble_closed_nif1.dat w]
set nf [molinfo top get numframes]

      puts "number of frames $nf"

set sf [atomselect top "sequence SVGFG"]
set drug [atomselect top "chain X"]

set H [atomselect top "chain X and name O2"]
set T [atomselect top "chain X and name O3"]

for { set i 0 } { $i <= $nf } { incr i } {

            $sf frame $i
            $drug frame $i
            set zpos [getz $sf $drug]

            $H frame $i
            set Hcoor [lindex [$H get {x y z}] 0]
            $T frame $i
            set Tcoor [lindex [$T get {x y z}] 0]

            set vlen [ veclength [vecsub $Hcoor $Tcoor]]

            set vdrug [vecnorm [vecsub $Hcoor $Tcoor]]

            set vz {0 0 1}
            set rotvec [veccross $vdrug $vz]
            set sine [veclength $rotvec]
            set cosine [vecdot $vdrug $vz]
            set th [theta $sine $cosine]
            set ph [phi $vdrug]
            if {$ph < 0 } {
               set ph [expr $ph +360]
            }

            puts $outfile [format "%6d\t%8f\t%8f\t%8f\t%8f\t%8f\t%8f" $i $zpos $sine $cosine $th $ph
$vlen]
         }

      close $outfile
```

```
$sf delete
$drug delete
$H delete
$T delete

mol delete all
```

E. Clustering Algorithm R Script

```
#### RScript to Calculate Symmetric RMSD
library(tidyverse)
library(animation)
library(rlist)
### Read in data file and cut
setwd("/Users/aiyana/Box/Work-In-Progress/workInProgress/l-sotalol/dock-herg-closed-lsot1/top-50")
dft <- read.table("ligand-tumble_closed_sotl1.dat",header=F)
temp <- dft[,c(2,5,7)]
data <- head(temp,-1)
data_scaled <- scale(data)

### Find max, min and diffs of each column
colMax <- function(data) sapply(data,max)
maxs <- colMax(data)
colMin <- function(data) sapply(data,min)
mins <- colMin(data)
diffs = maxs - mins

### Initialize variables
n_row = nrow(data)
n_col = ncol(data)
vars <- array(0,c(nrow(data),ncol(data),nrow(data)))
sims <- array(0,c(nrow(data),nrow(data)))
counts <- array(0,c(ncol(sims)))
cutoff = 0.05
size_min = 3

### Calculate squares of deviation for each variable
ref = 1
while (ref <= n_row){
  for (row in 1:n_row) {
    for (col in 1:n_col) {
      vars[row,col,ref] = ((data[ref,col]-data[row,col])/diffs[col])^2
    }
  }
  ref = ref + 1
}
```

```
### Calculate similarity (squareroot of sums)
ref = 1
sums = 0
while (ref <= n_row) {
  for (row in 1:n_row) {
    for (col in 1:n_col) {
      sums = sums + vars[row,col,ref]
    }
    sims[row,ref] = sqrt(sums)
    if (sims[row,ref] <= cutoff) {

    }
    sums = 0
  }
  ref = ref + 1
}

### Count number of neighbors within cutoff
neighbors <- list()
for (col in 1:ncol(sims)) {
  counts[col] = sum(sims[,col] <= cutoff)
  nbrs <- c()
  count = 0
  for (row in 1:nrow(sims)) {
    if (sims[row,col] <= cutoff) {
      count = count + 1
      nbrs[count] <- row
    }
  }
  neighbors[[col]] <- nbrs
}

### Find unique first elements
matches <- c()
count2 = 0
for (row in 1:(length(neighbors)-1)) {
  temp1 <- vector(,length(neighbors[[row]]))
  temp1 <- neighbors[[row]]
  for (second in (row+1):length(neighbors)) {
    temp2 <- vector(,length(neighbors[[second]]))
    temp2 <- neighbors[[second]]
    if (temp1[1] == temp2[1]) {
      count2 = count2 + 1
      matches[count2] <- second
    }
  }
}
matches_cull <- matches[!duplicated(matches)]
```

```
matches_sort <- sort(matches_cull, decreasing = TRUE)

for(values in 1:length(matches_sort)) {
  neighbors[[matches_sort[values]]] <- NULL
}

### Set min size of clusters
for(rows in length(neighbors):1) {
  if(length(neighbors[[rows]])<=size_min){
    neighbors[[rows]] <- NULL
  }
}

### Set lowest unique cluster as centroids
clusters <- array(0,as.integer(length(neighbors)))
for(i in 1:length(neighbors)) {
  temp1 <- vector(,length(neighbors[[i]]))
  temp1 <- neighbors[[i]]
  clusters[i] <- temp1[1]
}

### Build centroid array with scaled data
centroid <- array(0,c(length(clusters),ncol(data_scaled)))
for (col in 1:ncol(data_scaled)) {
  for (row in 1:length(clusters)) {
    centroid[row,col] <- data_scaled[clusters[row],col]
  }
}

### Kmeans optimization of clusters
op <- kmeans(data_scaled,centroid)
print(op)

### Find largest cluster number
op_cluster <- array(op$cluster)
op_size <- array(op$size)
largest_cluster_num <- which.max(op_size)

### Find lowest energy structure from largest cluster
lowest_energy <- 0
for(pose in 1:length(op_cluster)) {
  if(op_cluster[pose]==largest_cluster_num) {
    lowest_energy = pose
    break
  }
}

### Populate array with poses of largest cluster
```

```
largest_cluster <- vector(mode = "integer",length = max(op_size))
i=1
#pose=1
##print(length(op_cluster))
for(pose in 1:length(op_cluster)) {
  if(op_cluster[pose]==largest_cluster_num) {
    #cat("position, cluster, index: ", pose, op_cluster[pose], i)
    largest_cluster[i] = pose
    i=i+1
  }
}

### Create list with outliers removed
noOutliers <- NULL
for (row in 1:length(neighbors)) {
  noOutliers <- append(noOutliers, neighbors[[row]])
  }
noOutliers <- unique(noOutliers)

#print(op_cluster)

### Print Results for largest cluster only
### cat("The largest cluster has", op_size[5], "members.")
### cat("The lowest energy (representative) structure from the largest cluster is",
paste0(lowest_energy,"."))
### cat("The members of this cluster are", largest_cluster)

