Investigating the Molecular Mechanisms of Drug Induced Cardiac Arrhythmias

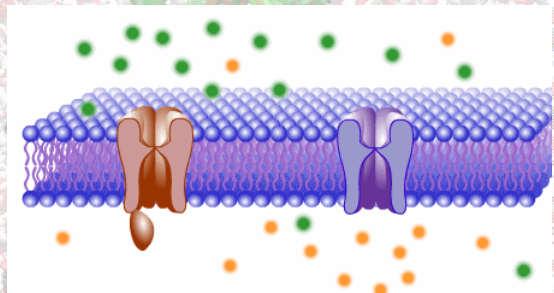
Blue Waters broadening participation allocation

Project PI: Prof. Colleen Clancy

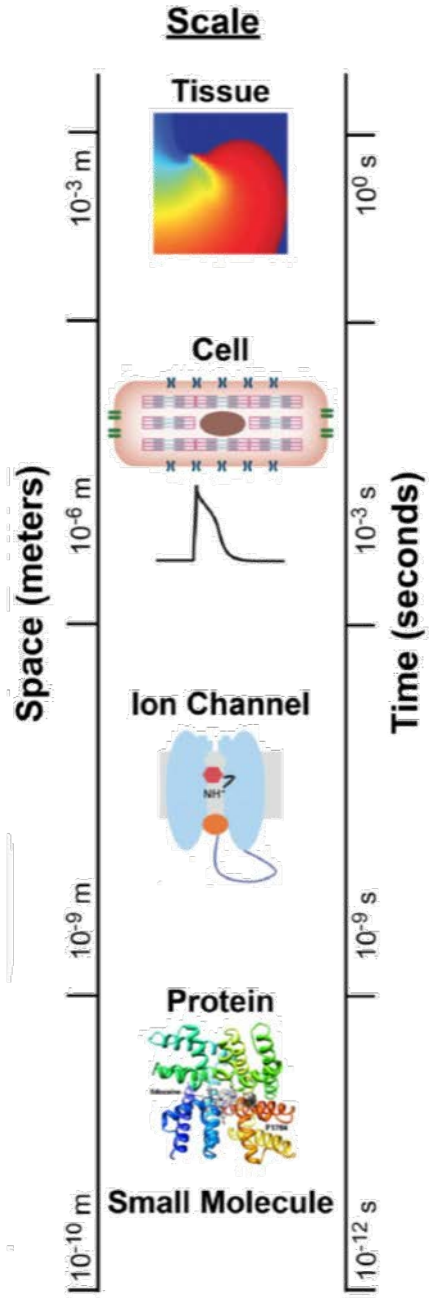
Presented by Igor Vorobyov (Co-PI)

University of California,
Davis

June 3, 2019



We use Blue Waters for multi-scale in silico pipelines of predictive safety pharmacoloty



In particular, we perform atomistic structural modeling and simulations of cardiac ion channels and their drug interactions.

Molecular modeling team leaders

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UC Davis

Prof. Vladimir Yarov-Yarovoy
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Prof. Sergei Noskov
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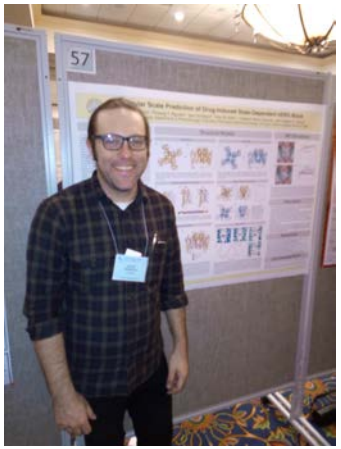
Prof. Toby Allen
RMIT U

UC Davis Postdocs & Biophysics Graduate Students



Dr. Kevin DeMarco

John Dawson

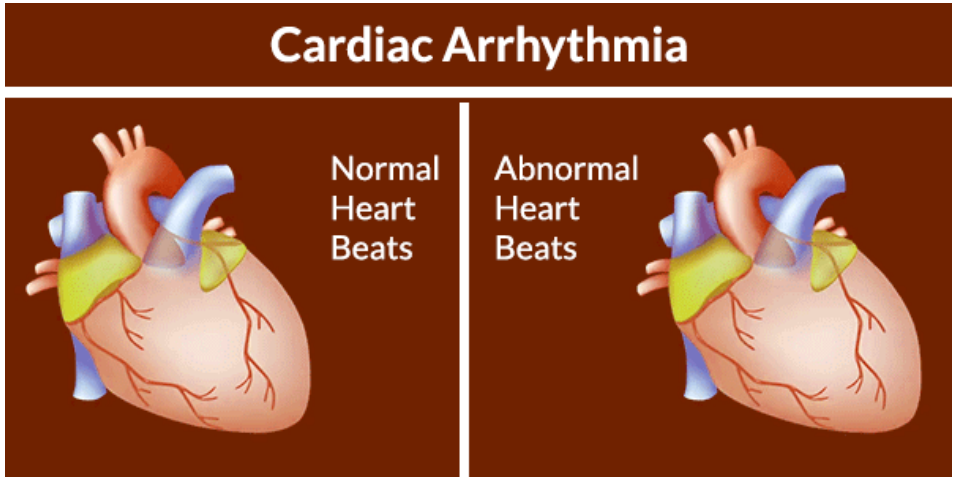
Dr. Phuong T Nguyen

Aiyana Emigh

Clancy, C.E. et al. Ann Biomed Eng. 2016

Why it matters: Cardiac Arrthythmias

Irregular, too slow or too fast heart rhythm

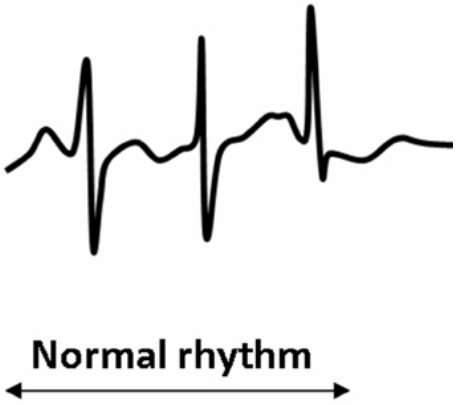


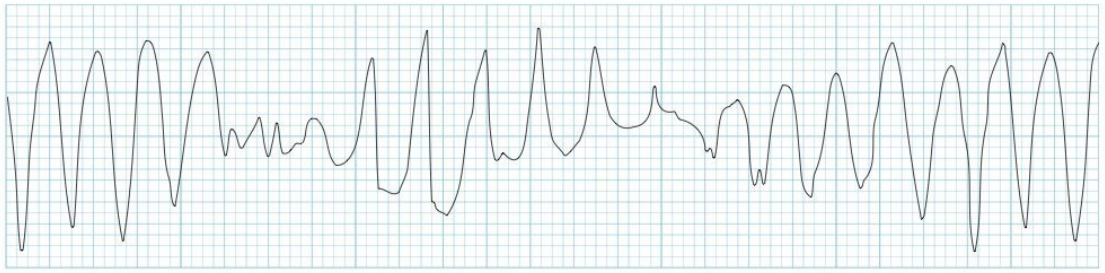
Affects millions of people worldwide.
~4 millions in USA (CDC)

A leading cause of sudden cardiac death. ~50% of cardiovascular or ~15% of ALL deaths globally.
~350K death in US per year (CDC)

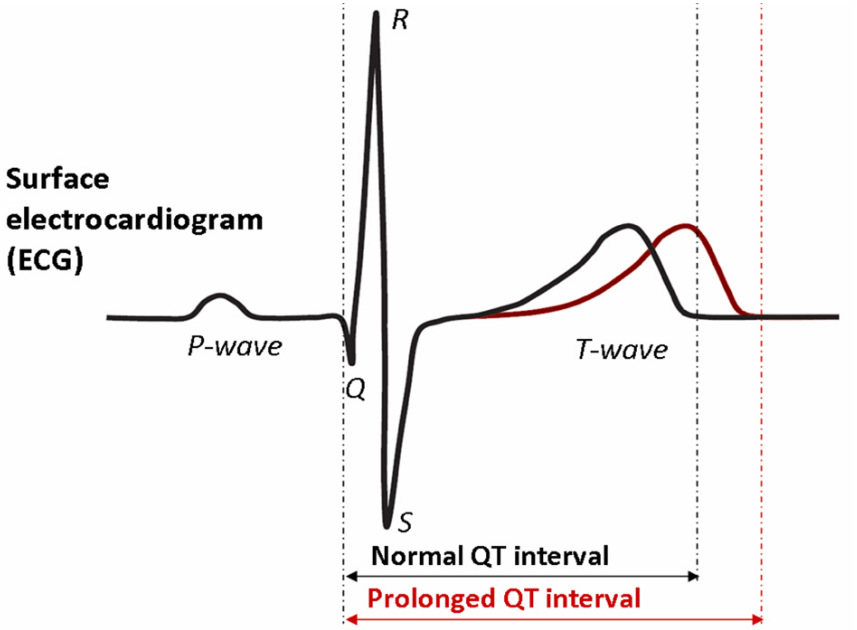
Arrhythmias can be detected at ECG

Torsades de Pointes (TdP) – a specific form of a Polymorphic ventricular tachycardia, often results from a long QT syndrome (LQTS)





Why it matters: Long QT syndrome (LQTS)



LQTS is one most prominent pro-arrhythmia markers

LQTS can be congenital (genetic mutations) or acquired (e.g. as a medicine side effect).

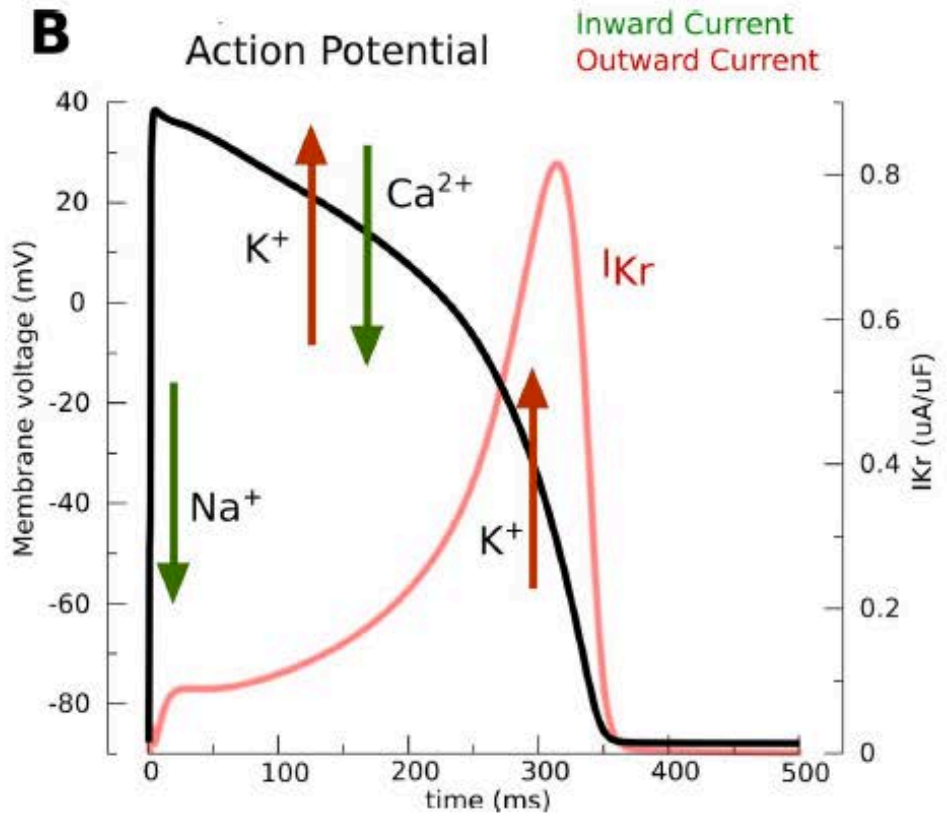
There are multiple subtypes of LQTS: 1, 2 or 3 are most common.

LQTS subtype	Culprit gene	Protein	Functional effect of mutation	Frequency of cases (%)
LQT1	*KCNQ1*[50]	Alpha-subunit of I_{Ks}	Loss-of-function, reduced I_{Ks}	30–35
LQT2	*KCNH2*[51]	Alpha-subunit of I_{Kr}	Loss-of-function, reduced I_{Kr}	25–30
LQT3	*SCN5A*[52]	Alpha-subunit of I_{Na}	Gain-of-function, increased late I_{Na} inward current	5–10

I_{Ks}, I_{Kr} and I_{Na} are different currents (generated by movement of K^+ and Na^+ ions across cellular membranes via voltage gated ion channels in cardiomyocytes).

Why it matters: hERG channel – major drug anti-target

I_{Kr} is a major repolarzing current in cardiomyocytes



I_{Kr} is mediated by a Kv11.1, a voltage-gated potassium channel encoded by hERG: human Ether a-go-go-Related Gene.

hERG belongs to EAG family.

EAG: ether-a-go-go gene in fruit flies (William Kaplan, 1969)

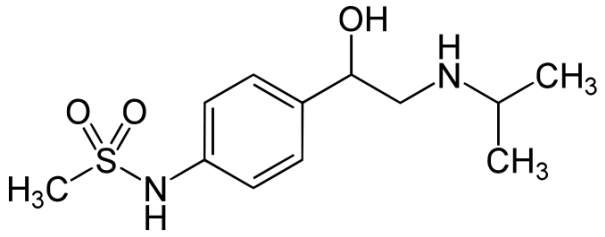
If I_{Kr} is reduced: repolarization is slowed, action potential is prolonged => LQTS

Wacker, Noskov, Perissinotti Cur. Top. Med. Chem. 2016.

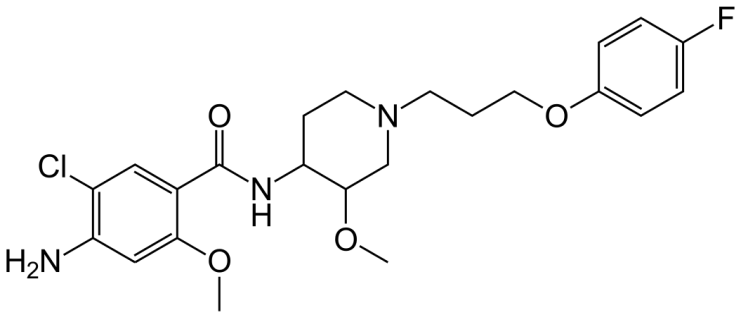
http://justlikecooking.blogspot.com/2012/02/herg-legs-drugs-and-heartbeats.html

Why it matters: hERG–drug interactions can lead to arrhythmia

Anti-arrhythmic drug d-Sotalol, hERG (I_{Kr}) blocker FAILED the Survival With ORal D-sotalol (SWORD) trial for patients surviving myocardial infarction in late 1990s: caused LQTS and TdP.



