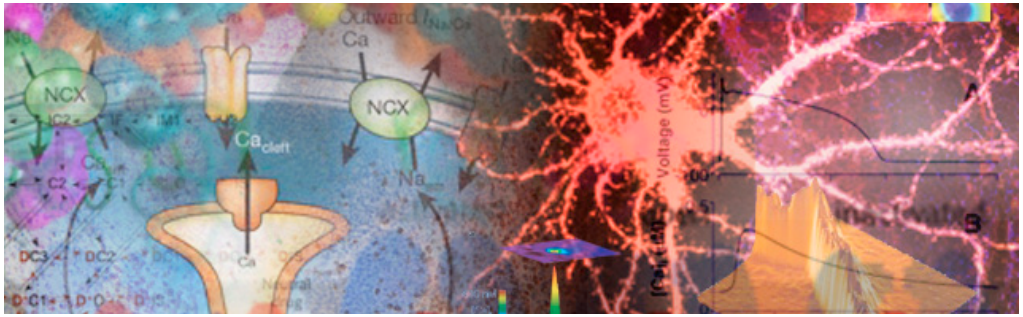
UCDAVIS
HEALTH SYSTEM
Department of Pharmacology



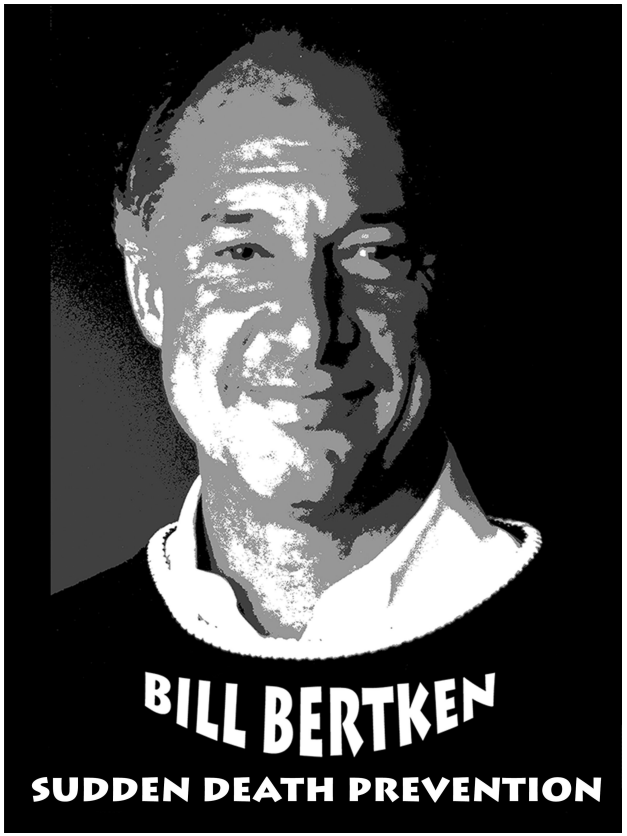
In silico evaluation of efficacy and safety of atrial fibrillation selective pharmacotherapy

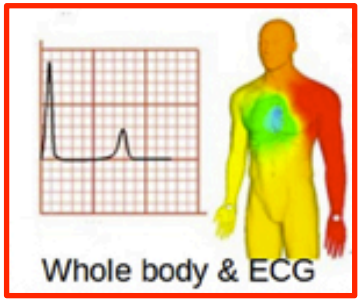
Ele Grandi, Ph.D.
Department of Pharmacology
University of California Davis

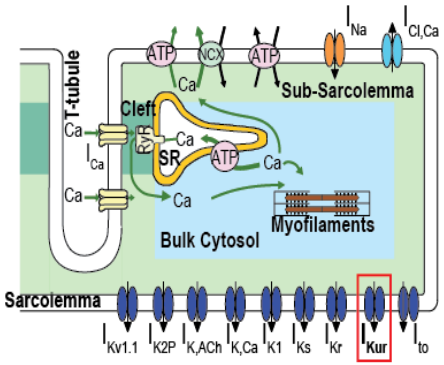
KITP

July 2, 2018

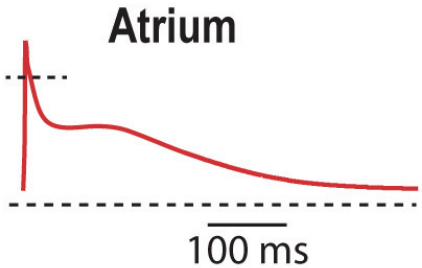
Antiarrhythmic drugs cause arrhythmia



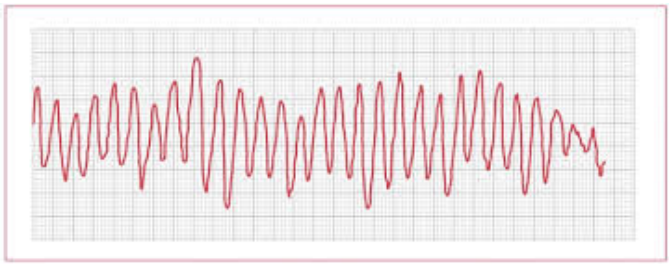


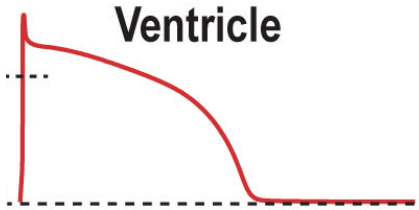


Atrial Fibrillation (AF)



Ventricular Fibrillation





AF-selective pharmacotherapy

Atrium-selective (*vs.* ventricle)

* **Atrium-specific K channel expression** (I_{Kur}, $I_{K,Ca}$, $I_{K,ACh}$, I_{TASK})

* **Atrio-ventricular differences in AP properties** (depolarized RMP, negative plateau, faster phase-1 and slower phase-3 repolarization)

* **Atrio-ventricular differences in I_{Na}**

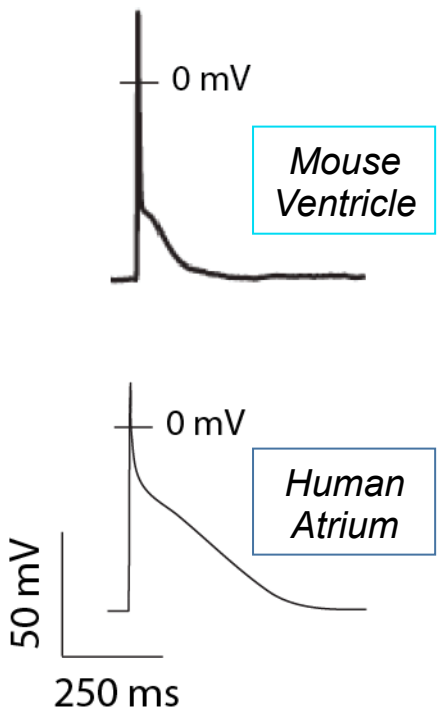
High rate-selective (*vs.* nSR)