### Create List of all clusters, print results
'%!in%' <- function(x,y)!('%in%'(x,y))
all_clusters <- vector(mode = "list", length = length(op_size))
for(index in 1:length(op_size)){
    all_clusters[[index]] <- which(op_cluster %in% index)
    for(element in 1:length(all_clusters[[index]])) {
      if(all_clusters[[index]][element] %!in% noOutliers){
        all_clusters[[index]][element] <- 0
      }
    }
}
all_clusters <- lapply(all_clusters,function(x) x[x!=0]) #How to remove zero elements?
sorted_clusters <- all_clusters[order(sapply(all_clusters,length),decreasing=T)]
print(sorted_clusters)
paste(sorted_clusters, collapse = ", ")
#testing <- paste(sorted_clusters[1], sep = ",")
#gsub()
```

II. Supplemental Figures

Figure 23 Analytical Plots of Amiodarone-hERG Channel Interactions

Left panels are box-and-whisker plots of top 50 interface scores (measured in Rosetta Energy Units) of amiodarone with each protein model. To compare each state to the open-state, wild-type, two sample for variance F-tests were done, followed by the corresponding t-tests assuming equal or unequal variances. Significance at p=0.5 is indicated by * and at p=0.001 by *** are indicated above their respective data sets. Right panels are probability density charts plotting probability versus interface score. Open-state wild-type is red, open-state F656A mutant is orange, open-state Y652A is yellow, open-state F656A/Y652A is green, and closed-state is blue.

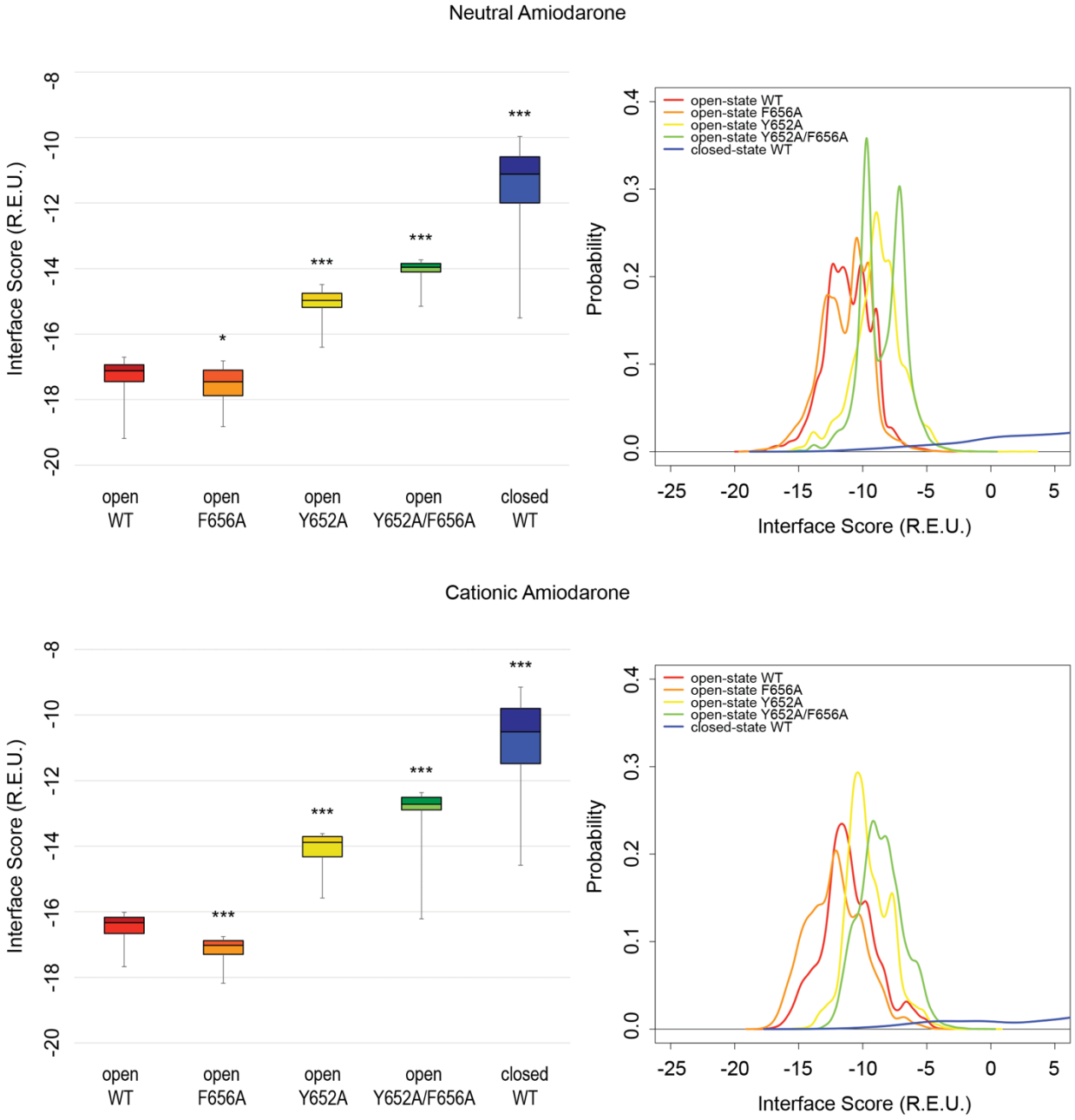


Figure 24 Analytical Plots of Nifekalant-hERG Channel Interactions

Left panels are box-and-whisker plots of the top 50 interface scores (measured in Rosetta Energy Units) of nifekalant with each protein model. To compare each state to the open WT, two sample for variance F-tests were done, followed by the corresponding t-tests assuming equal or unequal variances. Significance at p=0.01 is indicated by ** and at p=0.001 by *** are indicated above their respective data sets. Right panels are probability density charts plotting probability versus interface score of top 10,000. Open-state wild-type is red, open-state F656A mutant is orange, open-state Y652A is yellow, open-state F656A/Y652A is green, and closed-state is blue.

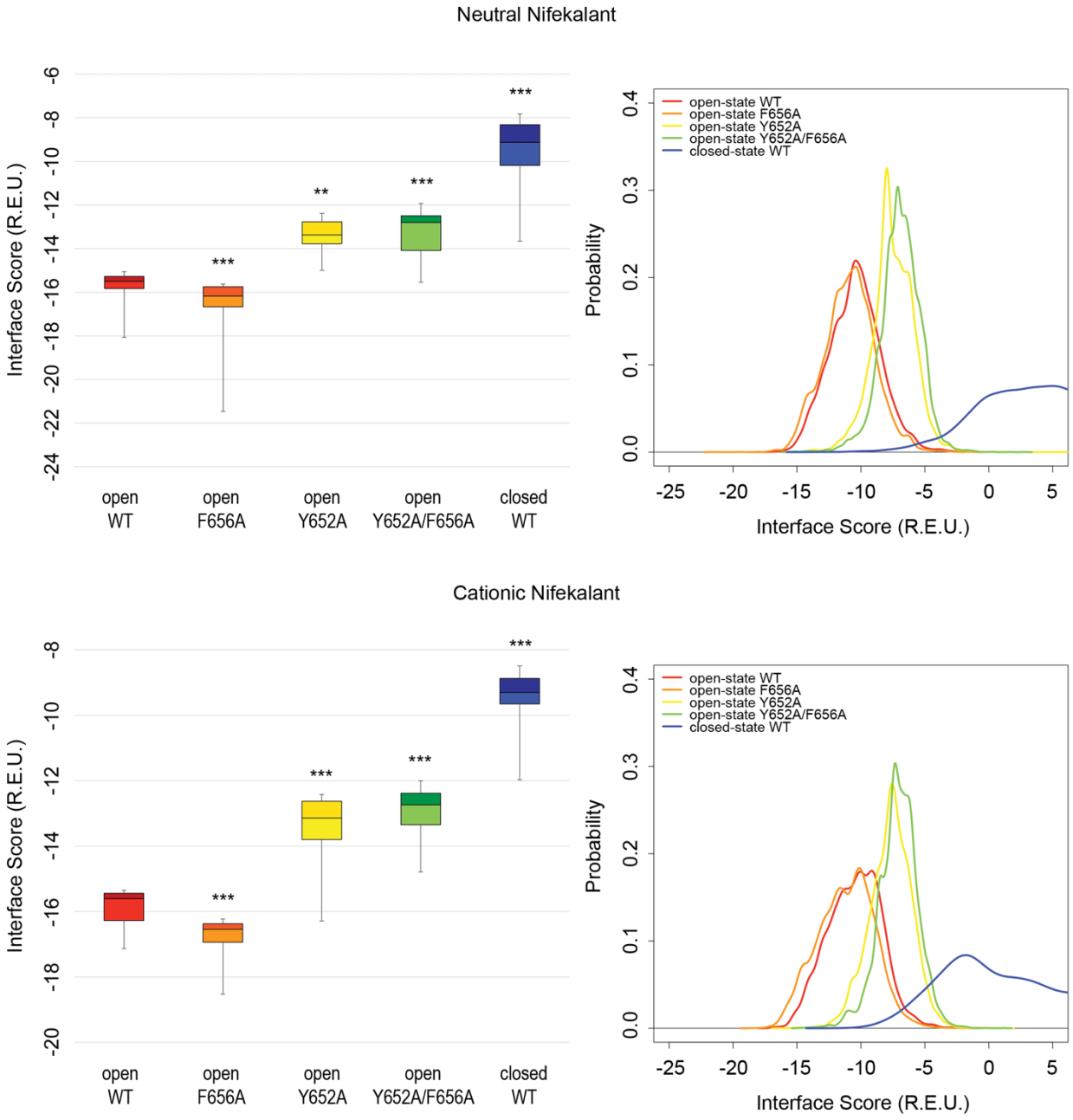


Figure 25 Analytical Plots of Flecainide-hERG Channel Interactions

Left panels are box-and-whisker plots of the top 50 interface scores (measured in Rosetta Energy Units) of flecainide with each protein model. To compare each state to the open WT, two sample for variance F-tests were done, followed by the corresponding t-tests assuming equal or unequal variances. Significance at p=0.001 is indicated by *** are indicated above their respective data sets. Right panels are probability density charts plotting probability versus interface score of top 10,000. Open-state wild-type is red, open-state F656A mutant is orange, open-state Y652A is yellow, open-state F656A/Y652A is green, and closed-state is blue.

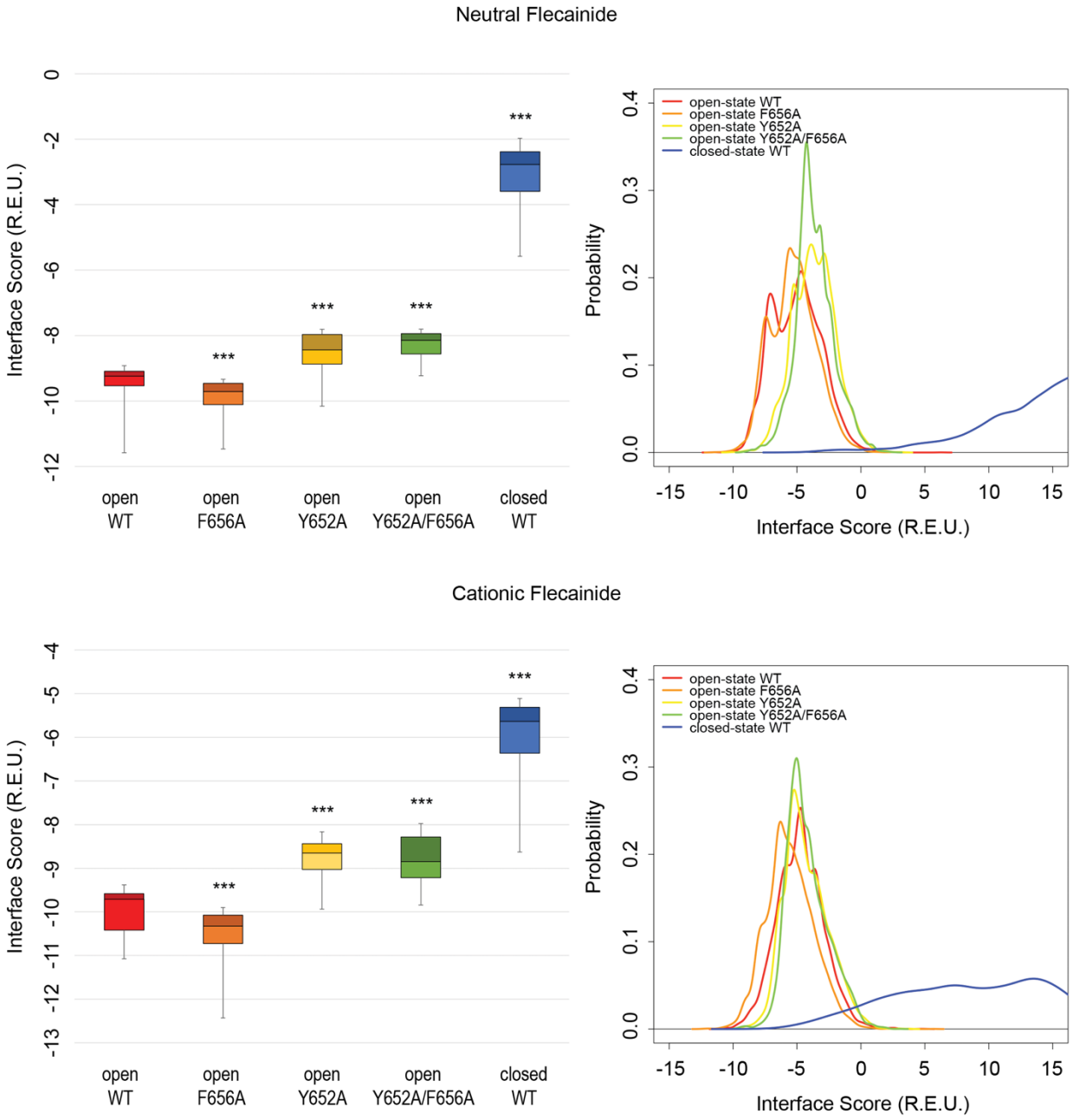


Figure 26 Analytical Plots of Moxifloxacin–hERG Channel Interactions

Left panels are box-and-whisker plots of the top 50 interface scores (measured in Rosetta Energy Units) of moxifloxacin with each protein model. To compare each state to the open WT, two sample for variance F-tests were done, followed by the corresponding t-tests assuming equal or unequal variances. Significance at p=0.05 is indicated by *, at p=0.01 by **, and at p=0.001 by *** are indicated above their respective data sets. Right panels are probability density charts plotting probability versus interface score of top 10,000. Open-state wild-type is red, open-state F656A mutant is orange, open-state Y652A is yellow, open-state F656A/Y652A is green, and closed-state is blue.

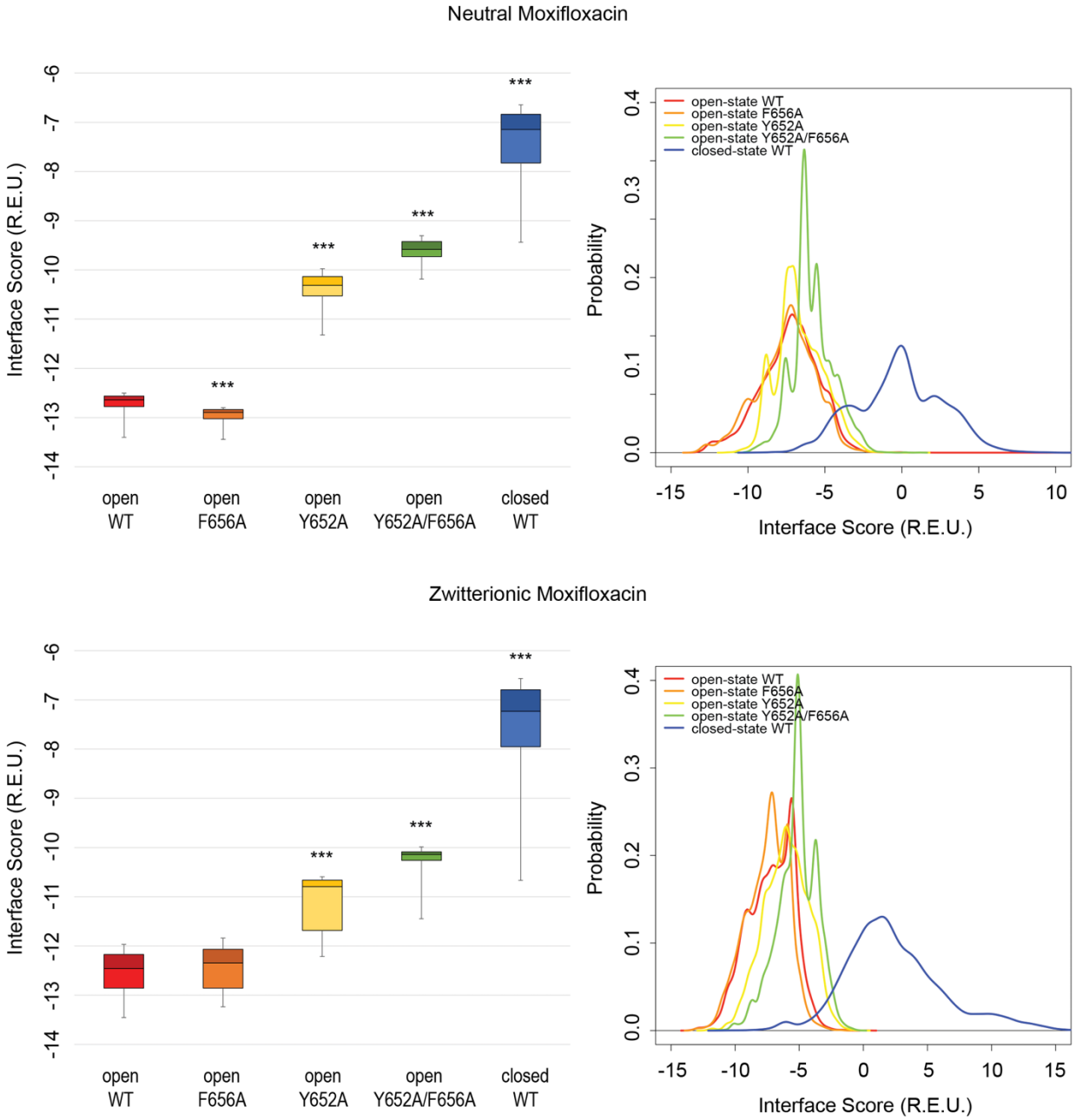


Figure 27 Analytical Plots of d-Sotalol–hERG Channel Interactions

Left panels are box-and-whisker plots of the top 50 interface scores (measured in Rosetta Energy Units) of d-sotalol with each protein model. To compare each state to the open WT, two sample for variance F-tests were done, followed by the corresponding t-tests assuming equal or unequal variances. Significance at p=0.05 is indicated by *, at p=0.01 by **, and at p=0.001 by *** are indicated above their respective data sets. Right panels are probability density charts plotting probability versus interface score of top 10,000. Open-state wild-type is red, open-state F656A mutant is orange, open-state Y652A is yellow, open-state F656A/Y652A is green, and closed-state is blue.

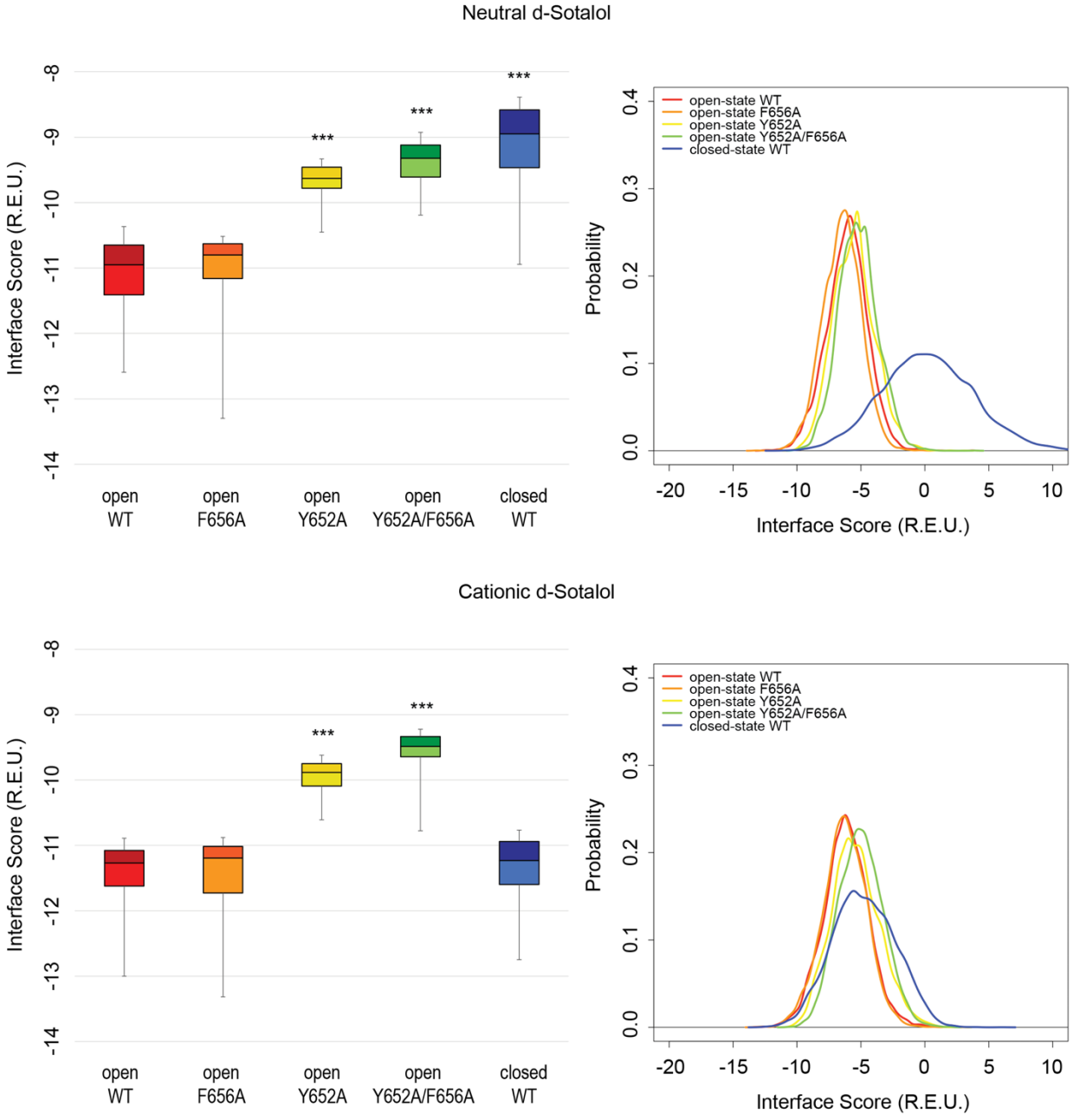


Figure 28 Analytical Plots of d-Sotalol–hERG Channel Interactions

Left panels are box-and-whisker plots of the top 50 interface scores (measured in Rosetta Energy Units) of l-sotalol with each protein model. To compare each state to the open WT, two sample for variance F-tests were done, followed by the corresponding t-tests assuming equal or unequal variances. Significance at p=0.05 is indicated by *, at p=0.01 by **, and at p=0.001 by *** are indicated above their respective data sets. Right panels are probability density charts plotting probability versus interface score of top 10,000. Open-state wild-type is red, open-state F656A mutant is orange, open-state Y652A is yellow, open-state F656A/Y652A is green, and closed-state is blue.

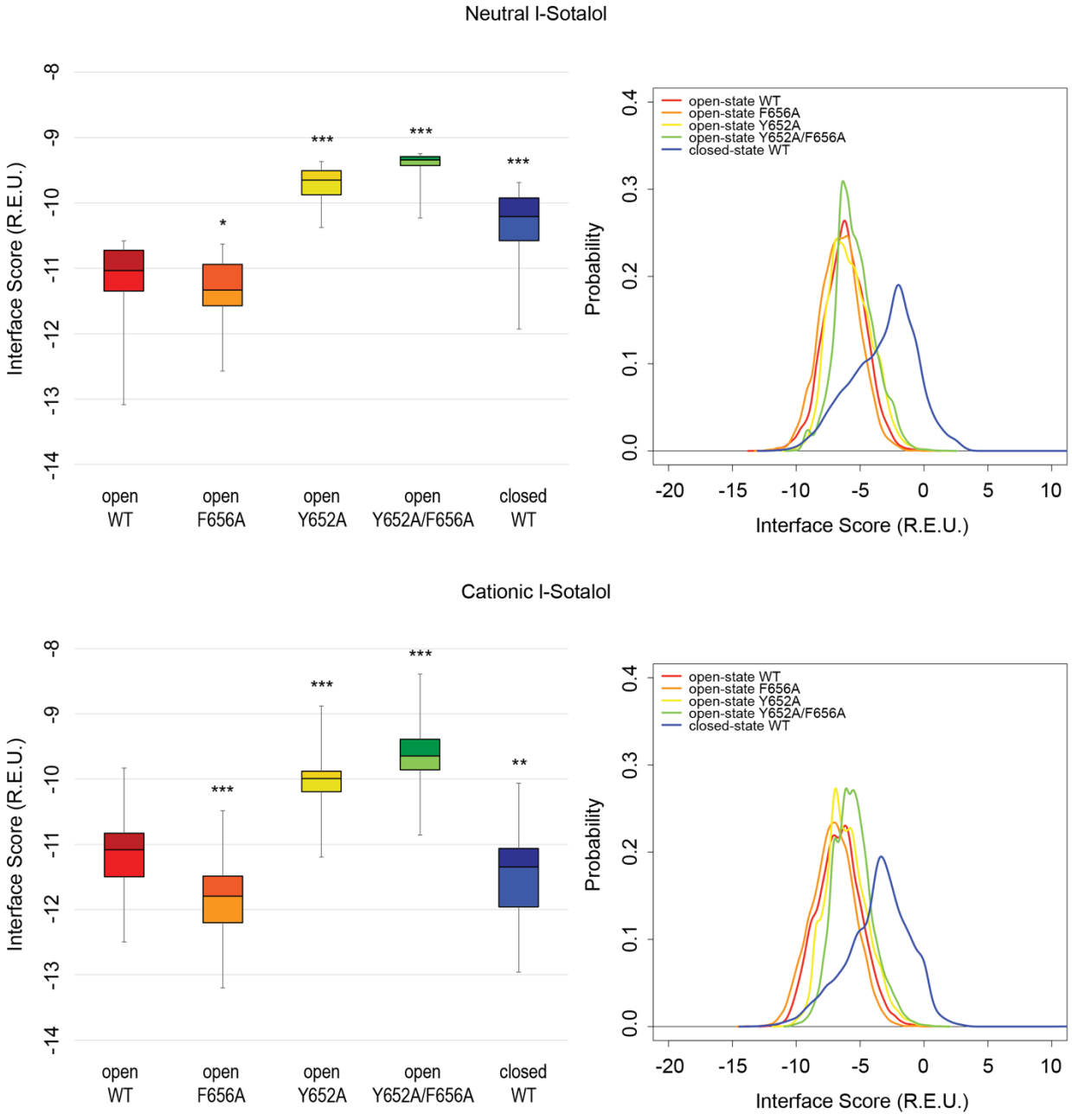


Figure 29 Analytical Plots of Dofetilide–hERG Channel Interactions

Left panels are box-and-whisker plots of the top 50 interface scores (measured in Rosetta Energy Units) of dofetilide with each protein model. To compare each state to the open WT, two sample for variance F-tests were done, followed by the corresponding t-tests assuming equal or unequal variances. Significance at p=0.05 is indicated by *, at p=0.01 by **, and at p=0.001 by *** are indicated above their respective data sets. Right panels are probability density charts plotting probability versus interface score of top 10,000. Open-state wild-type is red, open-state F656A mutant is orange, open-state Y652A is yellow, open-state F656A/Y652A is green, and closed-state is blue.

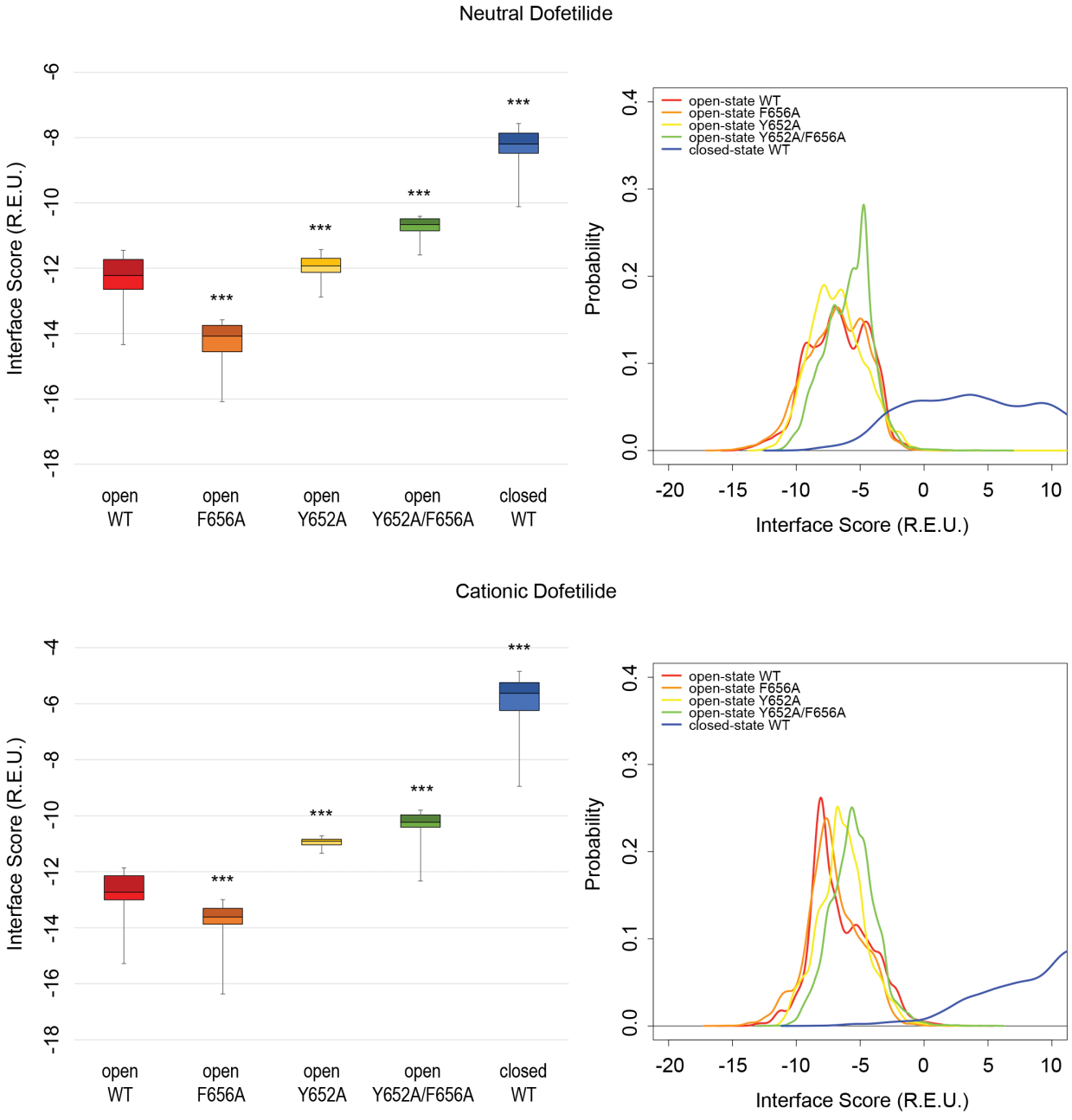


Figure 30 Interface Scores vs. RMSD Plots for top 1000 docked hERG-drug poses

Interface score plotted against root-mean-square deviation from the reference pose of the top 1,000 poses by interface score of the top 10,000 poses by total score. Clustering was done only on the top 50 poses by interface score as indicated by horizontal line. Black dots below line are unclustered poses within the top 50.

III. Portfolio

A. Personal History and Motivation

For many years, I questioned where I fit into the scientific and educational communities. As I explored careers as a scientist, research professor, K-12 teacher, and science communicator, I unexpectedly found my niche in policy and administration. While volunteering with the US Peace Corps (USPC) in The Gambia, West Africa, as a Math and Science Teacher Trainer, I gravitated toward opportunities to contribute to my Peace Corps community. I worked as a regional representative for the Gender and Development Committee, where I advocated for the programming needs of volunteers to address local gender inequalities in the North Bank Region. I also served as a peer advisor for the Volunteer Support Network (VSN), where I traveled to the homes of other Peace Corps volunteers (PCVs) in our country to help them resolve issues with host family dynamics, food insecurity, coworker relationships, and mental health. Within VSN, I was elected to be the first Training Coordinator, where I developed and implemented workshops to help train new peer mentors in conflict management, cultural sensitivity, and mental health support.

In this capacity as a peer mentor, through conversations with my fellow PCVs, I identified several important worldwide Peace Corps policies that negatively impacted volunteer access to treatment for substance abuse and lack of informing volunteers of their legal rights. I drafted two letters to D.C. headquarters—one to the Office of the Inspector General (OIG) and one to the President of the USPC—detailing the negative impact these policies were having on volunteers' health and safety. Also included in these letters were proposals for policy changes developed

through my discussions with all our volunteers in The Gambia. I then worked with our very supportive Peace Corps Director for The Gambia, Jennifer Goette, to submit these letters. Over the next year, Jennifer followed and participated in the conversations sparked by my letters and regularly provided me with updates about the administration's response. One year and 8 months later, the policy finally recognized drug addiction as a medical condition and changed the administrative response from immediate administrative separation to medical separation with coverage for treatment. Not long after, the OIG began including the rights of volunteers during investigations into the orientation materials for new volunteers. This was my first real experience working with an administration to advocate for my peers, and our success inspired me to continue working in this capacity to achieve positive change.

Returning from The Gambia two weeks before beginning my graduate studies, I began volunteering on campus and around Davis because of my belief that it is important to give back to the communities that support you. I began leading science outreach activities at festivals, local science centers, and the UCD Women's Resource Center. I helped with recruitment and orientation programs for my graduate program and listened to my fellow graduate students' stories about their experiences, motivations, struggles, and successes. Despite knowing that research was not my ultimate career goal, I absolutely thrived at the beginning of graduate school. I had joined a lab with a friendly environment and the three most engaging and supportive mentors. I had made friends, had a good support network, and found the coursework manageable and interesting. My program gave me the flexibility to pursue the communication, policy, and outreach aspects of science that would support my career path outside academia. At the same time, I started to see a decline in my mental and physical health. By the time I realized

it was physiological, rather than just existential, in origin, I could barely get out of bed most days. It took the next three years of fighting doctors to run tests and convincing them my symptoms were pathological before getting a diagnosis and a treatment and maintenance plan that worked for me. Balancing research, coursework, career development, and all the leadership responsibilities I had taken on while masking my struggles throughout my Ph.D. made me realize there were gaps in the administration of my graduate group. In speaking with other graduate students in my program, I realized my experience wasn't unique. Many students were struggling. Some weren't so lucky to have as involved, empathetic, and understanding advisors as I did. Students were falling through the cracks, and I felt I had the relevant experience and responsibility to contribute and advocate for my peers.

Many articles have been published over the past decade calling for a "rethinking" of the Ph.D., especially in the natural sciences, as the reasons for pursuing a Ph.D. evolve away from the way Ph.D. training was originally designed[172–178]. While most agree that there are issues with training, work-life balance, discrimination and harassment, and job prospects, steps to reconcile Ph.D. training with modern society at the systemic level are, by nature, difficult and slow to accomplish. An ever-growing proportion of graduate students are moving away from academic research career paths and towards other sectors (industry, government, non-profit, teaching-focused academia)[175]. So, what does that mean for current and prospective graduate students? I believe the environmental activist phrase from the 1970s–"think global, act local"–has pertinent applications here. While the global demand for academic scientists has plummeted[174,179] and we encourage state, national, and global funding schemas and education policy to adapt incrementally to the current state of affairs, program- and department-level shifts in culture,

expectations, and requirements are much more accessible to graduate students, program staff, and faculty.

Over the past 6 years, I have worked with our administration to push for changes within my graduate program to benefit graduate student education and well-being. I often talk about the UC Davis Biophysics Graduate Group as a double-edged sword–the best qualities of the program are also what can make students struggle. While the biophysics program at UC Davis is wonderful for its location, the large number of faculty, small size, and flexibility, these same qualities can result in the program having expensive and limited housing options, being overwhelming and hard to navigate, lacking diversity, and lacking adequate guidance and advising. In 2018 I co-founded the Student Chapter of the Biophysical Society, where I led discussions on the state of biophysics research and education on campus and how we can work to foster a stronger biophysics community and better support current students, post-docs, and faculty. I also served as my graduate group's representative to the Graduate Student Association and represented graduate student voices on the Graduate Council's Subcommittee on Academic Planning and Development. Additionally, I became Director of Professional Development for the graduate student organization ESTEME (Equity in Science, Technology, Engineering, Mathematics, and Entrepreneurship) to help provide career development resources to graduate students and career exploration outside the traditional academic pathways. During this time, I also developed a student survey based on established well-being surveys[173,180,181] to collect the stories of and data on the experiences of the graduate students in my program (Appendix II).

As someone pursuing a Ph.D. with my own non-traditional goals after graduation, I wanted to leave my program on a sustainable path towards equity, inclusion, transparency, and

accountability. Beyond the efforts that are already in progress–spearheaded by my peers and our program leadership–I believe documentation of the changes occurring is important for institutional memory. I also wanted to provide one example of how students can balance extracurricular activities with their degree requirements to expand their graduate education to meet their career development goals in the short term. The following portfolio summarizes the work I've done on my own and in collaboration with my passionate peers, faculty, and mentors to enhance the biophysics program and the non-traditional career training I participated in while completing my Ph.D.

B. Biophysics Graduate Student Experience Survey Data

Throughout my time at BPH, I engaged in conversations with faculty and students about their dissatisfaction with many parts of our program. However, I also had the pleasure of connecting with some of the most passionate and caring scientists I have ever met. I feel honored to consider the people of our program my friends, coworkers, and mentors. Because of this, I sought to help transform our program into something that everyone can be proud of and that meets the needs of a new generation of students.

To transform my conversations with students into concrete data, I designed and implemented a student experience survey–with help from my fellow students–informed by these conversations and adapted from student experience and mental health surveys from other universities. When I presented what we learned from the survey to the faculty, staff, and student of BPH, I believe it sparked needed discussion and revealed several areas for program reform that we began to address.

To put in context any trends in the data, we need to consider the timeline of some of the changes to the BPH program:

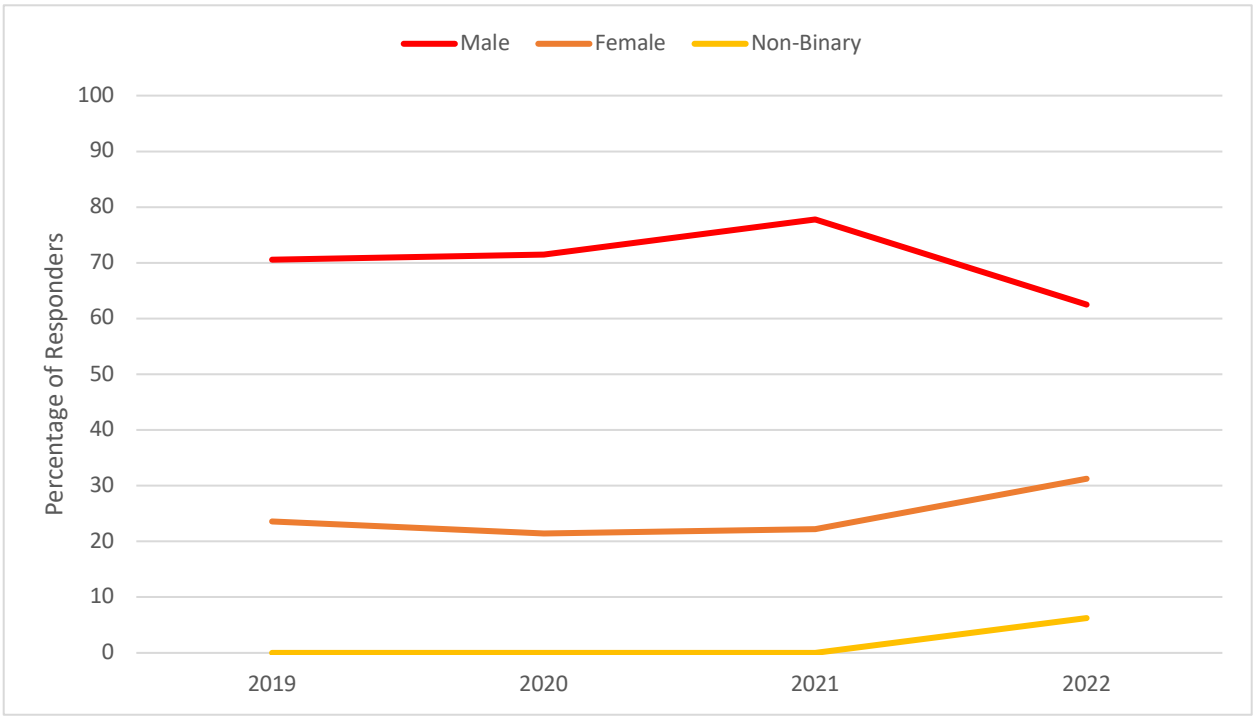
- 2018-2019
  - Updating website
  - New faculty as committee chairs