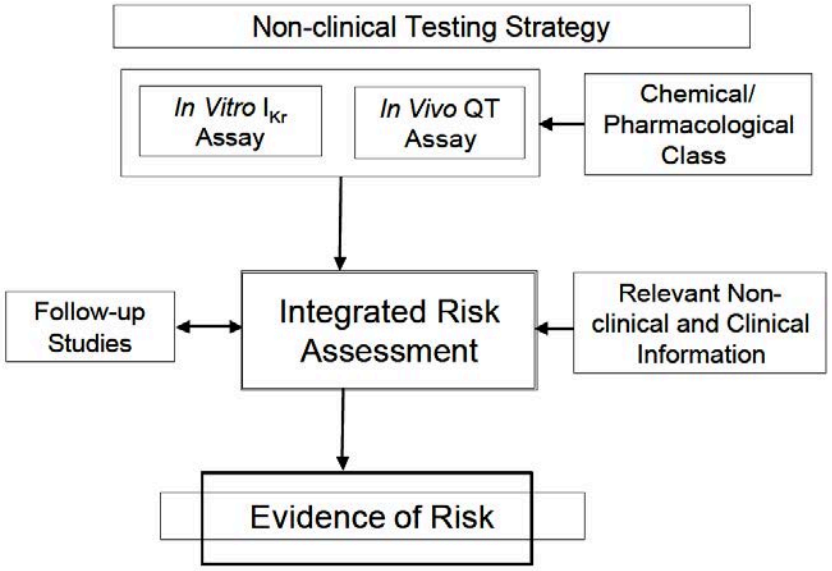
A gastroprokinetic agent cisapride was withdrawn in 2000 since it also caused LQTS and arrhythmias.



Up to 3% of all prescription drugs worldwide can cause arrhythmias

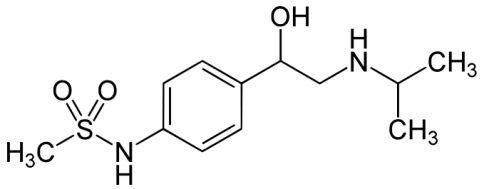
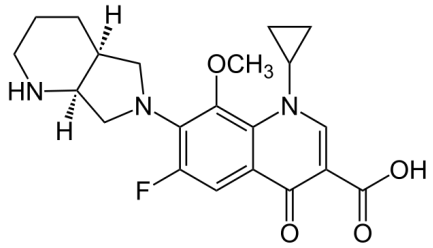
Now FDA mandates thorough QT studies for all newly developed drugs (since 2005).

Failed thorough QT studies is #1 reason for drug withdrawal from the market or pre-clinical testing.



https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM074963.pdf

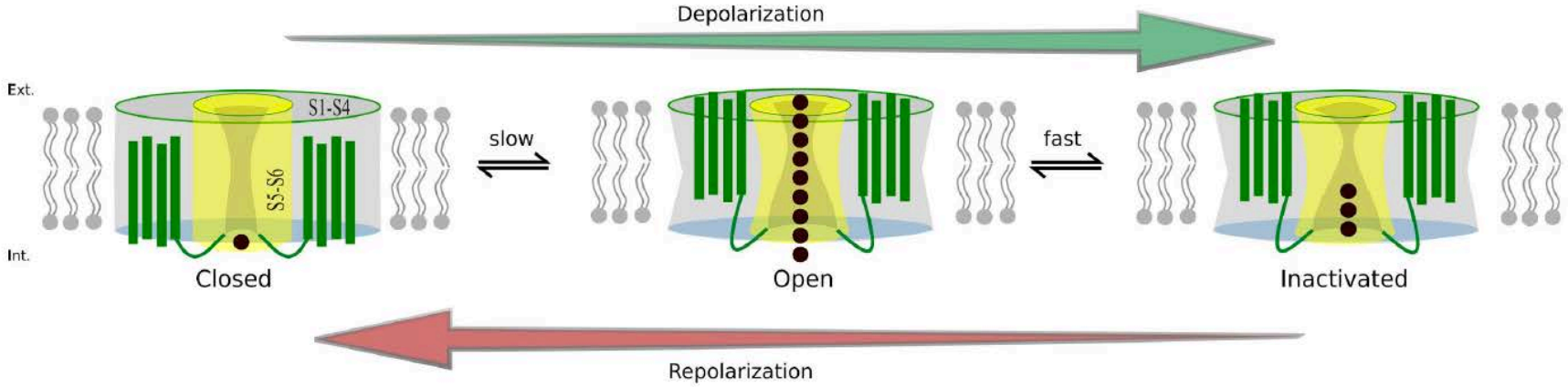
Key challenge: Not all hERG blockers are pro-arrhythmic.

	I_{kr} Block	QT prolongation	TdP arrhythmia
d-sotalol 	+	+	+
moxifloxacin 	+	+	−

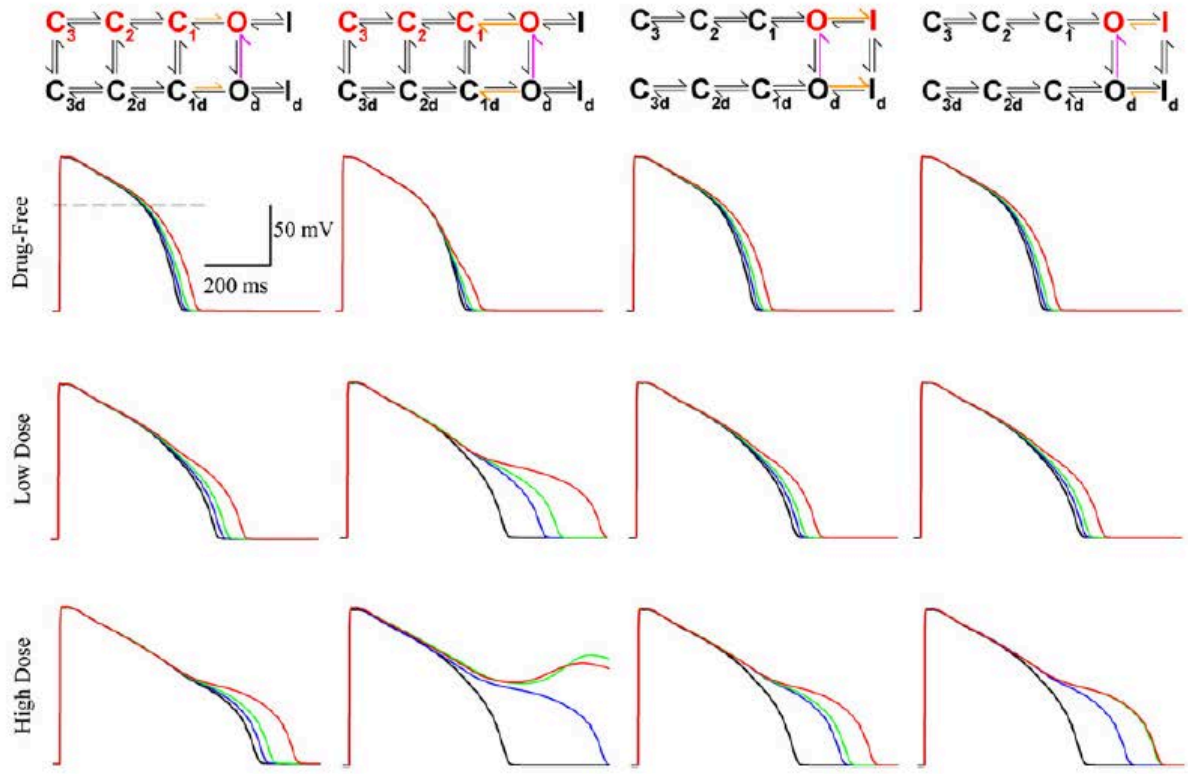
hERG block and QT prolongation are not selective criteria for pro-arrhythmia.

Ultimate goal: We need to develop a computational methodology, which can predict arrhythmogenesis from drug chemistry.

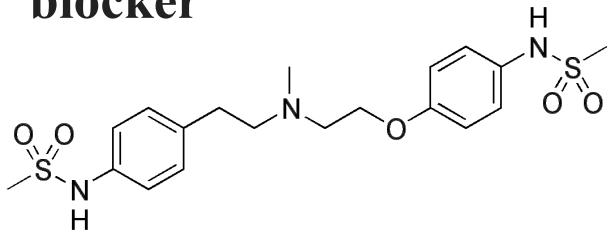
Key challenge: Different channel states



Wacker, Noskov, Perissinotti Cur. Top. Med. Chem. 2016.



Dofetilide: high-risk hERG blocker



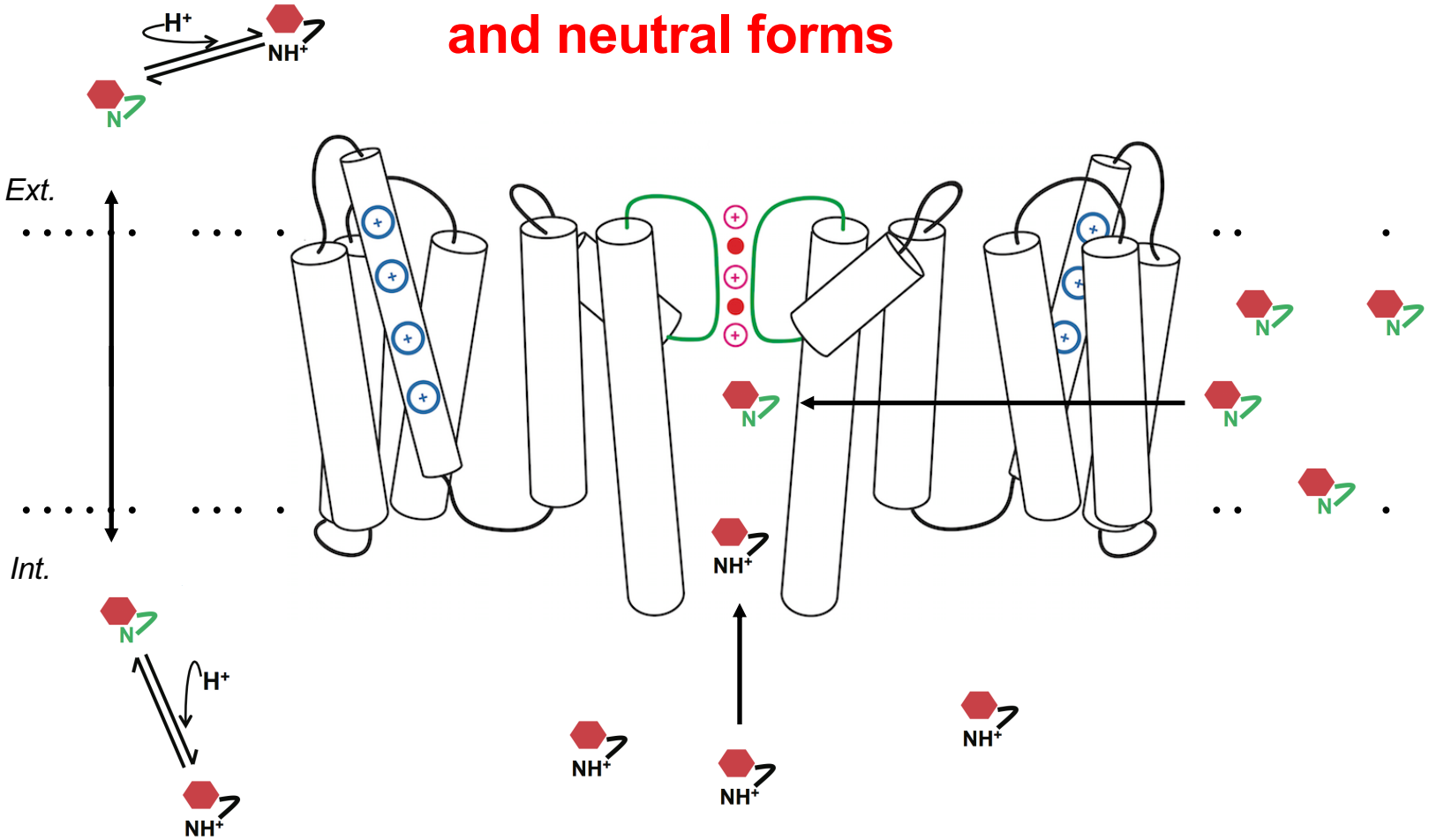
Based on kinetic models differential drug binding to hERG states can lead to arrhythmogenicity.

Romero et al J Mol Cell Cardiology. 2015.

Key challenge: different drug states & hERG–drug interactions

Many cardiovascular drugs: pK_a 7.8-8.5

Need to consider both ionized and neutral forms

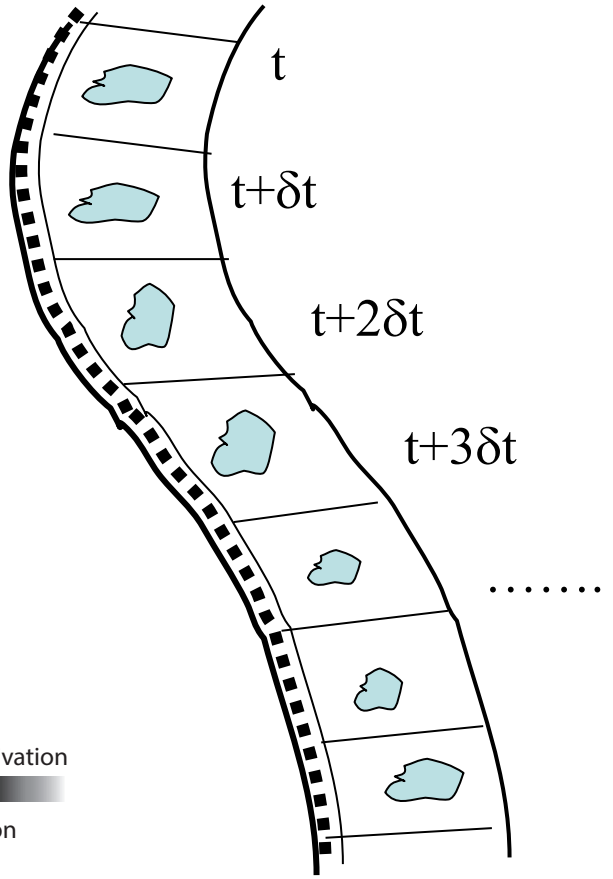


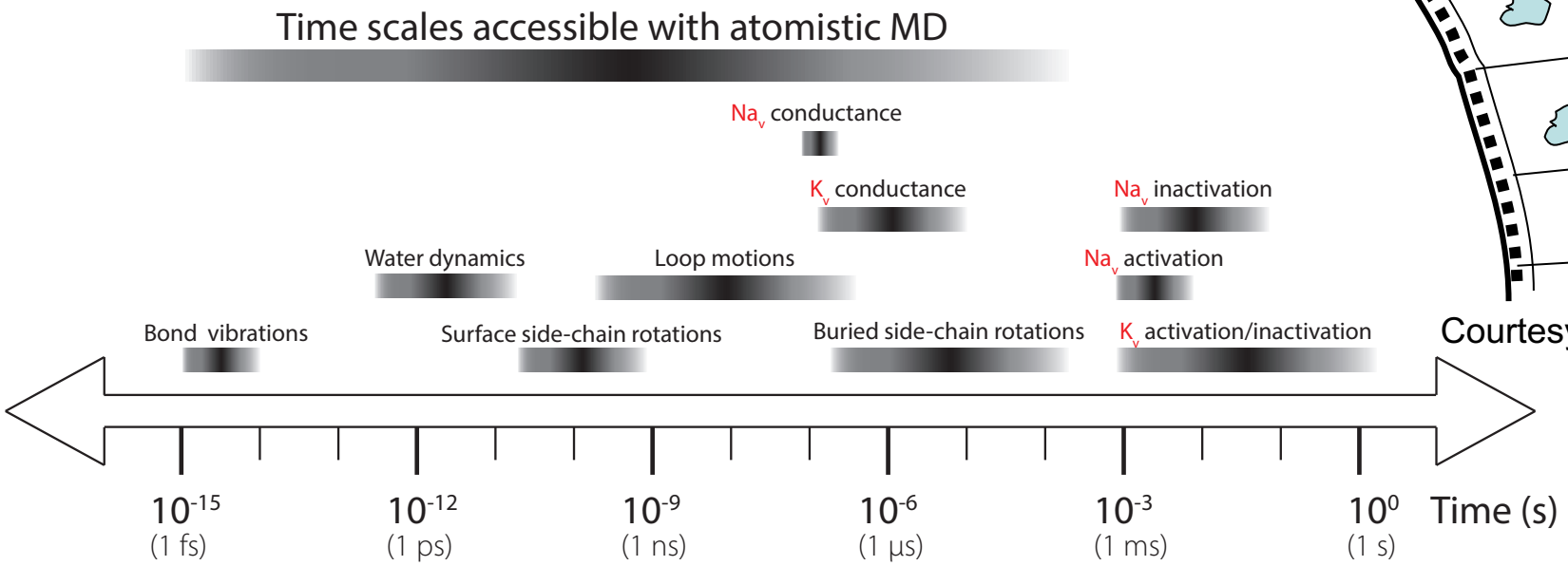
Key challenge: Molecular Dynamics (MD) time scales

- Generate step-by-step trajectory by numeric integration of Newton's equation of motion.

$$\mathbf{F} = m\mathbf{a} \quad F_x = -\,\mathrm{d}U / \mathrm{d}x$$

  - U is a potential energy function (empirical force field)
  - Typical time step $\delta t = 1 - 2$ fs (10^{-15} s)





Courtesy of Toby Allen

- Accessible times: ns – µs (10^{-9} –10^{-6} s) range, up to ~ 1 ms on specialized supercomputers.