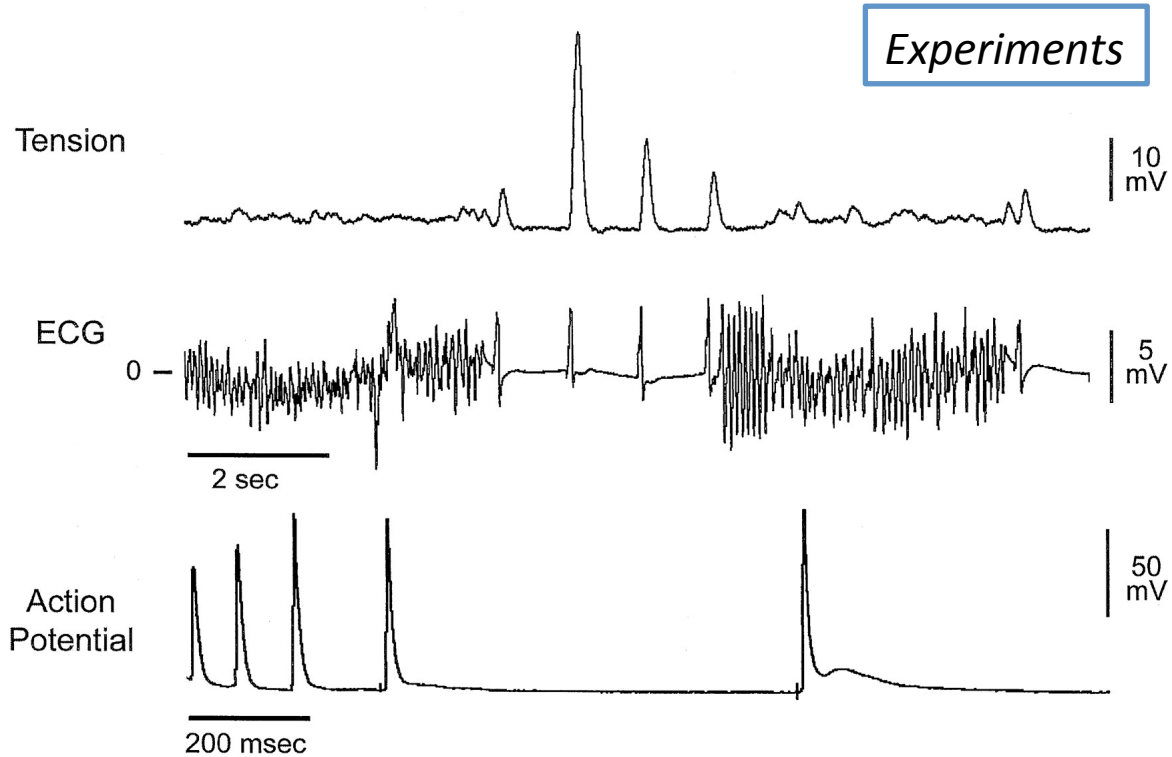
* **State-dependent block** (open vs. inactivated)

Stefano Morotti

Grandi and Maleckar, *Pharmacol Therap* 2016
Morotti et al., *J Mol Cell Cardiol* 2016

Phase-3 EADs underlie AF re-initiation





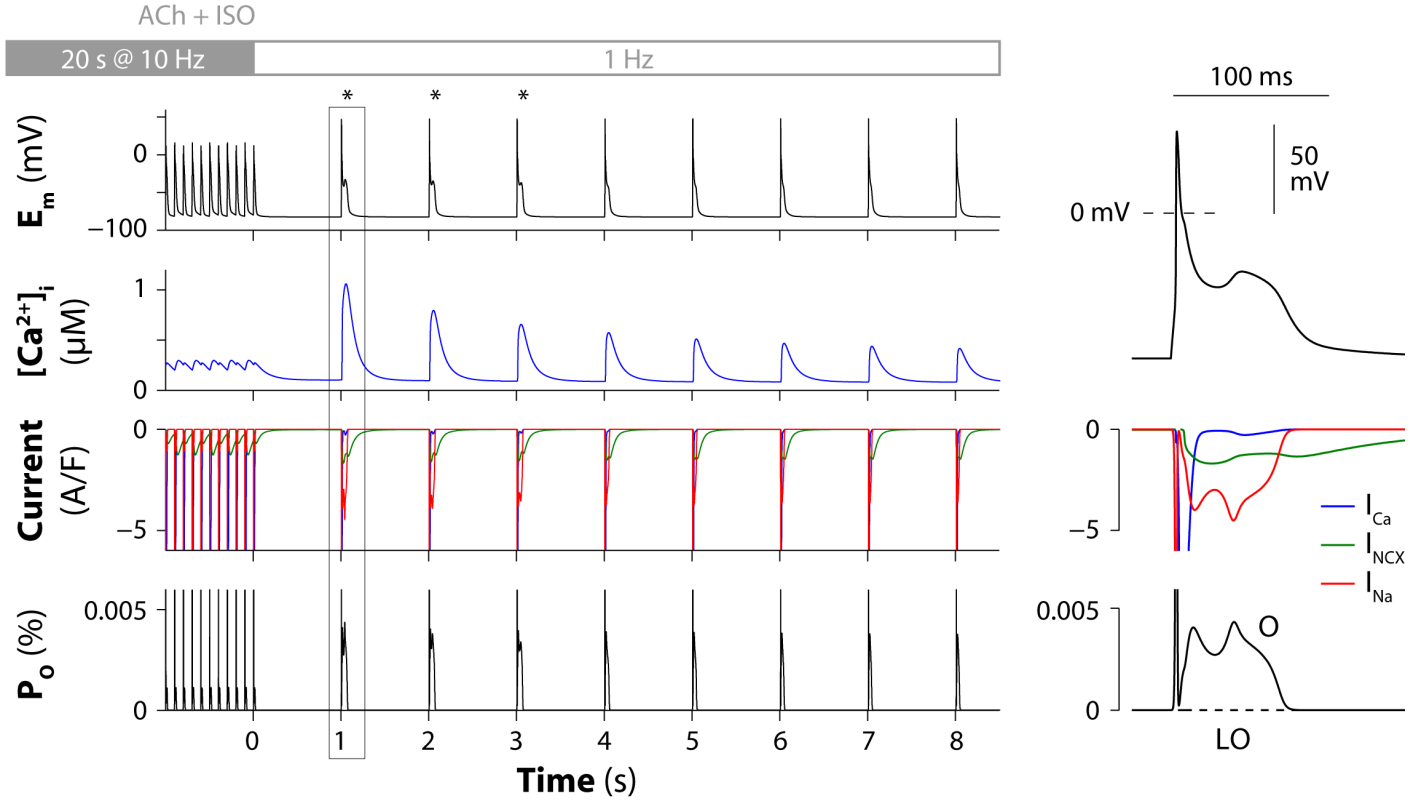
This mechanism may be dominant in atrial (and PV) myocytes, in agreement with a series of studies suggesting that Ca-induced triggered activity underlies AF re-initiation in canine atria exposed to combined sympathetic and parasympathetic stimulation

Burashnikov and Antzelevitch, *Circulation*. 2003

Canine Atrium and PV sleeves

N-E I_{Na} underlies EADs in human atria

Simulations



Morotti et al. *JMCC* 2016

Human Atrium

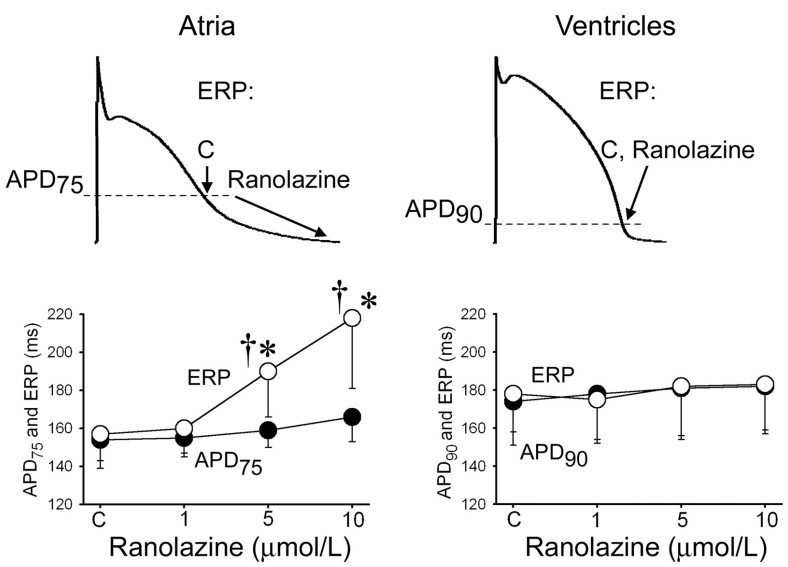
Ranolazine selectively blocks atrial peak I_{Na}

- Ranolazine is an open state blocker that unbinds rapidly from closed Na^+ channels, but is trapped in the inactivated state.