  - Founding of Student Chapter of the Biophysical Society
  - First student experience survey
- 2019-2020

  - Inclusion of navigating interviews with potential PIs in orientation materials for the first time
  - COVID-19 pandemic begins
- 2020-2021
  - Inclusion of mentoring expectations in orientation materials for the first time
  - Fully virtual orientation and rotations
  - Virtual recruitment
- 2021-2022
  - Selection of master advisor
  - Virtual recruitment
  - Revision of degree requirements and recommended courses

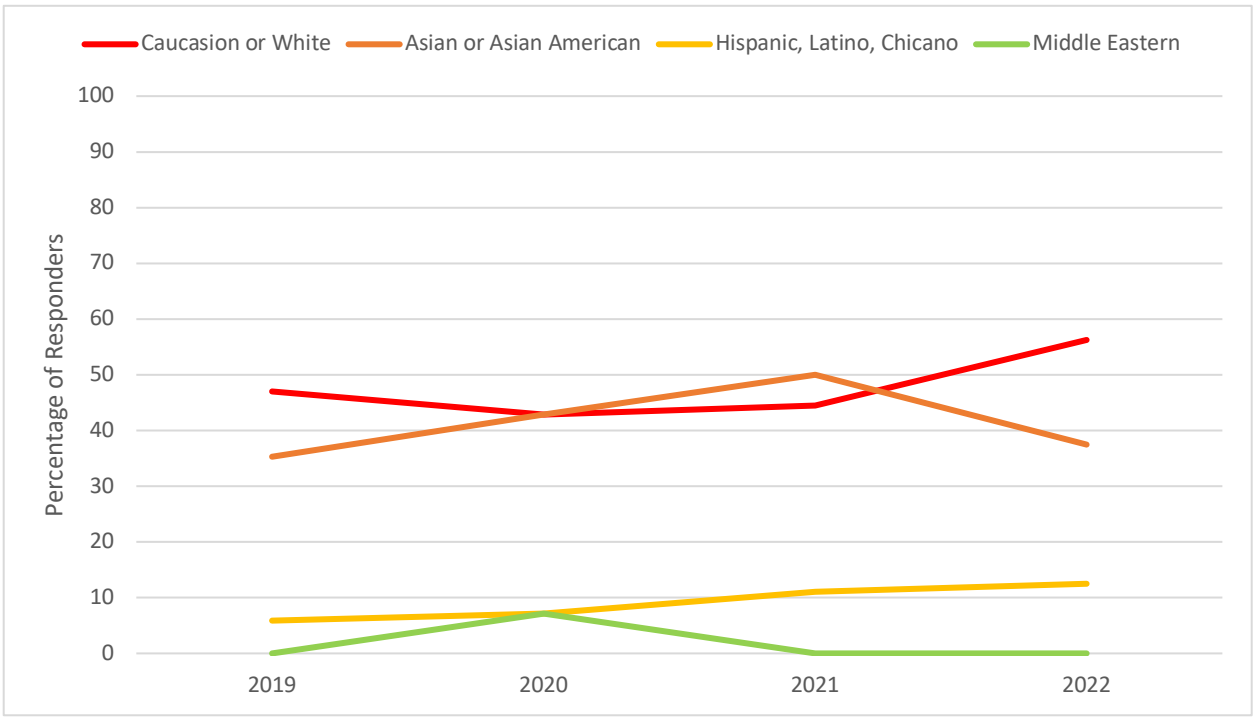
The following is the collected survey data from the past four years (2019-2022). Each year, 75-100% of BPH students participated in the survey. All data are presented as the percentage of responders for that year.

1. Demographic Data

Self-identified gender composition of survey respondents



Self-identified racial and ethnic composition of survey responders



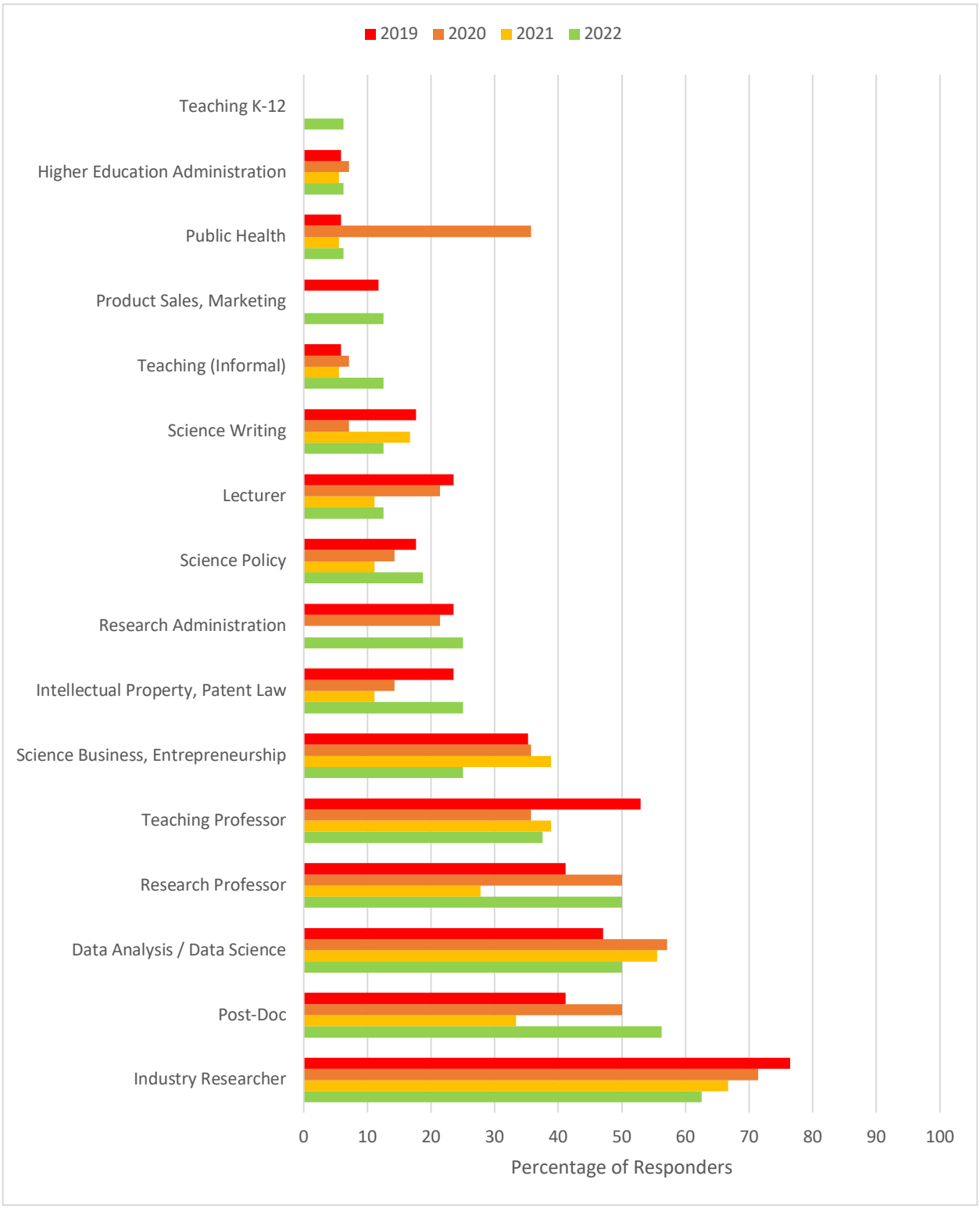
Additional demographic data

Note: “n/a” denotes a question added in a later year with no data collected previously.

	2019	2020	2021	2022
Total Number of Survey Participants	17	14	18	16
International Students	n/a	5	5	5
First Generation	n/a	n/a	n/a	5
Year in Program: 1	4	2	4	2
Year in Program: 2	5	3	2	4
Year in Program: 3	2	4	3	2
Year in Program: 4	2	2	3	2
Year in Program: 5	4	0	2	3
Year in Program: 6+	0	2	4	3
Do you plan on completing your PhD?: Yes	14	13	17	14
Do you plan on completing your PhD?: No	1	1	1	1
Do you plan on completing your PhD?: Undecided	2	0	0	1
Passed Qualifying Exam?: Yes	5	5	9	10
Passed Qualifying Exam?: No	12	9	9	6

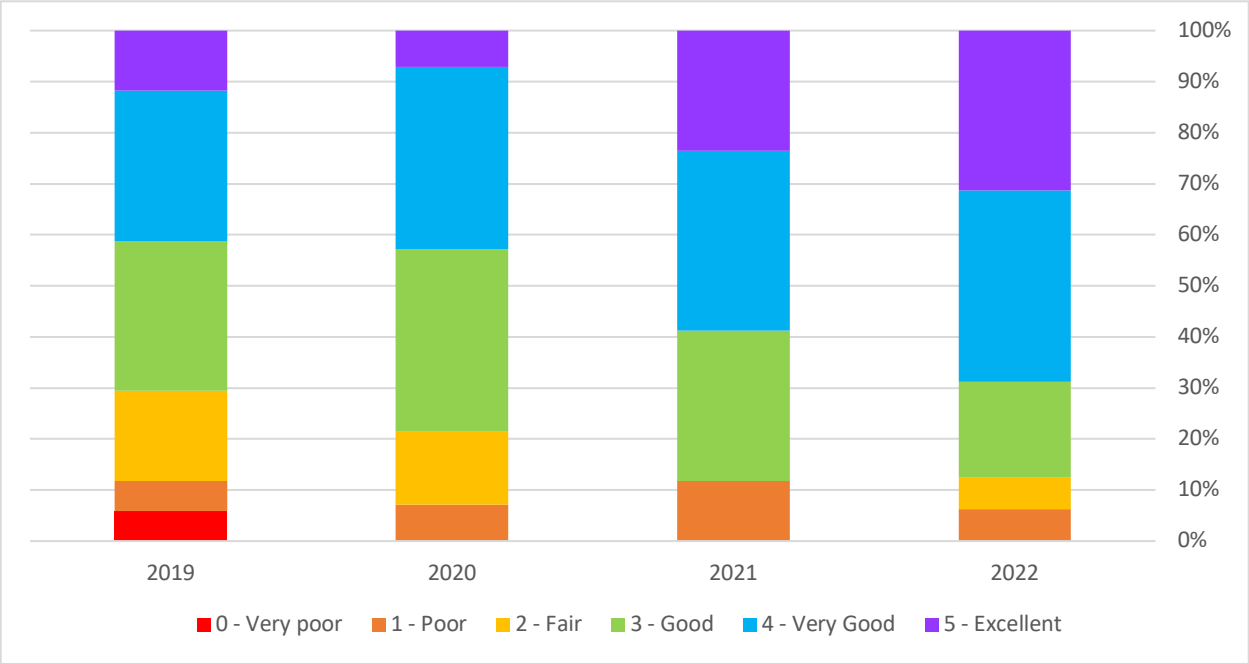
2. Career Interests

Reported interest of responders in various scientific careers

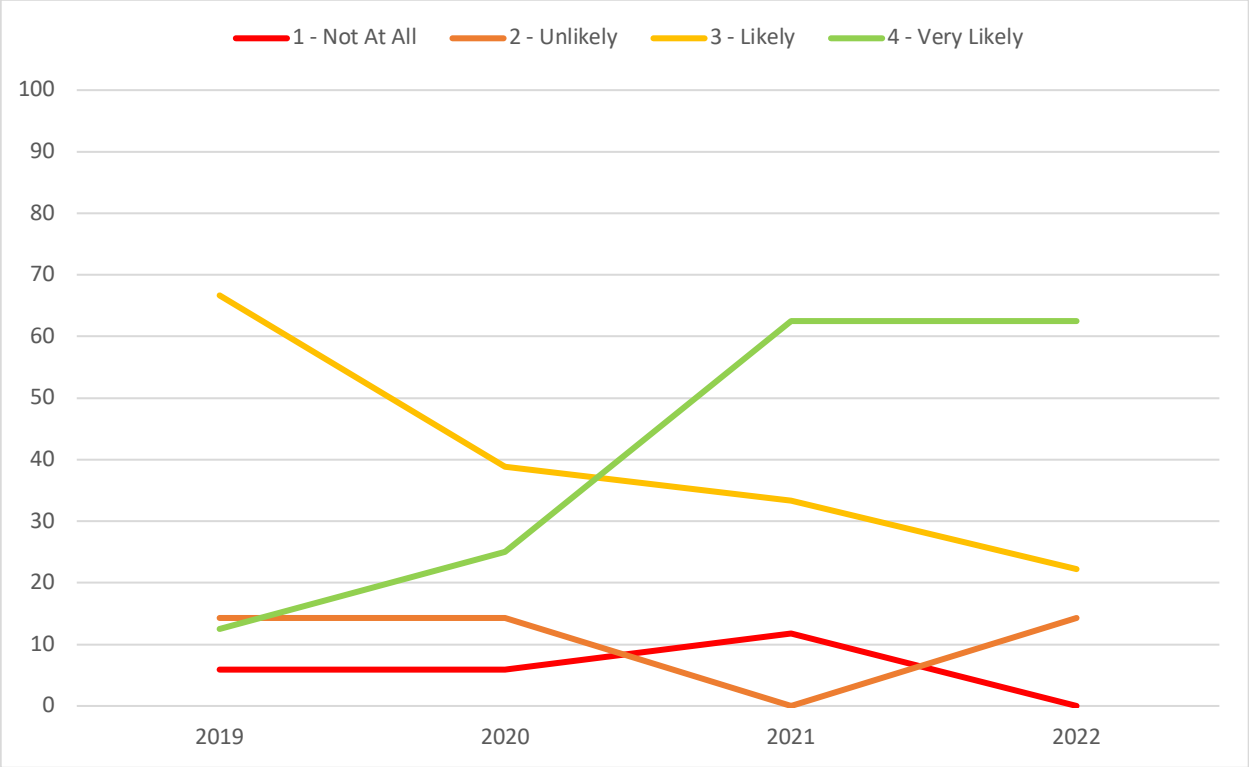


3. Academic Experiences

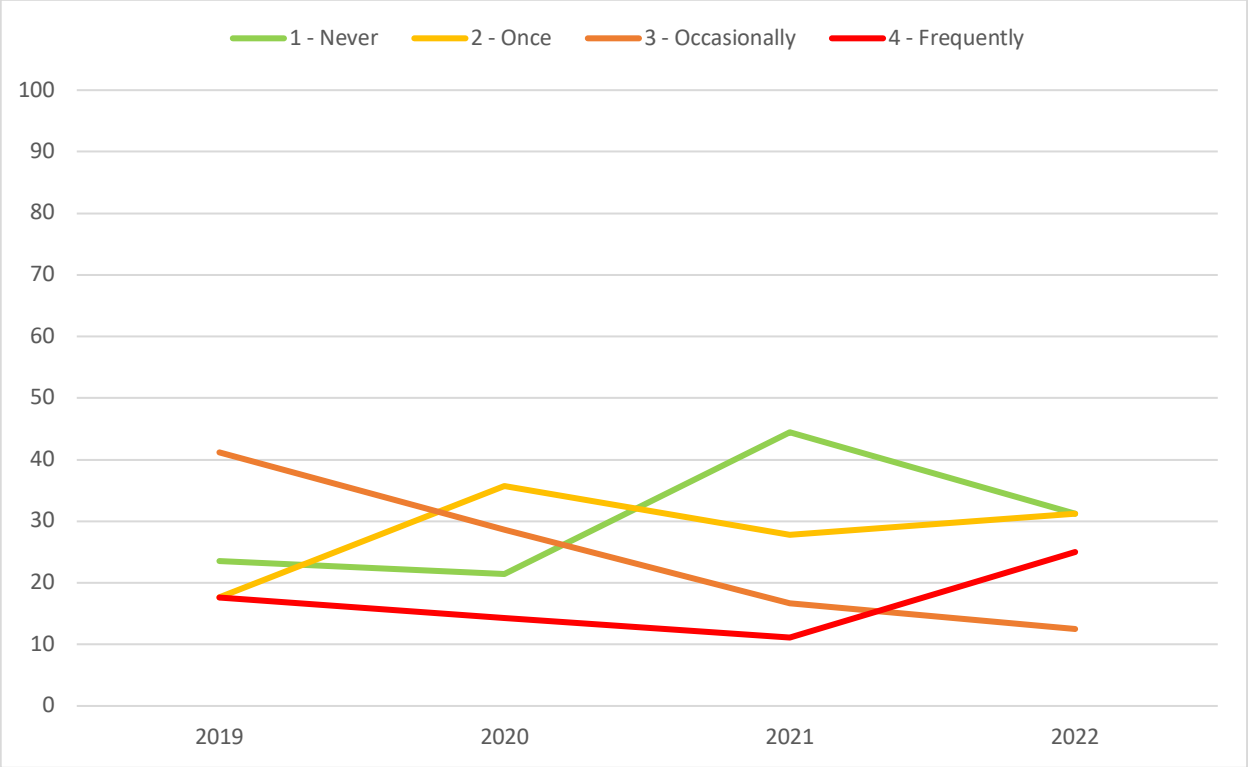
Perception of overall academic experiences



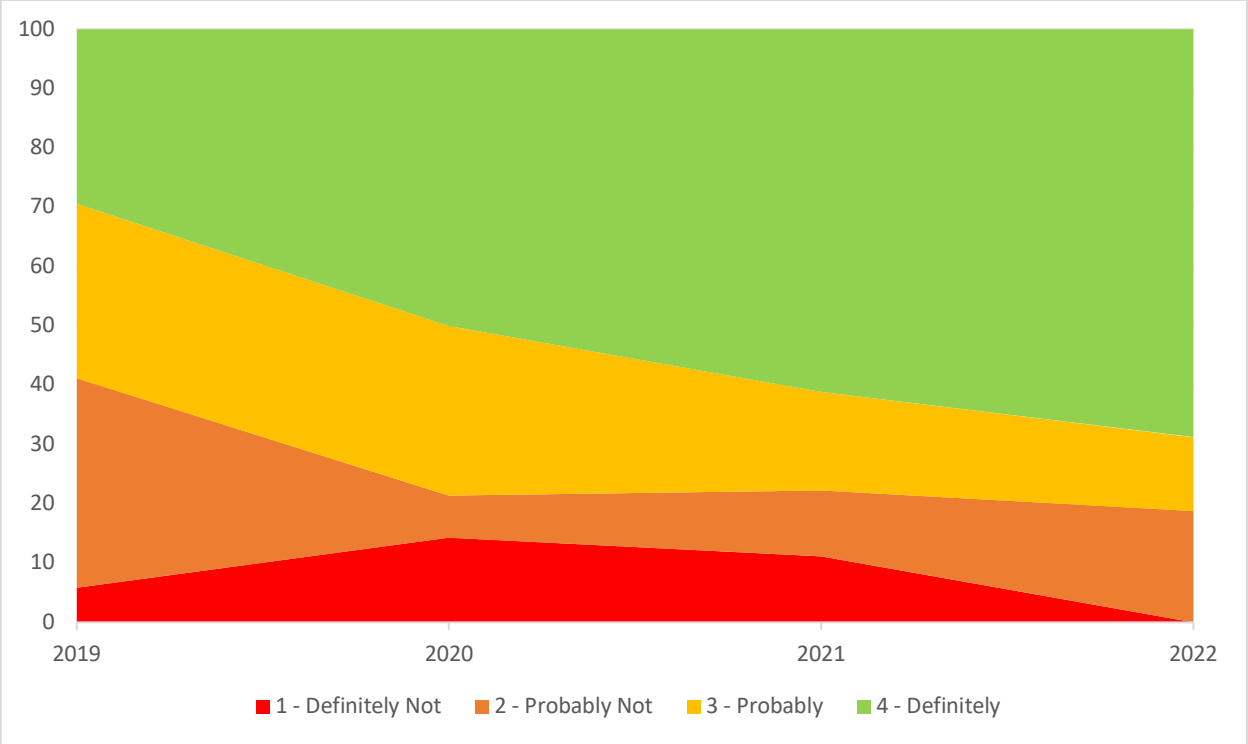
Likelihood of recommending the BPH program to potential students



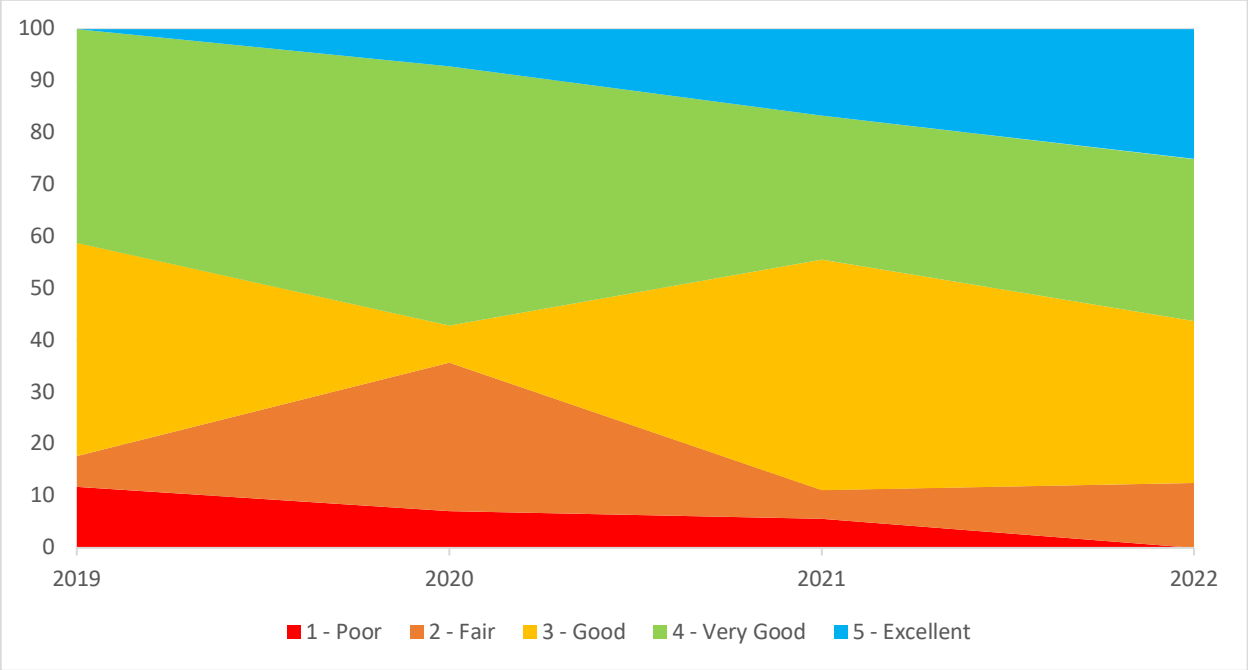
Frequency of considering dropping out of doctoral program



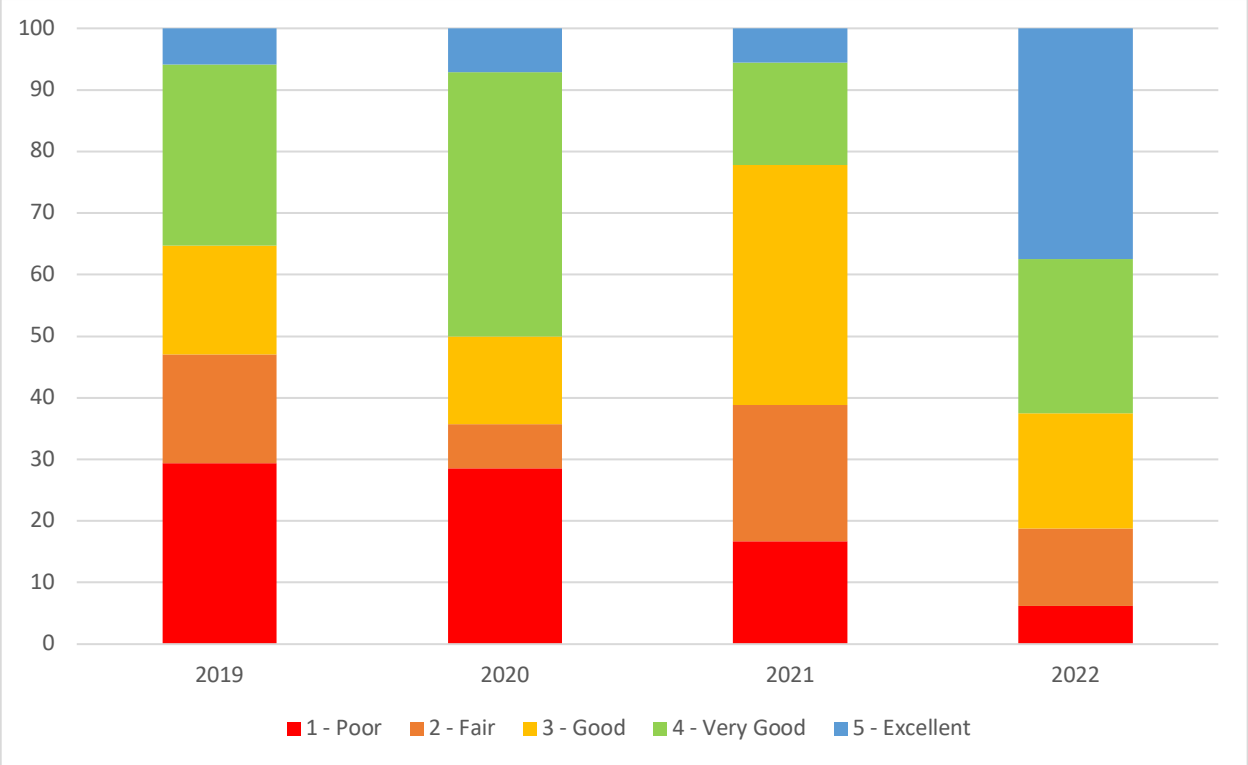
Likelihood of selecting BPH if repeating graduate studies



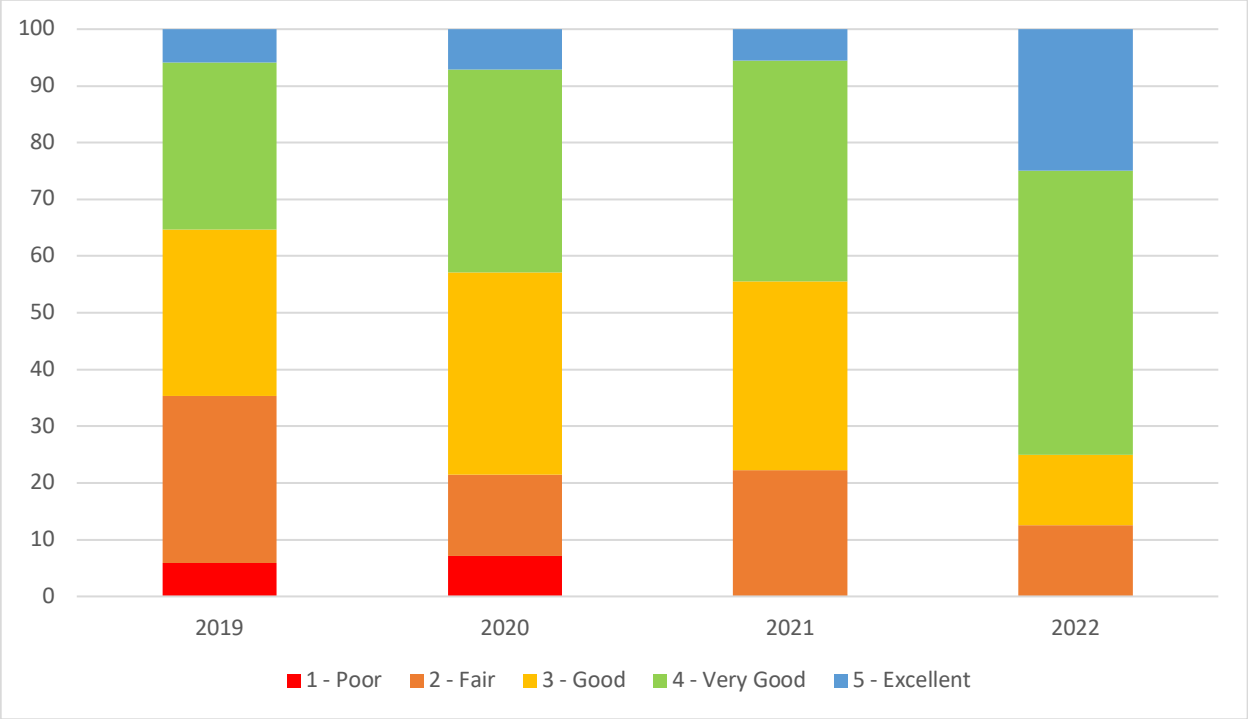
Quality of graduate-level teaching by faculty



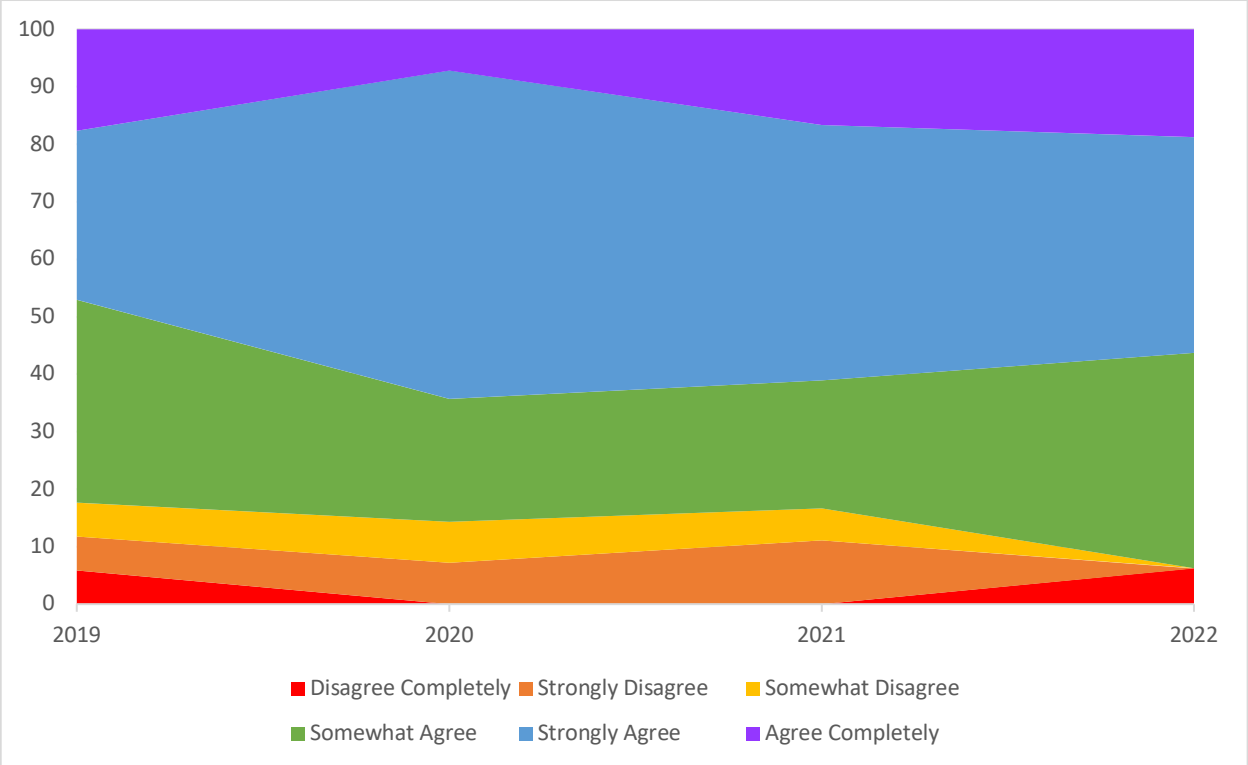
Helpfulness of staff members in my program



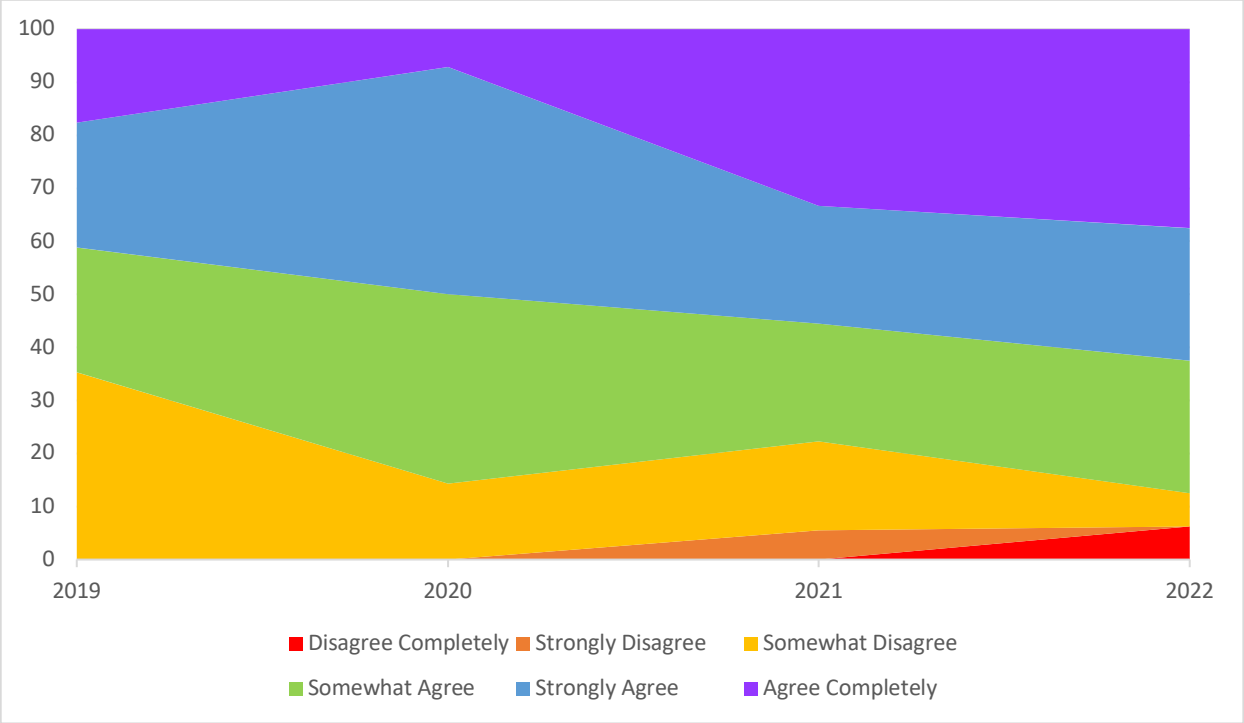
Overall program quality



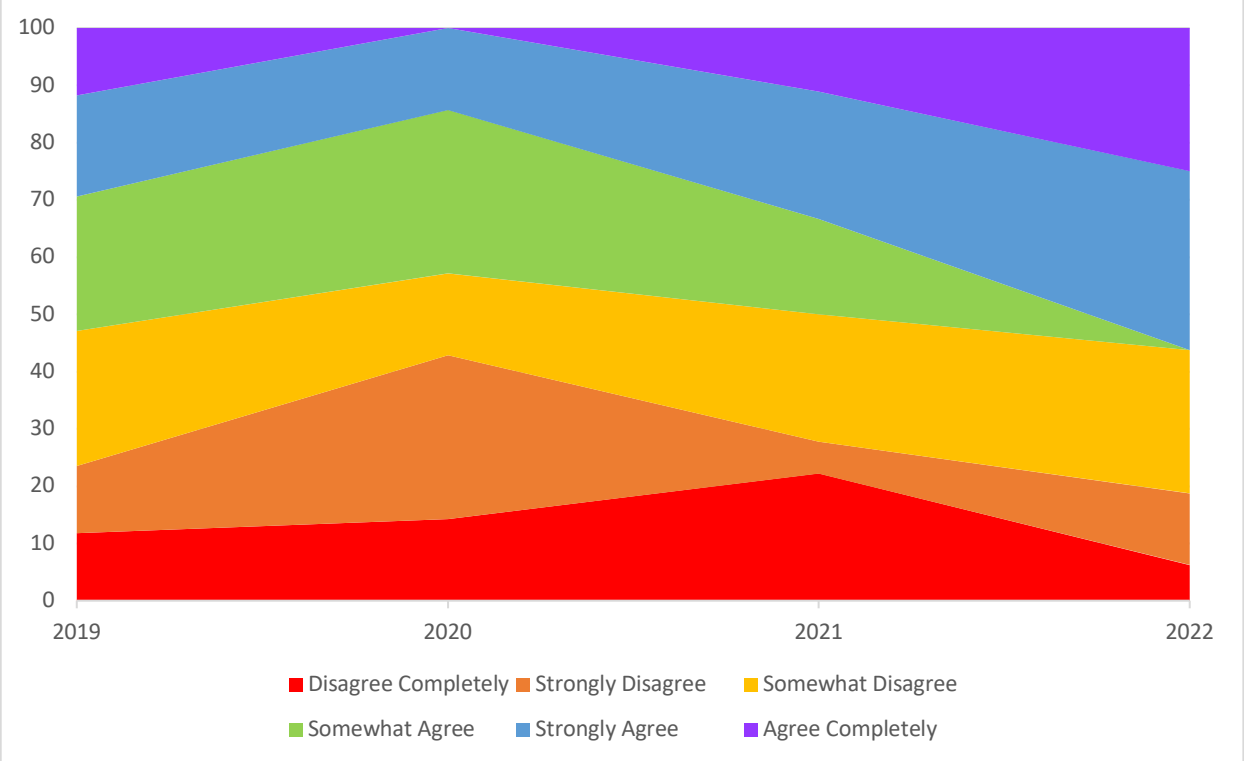
Faculty supportive of students



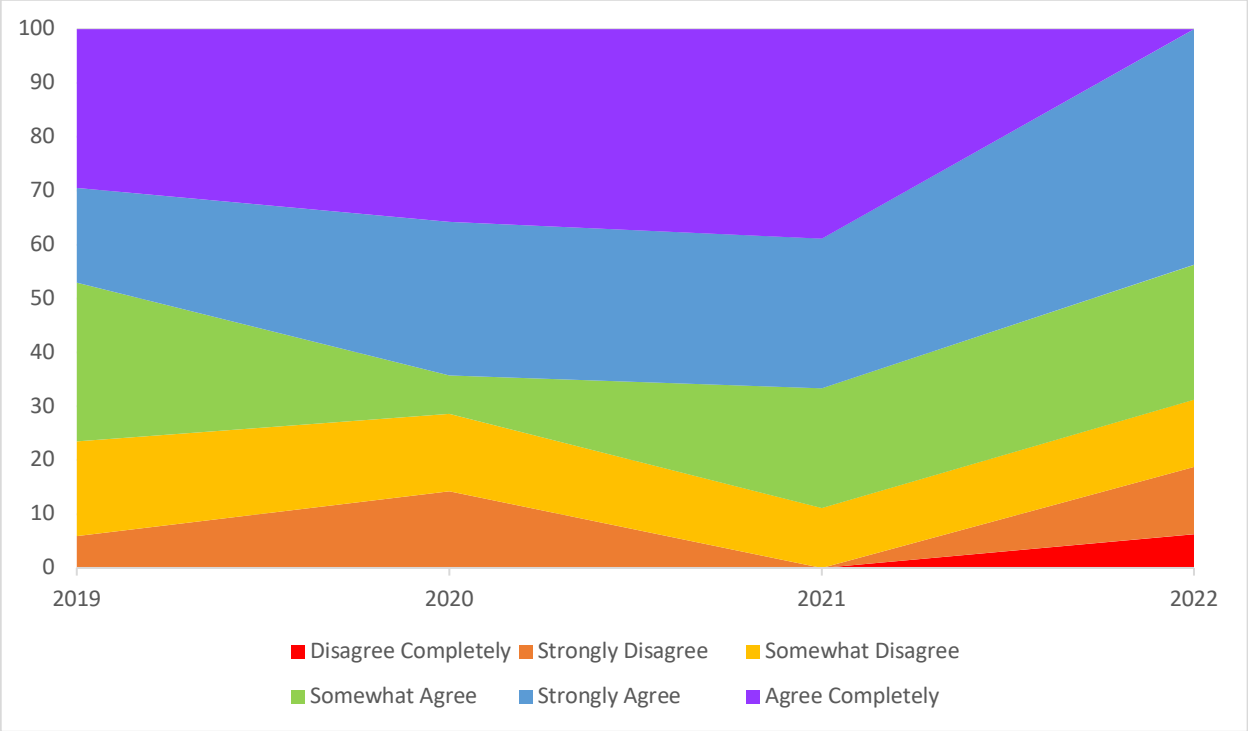
Experienced students mentor new students



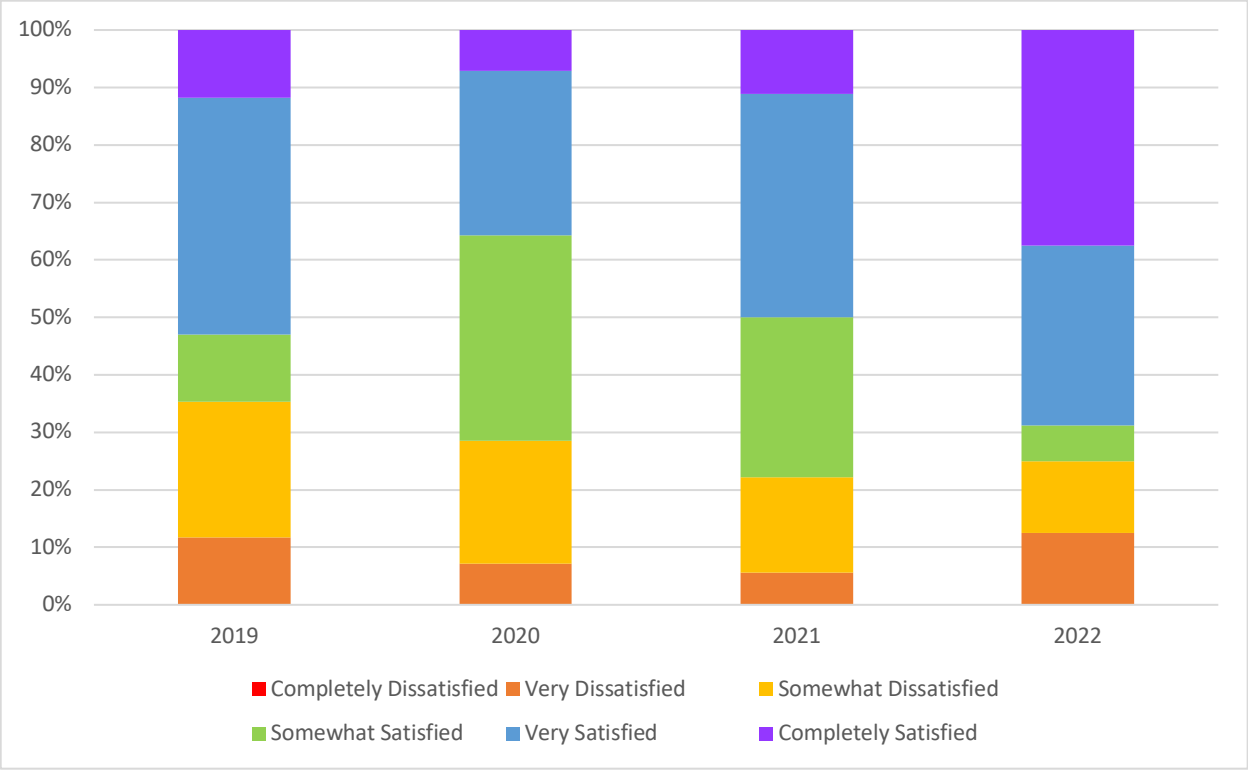
Program supports student efforts to find and attain funding



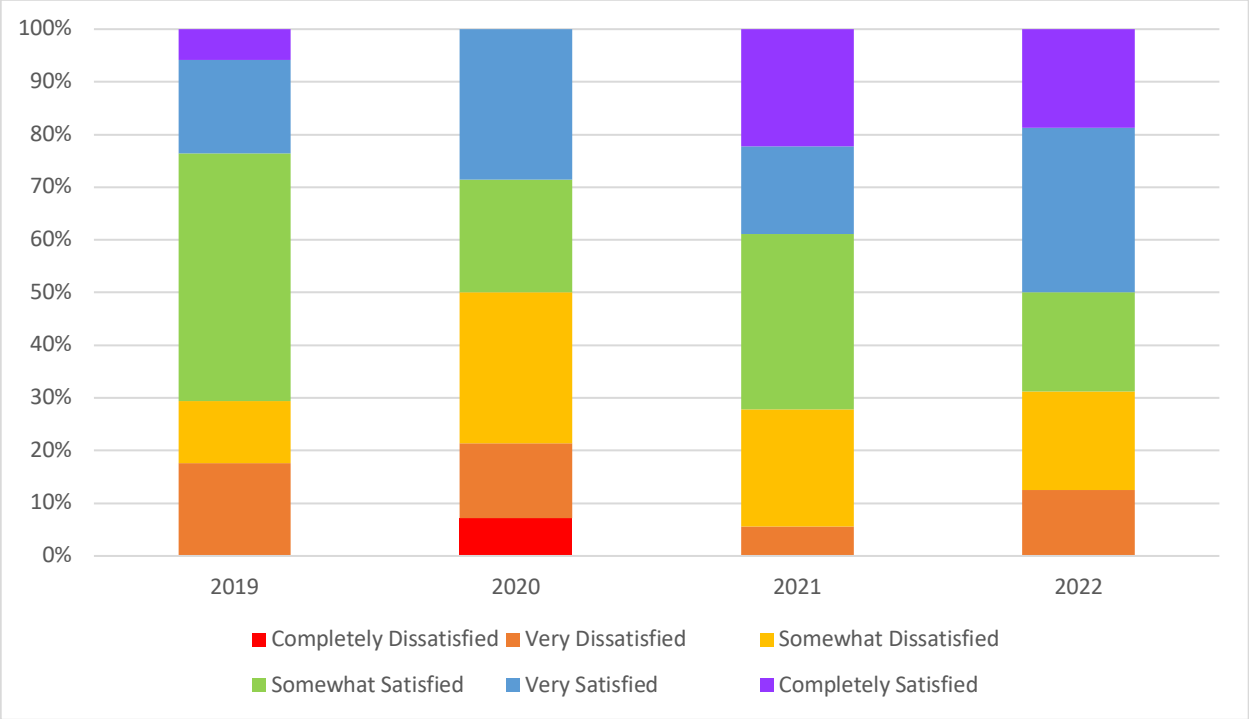
Supervisor provides actionable suggestions for student improvement



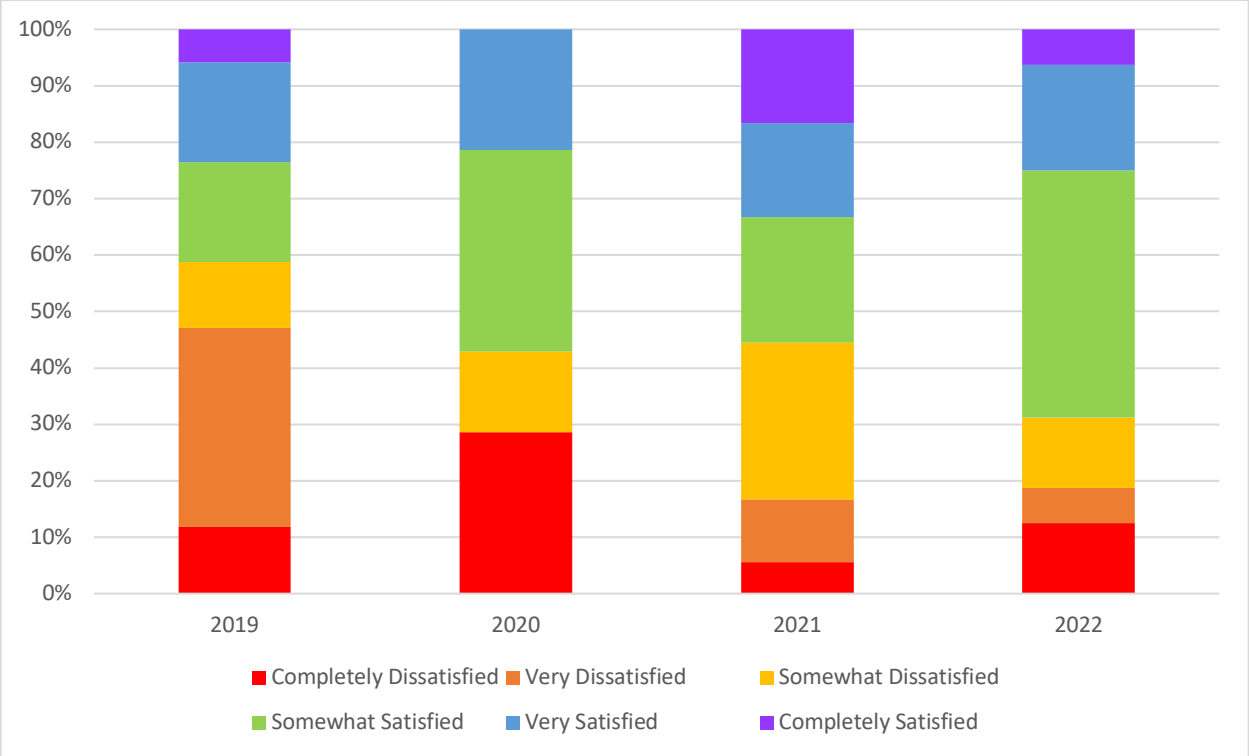
Satisfaction with conducting independent research



Satisfaction with guidance in preparing for an academic career

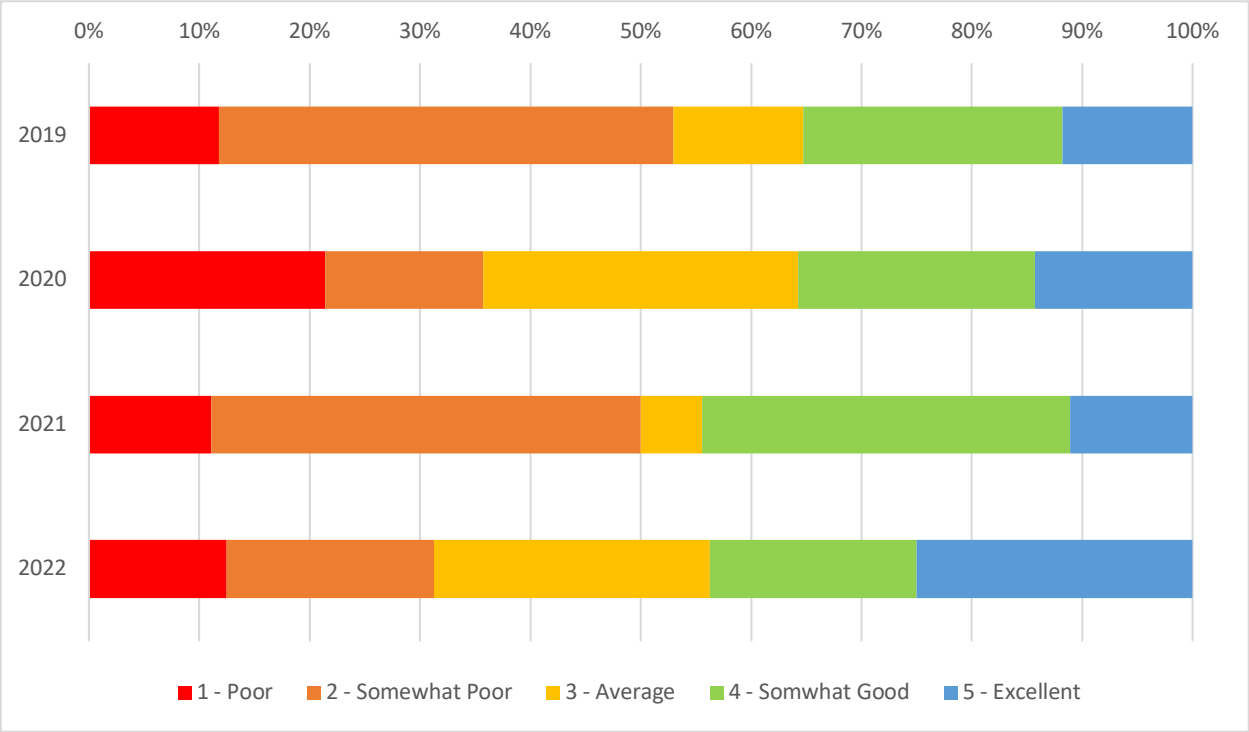


Satisfaction with guidance in preparing for a non-academic career

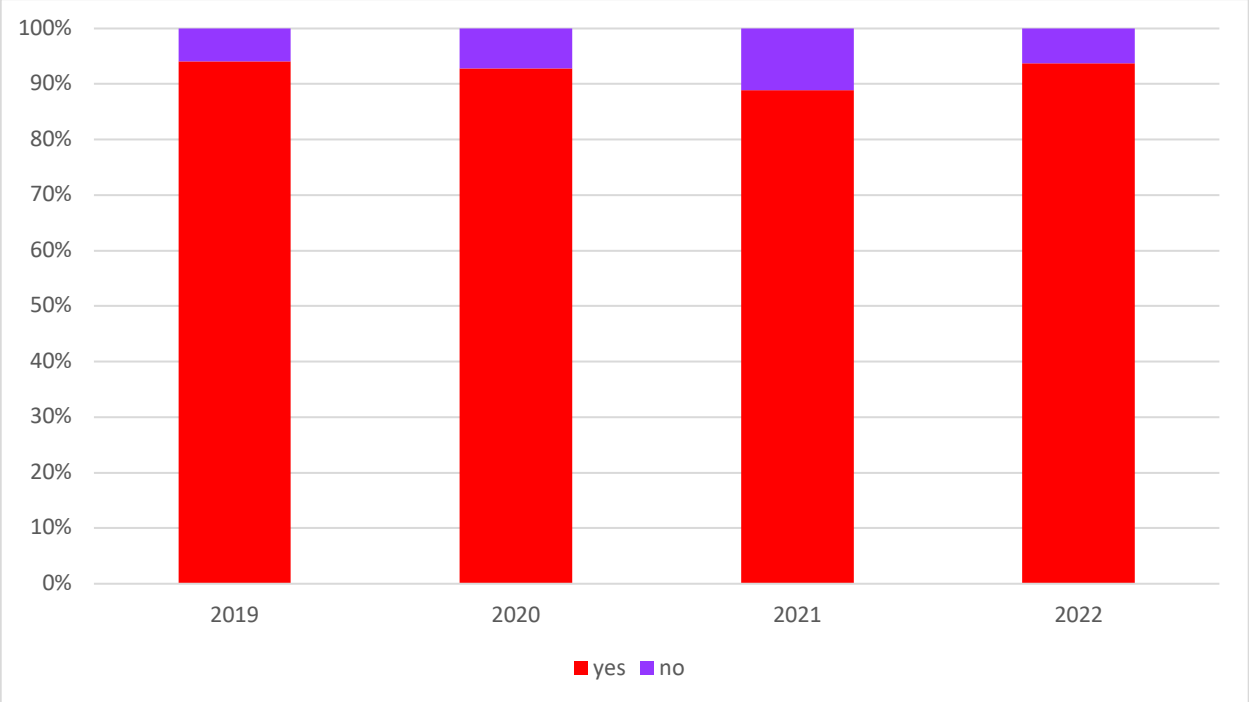


4. Mental Health

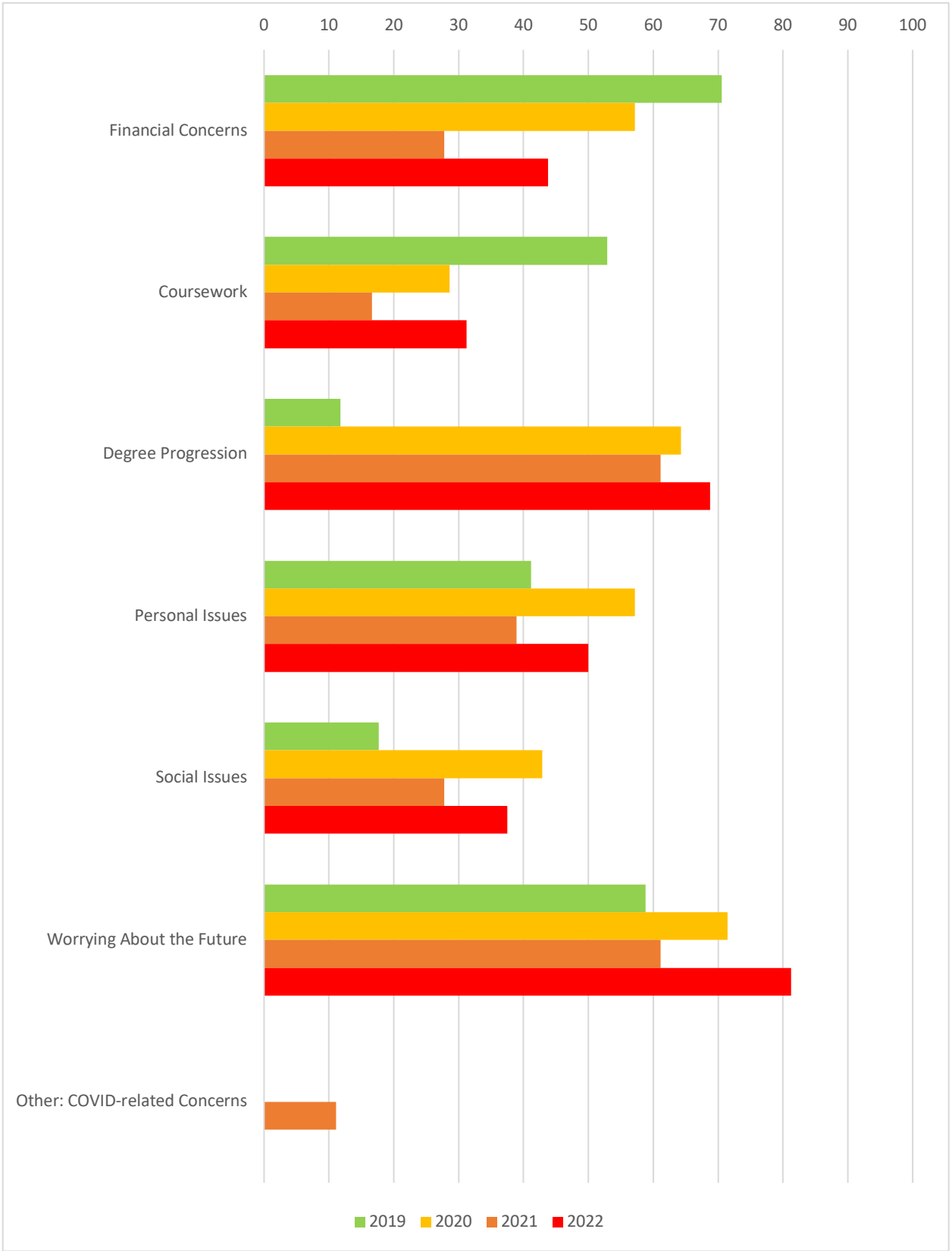
Rating overall mental health



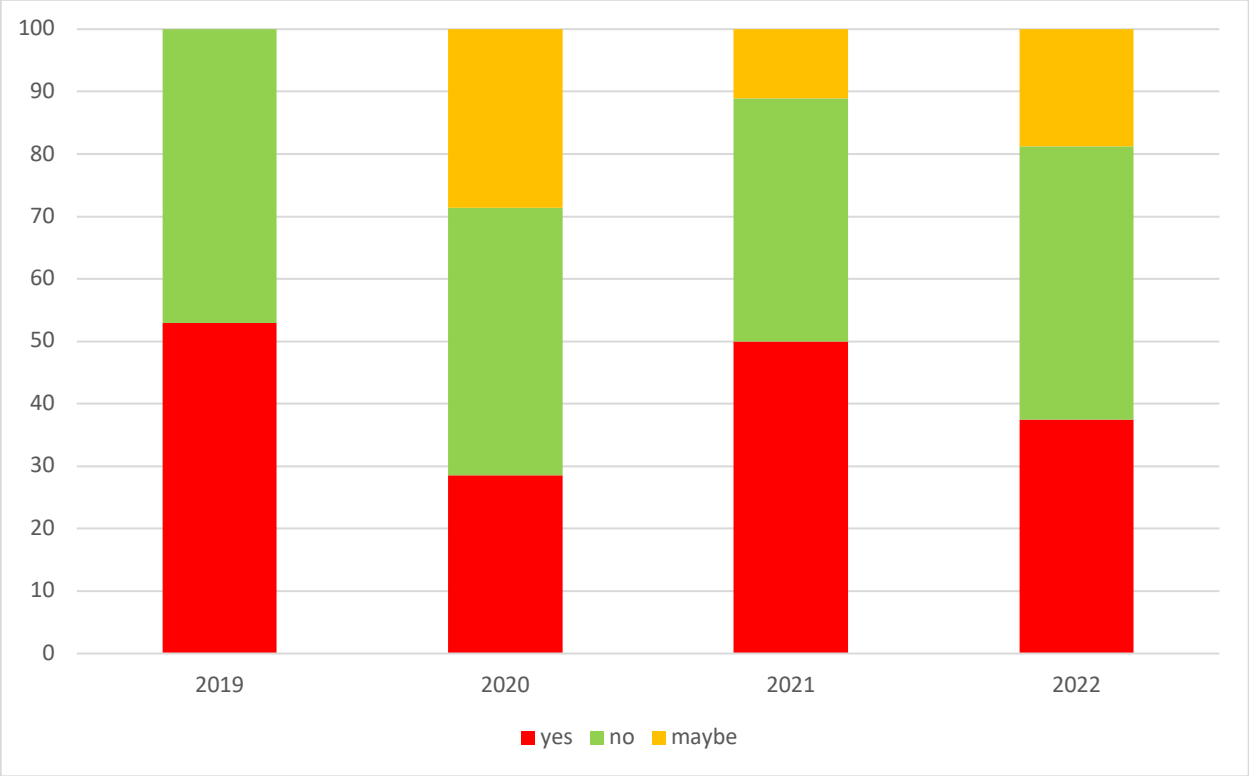
Feeling unusually stressed during graduate school



Causes of increased stress

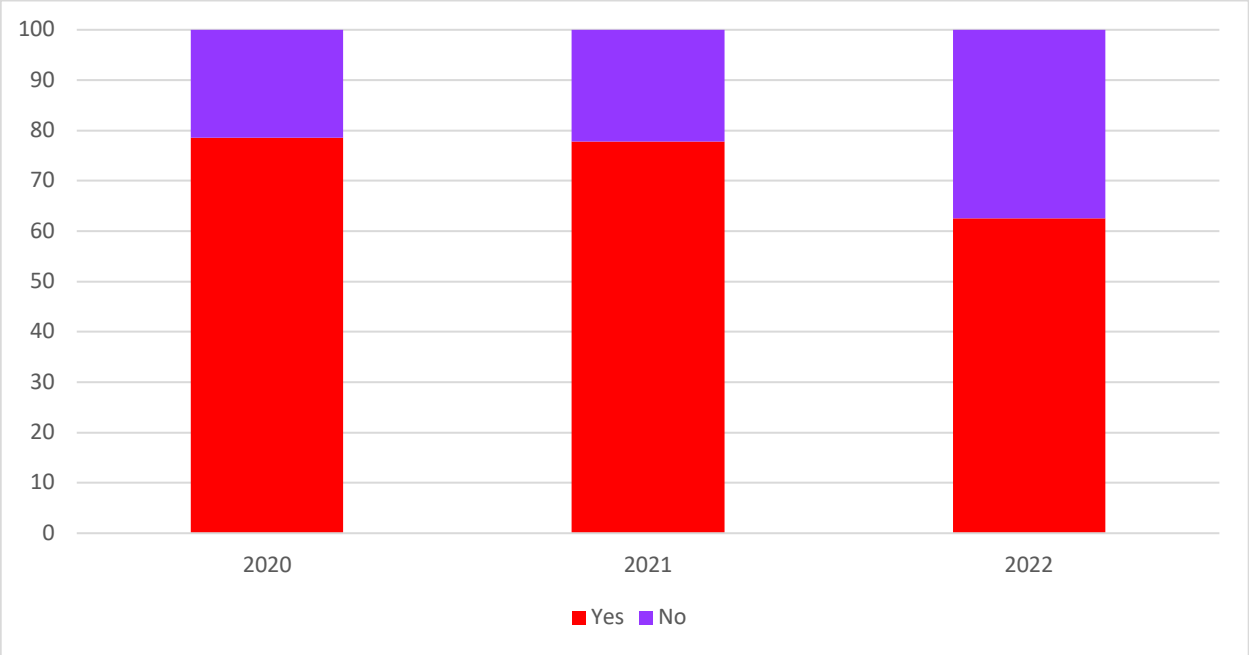


Developed or worsened mental health issues since beginning graduate school

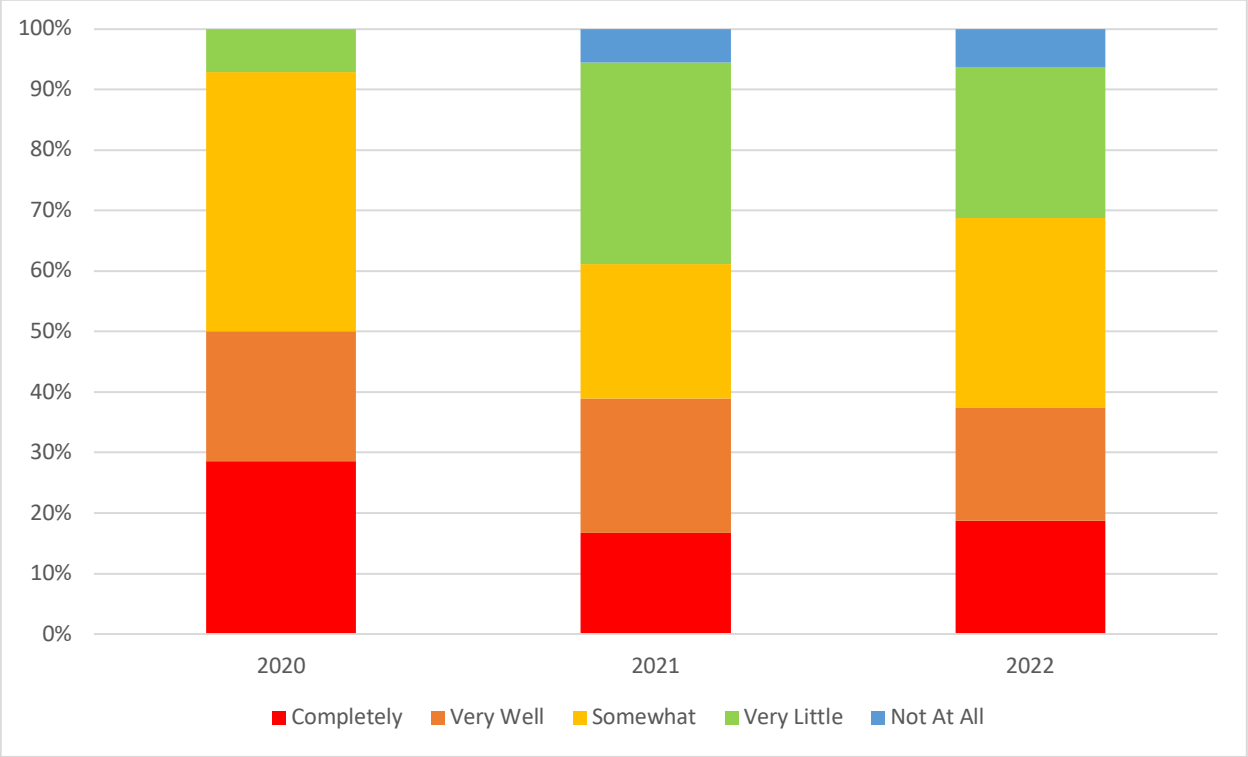


5. Financial Concerns

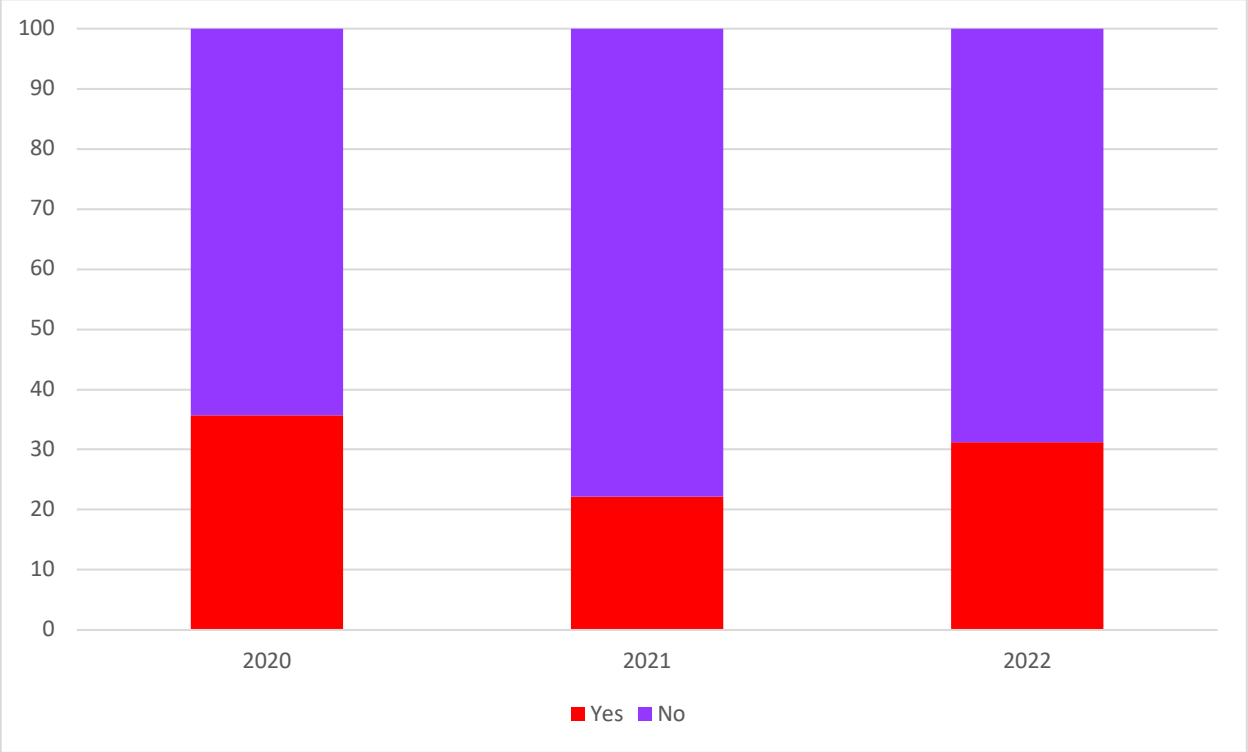
Worried about not having funding



Just getting by financially describes the student's situation



Need to supplement stipend with student loans, gifts, credit cards, second jobs, etc.



6. Student Experiences and Summary

The following lists summarize the feedback from student surveys from the free response sections where they were asked to describe their experiences with the curriculum, rotations, program support, and other facets of BPH.

Student identified challenges during the COVID-19 pandemic and in general:

- Communication Issues
- Isolation
- Time management
- Disruptions to advising
- Mental Health
- Lack of social events
- Few advising check-ins

Student recommendations to improve curriculum:

- Retain flexibility but provide additional structure
- Include computational and programming skills
- Include scientific reasoning, writing, and communication training
- Focus on techniques with more universal applications

Student asks for additional resources:

- More guidance on who has funding and is seeking graduate students
- More guidance on available teaching assistantships and funding resources
- More guidance for the first generation and international students
- Biophysics T32 program

- Biophysics retreat
- Mental health awareness from faculty
- Relationships with alumni of the program
- Clearer information on travel funding
- Tax help
- Larger stipend
- Housing vouchers

Key Takeaways:

- In-person preferable to virtual
- Students are drawn to small, flexible program with innovative research and great location
- Rotations, flexibility, friendliness, and networking has exceeded student expectations
- Faculty involvement and program guidance and support underwhelming to many
- Growth and improvements that have already happened are recognized and appreciated
- The website needs considerable updates and improvements

C. Graduate Program Orientation and Mentoring Documents

The following documents were put together in collaboration with fellow graduate students, building off previous orientation materials from other graduate groups. Student leadership found that the original welcome packet's introduction to laboratory rotations, program requirements, and resources lacked important details about reasonable program expectations and student mental health and well-being considerations. The updated Fall Welcome Packet, including the newly created Navigating Potential PI Interviews and Mentoring Roles and Expectations documents, was positively received by incoming students who reported that they found these materials useful in guiding their rotation and PI selection. Past students reported wishing this information had been communicated earlier in their graduate careers.

1. Biophysics Graduate Group 2021 Fall Welcome Packet

i. Title Page

ii. Table of Contents

iii. Welcome Letter

Greetings, Biophysicists!

We would like to congratulate you on your admission and welcome you to the BPH program at UC Davis. We received your Statement of Intent to Register (SIR) and looked forward to seeing you in the fall at UC Davis!

Please read the following pages carefully, as they contain a lot of important information to help you get prepared before the fall quarter begins. We have included information about setting up computing accounts, orientations and trainings, enrolling in classes, laboratory rotations, finding housing, and other helpful resources. Soon, you will be assigned an academic

advisor and graduate student mentor. If you are an international student, you will receive additional information about your student visa application, and you will work closely with Services for International Students and Scholars.

One reminder is that incoming students will not receive their first paycheck until November 1st. We understand this can cause some financial concerns for our students, so we encourage you to start planning for that delay in payment as early as possible. Graduate Studies requires you to provide them with final transcripts, including proof of graduation. Where to send your transcripts can be found on the following website: gradstudies.ucdavis.edu/official-transcripts. If you are a domestic student but not a California resident, you should begin establishing residency as soon as you arrive in Davis. It takes one full year to establish residency and qualify for resident tuition rates.

If you have any questions, please contact [insert Program Coordinator Contact Information].

We are excited that you selected the BPH program at UC Davis. We will be in touch!

Best,

Biophysics Graduate Group

iv. IMPORTANT DATES and DEADLINES

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
29	30	31	**1**	**2**	**3** *Register for Classes *Pre-register for New TA Orientation	**4**
5	**6**	**7**	**8**	**9** Holistic U	**10** Holistic U *International Graduate Student Orientation	**11**
12	**13** Graduate Student Orientation and Resource Fair	**14** Biophysical Society Student Chapter Fall Mixer	**15**	**16**	**17**	**18**
19	**20** *New TA Orientation	**21** *Biophysics Graduate Student Orientation *CBS Graduate Group Fall Welcome	**22** Instruction Begins	**23**	**24**	**25**

**Required*

September 3	*Pre-register for Orientation for New Teaching Assistants
	*Register for Classes
September 9-10	Graduate Diversity Orientation Program: Holistic U *(recommended)*
September 10	*International Grad Student Orientation *(for international students only)*
September 13	Graduate Studies Week of Welcome and Orientation
	*Sexual Violence Prevention Training
September 14	Student Chapter of the Biophysical Society Fall Mixer
September 20	*Orientation for New Teaching Assistants
September 21	*Biophysics Graduate Student Orientation
	*CBS Graduate Group Fall Welcome Reception
September 22	Instruction Begins

v. THE FIRST-YEAR CHECKLIST

Spring-Summer 2021

- Find Housing As Soon As Possible *(Information on Page 4)*
- Setup Computing Account *(Information on Page 5)*
- Get AggieCard *(Information on Page 5)*
- Register for Fall Classes by September 3 *(Information on Page 5)*
    - You will have the option to change your course registration after this date.
- Health Insurance Waiver
    - If you would like to opt out of the UC SHIP (Student Health Insurance Plan) and qualify for the waiver, the form must be completed by September 3.
- Setup First Lab Rotation *(Information on Pages 7-9)*
    - Helpful information from the BPH graduate students on choosing rotations and potential PIs can be found in the "Navigating Interview with Potential PIs" document that came with your Welcome Packet.
- Attend Graduate Student Orientations *(Information on Page 6)*
- Meet with Assigned Academic Advisor
    - Your academic advisor is [insert academic advisor contact information]. Set up a meeting before the school year begins to discuss elective course decisions,

laboratory rotations and any other academic concerns. It is recommended that you meet with your advisor multiple times during your first year as needed.

Fall Quarter 2021

- Explore Faculty Research Labs and Meet with Potential PIs
- Setup Second Lab Rotation
- Present First Rotation Project at Weekly Seminar on October 29
- Setup Third Lab Rotation
- Register for Winter Classes starting November 1
- Present Second Rotation Project at Weekly Seminar on December 3

Winter Quarter 2022

- Setup Fourth Lab Rotation
- Register for Spring Classes starting February 3
- Present Third Rotation Project at Weekly Seminar on February 4
- Choose Research Advisor and Complete Mentoring Contract
- Discuss and Finalize Spring Quarter Funding Situation With PI
    - Begin looking for teaching assistantship early in the quarter if necessary
- Present Fourth Rotation Project at Weekly Seminar on March 11

Spring Quarter 2022

- Register for Fall 2021 Classes
- Begin First Research Project Chosen With PI
- Apply for Fellowships, Grants, and Travel Awards
- Complete myIDP Assessment and Student Progress Report with Academic Advisor and/or Research Advisor (PI, Major Professor)

vi. HOUSING and TRANSPORTATION

The Graduate Student Guide contains useful information concerning transportation and housing. Once you have a UC Davis email address, you will be placed on the student listservs, and you are welcome to send an email message to the program coordinator if you are interested in finding a roommate/house-mate within the program/college.

Housing in Davis fills up very quickly, so it is important to start looking as soon as you can. As a friendly reminder: many rentals require the first and last month's rent and/or a security deposit when you sign your lease. Information to obtain California Residency can be found here. It takes one year to obtain California residency, so it is important to start this process soon after arriving in the Davis area.

If you are starting to look for housing, here are some helpful websites:

UCD Graduate Student Housing	https://housing.ucdavis.edu/graduate-and-professional-housing/
Davis Wiki – Renting	https://localwiki.org/davis/rental_housing_guide
Community Housing Listing	http://chl.ucdavis.edu/
Sacramento Area Craigslist	https://sacramento.craigslist.org/
UCD Grad and Professional Housing Facebook Group	https://www.facebook.com/groups/286762898448011/?ref=share
UCD Off-Campus Housing Facebook Group	https://www.facebook.com/groups/763250590497811/?ref=share
Davis Housing Facebook Group	https://www.facebook.com/groups/418689684823205/?ref=group_browse

Here are some other useful resources for transportation and furnishings:

UCD Go Club	https://goclub.ucdavis.edu/commuteoptions
UCD Transportation Options	https://taps.ucdavis.edu/transportation
UCD Bike Program	https://taps.ucdavis.edu/bicycle
Davis Wiki – Parking	https://localwiki.org/davis/Parking
Davis-Area Rideshare Facebook Group	https://www.facebook.com/groups/243044995791686/?ref=group_browse
UC Davis Sale or Trade Facebook Group	https://www.facebook.com/groups/655874594426741/?ref=group_browse
UC Davis Free and For Sale Facebook Group	https://www.facebook.com/groups/418689691489871/?ref=group_browse
Davis Buy Nothing	Search on Facebook - there are 5 groups in Davis, by geographical area

vii. HEALTH INSURANCE

All UC Davis students are required to have health insurance. To satisfy this requirement, all registered students are automatically enrolled in the University of California Student Health Insurance Plan (UC SHIP). UC SHIP is automatically paid from your university account after your stipend has been dispersed but before direct deposit into your personal account, just like all other student fees. If you already have comparable insurance coverage, and do not wish to be enrolled in UC SHIP, you must apply for a UC SHIP waiver by the posted waiver deadline date, September 1st, 2021.

viii. CREATE A COMPUTING ACCOUNT

All new students must establish a campus computing account and a UC Davis email account. Go to the Information Technology website. Follow the instructions for establishing a computing account, Kerberos ID (your campus login ID) and password. It may take up to 48 hours after submitting the Statement of Intent to Register (SIR) before you are able to access this function. This must be completed before registration for orientation and trainings.

iv. ORDER AGGIE CARD

You will need to order your AggieCard (the UC Davis identification card) to access certain services on campus. Go to the AggieCard website and follow the steps listed for a graduate student to obtain an AggieCard.

x. ACADEMIC ADVISOR

Your academic advisor is [Assigned Advisor Contact Information]. The academic advisor ensures that academic requirements are on track, and advises students on how to design a program of study and navigate the coursework requirements, find a lab, and work with their major professor. Academic advisors are appointed by Graduate Studies, and their signature verifies that academic milestones have been met. Academic advisors review and approve petitions to join Designated Emphasis programs, QE applications, candidacy forms, progress reports, petitions to add or drop courses, etc. More information on Graduate Program Roles and Responsibilities can be found here. Note that besides assisting students with course selection and administrative tasks, academic advisors can be a sounding board for major professor selection, and for handling delicate situations with faculty. You are expected to meet regularly with your academic advisor. **Please email your academic advisor to schedule a one-on-one meeting to introduce yourself before the quarter starts.**

xi. REGISTERING FOR CLASSES