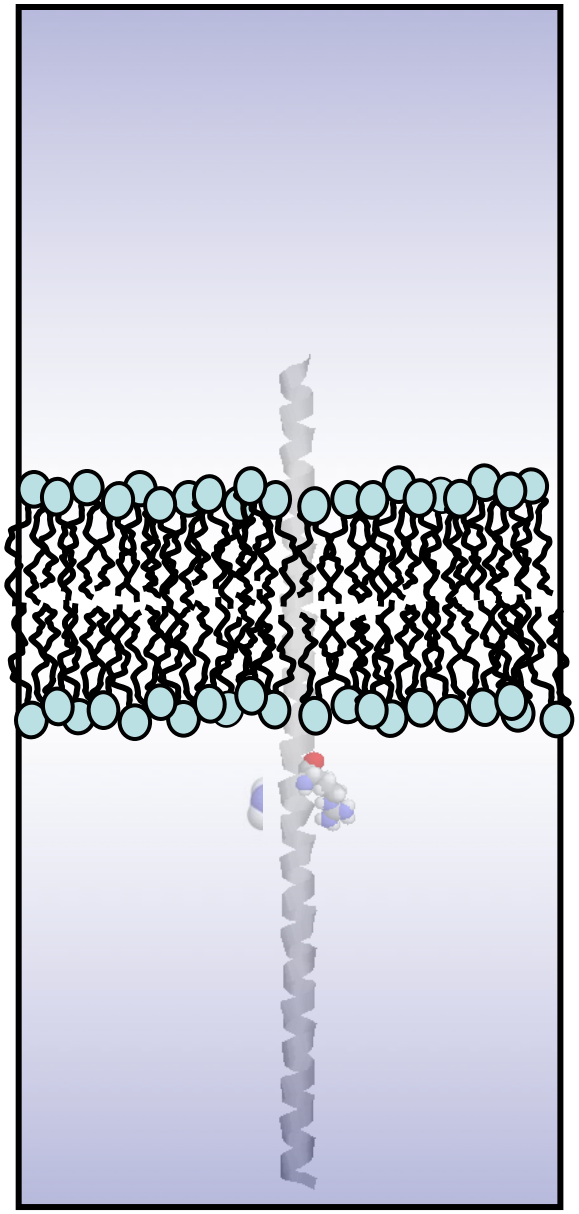
DeMarco et al. J Physiol vol. 597, no 3, p. 679, 2019

Key challenge: Compute free energy profile

Free energy or potential of mean force (PMF)

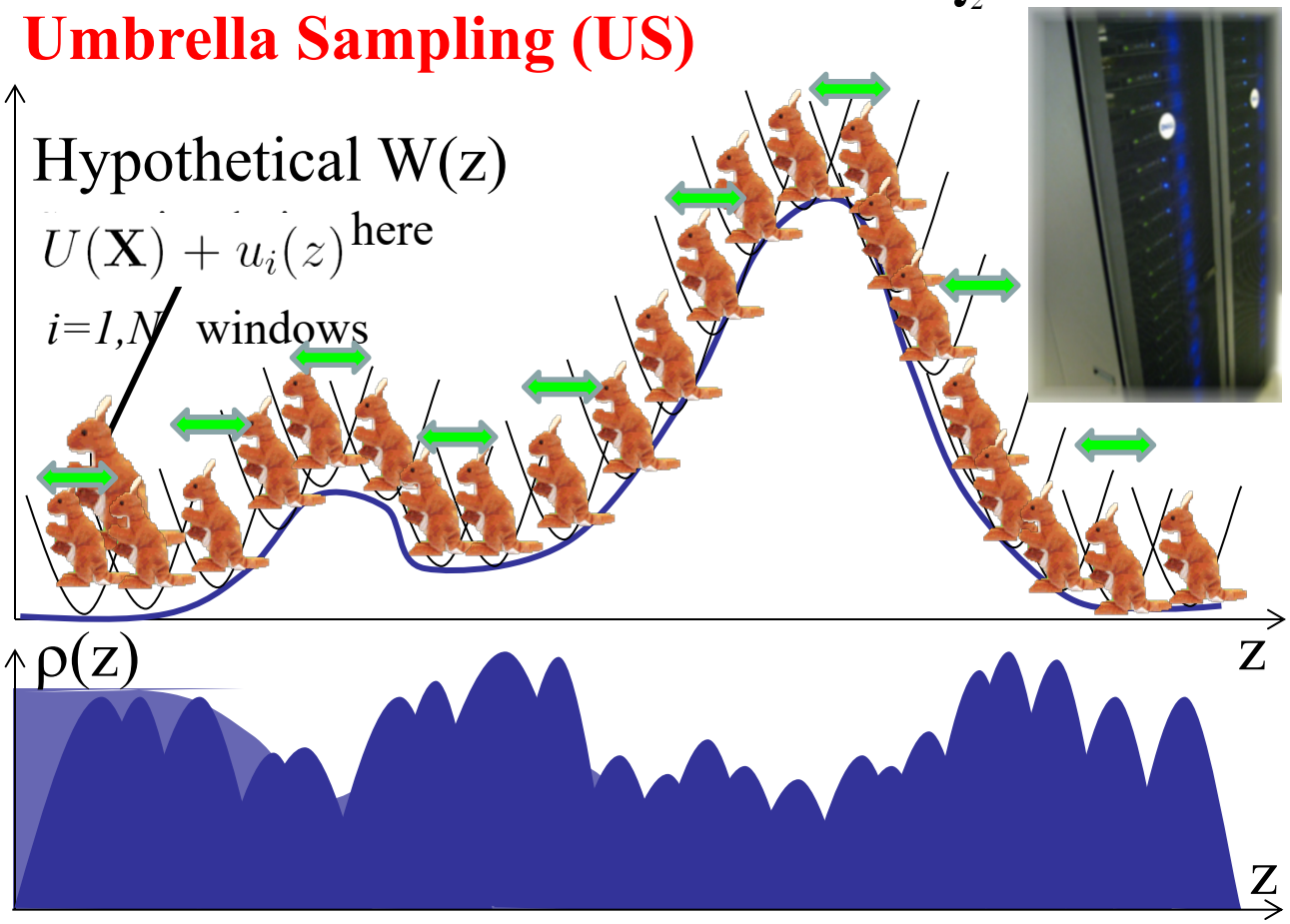
$$W(z) = -kT \ln \rho(z) + C$$

$$W(z) = -\int_{z'}^{z} d\zeta \langle F(\zeta) \rangle$$



Courtesy of Toby Allen

Umbrella Sampling (US)



Weighted histogram analysis method (WHAM) to get unbiased ρ(z) and compute W(z).

US Hamiltonian replica exchange – swap US windows using Metropolis criterion to accept.

Why Blue Waters?

Umbrella sampling (US) MD simulations: at least 80-90 individual simulations for different *z* positions of the drug.

US / Hamiltonian replica exchange (US/H-RE or REUX) MD simulations: need to be run all simultaneously. One or more runs per US window. Exchange rate increases for more runs.

System size: ~127,000 atoms or more for a typical ion channel + hydrated lipid membrane system.

Models: All-atom CHARMM force fields – CHARMM36 lipid and protein, general CHARMM force field (CGENFF) for drugs.

Simulation time: 10 ns equilibration + 30 ns production per US window or more, i.e. ~3,600 ns or more in total. For RE US 10 ns production per window was sufficient.

Performance: NAMD CUDA enabled, Blue Waters optimized, ~5 ns / day on SK nodes.

Bundled submission: one submission script for all or selected US windows.

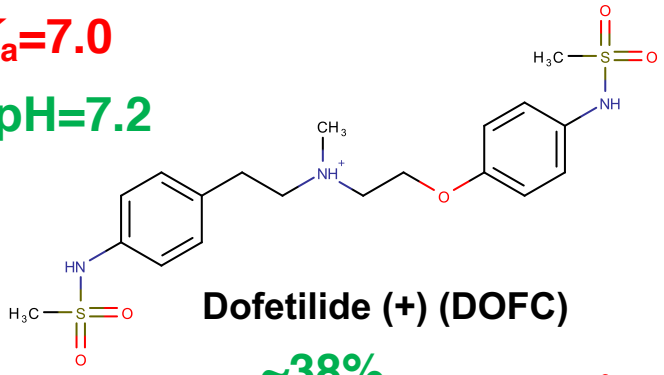
Rapid start: COMMTRANSPARENT option for US-MD (due to no inter-node exchanges)

For one system US MD can be run in about a week, faster for REUX MD.
~16K Blue Waters node hours for one US MD simulation.

Results: Dofetilide membrane partitioning

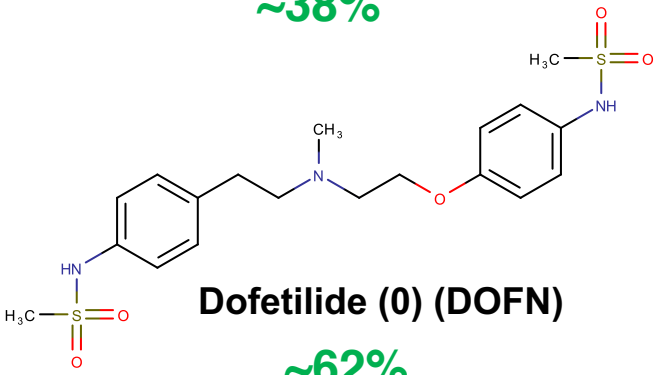
pK_a=7.0

At pH=7.2



Dofetilide (+) (DOFC)

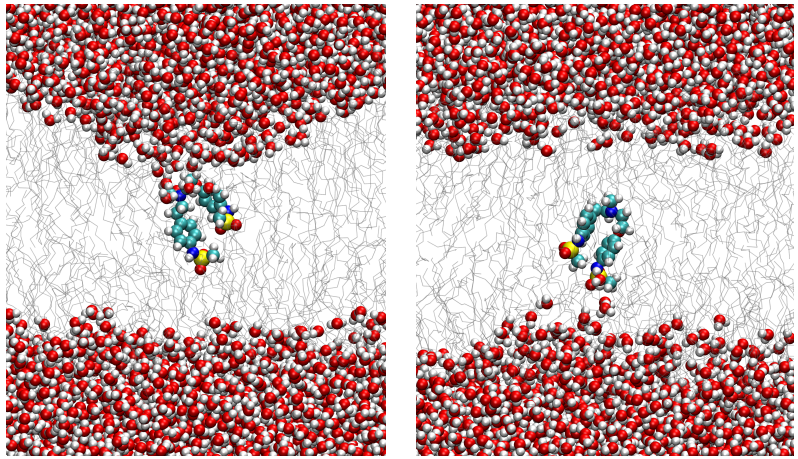
~38%



Dofetilide (0) (DOFN)

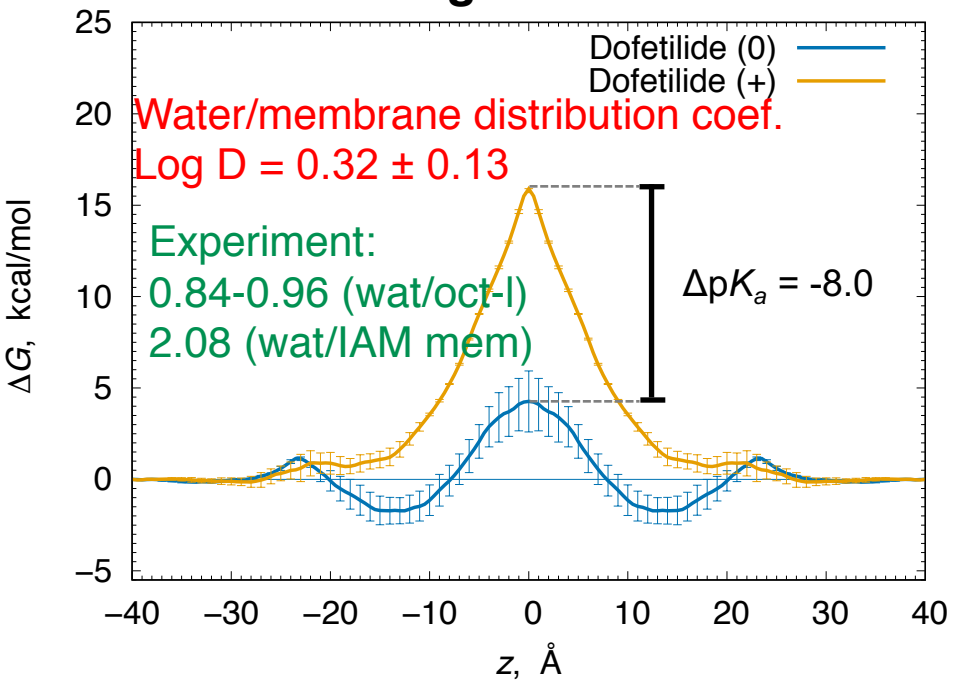
~62%

In the membrane center:

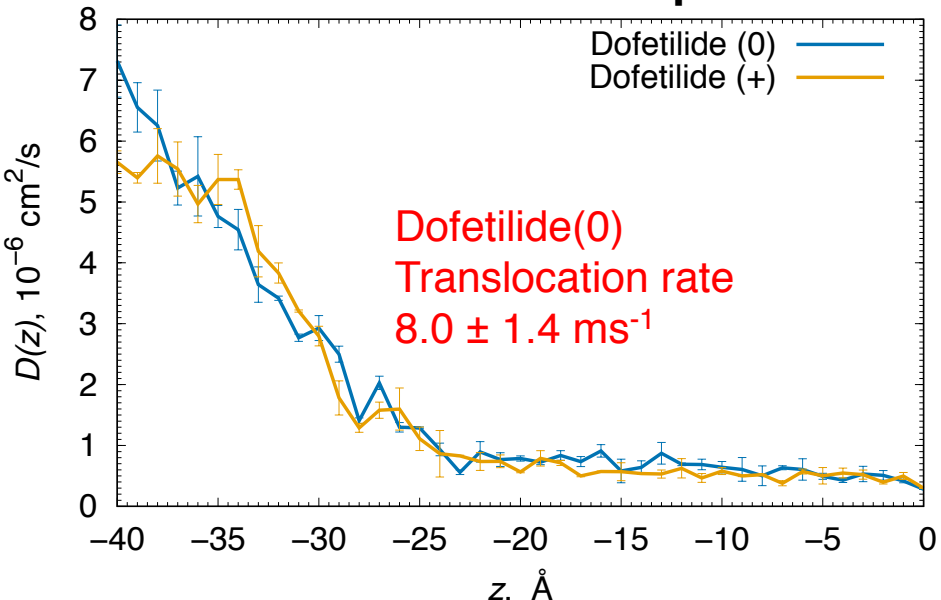


Dofetilide (+) (DOFC) Dofetilide (0) (DOFN)

Free energies or PMF



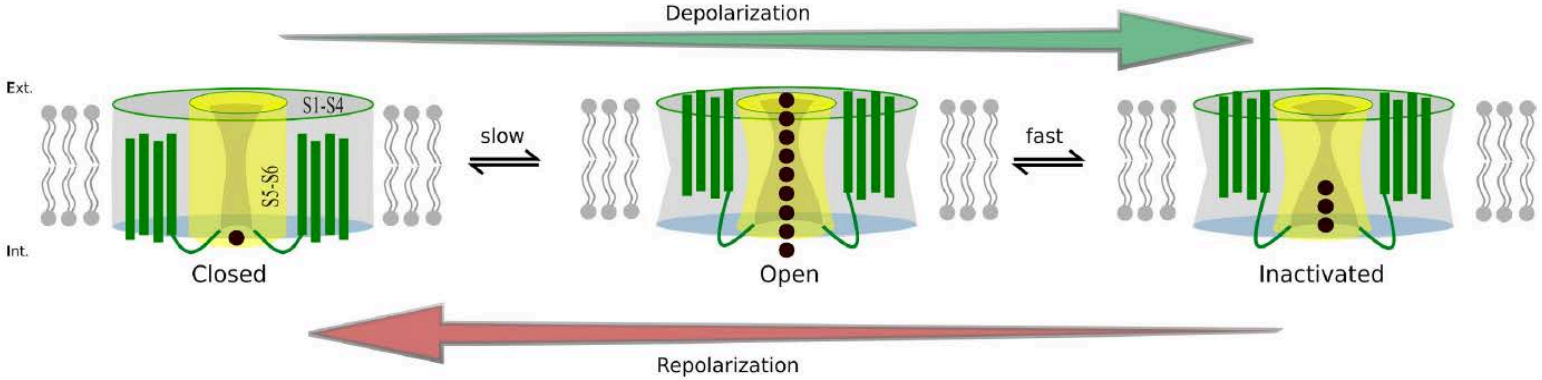
Diffusion coefficient profiles



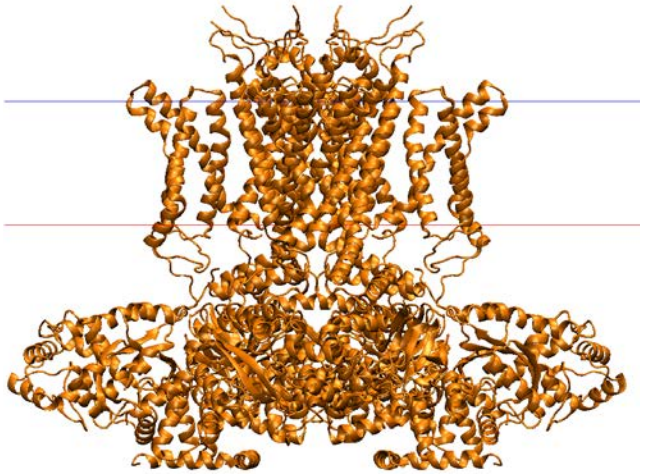
DeMarco et al. bioRxiv, p. 635441, 2019

Results: Structural models of hERG

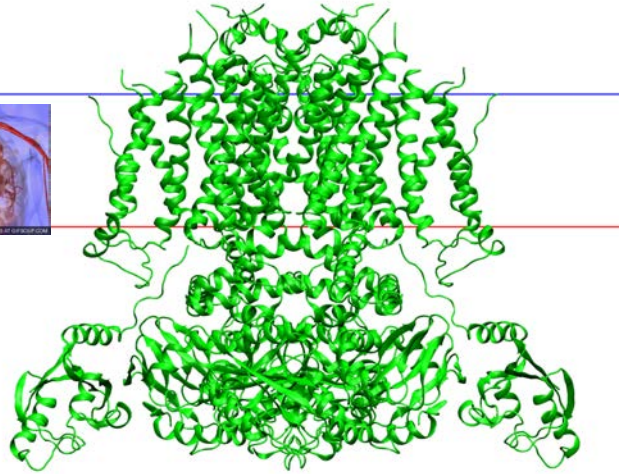
3 conformational states of hERG (I_{Kr}):



New eukaryotic ion channel structures (cryo-EM):



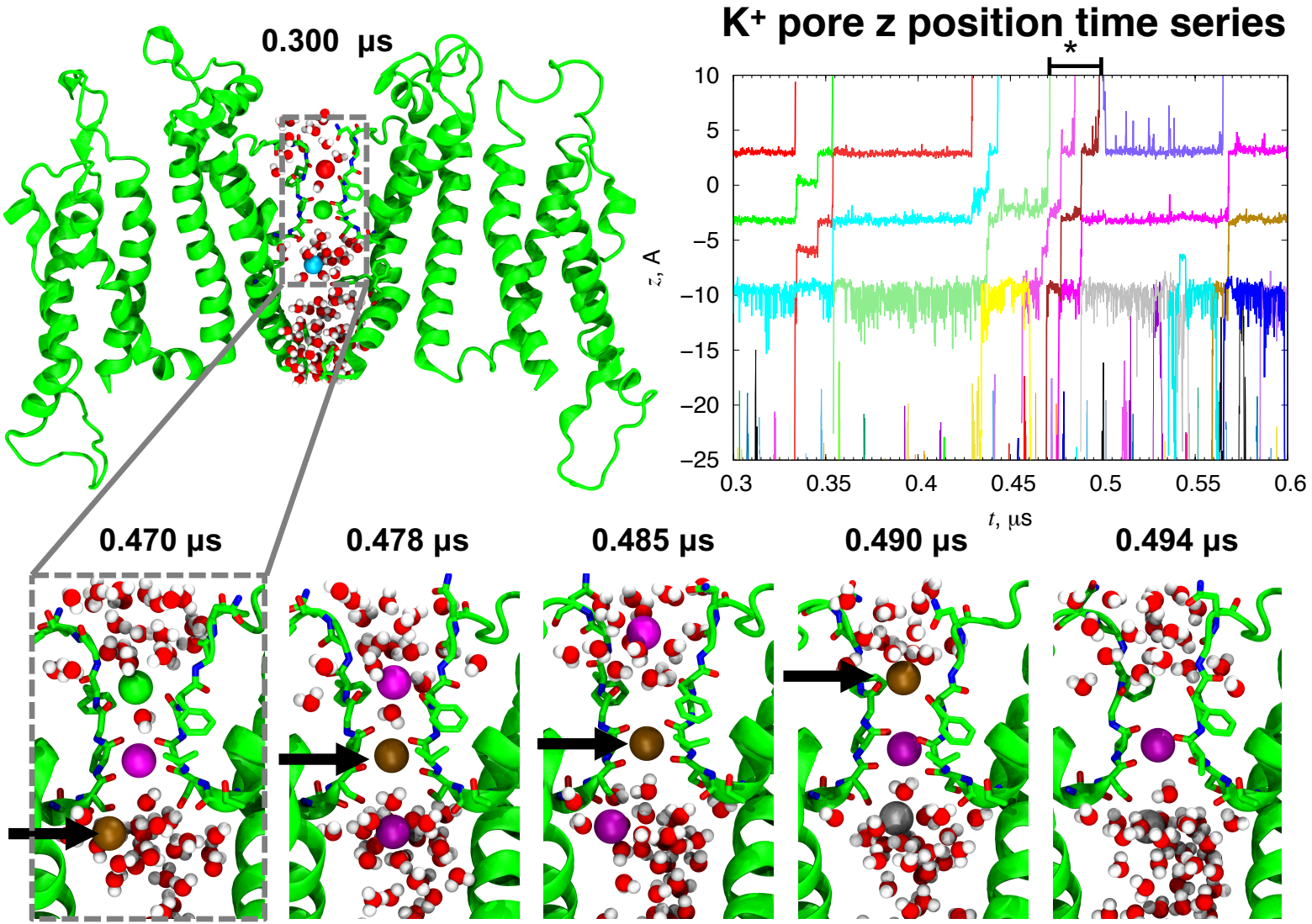
rEAG1 (rabbit K_v10.1) – closed state
~38% seq. identity to hERG
PDB: 5K7L (3.8 Å)
R. MacKinnon, August 2016



hERG (human K_v11.1, I_{Kr}) – open state (?)
PDB: 5VA2 (3.7 Å)
R. MacKinnon, May 2017

Results: Open hERG ion conduction

using published structure (PDB ID: 5VA2) and +750 mV applied voltage.

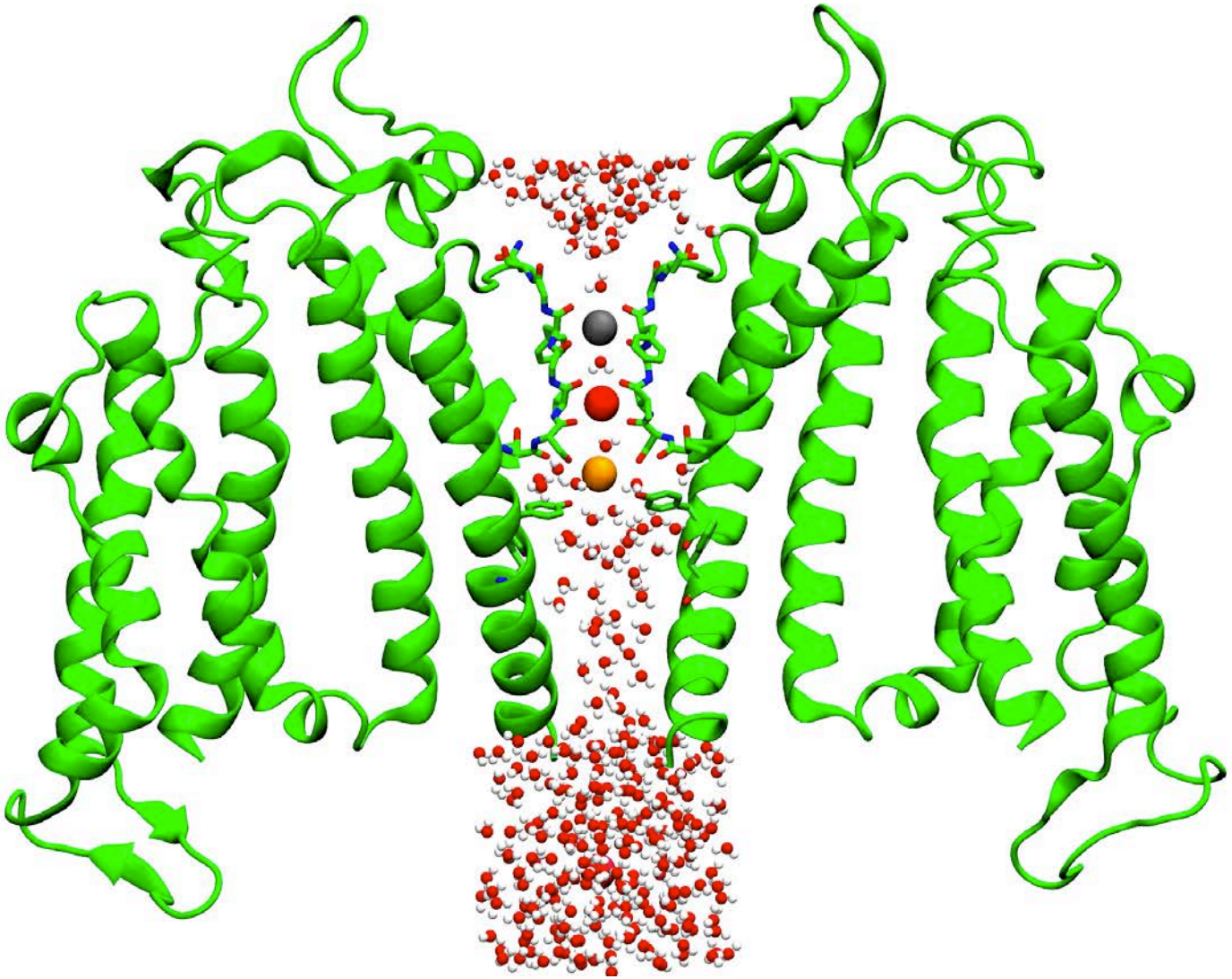


7 K^+ conduction events during 300 ns were observed.

DeMarco et al. bioRxiv, p. 635441, 2019

Results: Open hERG ion conduction

Open hERG K^+ conduction ~1,500 ns MD simulation with +750 mV applied electric field.