- While ranolazine binding/unbinding kinetics is similar in atria and ventricles, atrial-selective <u>peak</u> I_{Na} inhibition by ranolazine is due to:
  1. a more negative steady-state inactivation curve,
  2. less negative resting membrane potential,
  3. and shorter diastolic intervals at rapid rates in atria *vs.* ventricles.

- Ranolazine prolongs atrial ERP and slows atrial CV without affecting ventricular parameters

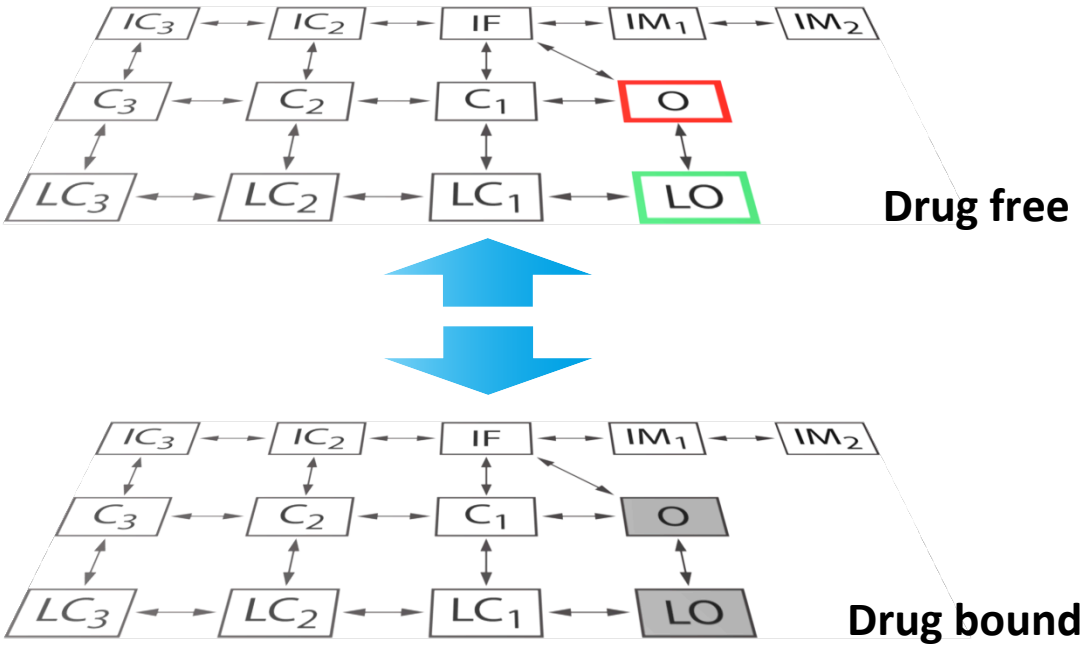
Can ranolazine prevent phase 3 EADs in human atrial myocytes?



Canine

Burashnikov et al., *Circulation*. 2007

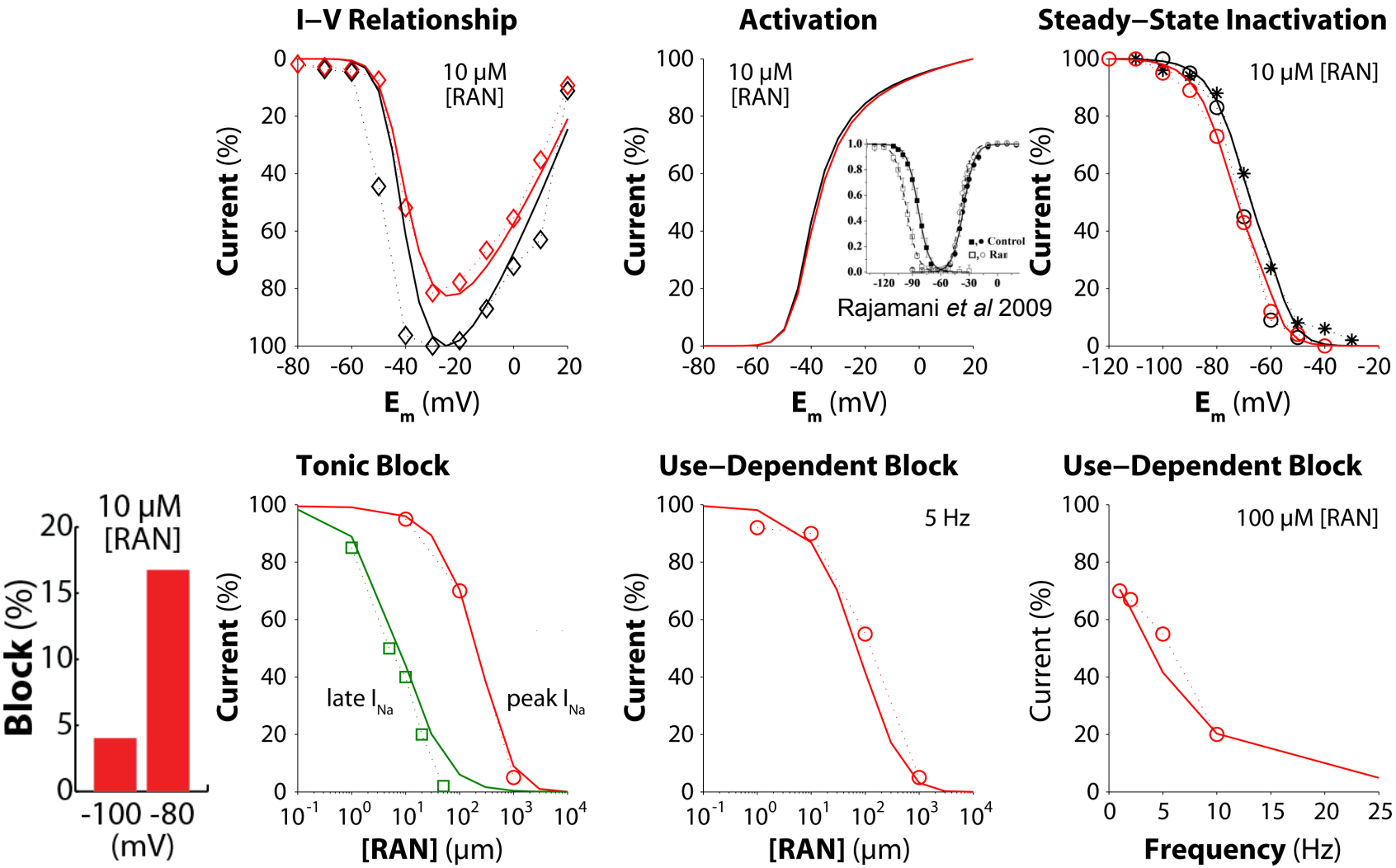
Ranolazine – I_{Na} kinetic model



Based on Moreno et al. *Circ Res* 2013 & Wagner et al. *Circ Arrhythm Electrophysiol* 2009