Registration will remain open until September 14th. You can build your Fall quarter schedule through Schedule Builder at any time without requiring a specific appointment time. You should be enrolled in the majority of your courses **by mid-August**. If you need to enroll in any undergraduate courses (numbered 1 to 199), be sure to enroll as early as possible because these classes become full very quickly. Complete instructions for using SISWEB will be included in the registration materials you will be receiving via email from the Registrar's Office mid-late July. If the system is saying that you are missing prerequisites, please do write a petition for exception, which will pop up when you attempt to register-this will save your spot in line. Faculty and staff are unable to approve those exceptions until after mid-August so please be patient.

Fall 2021 Required Courses

[required course lists]

First Year Recommended Courses

[recommended course lists]

A full course-load is 12 units minimum (and 16 units maximum). You may complete your schedule by making up any deficiencies in your background or by taking courses in other areas. You will mostly be engaged in course work and rotations during the first three quarters. Typically, you will commence your thesis research in the spring quarter of your first year. **We expect that you will remain on campus to work and study during the summers following your**

first year and that you will remain solely employed as a graduate student during your Ph.D. studies. If you are an international student, make sure you are meeting all visa requirements before committing to summer work.

xii. ORIENTATIONS and TRAININGS

All details about campus graduate student orientations can be found on the Office of Graduate Studies Orientation Page. Registration will open sometime in July.

1. Graduate Diversity Orientation Program (Holistic U, GDOP), September 9-10 **(encouraged but optional)**

This orientation focuses on diversity issues in higher education and success in graduate school. Register for GDOP at https://grad.ucdavis.edu/orientation after creating your computing account and ordering your AggieCard.

2. International Graduate Student Orientation (IGSO), September 10 **(mandatory for all international students)**

Register for IGSO at https://grad.ucdavis.edu/orientation after creating your computing account and ordering your AggieCard.

3. Graduate Studies Resource Fair, September 13 **(encouraged but optional)**

Register for the Graduate Studies Orientation (GSO) at https://grad.ucdavis.edu/orientation after creating your computing account and ordering your AggieCard.

4. Student Chapter of the Biophysical Society Fall Mixer, September 14 **(encouraged but optional)**

The first ever Fall Mixer hosted by the Student Chapter of the Biophysical Society at UC Davis will include research poster presentations and networking with light refreshments. Everyone in the UC Davis biophysics community is welcome to attend. Register at [insert link].

5. Orientation for New Teaching Assistants, September 20 **(mandatory)**

This orientation is required for all potential teaching assistants and is only offered once a year. You must pre-register by the September 3rd deadline here: http://cee.ucdavis.edu/tao/index.html. For more information, contact: cee@ucdavis.edu or 530-752-6050.

6. Biophysics Graduate Student Orientation, September 21 **(mandatory)**

Introduction to program expectations and presentations from faculty seeking rotation students. You will also meet your graduate student mentors and review your welcome materials.

7. CBS Graduate Group Fall Welcome, September 21 **(encouraged but optional)**

Mixer for all the graduate groups in the College of Biological Sciences held at the Life Sciences/Green Hall Courtyard. Light refreshments provided.

8. Sexual Violence Prevention Training (SVPT) (mandatory)

Register for SVPT at https://grad.ucdavis.edu/orientation after creating your computing account and ordering your AggieCard. SVPT is mandatory for all incoming graduate students. For more information on the training please send a message to the Sexual Violence Prevention program at SVPT@ucdavis.edu.

9. Laboratory Safety Training (mandatory)

Complete your mandatory online Laboratory Safety Training, preferably prior to arriving. The training takes 2-3 hours and includes testing. Be sure to save the certificate you receive, as it may need to be submitted later.

xiii. LABORATORY ROTATIONS

1st rotation: Wednesday, September 22–Friday, October 29

2nd rotation: Monday, November 1–Friday, December 3

Fall Quarter instruction ends on December 3; finals are December 6-10.

3rd rotation: Monday, January 3–Friday, February 4

4th rotation: Monday, February 7–Friday, March 11

Winter Quarter instruction ends on March 11; finals are March 14-18.

How will COVID-19 affect my rotations?

Currently, the plan is for a full return to in-person instruction and campus operations starting September 1. Please use this website to stay updated: https://campusready.ucdavis.edu/

What are laboratory rotations?

The purpose of laboratory rotations is to find a mentor for your PhD. Rotations are a wonderful thing. They are a period to try out hands-on experimental work in different laboratories. Two of the goals of your rotations are to "learn by doing" and to impress any professors with whom you might want to do a thesis. They are both a means of garnering new

technical skills and a dating period to find a lab to commit to. The most important aspect of every rotation is to identify a major professor who can support your research ambitions intellectually, interpersonally, and financially.

What are rotation expectations?

Rotations are a once in a lifetime opportunity to sample different areas of physiology research and to demonstrate your potential to future mentors and supporters - take full advantage. You should try to be in your rotation lab whenever possible. Your funding support is contingent on you actively engaging with a laboratory. Make sure to let your rotation mentor know when you have class or need to prepare for class so they'll know you are serious about being in the lab whenever you can. Training you to work in any specific laboratory environment is a significant commitment and energy investment on the part of your host laboratory. It's a tall order to get any significant science done in 5 weeks, but focus, careful thinking, and hard work can enable discoveries during a rotation that turn into a thesis. However, this does not need to be your goal, nor should it be expected. Rotations should be focused on finding out for yourself whether each lab would be a good fit for you.

Where to rotate?

You can rotate with BPH affiliated faculty: https://bph.ucdavis.edu/faculty , and with faculty who are willing to join the BPH group. We will also supply you with survey results from professors indicating whether they are interested in taking rotation students and supporting thesis student research. **Until you have a thesis laboratory confirmed, it is recommended to rotate only in labs that will be able to mentor and support your PhD research.** This list is a

good place to start considering labs to contact, but do not restrict yourself to it. We encourage you to contact any UC Davis faculty member whose interest sparks your imagination. To commence the rotation the professor must first join BPH; this is a straightforward process, and how most professors join BPH. Contact [program coordinator]] if you wish to do this.

You are responsible for setting up your own rotations. We advise you to begin setting up a first rotation immediately. Look at faculty web sites and read their publications. Contact professors you are interested in working with by email. If you get a response, great! If you do not, wait a week and try again. The first rule of emailing faculty is: a non-response means nothing. We are all incredibly busy. Write short informative messages with optional information attached or postscript. Make it easy for a professor to read and reply to your email in less than 2 minutes. Do not send this exact email, but feel free to use this template as a reference:

Dear Professor [Surname],

I am a first-year student in the Biophysics graduate program. I have laboratory experience with A and B. My curriculum vitae and graduate application are attached. From your website and publications, I've seen you are studying D, which I have a keen interest in. Might you have a moment to chat in person or by phone about the possibility of a laboratory rotation?

Sincerely,

[Your Name]

Your top priority is arranging a first rotation. Contact professors, rank order who you think you would most like to work with and starting with #1, ask if they might be willing to mentor your first rotation. Resist the temptation to commit to further rotations before you arrive. You will learn much more by meeting face to face and seeing their lab. When you have committed to a rotation, please inform the program coordinator.

Once you arrive in Davis, your goal is to find at least three more professors with whom you could rotate. You will be enrolled in a course requiring faculty meetings to facilitate and encourage this process. **Meet and talk with as many professors as possible as early as possible.** This should be an active selection process. Approach faculty in whose research you are interested. Ask them if they could recommend specific papers about their current research. You can also ask them if they are open to taking rotation students in the winter and whether they are open to taking a thesis student this year. Do not necessarily commit to a rotation at the first meeting, take time to reflect and consider your options before signing on. Before

agreeing to a rotation, do some investigating into what spending precious years in their lab might be like. **Talk to people in their labs.** Talk to their lab members privately and see if you could foresee happily spending forty plus hours a week in their company. Ask them in confidence whether they think you could be a good fit in the lab.

Ask the professor how they could financially support you through your thesis. Once rotations are over, your tuition and stipend need to come from somewhere. While you can work as a teaching assistant to support yourself, this will take time away from research. Some but not all students are awarded fellowships. Laboratory supplies are expensive. Think about how much certainty is in a laboratory's funding situation, and how you would feel if their uncertainties became your uncertainties. Four rotations may seem like a plethora of options, but choose wisely. Devote your time to laboratories you would be excited to join. Make these rotations a rich slice of life!

Suggestions for starting a rotation

- Ask the professor with whom you are rotating for background papers to read. The Professor may give you reprints from his/her own lab, may give you a list of references or may just give you some names or topics to search in PubMed or Google Scholar.
- Read the papers and **ask questions** about things you don't understand. Find out when lab meetings are held and go to them. Learn about the general area of your rotation lab's research beyond your individual project.

- Discuss and agree on a project outline with your professor. Make sure you can define the specific problem being addressed and the hypothesis being tested. Don't be satisfied with just doing tasks in the lab.
- Start your rotation early, if possible. Eagerness to engage in research never fails to impress.
- **Please complete the mandatory online training course entitled "UC Laboratory Safety Fundamentals" before you start your Fall quarter rotation.** You will need your UC login ID and passphrase. Ask your rotation mentor which safety courses are relevant to your research project, and complete them ASAP. Many of these courses are available online. http://safetyservices.ucdavis.edu/training/uc-laboratory-safety-fundamentals
- If you rotate in a lab in the Shriners Hospital in Sacramento, there are extensive background checks that need to be completed before you can rotate. Talk to the professor ASAP to start the process so your rotation starts on time.

How to rotate

Let your enthusiasm for research be palpable. Show up in the lab whenever you can. Take written notes on everything anyone in the lab tells you. Research the subject matter of your notes and come back with further questions. Read, read, read. Think, think, think. Understand what you are doing, what the reagents are, how the instrument works. Plan carefully for experiments. Treat equipment with the utmost care. Nothing will impress as much as experiments carried out thoughtfully and carefully. First impressions make deep imprints. Give these rotations 100% attention and you will be rewarded with an auspicious start to a

graduate thesis. Try to speak with current students of the lab about the mentor style of the PI; do they expect to meet daily/weekly/monthly? How hands on or hands off are they in designing experiments and experiments?

Timing

If you find a good match with a lab that has funding for you to do a research project, you may stay there for a second rotation, or join their lab permanently. If you find yourself in a rotation that is obviously not a good match, contact one of us. We may encourage you to move on to a new rotation early. Keep an open mind about what labs would be interesting. Graduate school is a time to broaden your interests and experience. Make the best use of this valuable time, learn passionately!

PROGRAM REQUIREMENTS

The full degree requirements (currently under revision) can be found here: https://programs.gs.ucdavis.edu/api/doc/2832

[degree requirement summary]

xiv. MENTAL HEALTH RESOURCES

Our program is working to support the efforts of UC Davis to create a culture of student mental health beyond the clinical setting that includes all members of the campus community who regularly interact with and support students. The Graduate Student Association has compiled a comprehensive list of mental health resources available to students that can be found here**.** Additionally, Each Aggie Matters is a mental health movement on campus and provides a calendar of all mental health related activities here. You can find a summary of the state of mental health on-campus and the Mental Health Task Force recommendations here and the full report here. Mental health is an intersectional issue and there are many non-mental health centered student groups who touch on mental health that focus on traditionally marginalized student populations. Below we highlight several of these resources and student interest groups:

Student Health and Counseling Services	https://shcs.ucdavis.edu	SHCS offers two major types of mental health resources: Counseling Services and Psychiatric Services. Counseling Services provides issue-focused, short-term care, typically eight sessions or less. Within this time, the therapist and student will determine whether a referral to an outside provider is necessary. Psychiatric services

		include psychiatric assessment, medication management, and medication monitoring.
24-Hour Phone and e-Messaging Hotline	530-752-2349	This phone line and e-messaging service can provide both crisis assessment and counseling services.
LGBTQIA Resource Center	https://lgbtqia.ucdavis.edu	The LGBTQIA Resource Center promotes education as well as space for self-exploration about all sexes, genders and sexualities and their intersections with other identities. The center provides a wide range of resources and support.
Student Disability Center	https://sdc.ucdavis.edu/	The SDC is staffed by a team of professionals who have expertise in the education of students with disabilities. SDC Specialists approve services and coordinate accommodations to ensure equal access to the University's educational programs.
Graduate Diversity Resources	https://grad.ucdavis.edu/about-us/priorities-initiatives/diversity	UC Davis values a diversity of viewpoints, backgrounds, and experiences among its graduate student population and remains

		committed to facilitating a campus atmosphere well suited to this diversity. As part of this commitment UC Davis offers numerous services, workshops, and training, as well as key faculty, staff, and students situated to promote and address the needs of diverse students and those allied in this mission.