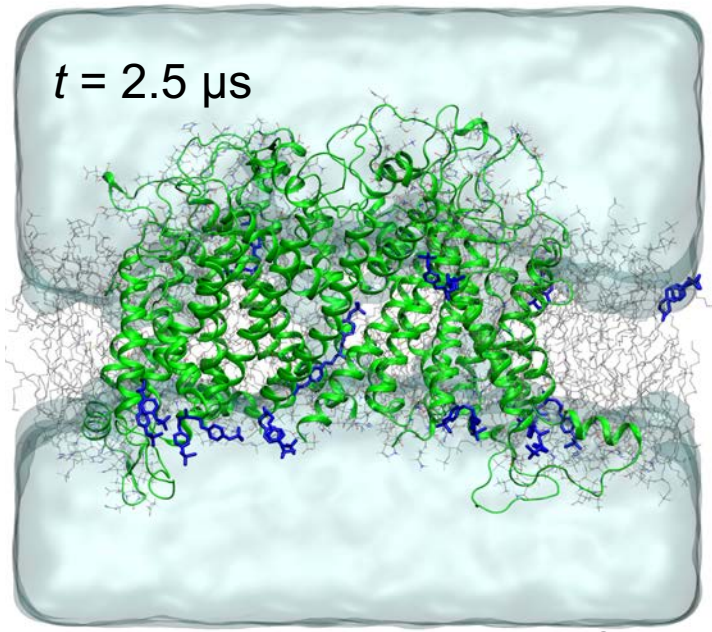


DeMarco et al. bioRxiv, p. 635441, 2019

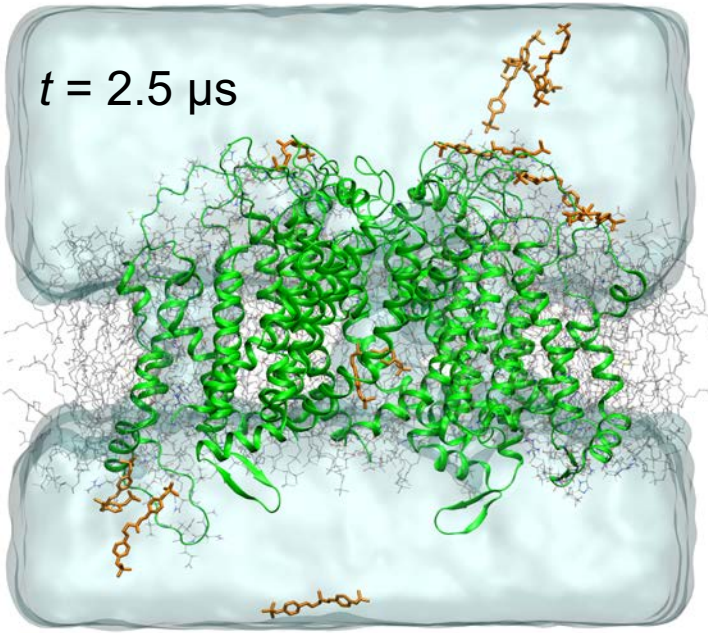
Results: Open hERG – dofetilide "flooding" MD

"Flooding" MD simulations of WT hERG channel embedded in a POPC bilayer and soaked by 0.15 M aqueous KCl solution with 0.025 M of drug (20 molecules)

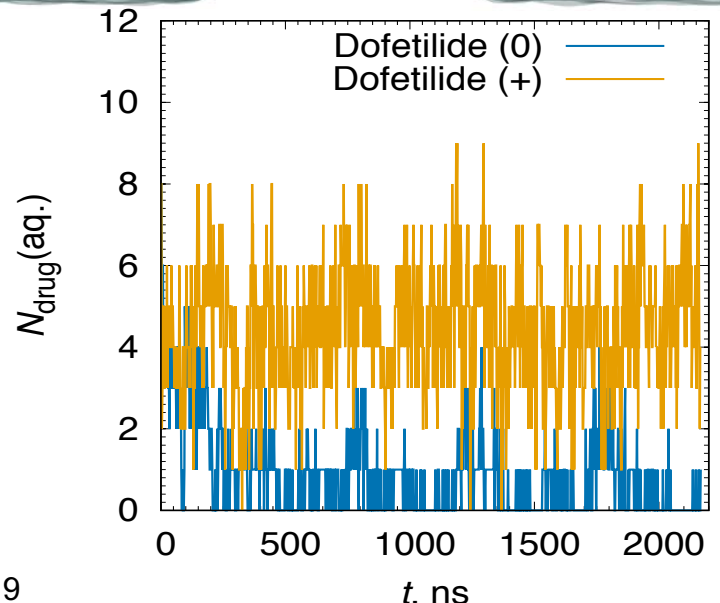
Dofetilide (0)



Dofetilide (+)



Most dofetilide(0) molecules end up in the membrane or protein bound

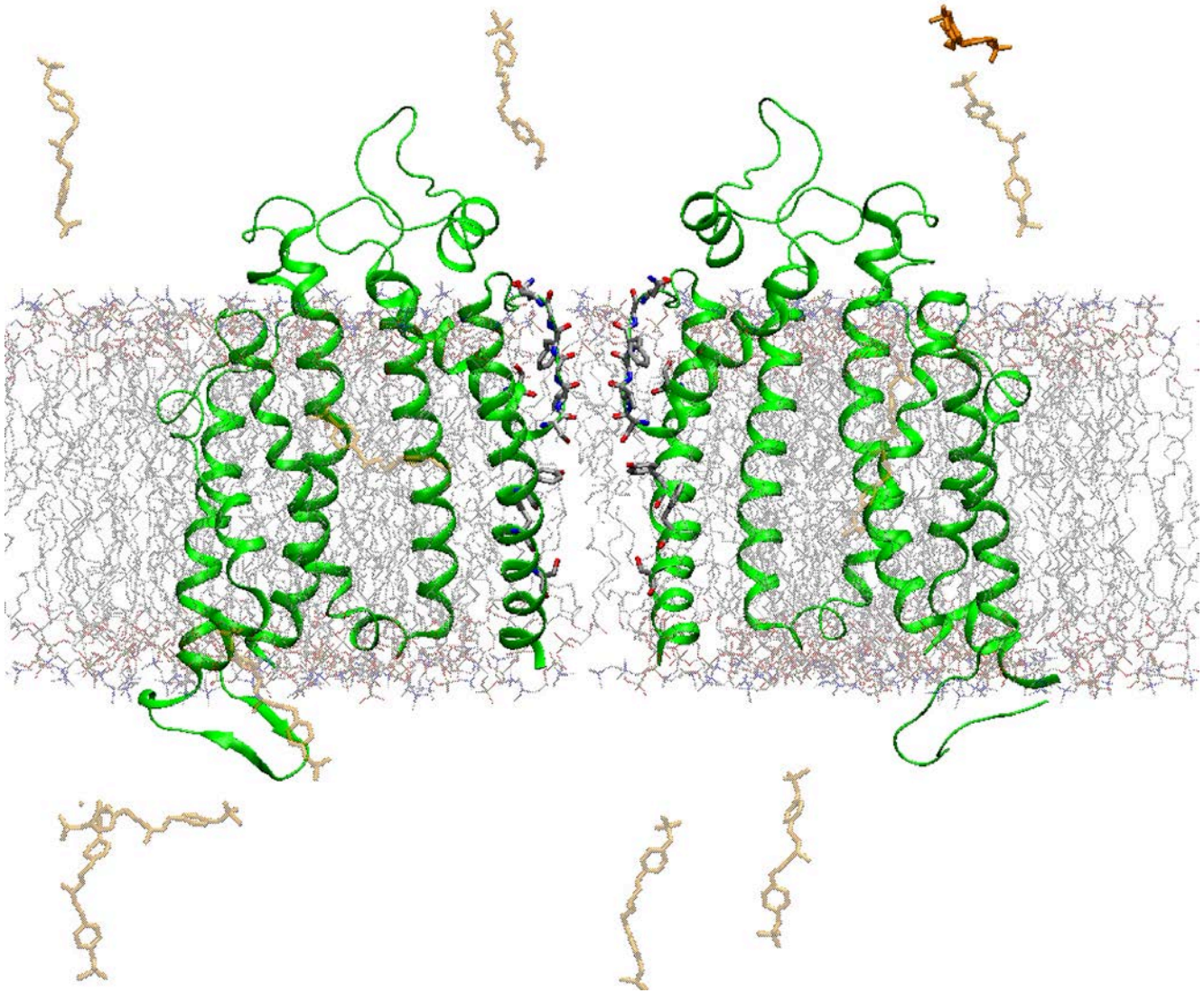


Most dofetilide(+) molecules remain in bulk water or transiently interact with protein

DeMarco et al. bioRxiv, p. 635441, 2019

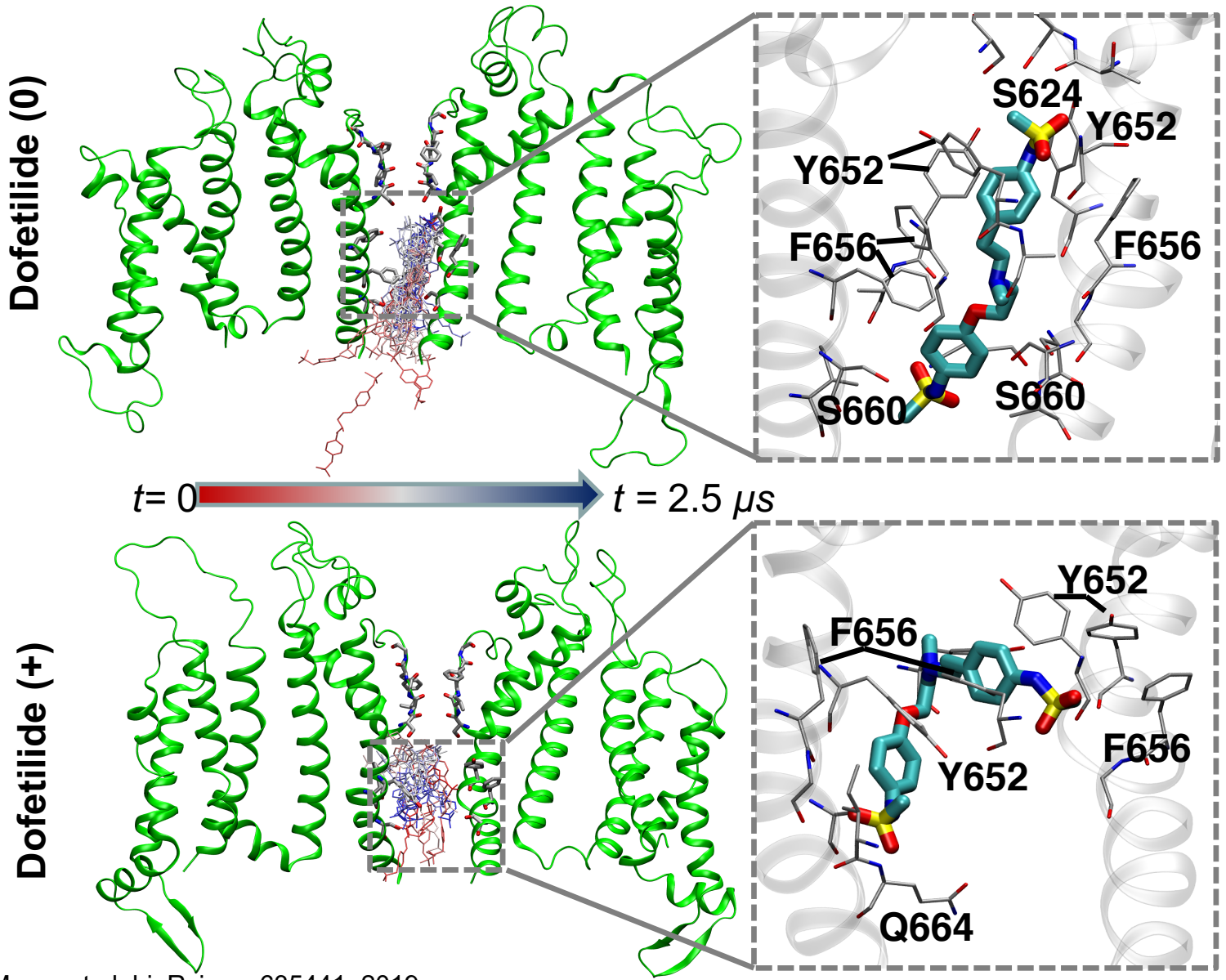
Results: Open hERG – dofetilide enhanced sampling MD

Open hERG – dofetilide(0) “flooding” MD simulation (90 ns out of 2,5000 ns)



Resuts: Open hERG – dofetilide binding from “flooding” MD

For both drug forms one dofetilide molecule moves into channel pore and stays there for the rest of 2.5 μs simulation.



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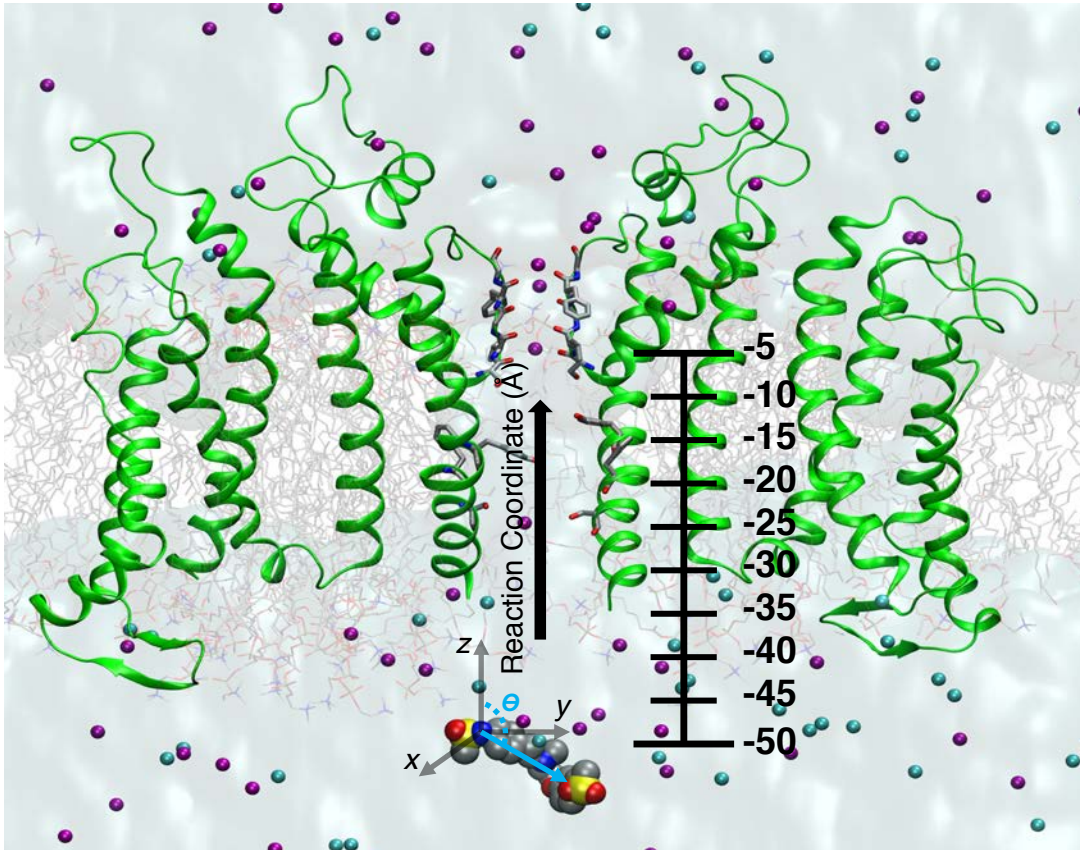
Results: Open hERG – dofetilide enhanced sampling MD

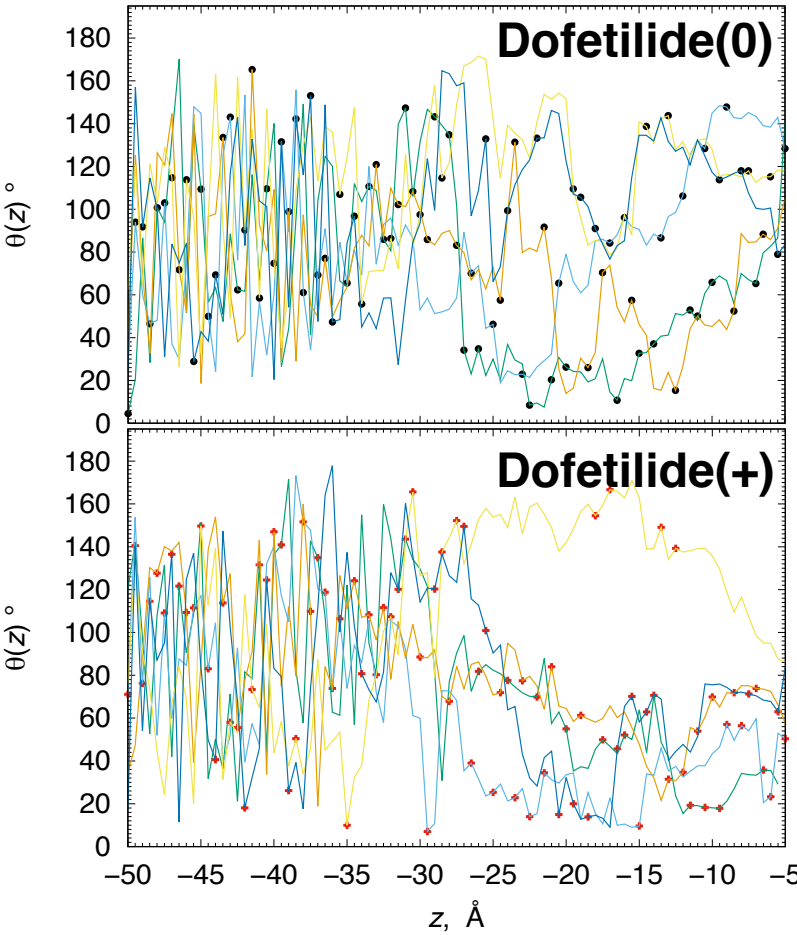
To obtain quantitative estimates, umbrella sampling MD simulations were used.