Human

Ranolazine – I_{Na} kinetic model



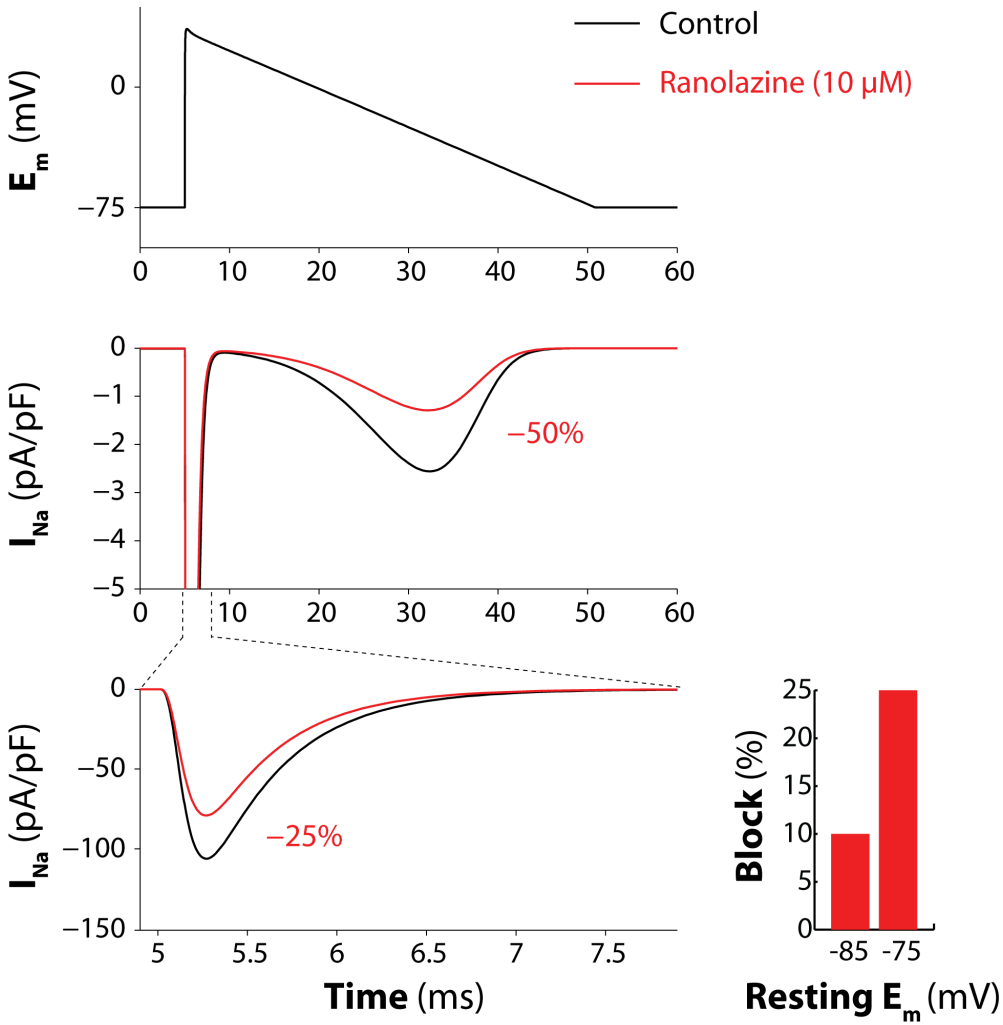
Simulations — control; RAN

Experiments — Wagner *et al* 2009; Antzelevitch *et al* 2004; Moreno *et al* 2013; Sossalla *et al* 2010

Human

Ranolazine blocks non-equilibrium I_{Na} better than peak

Simulations

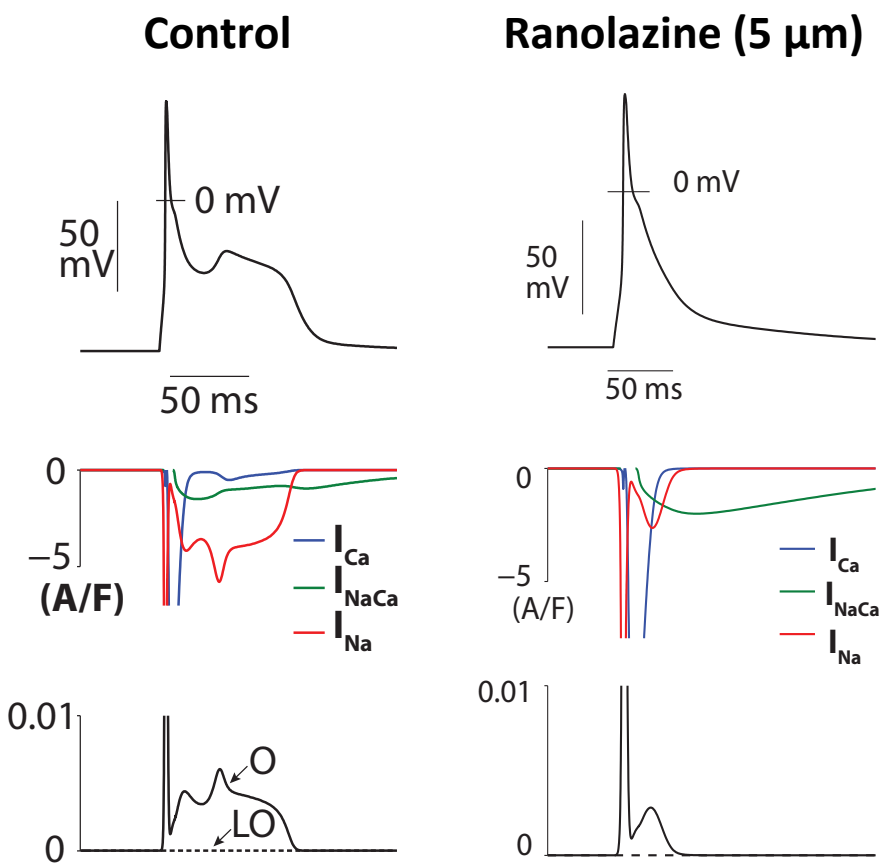


Based on Moreno et al. *Circ Res* 2013 & Wagner et al. *Circ Arrhythm Electrophysiol* 2009

Human

Ranolazine prevents phase 3 EADs in human atrial myocytes

Simulations

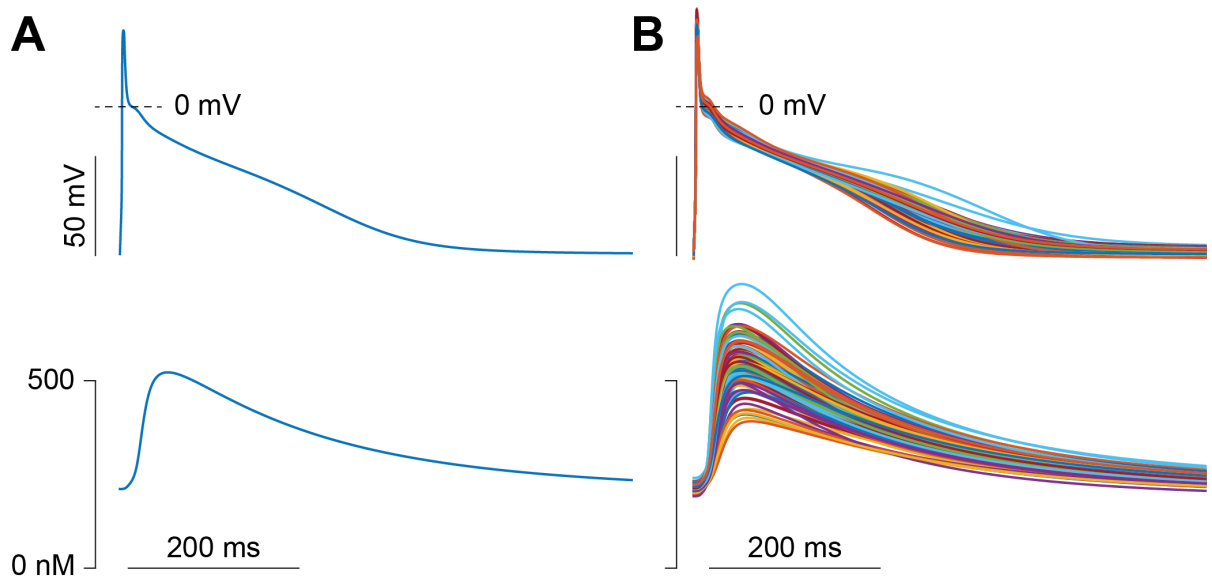


Based on Grandi et al. *Circ Res* 2011 & Wagner et al. *Circ Arrhythm Electrophysiol 2009*

Human Atrium

How does variability affect EAD occurrence?