2. Navigating Interviews with Potential Major Professors

To the New Biophysics Students:

As you've been informed throughout your interview, recruitment, and orientation process, choosing your major professor for your thesis is one of the most important decisions you will make in grad school. You will be working for and with this individual for the next 4-6 years. They will play a critical role in your development as a scientist and shaping your early career pursuits.

Picking a major professor can be a daunting process but we hope this guide will help you navigate conversations with potential advisors and highlight some important factors to consider when making your selection. This set is not meant to be followed to the letter, as a good major professor-student relationship depends primarily on your personal needs, but it is reflective of the experiences of many of our current graduate students and what we found useful or wish we had known when we were in your shoes.

Important Topics to Cover

1. Rotations and Potential Projects
2. Funding
3. Expectations
    1. Academic background
    2. Available Now and Potential Future Projects
    3. Time commitment/Working style in lab
    4. Time to finish, publications expectations, career mentorship
4. Mentoring Style
5. Mentoring Agreement

1. Rotations and Potential Research Projects

It can be daunting to try to decide on a research area and rotation. There is a broad array of biophysics research going on at UC Davis. The best advice is to choose a rotation that you find interesting in an area that you are thinking about for your graduate research. The project should suit your career goals, and fit well with your skills and talents. For example, if you love working hands-on in a "wet" lab, a purely theoretical or computational research project may not be well suited for you. However, if you're not sure, rotations are a great way to explore new areas. Beyond broad brush strokes of figuring out a dry or wet lab, there isn't one choice that is best for your academic and research success, rather there are different options to

work with different possible major professors that lead to different opportunities. The first place to start is with faculty websites. Does their general research area interest you? What approaches do they use? Note: websites by their very nature are out of date. The papers that came out are likely from work that was accomplished 6 months to a year ago and the group could have moved into a different but aligned research area. Still, doing a "web of science" search of the most recent work of the professor is a great first step. Once you narrow your potential faculty down to five or so, take the time to talk to students and postdocs in their group to see what is currently happening in the lab. Still interested? See if the faculty member is taking rotation students, has funded research projects, and test the waters.

2. Funding

One of the biggest (unnecessary) stressors for graduate students is where their funding is coming from. There are several ways to be funded throughout your graduate career. The primary forms of funding are teaching assistantships, grants your major professor earns, and grants/fellowships you earn as an individual. When conversing with a potential PI, it is extremely important that they understand that they are responsible for helping you find funding and/or providing you with funding. It may feel weird, but absolutely ask forthright "Do you have funding to support me?" Furthermore, do they have funding to support you throughout the majority of or your whole PhD? Will they help you look for and apply for personal grants, fellowships, etc.? Do they expect you to TA, and if so, will they help you find a position and how frequently are you expected to TA? In the biophysics graduate group, you are required to TA at least one quarter, and student experiences range from just one quarter to once a year to every quarter. Know which one you are okay with, and communicate that with

your potential PI. For example, if a major professor tells you that you will have to TA every quarter, consider whether that will impede your ability to complete your research in a timely manner, and ask whether it will be up to you to find these teaching positions. Some students love teaching and are frequently enthusiastic, while others are not; neither preference is right or wrong, but being on the same page is important. Also ask them if they would be willing to help you apply for fellowships, and whether the lab currently has funding.

2. Expectations

a. Academic background

The Biophysics Graduate Group is highly interdisciplinary, an attribute we take great pride in. Our professors span a broad range of research interests; one could argue that almost any science professor on campus could be considered doing biophysics research. Our students also come into the program with a broad range of backgrounds, and picking the right topic to study can be challenging. You should consider whether you want to stay close to what you have experience with or whether you want to learn new topics and techniques. You will want to communicate these expectations with potential advisors, especially if their field is new to you. As you are entering graduate school with a minimum of a bachelor's degree, you are expected to have a fair handle on the fundamentals of the science you will be studying. Gaps in knowledge are okay, but your major professor should know where these gaps lie. When talking to a potential advisor, inquire what knowledge you are expected to start with, and whether they are willing to teach you or wait for you to learn what you are lacking (e.g., If you join a computationally focused lab, are you expected to already have programming experience, or can

you learn it as you progress with your studies?). Some professors are more supportive of students pursuing new tracks than others, and it will be an immense source of stress for you if your major professor expects you to produce results faster than your starting knowledge allows. Graduate school is supposed to be a learning experience, so if a professor expects you to already be an expert, be sure that you really want to do that research.

b. Available Now and Potential Future Projects

Some projects in a lab may already be funded and a major professor is actively looking for students to work on these projects. Other times, the major professor has submitted a grant application and is anticipating project funding but cannot immediately commit to having available funding. This is an important conversation to have with a PI. Are you willing to wait for confirmation and possible need to TA in the meantime with the potential for funding later? Or are you uncomfortable with the uncertainty? Additionally, laboratories are restricted by the projects they have funding for. If you are mainly interested in working on a specific, currently unfunded project, would you be still happy in that lab if you could only work on a different project and the one you want never gets funding or fails to show promise during collection of preliminary results? Whatever the project and its funding-status, it's important to discuss with your potential major professor the specific aims of the project and what the expected timeline is. What are the component parts? What are the main goals? What knowledge and skills will/may you need to complete the project? How long should each part take? What are possible roadblocks to a timely completion? What happens if the project takes much longer than expected? How might this affect your graduation? You and your major professor may not be able to answer all these questions right away. However, they are important to keep in mind and

can help guide your questions of students currently in the lab and their experience with past projects.

c. Time commitment/Working style in lab

Every graduate student works differently, and every major professor has different expectations for how you work in the lab. Do they expect a 40-, 50-, or 60-hour work week, or do they just expect you to keep up with your research? Do they expect you to work exclusively in the lab, or can you work from home if your research topic allows it? Are they supportive of taking vacation time? Know what environment you work best in and communicate that with the PIs you talk to.

d. Time to finish, publications expectations, career mentorship

Graduate school is a steppingstone to a career in or adjacent to science, and should be treated as such. Ask a potential major professor on average how many years their students take to complete their degree. If they already have an established research group, this is a great question to ask current students who know some of the history. How many publications do students typically produce during their degree? Does the major professor support students pursue non-academic jobs such as industry, education, science policy, or science communication? This question is especially important if you are unsure of what you want to do after graduating. If a major professor won't support your career goals, working for them may be fruitless when you need them for a reference or a contact as you enter the professional world. Ask your potential advisor what steps they take throughout a student's degree to mentor them toward finding a career. Do they encourage students attending and presenting at conferences?

Do they encourage participation in extracurricular career events and workshops on campus? Do they meet regularly with their students to discuss progress and goals?

3. Mentoring style

Ask your potential advisors about their mentoring style (this may sound redundant–the theme is present in the above sections). Are they hands on, designing experiments for or with you, assisting in writing, choosing projects, etc.? Or are they hands off, touching base every month or so but largely letting you direct your research? The answer is probably somewhere in between, but it is important to select a major professor whose mentoring style matches your learning needs. If you need more guidance and communication from your PI, make that clear. If you prefer to figure things out on your own, pick a major professor who will give you the freedom to do that. One of the biggest day-to-day struggles for a graduate student occurs when a PI's mentoring and the student's needs don't match up, so it is important to be upfront about expectations for this dynamic, and to communicate throughout your degree if your styles aren't meshing well. A simple aspect can be how often they hold group meetings and whether you can have a separate meeting with them and how frequently. Many advisors are willing to meet individual student needs, but only if you communicate what your needs are!

4. Mentoring Agreement

The form on the following page is meant to be used for check-ins or updates with your future major professor to make sure you are each on the same page. It can be used formally or informally–you can share this with your major professor and fill it out together or merely use it to guide your own questions. It can also be adapted as a guideline for interviewing a potential

PI. Feel free to modify as necessary to meet your individual needs. Keep in mind that your goals or needs might change throughout your graduate school career so semi-regular check-ins or updates of this agreement are beneficial.

We hope these conversation topics have provided you with some factors to consider when talking to and selecting your future advisor. Your major professor is supposed to be your biggest confidant, supporter, and guide throughout grad school, and the easiest way to ensure a good relationship is to communicate expectations early and often. Additional resources can be found on the University of California Davis Graduate Studies website here: https://grad.ucdavis.edu/resources/mentoring/mentoring-resources. A recommended post to read from the "Tenure, She Wrote" blog on toxic academic mentorship can be found here: https://tenureshewrote.wordpress.com/2013/08/12/toxic-academic-mentors. If you have any questions or concerns, contact your fellow BPH graduate students.

Good luck with your search!

Best,

Biophysics Graduate Students

University of California Davis

Mentorship Agreement

I. Student and major professor Feedback

A. Student/major professor is doing _____ well.

B. Student/major professor can improve upon _____.

This section should elaborate on what the student or major professor is doing well and what they can improve upon in terms of research and mentorship.

II. The mentor will provide _____

This section should list technical, non-technical, financial, career, mental health, or other supports that are the responsibility of the mentor to provide the student.

III. Goals

1. Short-term goals (0-12 months): _____
2. Medium-term goals (1-5 years): _____
3. Long-term goals (5-10 years): _____

This section should focus on big-picture goals such as skills to develop, presentations and professional meetings, publication and authorship plans, professional development, and more.

IV. Milestones: _____

This section should contain concrete items that let students know they are on track to achieve their goals.

V. Plan of Attack: _____

This section should describe the day-to-day and quarterly activities necessary to accomplish milestones and achieve goals.

VI. Data Management: _____

This section should include a plan for both when and where to backup and/or make copies of both digital and non-digital data and protocols.

VII. Funding: _____

This section should detail where stipend, tuition and fees, research, and travel support should come from.

VIII. Courses: _____

This section should list the courses needed to be taken to fulfill program requirements, major professor expectations, career development needs of the student, and ethics requirements for NIH grants.

IX. Communication and Meetings: _____

This section should define how often students and PIs should meet in person, the preferred mode(s) of communication, and the methods for document editing and revision.

3. Mentoring Roles and Expectations

This document is intended to be modified by mentors and mentees as they lay out their expectations for each other. The goal of this is to get mentors and mentees on the same page to help students succeed in their educational endeavors.

Section 1: Example Expectations

The most important expectations I have are that you:

- Work on what you are passionate about to the extent possible given my research expertise and available funding.
- Adhere to the highest possible standards for ethical conduct of research, ethical treatment of human subjects, and professional behavior.
    - Consistent with the NIH mandate, UC Davis provides training in the Responsible Conduct of Research and Professional Ethics. Find more information here on how to meet these requirements or complete one of these courses: BIM 209, 464 RCR (also called CLH 204), GGG 296, PLP 298, or PMI 250.
- Show respect and courtesy to your colleagues and trainees, regardless of race, nationality, age, gender, sexual orientation, or political beliefs.
- If you find yourself in conflict with or the subject of discrimination or harassment by anyone, contact the appropriate entity or who you feel most comfortable with to get help.
    - I am open and available to hear your concerns and provide support where I can.

    - Many campus resources are available: Student Health and Counseling Services, Campus Dialogue and Deliberation, Report Hate and Bias, Sexual Violence Prevention and Response, Center for Advocacy, Resources and Education, and other reporting resources.
- Tell me as soon as possible if there are technical or logistical issues I can help you resolve or if you need additional support or guidance to advance your research progress.
- Schedule regular check-in meetings to ensure open communication and that we are meeting our mutual expectations. We can use this time to put together a professional development plan and identify areas for improvement.