Multiple starting points from simulations of drug slowly pulled into the pore to randomize initial drug orientations.

Simulation system for pulling/umbrella sampling:



Drug orientations:



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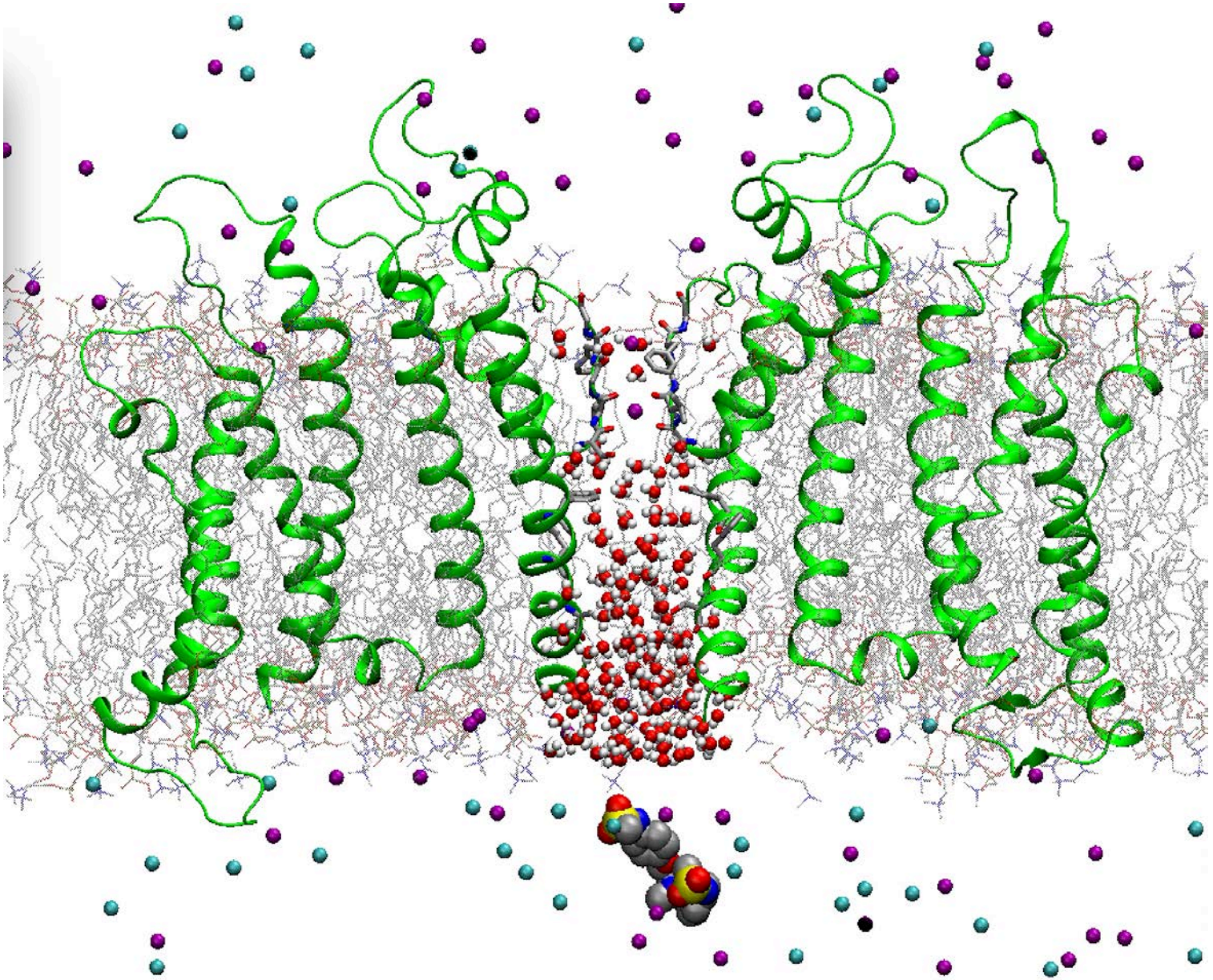
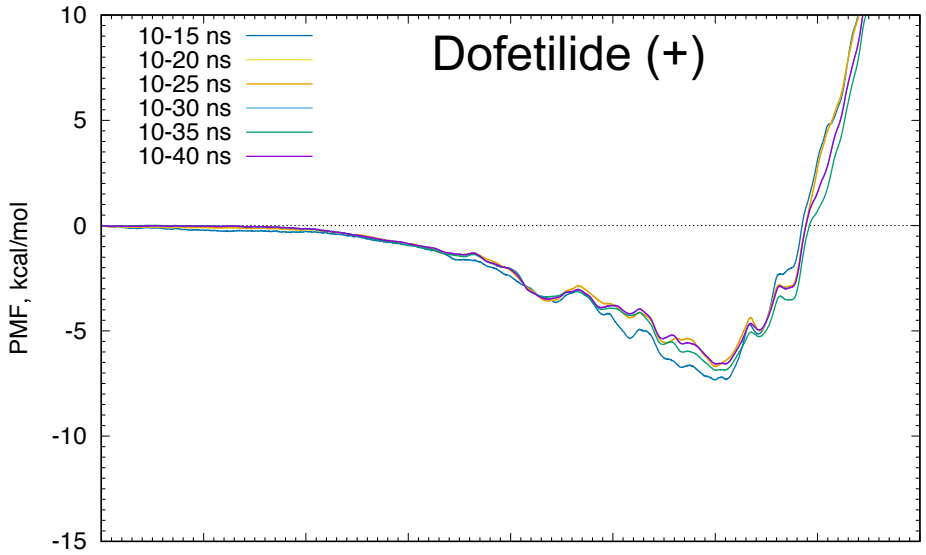
Results: Open hERG – dofetilide enhanced sampling MD

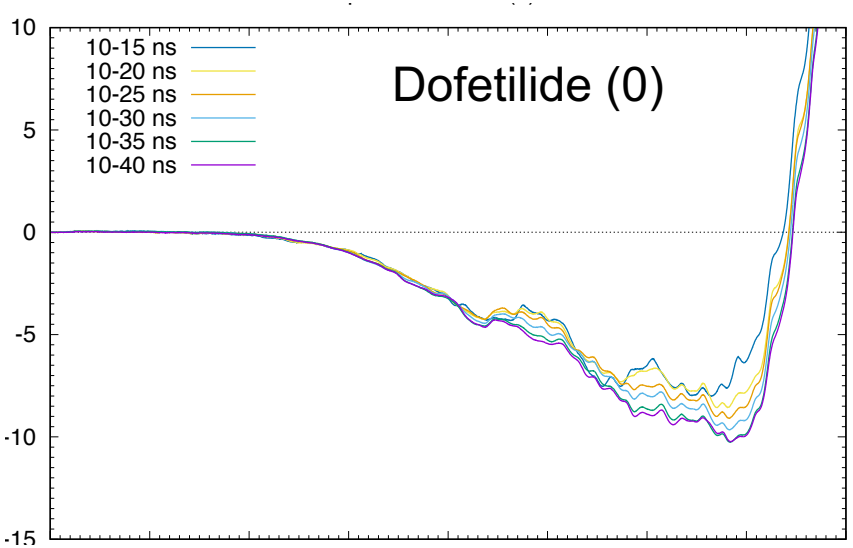
Open hERG – dofetilide(0) 90 ns pulling (steered MD) simulation



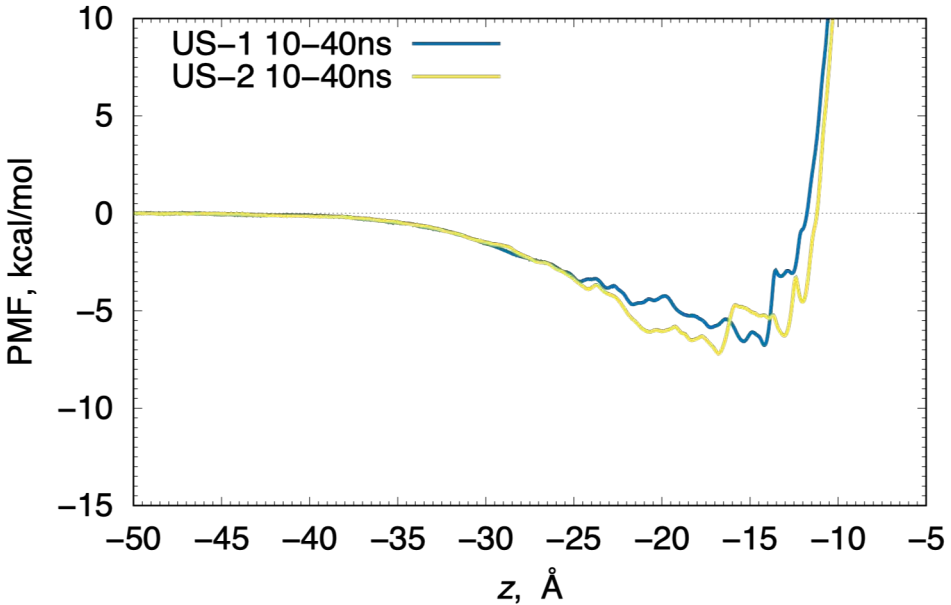
Results: Open hERG – dofetilide US MD convergence

Block averaging:

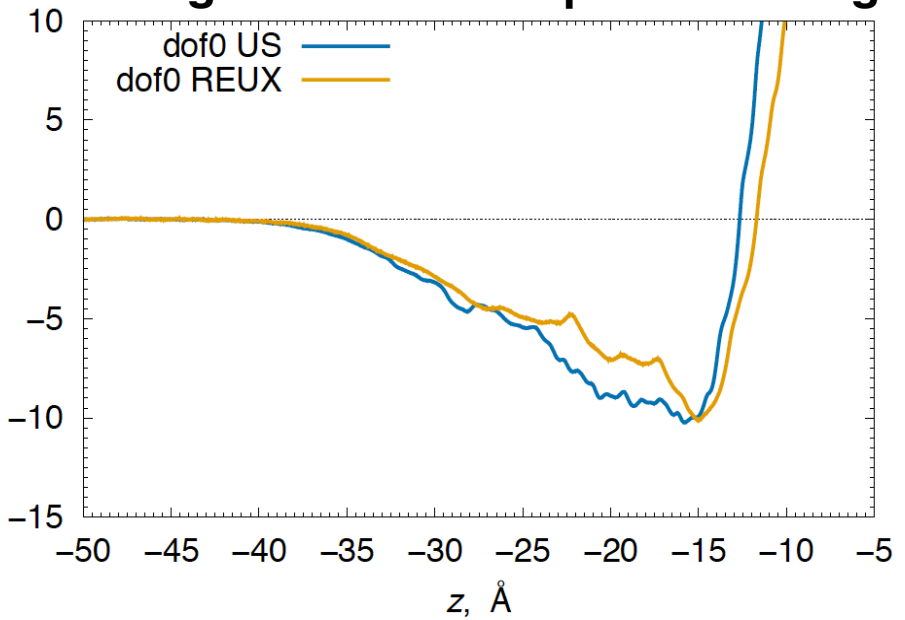




For dofetilide(0): 2 separate simulations

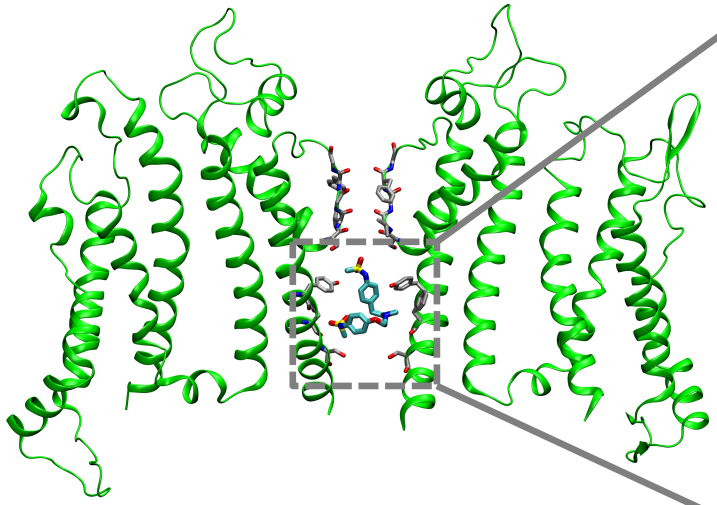


or using Hamiltonian replica exchange

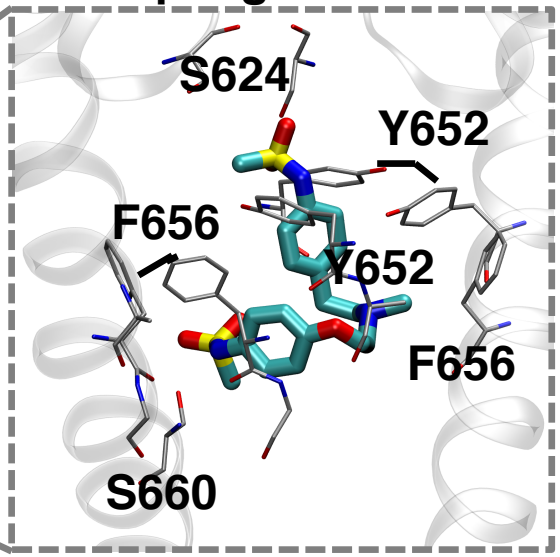


Results: Open hERG – dofetilide binding poses from MD

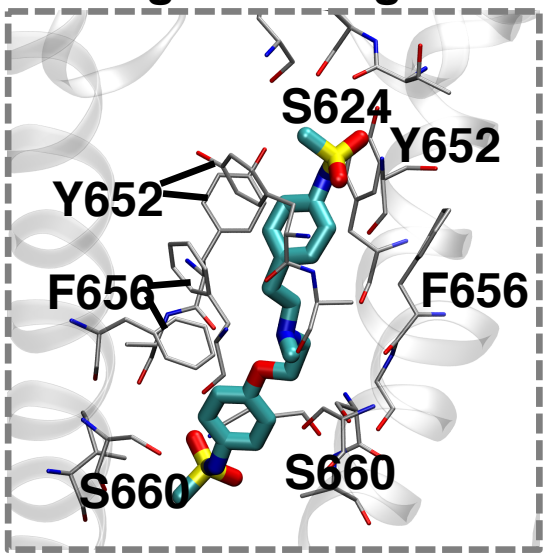
hERG + Dofetilide (0)