Baseline model

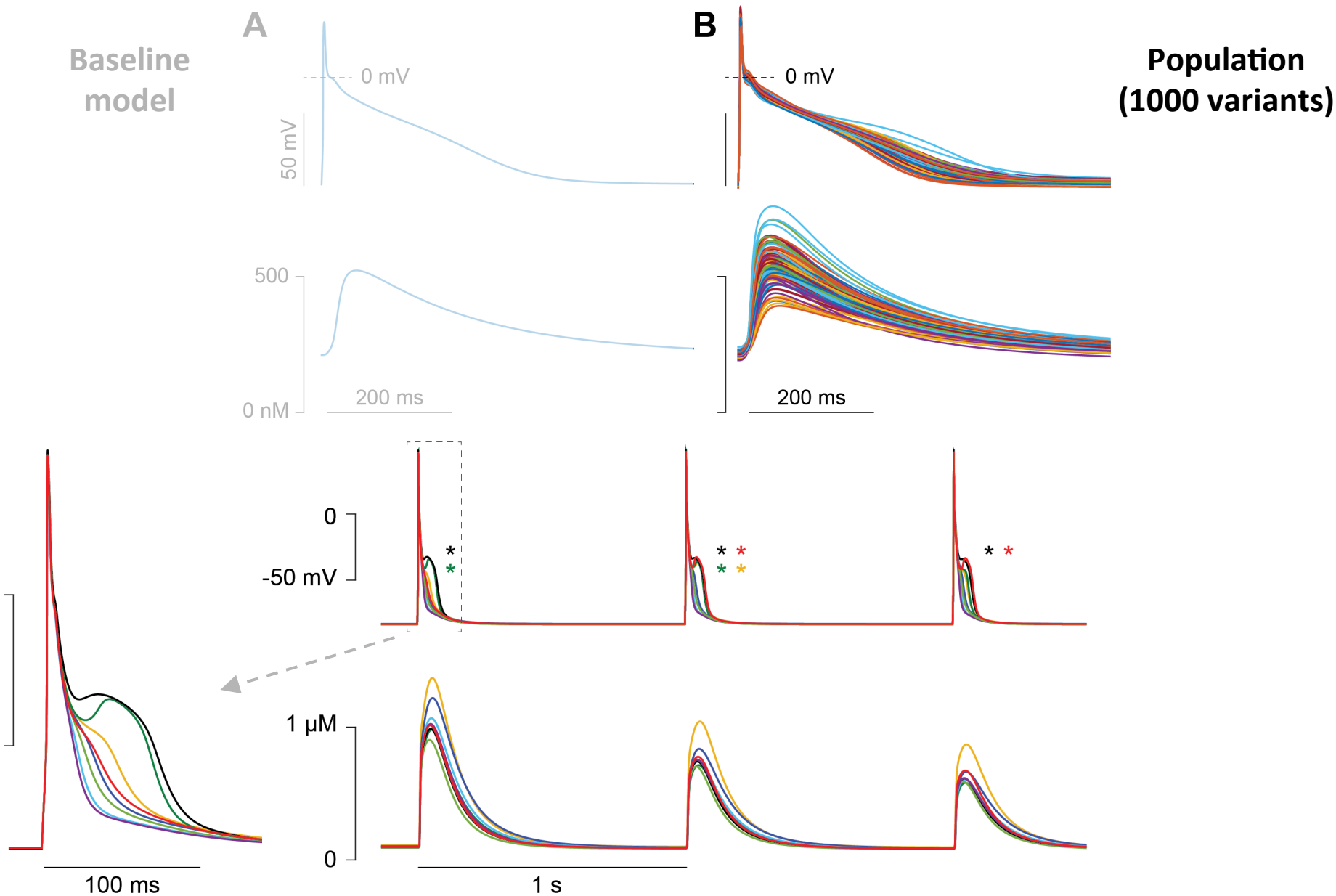


Population (1000 variants)