- [Insert additional items here as needed]

The most important responsibilities I have are to:

- Work with you to develop ideas that you are excited about to the extent possible given my expertise and research funding limitations.
- Adhere to the highest possible standards for ethical conduct of research, ethical treatment of human subjects, and professional behavior
- Show respect and courtesy to all lab members, regardless of race, nationality, age, sexual orientation, gender, or political beliefs and ensure that lab is a safe space for everyone.
- Encourage your professional development and transition to independence and provide a vision for a satisfying and successful career.

- Provide direct, honest feedback about your progress and performance.
- Provide support through active and empathetic listening, expression of positive expectations, and self-disclosure as appropriate.
- Challenge you through setting tasks, high standards, modeling and providing a mirror.
- [Insert additional items here as needed]

To succeed as a scientist and position yourself as an independent researcher in the coming years, you will need to:

- Be willing to perform experiments, analyze and understand the results, draw conclusions, and develop ideas.
- All datasets are noisy, and no scientific explanation is perfect. That said, as a scientist, your responsibility is to think about and interpret results, not just regurgitate data.
- Actively read and critically think about the relevant scientific literature and literature in other fields that may be pertinent. Especially make sure that you seek out and cite work that is highly relevant to your projects and that you have read all the work that you cite.
- Be willing to write up and publish your results.
- Maintain a healthy life outside of work by making time for family, friends, and relationships, and by pursuing hobbies or activities that energize you.
- Be willing to work long hours when necessary.
- Advise, collaborate with, and learn from graduate students, postdocs, and other researchers.
- Learn to mentor and advise research assistants who may be helping with your projects

- Actively pursue opportunities for professional development, networking, awards, and funding.
- Be willing to face, overcome, and learn from adversity, including experiments that don't work out or disappointing decisions on papers or grants.
- [Insert additional items here as needed]

My Expectations and Goals for You

Year 1

- Focus on coursework and learning as much as you can from your rotation projects
- Familiarize yourself with relevant literature and techniques
- Fulfill the NIH Responsible Conduct of Research requirements
- Regularly attend seminars and actively participate in lab meetings
- Apply for fellowships or grants to support your research
- Actively pursue professional development opportunities
- Regularly meet with PI to check in and discuss progress

Year 2

- Complete required coursework and relevant electives
- Work with PI to conceive of a project and work on data collection and analysis
- Deepen your understanding of the relevant literature
- Participate in a journal club
- Prepare for your qualifying exam
- Regularly attend seminars and actively participate in lab meetings

- Apply for fellowships or grants to support your research
- Actively pursue professional development opportunities
- Regularly meet with PI to check in and discuss progress

Year 3

- Pass your qualifying examination
- Complete data collection and analysis and prepare manuscript of your first project
- Begin work on dissertation project
- Deepen your understanding of the relevant literature
- Regularly attend seminars and actively participate in lab meetings
- Apply for fellowships or grants to support your research
- Actively pursue professional development opportunities
- Regularly meet with PI to check in and discuss progress

Year 4

- Make progress on dissertation project
- Complete data collection and analysis and prepare manuscript of your second project
- Deepen your understanding of the relevant literature
- Regularly attend seminars and actively participate in lab meetings
- Apply for fellowships or grants to support your research
- Actively pursue professional development opportunities
- Regularly meet with PI to check in and discuss progress

Year 5

- Complete data collection and analysis and prepare manuscript of your third project
- Deepen your understanding of the relevant literature
- Regularly attend seminars and actively participate in lab meetings
- Actively pursue professional development opportunities
- Regularly meet with PI to check in and discuss progress
- Submit dissertation project and complete exit seminar

Section 2: The "Mentoring Up" Philosophy

"Mentoring up" is a concept that empowers mentees to be active participants in their mentoring relationships by shifting the emphasis from the mentors' responsibilities in the mentor-mentee relationship to equal emphasis on the mentees' contributions. A full background and explanation can be found in a book chapter from "The Mentoring Continuum: From Graduate School Through Tenure" entitled "Mentoring Up: Learning to Manage Your Mentoring Relationships" written by former UC Davis Graduate Diversity Officer Steve Lee and his collaborators. Detailed below are the core principles and descriptions identified in this framework that can provide a foundation to understand the various aspects of an effective mentoring relationship that can mutually benefit the mentee and mentor. Recognition that both the mentor and the mentee must gain mentoring knowledge and skills and intentionally engage in effective mentoring practices is important. However, the list focuses on skills the mentee can use to make their mentoring relationship more effective:

1. **Maintaining Effective Communication.** Good communication is a key element of any relationship, and a mentoring relationship is no exception. It is critical that mentors and

mentees seek to understand their own and the other's communication styles, and take time to practice communication skills.

a) Determine your mentor's preferred medium of communication (face-to-face, phone, or email) and acknowledge if it differs from your own personal preference.
b) Schedule a regular time to meet or check in with your mentor.
c) Track and share progress toward project and professional goals–verbally and in writing.
d) Identify challenges and request your mentor's advice/intervention when appropriate.
e) Prepare for meetings with your mentor by articulating specifically what you want to get out of the meeting and how you will follow up after the meeting.

2. **Aligning Expectations.** Another key element of effective mentor-mentee relationships is a shared understanding of what each person expects from the relationship. Problems and disappointment often arise from misunderstandings about expectations. Importantly, expectations change over time, so reflection, clear communication, and realignment of expectations are needed on a regular basis.

    a) Ask your mentor for his or her expectations regarding:

        - mentees at your stage of career generally.
        - you as an individual scholar.
        - the research projects.

b) Share your expectations regarding:

- your career as a scholar and professional.
- the research projects.

c) Ask others in the research group, who know your mentor better, about the mentor's explicit and implicit expectations.

d) Write down the expectations you agree to and revisit them often with your mentor. Use a mentor-mentee contract to formalize the expectations.

3. Assessing Understanding. Determining what you understand as well as if someone truly understands you is not easy, yet is critical to a productive mentor-mentee relationship. Developing strategies to self-assess and assess others' understanding is an important part of being an effective mentor and mentee.

- Ask questions when you do not understand something. If you are afraid to ask your mentor directly, start by asking your peers.
- Talk and write about your project; Ask peers and mentors in the field for feedback.
- Ask peers and mentors to share their perspectives on your work and its meaning in the context of the field more broadly.
- Explain your project to someone who is new to the field and help them to understand your project and its significance.

4. Addressing Equity and Inclusion. Diversity along a range of dimensions offers both challenges and opportunities to any relationship. Learning to identify, reflect upon, learn from,

and engage with diverse perspectives is critical to forming and maintaining an effective mentoring relationship.

- Be open to seeking out and valuing different perspectives.
- Engage in honest conversation about individual differences with mentor and coworkers.
- Contribute positively to shared understandings and solutions to problems.
- Talk to peers and mentors when you feel conflicted about the ways in which your personal identity intersects with your academic identity.

5. Fostering Independence. An important goal in any mentoring relationship is helping the mentee become independent; yet defining what an independent mentee knows and can do is not often articulated by either the mentor or the mentee. Identifying milestones towards independence and setting goals are key strategies to fostering independence in a mentoring relationship.

- With your mentor, define what it takes to do independent work in your field.
- Define a series of milestones to independence with your mentor and set goals for meeting these milestones as part of your research plan.
- Ask peers and mentors to share with you their strategies for achieving independence.

6. Promoting Professional Development. The ultimate goal of most mentoring situations is to enable the mentee to identify and achieve some academic and professional outcomes after the

training period. It is the responsibility of both the mentor and mentee to identify and articulate these goals and to strive towards them together.

- Create an Individual Development Plan (IDP) to set goals and guide your professional development, using resources such as Science Career's myIDP website.
- Seek out and engage multiple mentors to help you achieve your professional goals.
- Ask peers and mentors to discuss with you the fears and reservations you may have about pursuing a certain career path.

7. Ethics. Mentors and mentees must engage in and model ethical behavior, while openly discussing issues dealing with gray areas. Moreover, it can be important to acknowledge when a mentoring relationship includes an unequal power dynamic and any additional ethical considerations it raises.

- Take responsibility for your own behavior.
- Seek out formal and informal ways to understand the norms of practice in your field.
- Learn about ethical issues associated with your work and proactively address them.
- Learn about UC Davis's policies for dealing with unethical behavior.

D. Science Communication

One of the most undervalued skills in scientific graduate education is communication to public audiences. While the ability to communicate your science at academic conferences or in grant applications and publications are recognized as necessary, the ability to communicate to non-expert audiences remains an afterthought in graduate education, despite the current societal climate and the rise of misinformation/disinformation increasing real-world impacts on scientific communication, funding, and acceptance. Arguments for inclusion of science communication to the general public within formal scientific training at both undergraduate and graduate levels have been published in various journals over the past many years[182–188]. Barriers to inclusion of non-academic science communication in graduate curriculum include faculty resistance, bureaucratic impediments to changing curriculum requirements and new-course creation, and most importantly, lack of faculty availability to consistently offer the courses–a necessity for degree requirement inclusion. Not only is non-academic science communication important for those on academic career pathways to better communicate their science to funders and the press, but it is also one of the most transferrable and important skills for those on non-traditional science career pathways.

I aim for the following documentation of my public-targeted communications to serve as an example of ways students can gain this critical experience outside of their prescribed curriculum and research training.

1. "Scientists, the public is not your enemy"

Published in the Davis Enterprise, November 10, 2018

Despite popular belief, the public is not at war with science. Individuals who are resistant to scientifically sound positions are not necessarily uninformed, uneducated, or irrational. In fact, Pew Research Center studies show that 67 percent of people believe science has positively impacted the world and that confidence in scientists has remained steady over the past 50 years.

While it is true that individuals that espouse anti-GMO or anti-vaccine positions often cite flawed scientific studies to support their claims, they are not being purposefully anti-science. Instead, the likely main motivator of this response is emotional–expressing a larger distrust of our society's institutions and concern at the rapid pace of technological change. Instead, the scientific community needs to move beyond alienating rhetoric—like a "war on science"—and toward proactive engagement with the public.

Our dilemma with public acceptance of genetically modified organisms illustrates this phenomenon. GM crops are essential to survival. They allow for nutrient-rich, insect-resistant crops that decrease exposure to carcinogenic pesticides and increase crop yield. Without GM crops, we are at risk of failing to feed our ever-growing world population.

But despite evidence supporting the benefits of GM crops, social stigma and fear about safety dominate public opinion and the gap between scientists' and the public's views is only growing. Even advances in crop gene-editing technologies — like the highly touted CRISPR technology — are at increasing risk of overregulation, which may stifle scientific innovation and harm efforts

to positively impact global hunger and climate change. So, what can we do to address these urgent problems?

First, teachers and scientists must challenge students to think critically in our classrooms and labs. We need to give students the means to dissect misleading information and statistics, avoid confirmation bias, encourage skepticism, and value truth. Too much of our curriculum focuses on broad memorization of facts and does not teach students to think critically or inspire them to research topics for themselves.

As examined in an insightful TED Talk by Dan Meyer titled, "Math Class Needs A Makeover," students are not effectively retaining information and textbooks and teachers may be to blame. It is simple to see this phenomenon. I challenge you to look in almost any textbook. You will see paint-by-numbers modules that spoon-feed students the questions to ask and the answers to find.

Instead of students and teachers working to determine why a problem needs solving in the first place, we rush to find the solution so tests can be passed, and courses can be completed. We cannot expect our youth to become informed, civically-minded citizens if we don't help them develop the skills to do so.

Second, scientists must come out from behind the closed doors of the laboratory and into the public eye. People don't trust what they can't see and understand. We can't leave all the science communication to a handful of science celebrities and popular science writers. It is not enough. Scientific Societies — like the Biophysical Society, of which I am member — are

increasingly developing communication workshops to help our community succinctly explain our research and why it is important.

After all, California receives nearly $4 billion in biomedical research funding from the National Institutes of Health and these dollars are helping to create jobs in Davis and across the state. It is vital that we get involved to ensure our nation and our state continue to be leaders in science and economic growth.