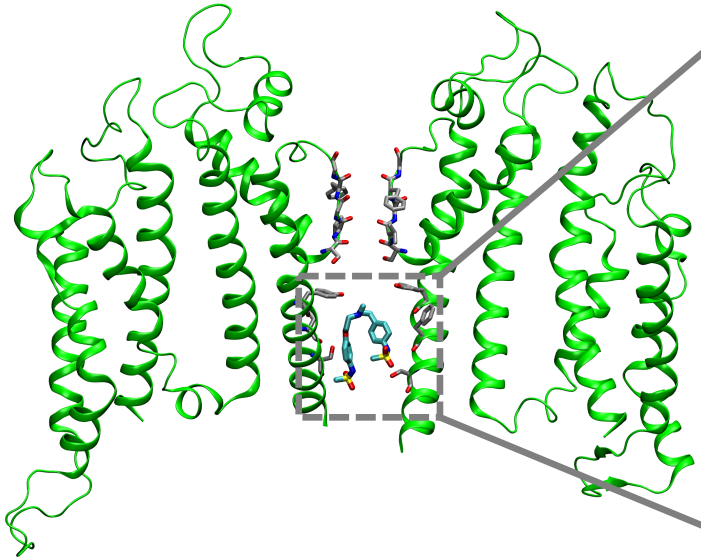
Umbrella sampling



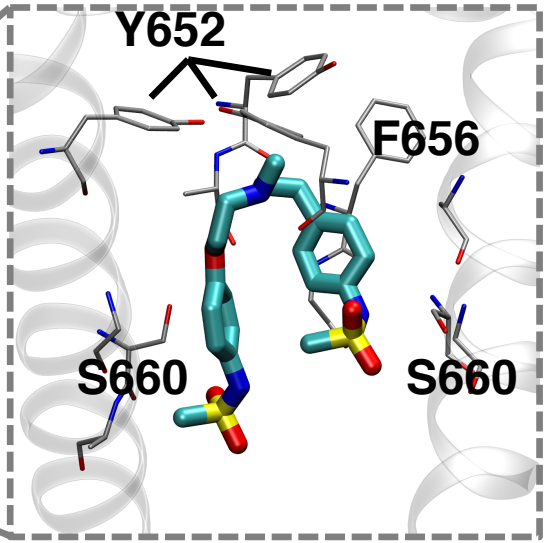
Drug "flooding"



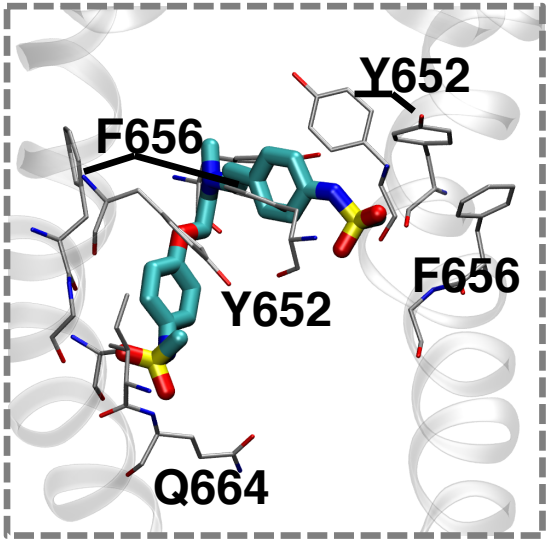
hERG + Dofetilide (+)



Umbrella sampling



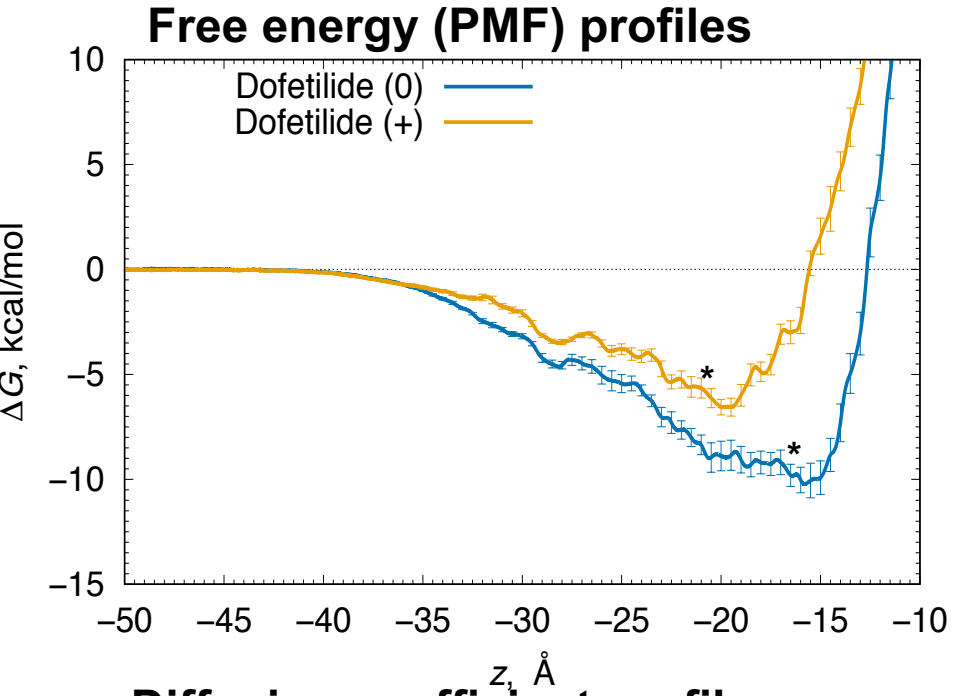
Drug "flooding"

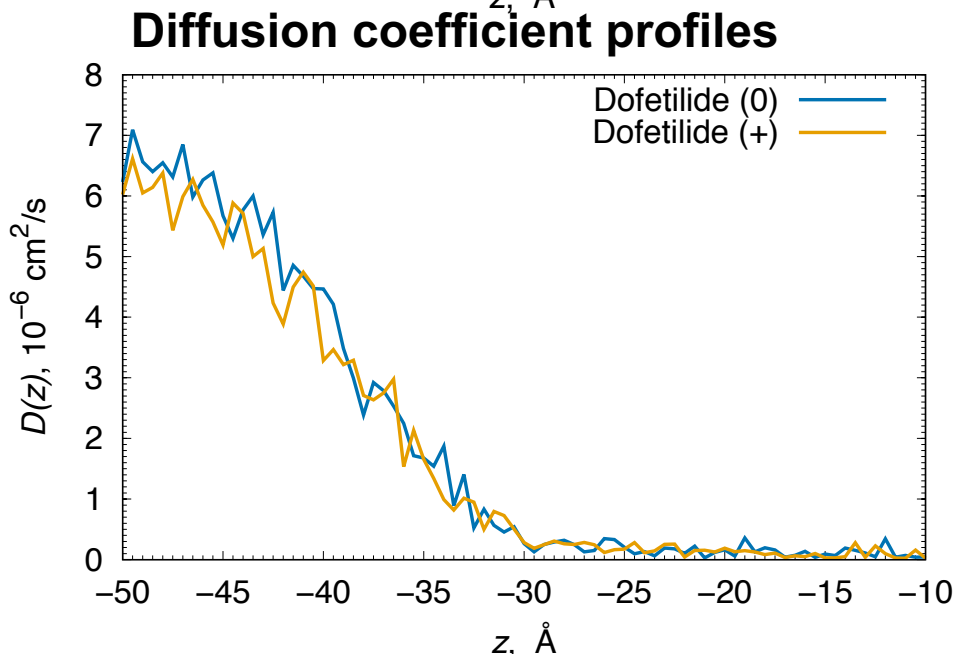


Similar binding sites from unbiased and US MD.

DeMarco et al. bioRxiv, p. 635441, 2019

Accomplishment: Open hERG–dofetilide energetics & kinetics





Drug dissociation coefficients:

Dofetilide (+) K_d = 65 μM

Dofetilide (0) K_d = 0.16 μM

Overall K_d = 25 μM

Experimental IC_{50} or K_d 3.5 - 11 μM

Good agreement with experimental IC_{50}.

Drug association and dissociation ("on" and "off") rates:

Dofetilide (+) k_{on} = 110 $\mu M^{-1}\ s^{-1}$;
k_{off} = $3.5 \times 10^4\ s^{-1}$

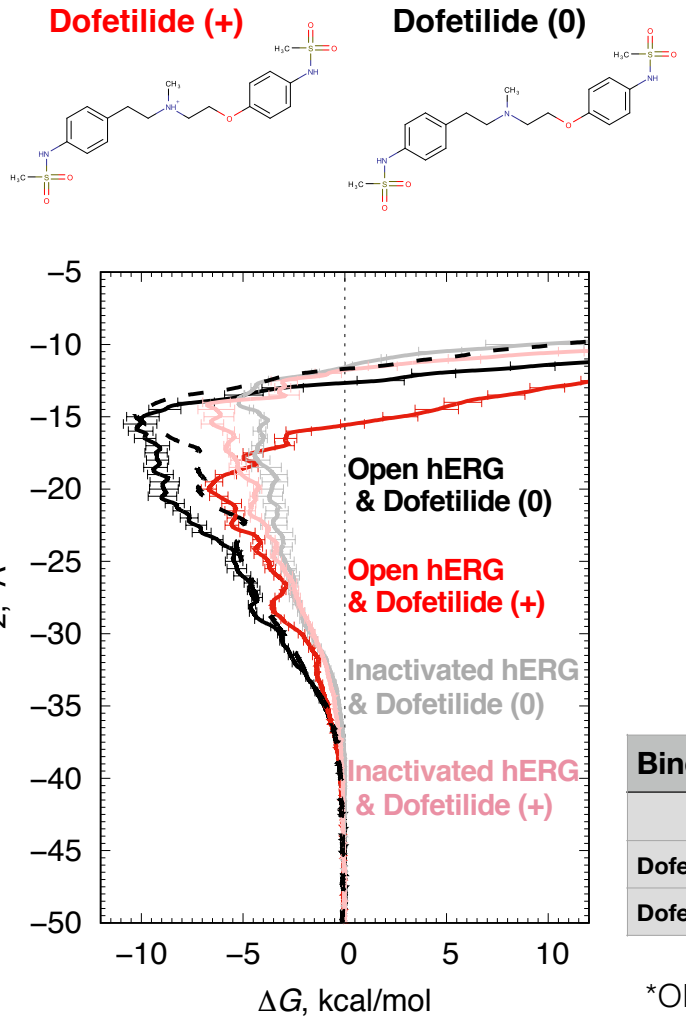
Dofetilide (0) k_{on} = 670 $\mu M^{-1}\ s^{-1}$;
k_{off} = 110 s^{-1}

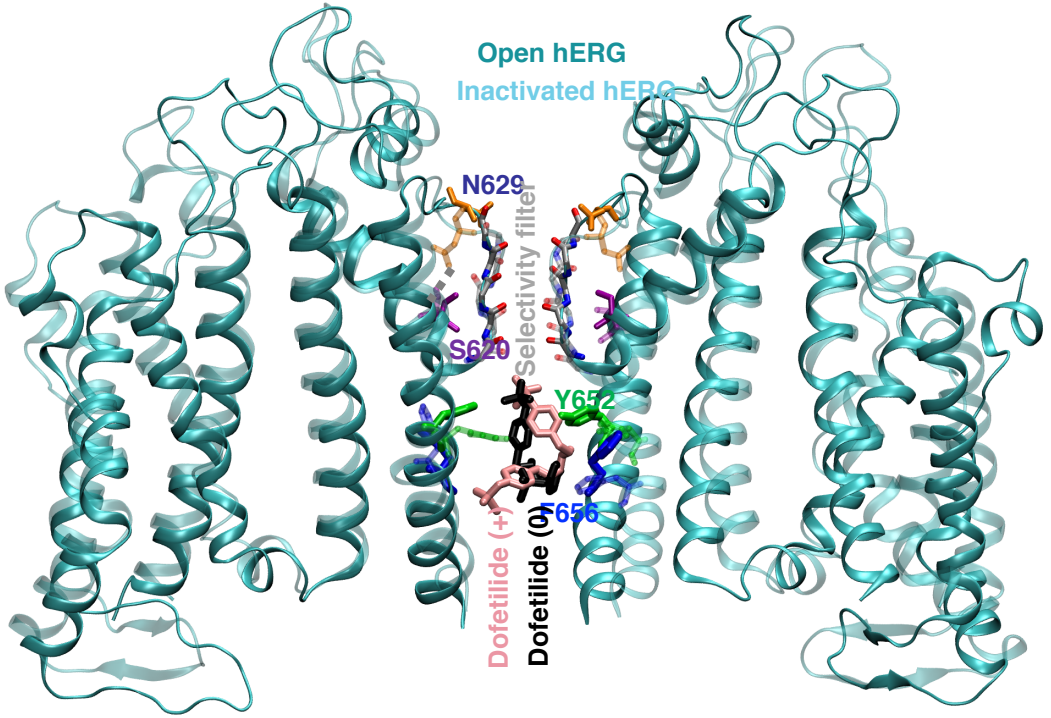
Rates are used for functional modeling.

DeMarco et al. bioRxiv, p. 635441, 2019

Key challenge: inactivated hERG – dofetilide interactions

There is no inactivated hERG structure. Previous homology modeling / experiments suggested intrasubunit N629···S620 hydrogen bond is important.