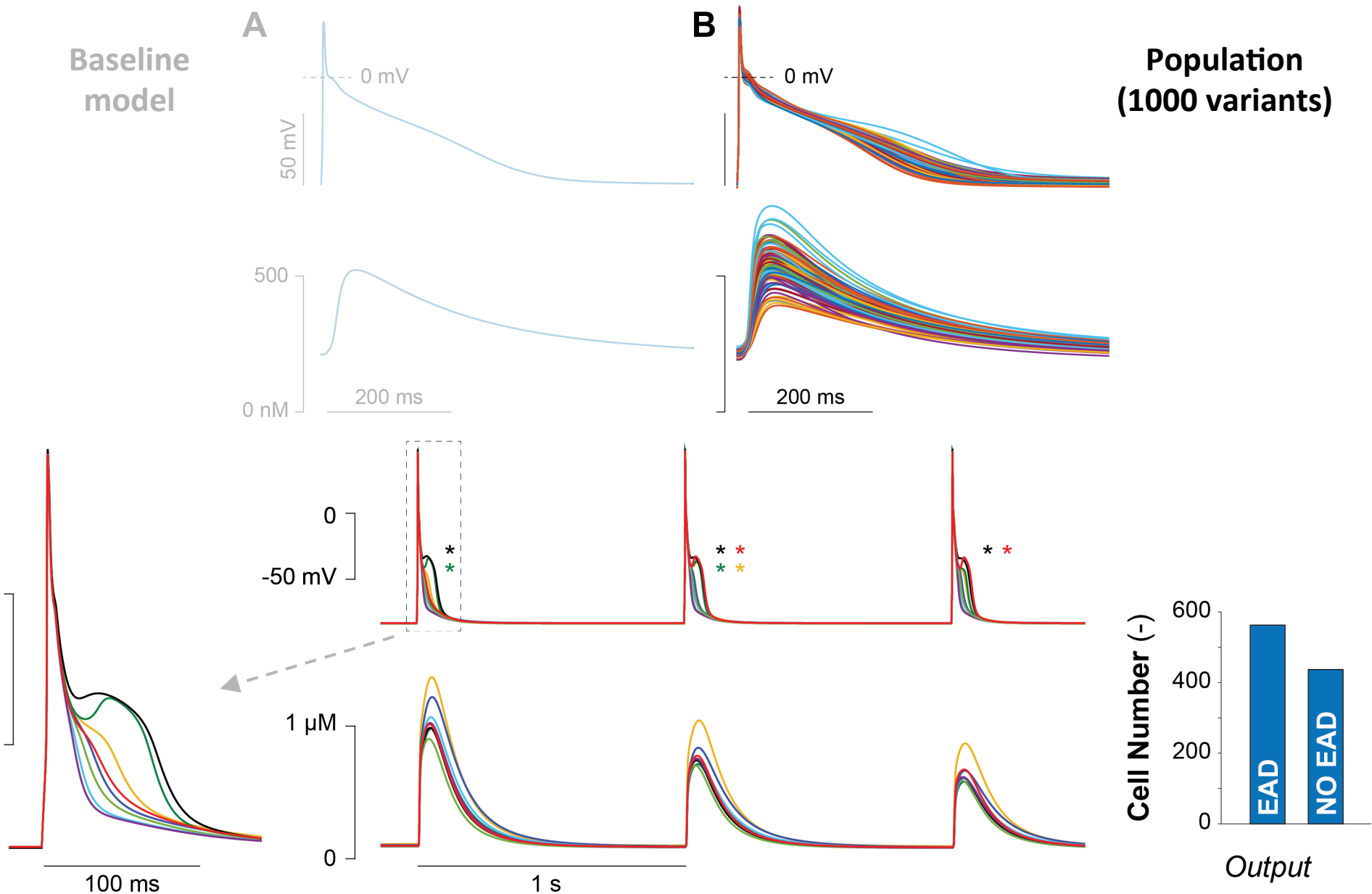
Morotti and Grandi, MethodsX 2017

Human Atrium

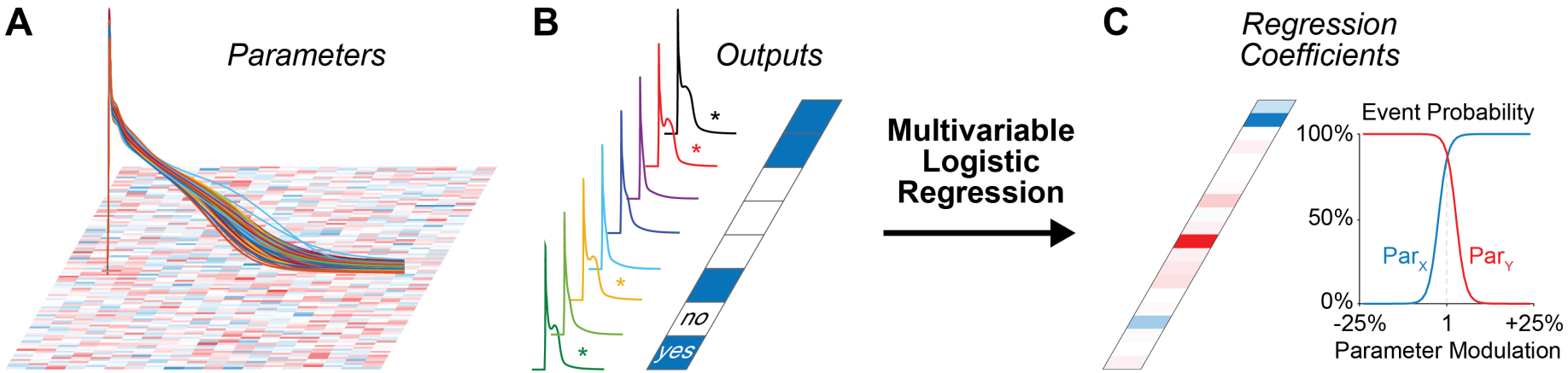
How does variability affect EAD occurrence?



How does variability affect EAD occurrence?



What conductances influence EAD occurrence?



Morotti and Grandi, MethodsX 2017

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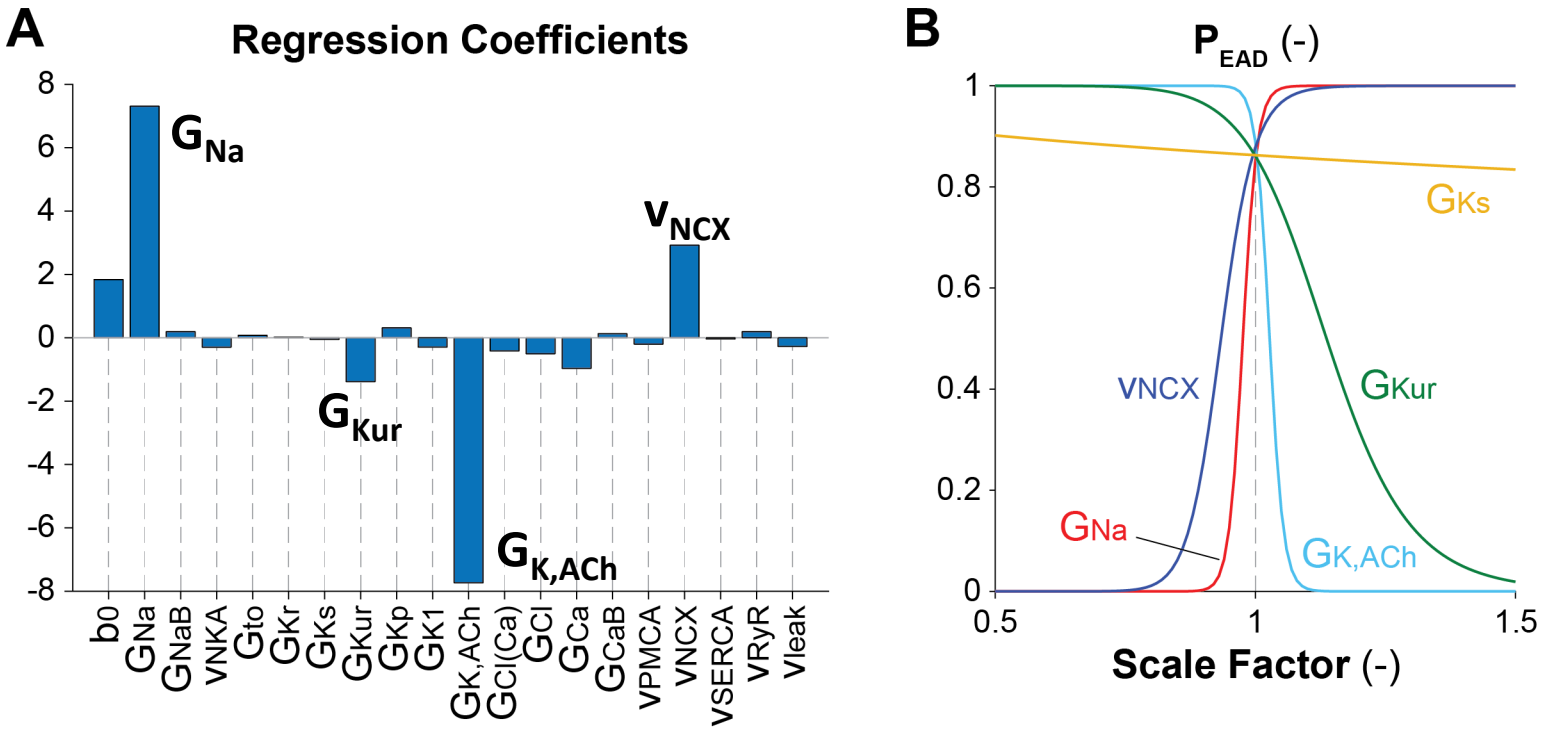
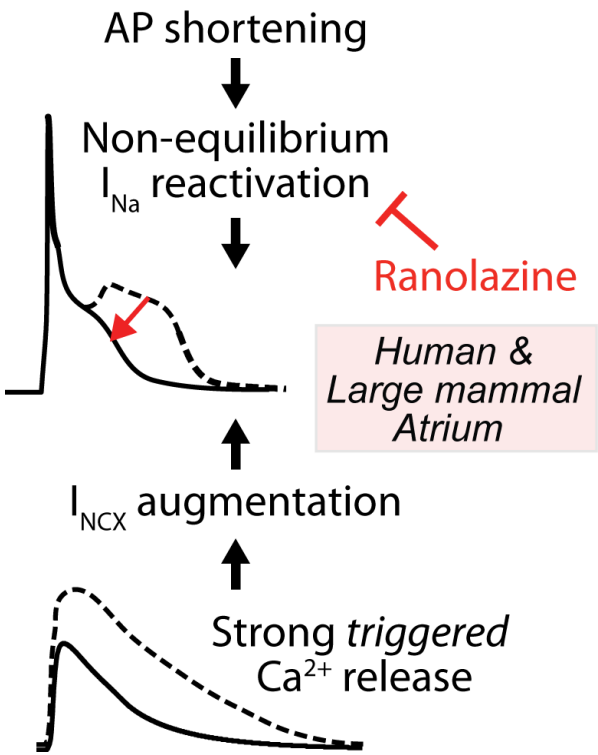


- Phase-3 EAD formation is sensitive to I_{Na}, I_{NCX} (+), $I_{K,ACh}$, I_{Kur} (-)
- These results match our mechanistic predictions

Morotti and Grandi, MethodsX 2017

Summary (i)

- Phase-3 EADs in human atrium are favored by APD shortening and cellular Ca^{2+} overload
- Ranolazine prevents EADs in an atrial-selective manner
- Statistical analysis of a virtual *in silico* cell population confirms our mechanistic predictions



AF-selective pharmacotherapy

Atrium-selective (*vs.* ventricle)

* **Atrium-specific K channel expression** (I_{Kur}, $I_{K,Ca}$, $I_{K,ACh}$, I_{TASK})

* **Atrio-ventricular differences in AP properties** (depolarized RMP, negative plateau, faster phase-1 and slower phase-3 repolarization)