Lastly, we must listen to, empathize with and value concerns expressed by the public. Their fears and concerns are real. The increasing morbidities of certain diseases are real, even if the causes are unclear or misattributed. People need to be heard and feel empowered. Science is our best tool for enabling life-saving discoveries, stimulating the economy, creating jobs, and spurring innovation. It is important that we convey the importance of federally supported basic science research in furthering our quality of life. Yet, these investments and potential breakthroughs will mean little if public concerns are not addressed.

If we as a scientific community can come together, move our work into the public sphere, and thoughtfully engage public interest, we can make a difference. We need to change the conversation and end divisive rhetoric like a “war on science.” Instead, scientists and the public must develop practical compromises and solutions. Those facing hunger or disease around the world are depending on us to develop a new scientific consensus.

2. "Diversity in STEM Conference: An Interview and Reflection"

Published in BioScope, April 2, 2020

Note: Interwoven into this article are parts of an interview held on Feb 12th with Alexus Roberts, third-year PhD candidate in Population Biology, who was one of the lead organizers for the Diversity in STEM Conference.

In the midst of university controversy over the valuation of faculty diversity statements in their application process, the students of UC Davis held their newly-expanded, annual Diversity in STEM Conference (DISC) on January 25th with the purpose of "[honoring] the progress that has been made towards diversity and inclusion on campus, in the industry, and beyond."

***Me**: How well did the events of the day meet your mission? Did you achieve everything you wanted to achieve?*

***Alexus**: I think based on our mission and our purpose statements we were talking about bringing together marginalized students to create community… and [talk] about the barriers to marginalized students and how you overcome them. I think the panel alone addressed that. And then, honoring the progress that has been made on campus… those are really broad goals. We definitely addressed them, but I think we have to collect feedback from people [over the next] couple years and … see if that [led] to internships or job opportunities. I think as a committee one of our goals is to create a pipeline for marginalized students to graduate from higher education on their own terms… That [will take] time.*

Held in the ARC Ballrooms and filled with more food than everyone could eat, the day began with breakfast and time to mingle before sitting down for the first speaker. After splitting a cinnamon roll with the keynote speaker, Dr. Renetta Tull, because we both had a craving we didn't want to indulge, I sat down to listen to a very inspiring morning of speeches and panel discussions.

Dr. Tull's keynote address "Joy in the Journey" outlined the meandering path she took to her current position as Vice Chancellor of Diversity, Equity, and Inclusion. The title was inspired by a quote from Representative Ayanna Pressley of Massachusetts, "It's alright to stand in joy…joy is a necessary act of resistance." It was very clear from her talk that Dr. Tull intimately understands the mental health concerns of graduate students and personally experienced the bias and discrimination women and minorities face, especially within the STEM community, and wants to be an ally to current students who may be struggling. Her story highlighted that success may not always look like we imagined it to, and we may need to rethink our trajectory, but if we focus on the problems that are important to us, we can find our way. One part Dr. Tull shared that stuck in my mind was a section of a poem she wrote on her flight home from a speaking engagement on diversity in Latin America:

I am out of the box,
the voice you didn't know you needed to hear,
together we are better,
join me in the struggle to lift others up.

This consistent message throughout her story was the need for resilience, mentorship, and community. "[Reshaping] environments [people exist in] can mean something. It can inspire." We need to choose to surround ourselves with people who are going to build us up.

***Me**: What did you enjoy most about the day?*

***Alexus**: There was a certain point during the luncheon where I had to go up [on stage] and tell everyone … 'here is what's happening.' And just seeing everyone out there talking, smiling, and laughing, seeing them lining up to talk to the panelists–it was really cool. [The DISC Organizers] are really making a difference and making a space for community … [and] connections.*

This message of community was echoed throughout the remaining morning sessions. Two panels – "Transitioning from College to the Workplace" and "Navigating the Workplace in STEM" – consisted of four speakers* each plus the lead panelist and moderator, Dr. Devin Horton. I could fill up several pages with the stories, advice, clarifications, solidarity and support the speakers packed into this hour, but to sum it up through quotes by the panelists:

1. "Don't measure by how much help someone appears to need." –Lakshmi Sharma
2. "The system is not fair, and you have to find ways to change it, but don't let it compromise your mental health." –Colleen Bronner
3. "There is so much [we] don't know and don't realize. Mentors can help you figure out how prepared you are." –Gwladys Keubon
4. On dealing with imposter syndrome, adopt the attitude: "I don't know it yet, but I will." –Amanda Dang
5. "Resources may have been there but not the knowledge of them or the thought I deserved them. Have the confidence to go after them. Move the resources closer to you." –Barbara Blanco

6. “My culture tells you to be humble and that if you put your head down and work someone will notice you. This doesn’t work. [We need to] encourage people who are doing well and give them the opportunity to be leaders. Doors open up from achievements but also from advertising them.” –Carlos Gonzalez
7. “Always be willing to learn and grow no matter what position you are in.” –Linda Finley
8. “The reimbursement system is b***s***.” –Crystal Rogers

An important distinction made during this time that I don’t think is discussed enough is on the difference between **mentors** and **sponsors**. A mentor helps you through advice and support. A sponsor advocates for you, even when you aren’t in the room. During the Q&A, one student attendee spoke about his struggle with finding a sponsor. The response: *Sponsorship requires trust; Invest time in them, in who they are and their personality.* The truth is that these sponsor-sponsee relationships are investments and, when networking, the number one piece of advice someone can give you is to find what you can do for your sponsor. It’s not just about what they can do for you. Demonstrating what you can do for the sponsor and your developing relationship with them will encourage the sponsor to advocate for you when you are not in the room.

***Me**: What challenges did you face?*

***Alexus**: Everyone [who was planning this event] is a student. So, making sure that we actually made time for all of this was difficult. I definitely know there was a good two weeks when I got back from winter break where I didn’t focus on anything else besides this conference. We had already been planning since June, but [this] was the time when everyone needed updates … Additionally, [the organizers] are strong visionaries and leaders … and when you have*

[many of these people] in a room together, there is a lot of back and forth about how we [wanted] things to be—making sure that everyone was happy with how this looked can cause tension sometimes. The last thing was student turnout. We had 100 students come which was incredible. And all the students that were there really gave some high praise and admiration for the conference overall. But, to put in all that work and have 100 of the 200 that RSVP'd not show up was [frustrating]. I think people are busy but if you are involved in planning an event then, you know, that handful of people not showing up is difficult.

Another important discussion graduate students need to have is on the balance between hard work and mental health. So many students come in and acquiesce to professors' expectations or demands, or they don't feel like they have the right to pursue their interests outside of their lab work. Complicated by a power imbalance and centuries of tradition, the relationship between PI and student can be wonderfully supportive and productive but it can also be very contentious.

Also asked in the Q&A was how to bridge the gap between working hard and maintaining mental health. The response: *Taking care of yourself improves your work. Set boundaries and priorities, and commit to your hobbies.* Most importantly: *Learn to say no. "No" is a complete sentence.* This advice should be given to every graduate student walking on to campus and will continue to be relevant as we move on in our career. Women and minorities are often asked to contribute more of their time on average to serve on committees and be present because of these efforts to diversify. This means the people often struggling hardest to stay afloat are the same people with more pressure and responsibilities.

***Me**: One point brought up in the panels was how people may have access to resources but didn't know they were there. What are your thoughts on trying to make all the resources known to people, whether on campus or in general?*

***Alexus**: I think it's difficult because we have all these different mediums to connect with students and make sure that what you have to offer is out there. The people providing the resources… have to do their part to advertise it and make it accessible to people… I know it can be very overwhelming to try to look for all those things and when you are struggling it is very hard to be like 'I can go, and I can do this thing.'*

After the lunch break and conversations with the interesting women at my table, we split up for an afternoon of workshops. There were three options for each of the two workshop sessions divided into two tracks: graduate and professional. For my first session, I attended the "Conflict Management" professional track option led by two student interns from the Center for Leadership and Learning which began with an exercise where we individually chose four words that we associated with the word "power." I chose (1). Money, (2) Politics, (3) Corruption, and (4) Confidence. We then paired up and were tasked with narrowing down our combined eight words back down to four by advocating for more of our words to be included in the final set than our partner's. Then, our pairs combined with another group and repeated the process but this time arguing for more of the other group's words to be included in the final set. This activity was accompanied by debrief questions about what it revealed about your approach to conflict and was followed up by a quick conflict management style assessment similar to the one linked here that assigned an animal (I'm an owl apparently) to different approaches to conflict. The last session before the career fair, "Stories from Professionals," consisted of guest

speakers talking about their career paths and experiences. Their advice reiterated many points made during the morning panels but could be boiled down to *reach out and make as many connections as possible*. Not only do opportunities open when you meet new people, but it exposes you to differing viewpoints that improve collaboration and broaden your perspective.

***Me**: What did you find disappointing about the conference?*

***Alexus**: From a little feedback from people, making sure that this is useful for graduate students. All the undergrads really seemed to enjoy it, but I want to make sure we are serving the general UC Davis student body. So, making sure that the people we are inviting to lead our workshops are aware that we have grads and undergrads. [However,] I think Dr. Tull's keynote speech and the panelists really addressed everyone.*

To close the day, there was a career fair composed of several industry sponsors plus a few departments and campus resource centers. The room felt lively with conversation, but I couldn't help but notice the lack of diversity in the organizations attending – nearly all were engineering-based. I don't happen to be personally interested in working in industry (although I did stop by the US Army Corps of Engineers booth to say hello since my dad and uncle worked for them for forty years) so I gravitated towards speaking with the representatives from the departments and resource centers. I mostly spent time speaking with the wonderful Nicole Rabaud, the Director of Graduate Academic Programs for the College of Biological Sciences. We spoke for nearly an hour about the state of graduate education (and more specifically my biophysics graduate group) and pathways for influence and reform. A few important reminders inspired by our conversation: (1) there are several exciting science policy fellowships opportunities in Sacramento including CCST and Capital Fellows, (2) Aggie Compass is available

24/7 for helping meet your basic needs, and (3) A "Buy Nothing" Facebook group exists for the Davis area.

***Me**: Will you be doing it again next year? If so, what will you be changing?*

***Alexus**: Yes, we are meeting [soon] to talk about next steps and plans for next year. We are looking to make DISC an actual organization. Having people focused just on DISC will be good. This year, we had the presidents of all of the organizations that were involved be the representatives on the committee. I was with ESTEME and also [focusing] on DISC as well, which means that often something else had to go. It's not like we could drop off [the responsibility to] our clubs. So, for me, that was my research sometimes.*

Overall, I really enjoyed the day and I look forward to attending this event next year. The DISC conference is a great celebration of the diversity of people in STEM at UC Davis and an important reminder about the value of community.

3. "(Ethically) Talking Science"

Published in BioScope, July 24, 2019

What does it mean to ethically communicate your science? What are our responsibilities as graduate students doing scientific research? What policies govern our actions? Although these questions seem straightforward, the answers are deceptively elusive.

Since starting graduate school, I've participated in several programs that emphasize the importance of everyone sharing their science and engaging with people outside of their immediate scientific community to hone my science communication skills. I even published an article last year in the Davis Enterprise calling for more public engagement by scientists. What was missing in nearly all of these discussions was whether we as scientists are

communicating about our work responsibly – ethically. How are we depicting our research? Are we inflating our results? Are we misrepresenting reality? Are we open about our biases?

Take the "CRISPR Babies" controversy as an example: scientists have decried the ill-advised embryonic genome editing as an unacceptable ethics violation. However, their responses to this controversy do not actually address ethical concerns. Leading CRISPR scientists seem to be more concerned with their ability to continue their research rather than the ethical question: Should their research be done at all?

An article in Discover Magazine does a good job of highlighting the failures of our current system to regulate ethical violations. Whose ethics are being upheld? Are we asking the right people the right questions? At the most recent International Summit on Human Genome Editing (where the "CRISPR babies" were announced), many presenting researchers disclosed their private business ventures at the start of their talks. Researchers require immense funding to achieve tenure and status within the scientific community, so how much of their communication is biased by their desire to receive grants? Are ethics the number one concern of these scientists upon whom we rely for self-regulation?