Binding Data	Open hERG		Inactivated hERG	
	ΔG_{bind} (kcal mol^{-1})	K_D (μM)	ΔG_{bind} (kcal mol^{-1})	K_D (μM)
Dofetilide (0)	-9.64, -9.19*	0.16, 0.32*	-4.71	480
Dofetilide (+)	-5.94	65.09	-6.04	55.1

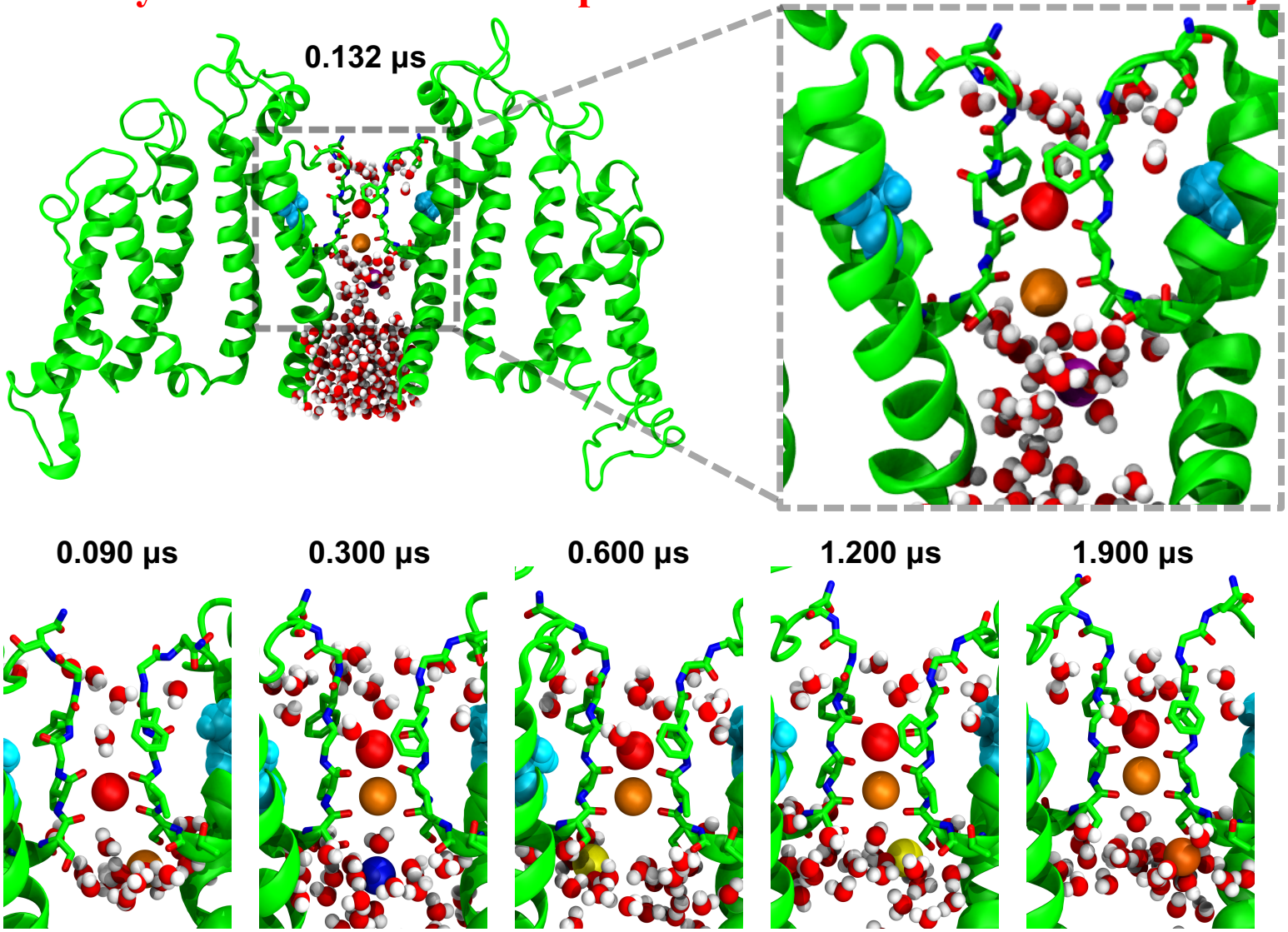
*Obtained from US/H-REMD simulations

Experimental IC_{50} for inactivated state is in ~nM range. Our estimate is ~320 μM. The structure is not stable. N629···S620 hydrogen bonds break during MD.

Key challenge: Inactivated S641A hERG mutant model

Experimentally: fast inactivation comp. to WT

MD: "Pinched" selectivity filter

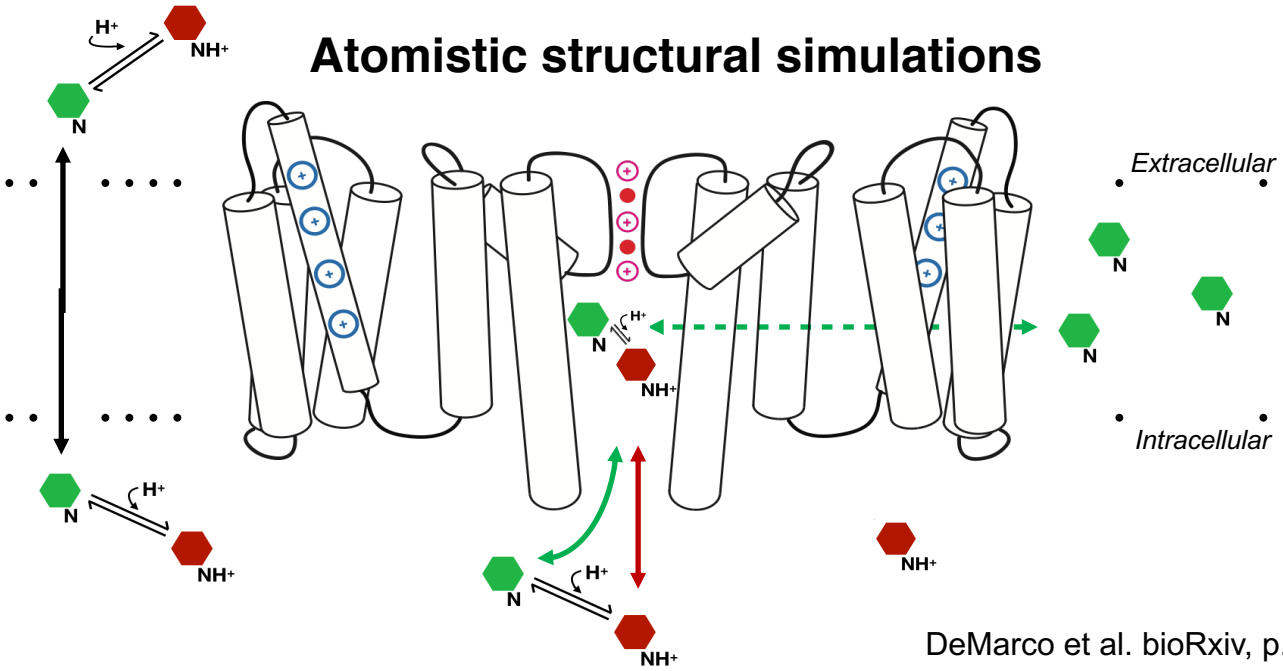


MD: No ion conduction in 2 microsecond-long simulation.

But experimentally this mutant does not have high affinity drug binding.

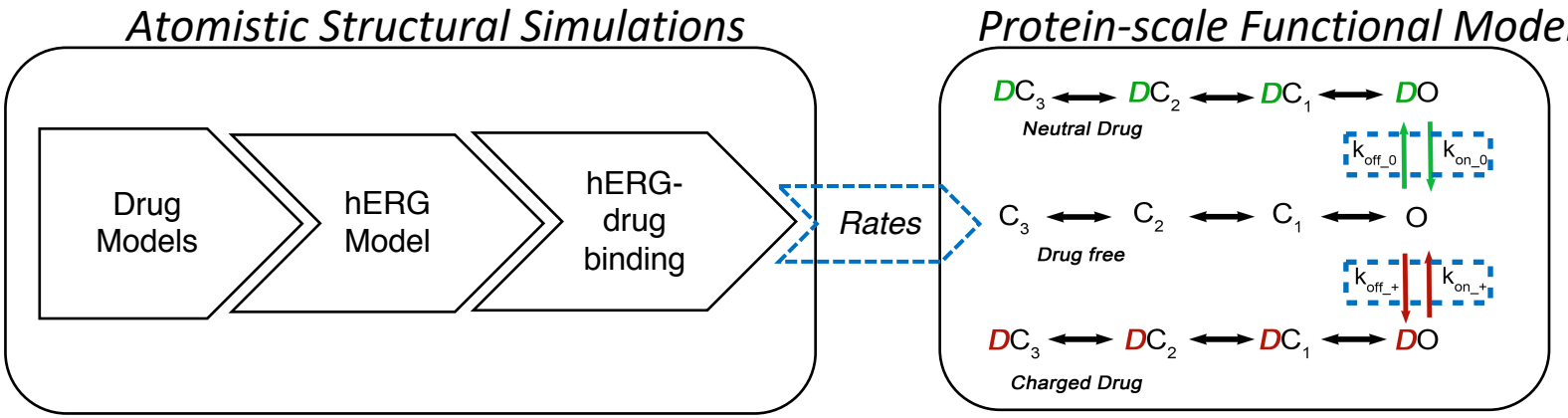
DeMarco et al. bioRxiv, p. 635441, 2019

Accomplishment: direct link to functional models



DeMarco et al. bioRxiv, p. 635441, 2019

Novel link between structural and functional models

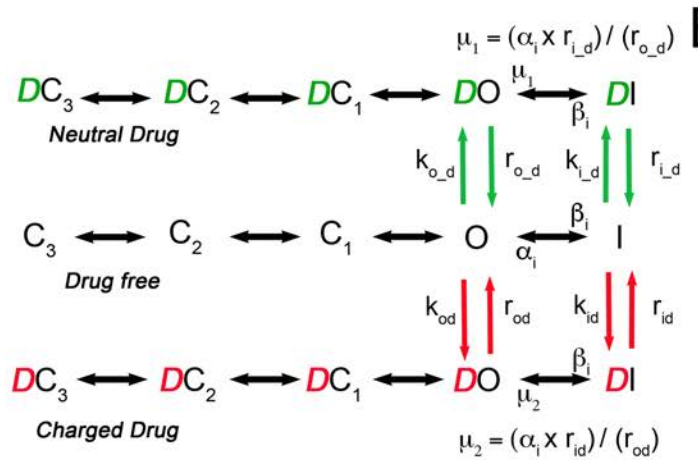


Open hERG–drug "on" and "off" rates computed via MD in in are used directly as functional model parameters.

Dofetilide arrhythmia proclivity using functional models

New dofetilide – hERG functional model using data from atomistic MD

D



	Neutral Drug		Charged Drug	
Transition rates	Open (O_d)	Inactivated (i_d)	Open (Od)	Inactivated (id)
k_x ($\mu M^{-1} s^{-1}$)	6.66E+02	1.72E+03	5.34E+02	1.51E+06
r_x (s^{-1})	1.06E+02	3.9	3.47E+04	1.4E+06

E

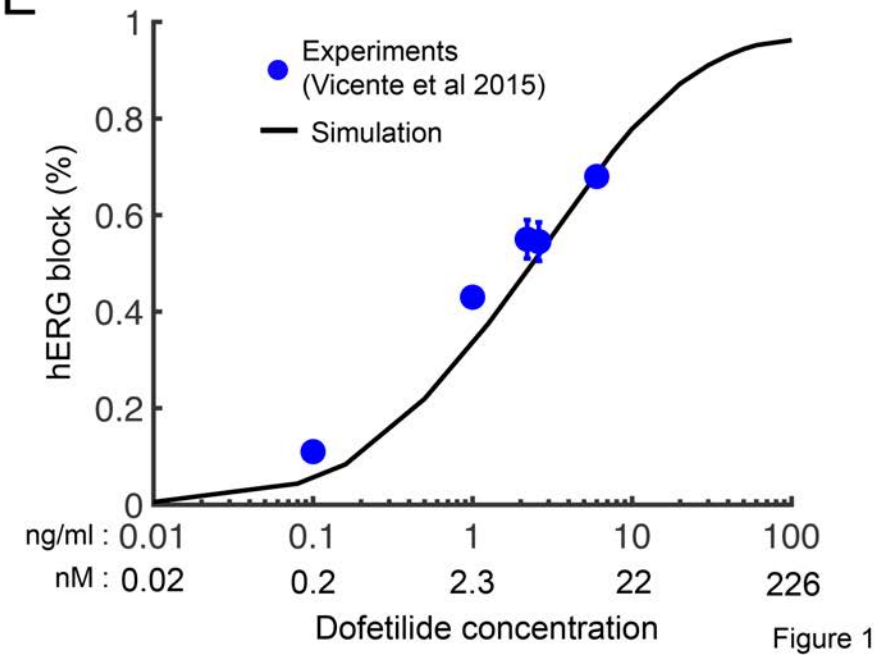
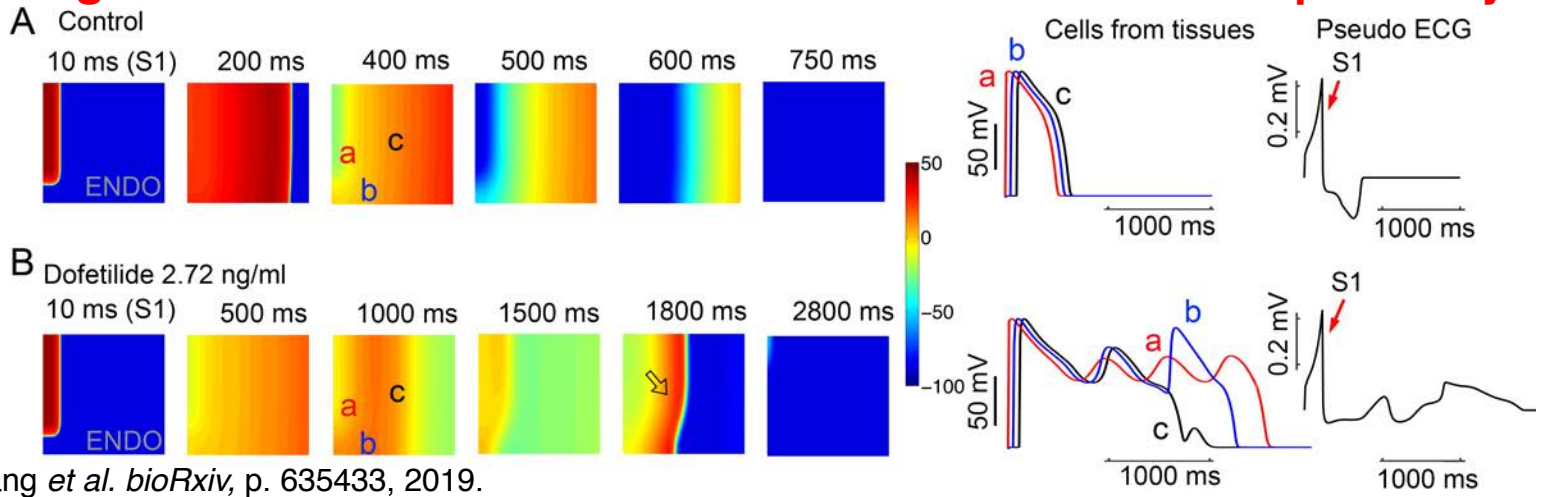


Figure 1

Using this model in 2D cardiac tissue simulations reveals pro-arrhythmia markers.



Yang *et al. bioRxiv,* p. 635433, 2019.

Conclusions

- Atomistic MD simulations on Blue Waters are useful to provide structural-level information for ion channel function and drug affinities and crucial to be able to predict drug mechanism of action based on its chemistry.

- These simulations rely on high-resolution ion channel structures or high accuracy homology models, accurate drug parameters as well as good performance on highly parallel architectures such as Blue Waters.

- Drug binding affinities and kinetics from atomistic MD simulations on Blue Waters were used to populate protein- and cell-based kinetic models and predict molecular-level mechanisms for arrhythmogenesis.

- Next: other drugs, hormones, consider alternative mechanisms (channel gating modification), multi-channel block.

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