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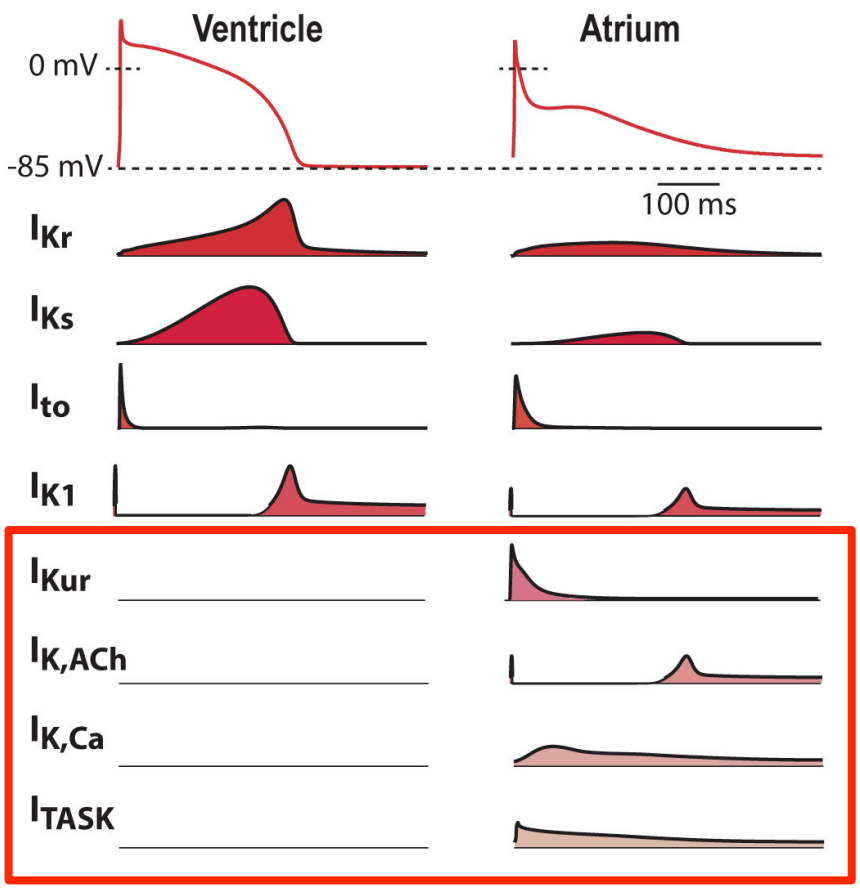

Grandi and Maleckar, *Pharmacol Therap* 2016
Grandi et al., *J Physiol* 2017

AF-selective pharmacotherapy

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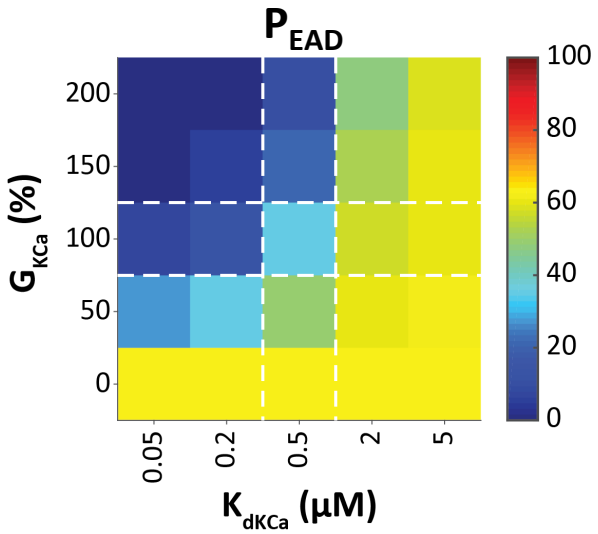

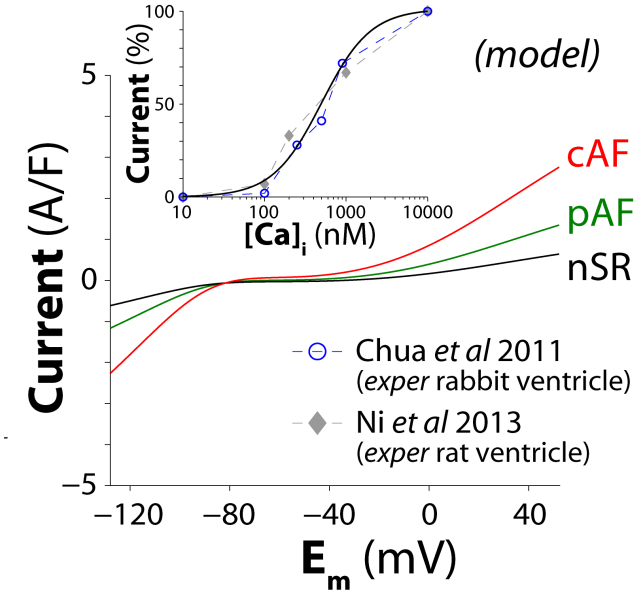

	w/ I_{SK}	w/out I_{SK}
APD prolongation		✔
ERP shortening	✔	
Phase-2 EADs		✔
Phase-3 EADs		✔
DADs		✔
Triggered activity		✔
Alternans	✔	

Grandi and Maleckar, *Pharmacol Therap* 2016
Morotti et al., *Biophys J abstract* 2018

AF-selective pharmacotherapy

Atrium-selective (*vs.* ventricle)

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High rate-selective (*vs.* nSR)

* **State-dependent block** (open vs. inactivated)

→ **AF-selective I_{Kur} block**

Nick Ellinwood

Stefano Morotti

Dobromir Dobrev

Grandi and Maleckar, *Pharmacol Therap* 2016
Ellinwood et al., *Chaos* 2017 & *Front Pharmacol* 2017

Improvement of current guidelines for preclinical assessment of candidate drugs

We can employ a computational pharmacology approach to identify an optimal set of drug binding characteristics, such as **state-dependent affinity and binding kinetics**, that can maximize AF-selective antiarrhythmic therapy.

"The new CIPA paradigm will be driven by a suite of mechanistically based *in vitro* assays coupled to *in silico* reconstructions of cellular cardiac electrophysiologic activity…"

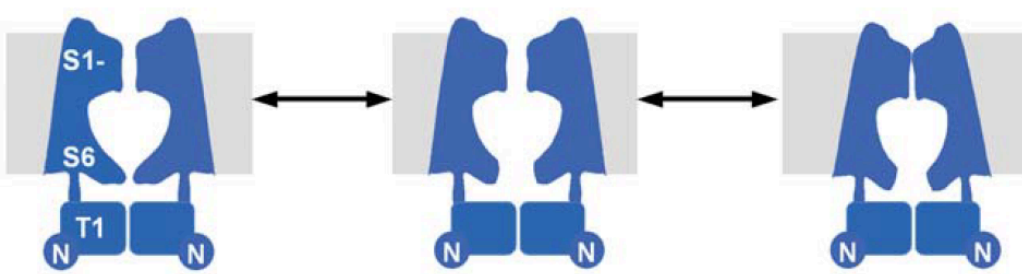
Modeling approach

We can employ a computational pharmacology approach to identify an optimal set of drug binding characteristics, such as **state-dependent affinity and binding kinetics**, that can maximize AF-selective antiarrhythmic therapy.