The long history of scientific misconduct led me to investigate our ethical policies at UC Davis. To narrow the broad scope of ethics, I focused only on the requirements surrounding the reporting of funding sources and conflicts of interest by campus researchers. The policies are detailed across an unmanageable number of web pages, documents, and training videos. After my first passthrough, I learned PIs are required to self-report new funding sources or conflicts of interest to an internal review committee of fellow professors.

I emailed the Conflict of Interest Committee (COIC) to confirm my interpretation of the self-reporting policies and was informed that the "complex subject" would be better suited to a phone conversation than emailed correspondence. In this phone call, I explained my inquiry into the university's guiding policy around financial conflict of interest disclosures for scientists. Surprisingly, I was asked whether this phone call was "on the record" and met with repeated assurances that graduate students do not typically meet disclosure requirements. Seeking guidance in person proved to be almost as challenging.

My correspondence with the COIC ultimately confirmed that there is no blanket requirement for reporting funding sources or conflicts of interest for anyone. They reasoned that specific conferences or journals may have their own reporting requirements and did not want to risk conflict with these policies.

Yet, this policy is not reflected across the University of California system. For example, UC Irvine requires "disclosure of related financial interests in publications and presentations to promote transparency" regardless of the venue or publication requirements. It stands to reason that a venue or publication would have very little impact on the overall responsibility of researchers to disclose funding sources and financial conflicts of interest. Current UC Davis COIC policy might be reinforced by requirements similar to UC Irvine.

The scientific community is at a critical stage. As the University of California cancels its subscription contract with Elsevier and we push for more open access to scientific research, we also need to push for transparency in other areas such as ethics. Are our policies adequate? Whose interests are being served? What can graduate students do today to promote research

transparency? Are we critically examining our lab practices? We should be doing everything we can to practice and communicate our science in an ethical manner.

An important (but unfortunately not well-known) resource available to our community is the Ethics Commons. This multidisciplinary group comprises faculty from the entire UC Davis campus who serve as a resource to help us think about the "transformation and integration of ethical considerations in research, education, and public engagement." Let's make ethics an integral part of how we do science *and* share it.

4. "What can you do in an hour?"

Published in BioScope, April 2, 2019

Earlier last month, the White House released the president's FY2020 budget proposal. For those of us supported by non-defense federal funding sources, this proposal should worry you: the budget asks for a **$54 billion (9%) drop in spending** for R&D programs. A detailed analysis of the proposed R&D budget can be found here. However, there is hope.

This past week, I was selected by the UC Davis Government and Community Relations office to be one of two students sponsored by UC Davis to attend the annual AAAS CASE Workshop in Washington DC. This program included three days of workshops on science policy, advocacy, and communication, followed by a day of meetings with the offices of congressional members.

On our day of meetings, the California student delegation met with the offices of Senator Feinstein, Senator Harris, Speaker Pelosi, and House Minority Leader McCarthy. The UC Davis students also met with Representatives Garamendi, Bera, and Matsui, representing the greater Sacramento area. Every office expressed its fervent support for our research

efforts. While #MakingOurCASE for federal science funding, a legislative staffer in Pelosi's office stated that the president's budget was **"not a starting point for negotiations."** There is strong bipartisan support for science funding.

However, this doesn't mean we can sit back and relax. Although general support for science is strong, research on key politicized issues (such as climate change) is still controversial. And, with the Democrat majority in the House for the first time in 8 years, the funding of many important social programs is high priority and means a tighter budget. So, the next question is: how can you get involved?

One of the most important workshop sessions I attended this week was led by Erin Heath, the Associate Director of Government Relations at AAAS. What I found significant during her talk was her recognition that graduate students are extremely busy–we don't have much time to spare, and there is often an energetic barrier to trying unfamiliar things. She broke down her presentation into segments of what we could do in an hour, day, week, year, or lifetime to participate in science advocacy efforts. In just one hour, you can:

- **Vote:** This opportunity may only come up every once in a while, but it is one of the most important things you can do: help elect future leaders who are responsive to the needs of our community and will advocate on our behalf. It is easier than ever before to be an informed voter.
- **Learn:** Do you not feel informed on a topic? Are you unsure who your representatives are? Do you want to know what is happening in science policy? Spend a free hour researching, sign up for updates from science policy news sources, watch a webinar,

discuss issues with people in your community, or check out resources available through your scientific society. The first barrier to action is the lack of information.

- **Reach Out:** This is **the crucial time of year** for science advocacy efforts. The Senate and House have just started holding appropriations hearings that will decide next year's funding levels. Reaching out to your representatives and senators and telling them your story and why science funding is important to you and your district can be very powerful. This can be done with a quick phone call, email, or visit to their local offices. Alternatively (or additionally), you can meet with someone from our government relations office at UC Davis to share your story and talk to them about how to get involved.

The ability to make a difference is within reach. Stay informed, speak out, and take action.

5. Additional Writing Experience

- **Co-Author** "2021 Annual Report to the California Legislature" *California Initiative to Advance Precision Medicine, Governor's Office of Planning and Research* (2022). https://opr.ca.gov/ciapm/activity/publications.html
- **Co-Author** "2020 Annual Report to the California Legislature" *California Initiative to Advance Precision Medicine, Governor's Office of Planning and Research* (2021). https://opr.ca.gov/ciapm/activity/publications.html
- **Reference Manager** "Roadmap for Resilience: The California Surgeon General's Report on Adverse Childhood Experiences, Toxic Stress, and Health" *Office of the California Surgeon General* (2020). https://osg.ca.gov/sg-report/

6. Public Speaking Experience

- **Panelist** ”Biophysics & Graduate School” UCD Biophysics Seminar (2021)
- **Panelist** ”Unique K-12 Teaching Experiences” EDU 120 Philosophical, Social Foundations of Education (2019)
- **Speaker** “Utilizing Local Expertise” UWP 011 Popular Science and Technology Writing (2019)
- **Speaker** ”The Heart of It: Modeling for Heart Health and Drug Interactions” Sacramento Science Distilled by Science Says and CapSciComm (2019). https://capscicomm.org/2019/01/05/science-cafe-events-in-the-sacramento-region-for-january/
- **Speaker** “Education Technology in Non-Traditional Teaching Environments” EDU 180 Technology in Education (2018)
- **Speaker** ”Life of a Scientist ” Dinner with a Scientist by Powerhouse Science Center (2018)
- **Panelist** ”Graduate Student Experience ” Undergraduate Mentorship Program hosted by Equity in STEM and Entrepreneurship Graduate Student Organization (2018)

E. Leadership, Extracurriculars, and Professional Development

The lack of emphasis on communication skills in graduate science education can be expanded to most soft, transferable skills. A 2015 report by the New York Academy of Sciences, "The Global STEM Paradox," highlighted a global crisis in STEM education: while numbers of STEM graduates are at an all-time high, they lack the soft skills needed to get hired for most careers. You can find this sentiment oft repeated in articles published over the last decade and increasing calls for strengthened partnerships between academia and industry to bridge this gap between requisite education for employability and skills upon graduation[189–199].

While there are many opportunities for graduate students for internships, fellowships, workshops, seminars, and volunteer experience that can help students develop these skills, many barriers are in place that discourage graduate students from taking advantage of these resources. Graduate student mental health is in crisis[200–204]. Academic science research culture tends to reward poor work-life balance and unrealistic expectations regarding productivity[205,206]. The design of the graduate education system is innately hierarchical with power imbalances that can become exploitative or abusive situations if not properly supervised[204,207–209]. Without adequate encouragement and valuation from a student's major professor, spending time away from lab and coursework can feel inaccessible, guilt-laden, or come with a fear of repercussions[210]. A graduate program culture that actively encourages and supports professional development opportunities, transparency, and accountability of administration, and advising independent of the major professor are all needed to ensure students get the opportunity to engage in the necessary career development.

The following sections summarize my extracurricular, leadership, and professional development activities that supported the development of my soft, transferrable skills and career goals. I hope this can inspire students to realize the full potential of their graduate studies and make the time and effort to pursue all the amazing resources and opportunities they have to achieve their goals.

1. Wellness, Inclusion, and Social Equity (WISE) Initiative
    - Co-founded a faculty and student joint-initiative focused on instilling the principles of social equity and well-being into partnering graduate groups
    - Curated a series of seminars, trainings, discussions, and activities for cohorts of students, faculty, and staff
2. California Initiative to Advance Precision Medicine
    - Designed and coordinated the creation of a Precision Medicine Primer and Educational Resource to be highlighted on CIAPM's website
    - Supported organization, processing, and selections efforts for an RFP on Adverse Childhood Experiences with a Precision Medicine approach as a Scientific Review Officer in partnership with California's Surgeon General
    - Authored case studies on historical ethics violations of science research to support administration and projects of the California Precision Medicine Advisory Council Working Group on Equitable Consent
    - Assembled, organized, and quantified data on state legislators and program activities to support legislative outreach efforts, assessment of program impact, and annual report to the state legislature

- Reference manager for the “Roadmap for Resilience: The California Surgeon General’s Report on Adverse Childhood Experiences, Toxic Stress, and Health” *Office of the California Surgeon General (2020)*
- Co-author of the 2020 and 2021 Annual Reports to the California Legislature” *California Initiative to Advance Precision Medicine, Governor’s Office of Planning and Research*

3. Biophysics Graduate Group Student Leadership
    - Designed and annually implemented the group’s first Student Experience Survey to collect data on student well-being and program success
    - Presented action plan based on survey results to faculty and staff proposing potential changes to curriculum and mentorship practices to better support student mental health, degree progress, and learning
    - Organized research mixers and social events to support student and faculty community building
    - Advocated for peer needs in discussions on graduate education, mental health, equity efforts, and unmet needs
4. AAAS Catalyzing Advocacy in Science and Engineering Workshop
    - One of two chosen to represent UCD during meetings with staff of California senators and representatives to advocate for increased STEM research funding
    - Attended 2 days of workshops from science policy and advocacy experts about the role of science in policymaking and the federal policy-making process
5. Teaching Assistantship and Public Speaking

- Revise and present lectures, respond to student questions and concerns, and provide detailed feedback on science writing and analysis assignments
- Facilitate discussions on current and controversial topics in nutrition research
- Invited Panelist at ”Unique K-12 Teaching Experiences” EDU 120 Philosophical, Social Foundations of Education
- Invited Speaker at “Utilizing Expertise” UWP 011 Popular Science and Technology Writing
- Speaker at ”The Heart of It: Modeling for Heart Health and Drug Interactions” Sacramento Science Distilled by Science Says and CapSciComm (2019)
- Invited Speaker at “Education Technology in Non-Traditional Teaching Environments” EDU 180 Technology in Education (2018)
- Speaker at ”Life of a Scientist ” Dinner with a Scientist by Powerhouse Science Center (2018)
- Panelist at ”Graduate Student Experience ” Undergraduate Mentorship Program hosted by Equity in STEM and Entrepreneurship Graduate Student Organization (2018)

6. Student Chapter of the Biophysical Society
    - Conceptualized, organized, fundraised, and virtually hosted the first-ever Biophysics Conference at UCD with two keynote speakers, graduate and postdoctoral speakers, undergraduate and graduate student poster competition, and collaborative agenda-setting group discussions on supporting and enhancing the biophysics community

- Designed and organized the first interdepartmental Biophysics Fall Research Mixer at UCD to facilitate student-faculty interactions and support incoming graduate students

7. Equity in STEM and Entrepreneurship (ESTEME) Graduate Student Organization
   - Established seminar series on pathways to non-traditional STEM careers and what DEI initiatives look like in these industries
   - Organize workshops for grad students on cover letter and CV/Resume writing
   - Designed and led interactive science activities at local science festivals
   - Activity creator, team leader and support for biweekly after-school science club
8. Extracurricular Coursework
   - EDU 264 Science Education Reform
   - CRD 209 Critical Social Sciences Perspectives of Agriculture
   - UWP 011 Analysis of Popular Science Writing
9. Awards and Training Programs
   - NIH T32 Training in Basic and Translational Cardiovascular Medicine*
   - SIMULA Summer School in Computational Biology in Oslo, Norway*
   - NIH TCGB/MMBioS Hands-On Workshop on Computational Biophysics at Carnegie Mellon's Pittsburgh Supercomputing Center
   - AAAS Catalyzing Advocacy in Science and Engineering
   - Science Communication Fellowship by UCD and Powerhouse Science Center
   - NIH FUTURE Career Skills and Exploration Program

- Student Scholarship to the International Conference on Mathematical and Multiscale Modeling in Biology in Guanacaste, Costa Rica (2019)
- UCD Graduate Student Travel Award (2019)
- Student Scholarship to Grace Hopper Conference for Women in Computing (2017)

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