K_v1.5 channel drug-binding model

$$C_1 \underset{\beta_1}{\overset{\alpha_1}{\rightleftarrows}} C_2 \underset{\beta_2}{\overset{\alpha_2}{\rightleftarrows}} C_3 \underset{\beta_3}{\overset{\alpha_3}{\rightleftarrows}} C_4 \underset{\beta_4}{\overset{\alpha_4}{\rightleftarrows}} O \underset{K_b}{\overset{K_f}{\rightleftarrows}} I$$

Closed **Open** **Inactivated**



Ravens and Wettwer 2010

Modeling approach

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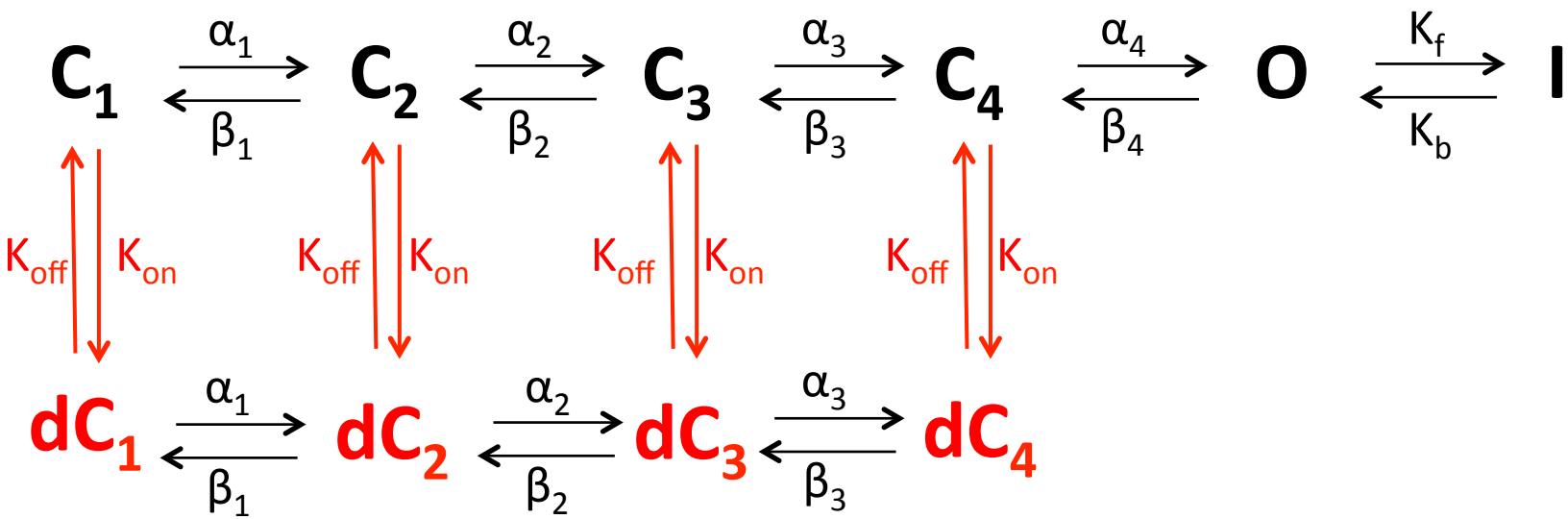
K_v1.5 channel drug-binding model

C Blocker



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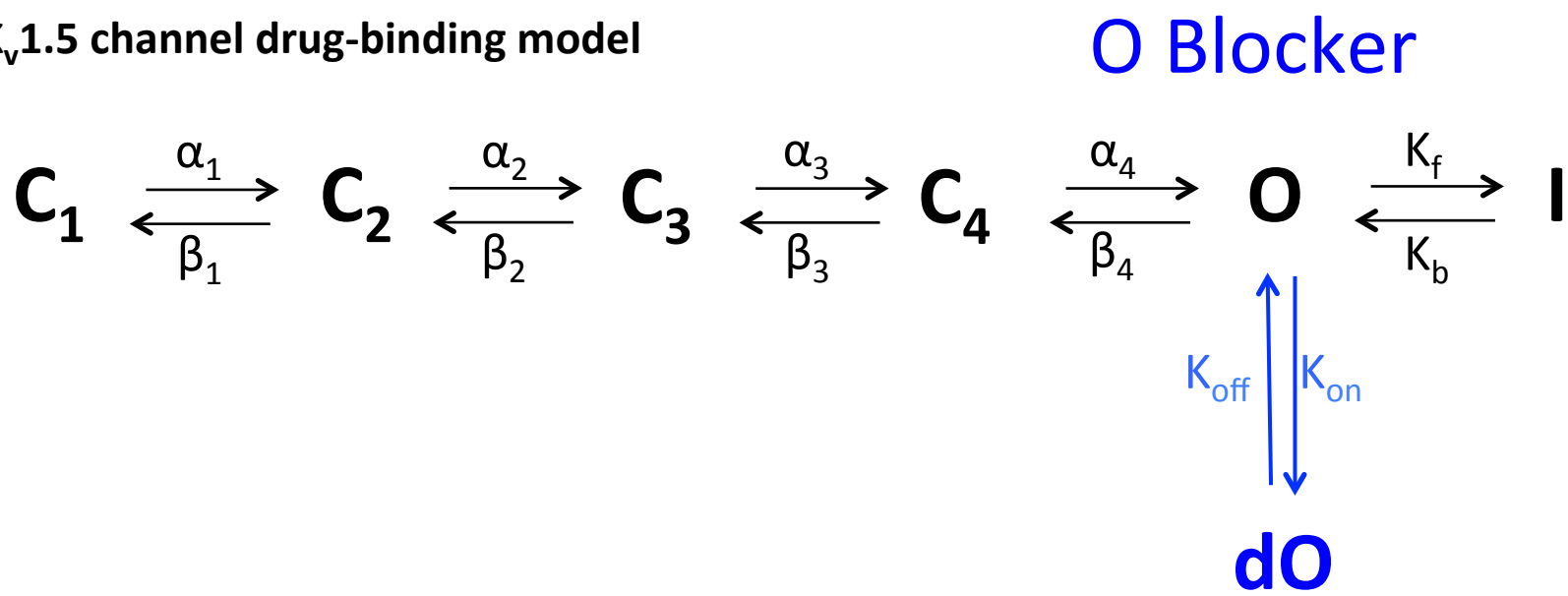

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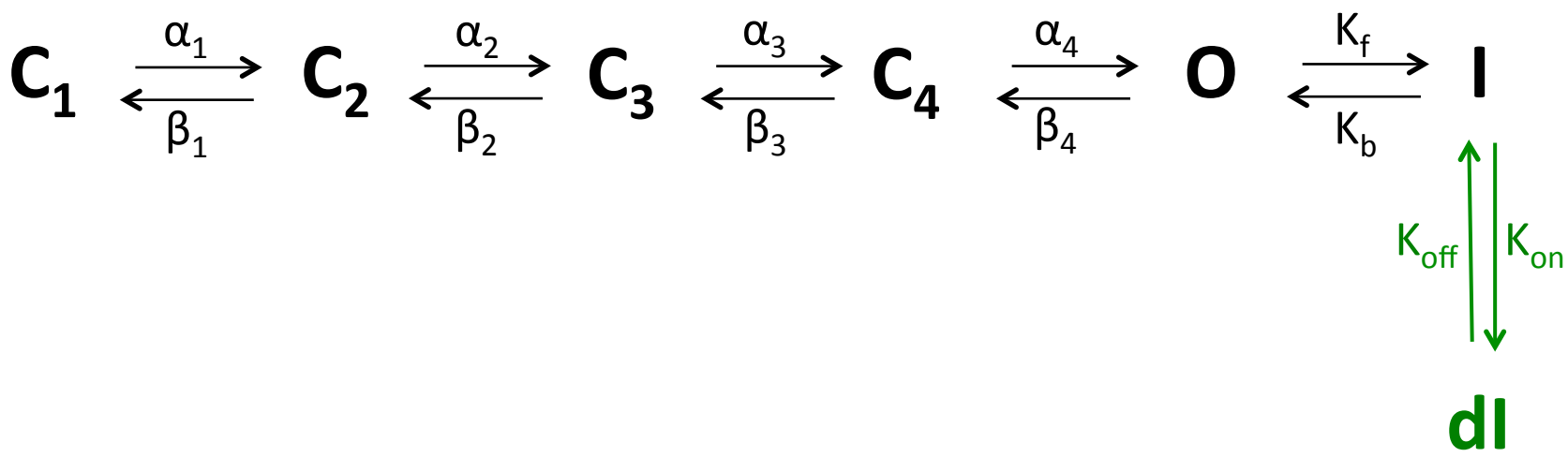

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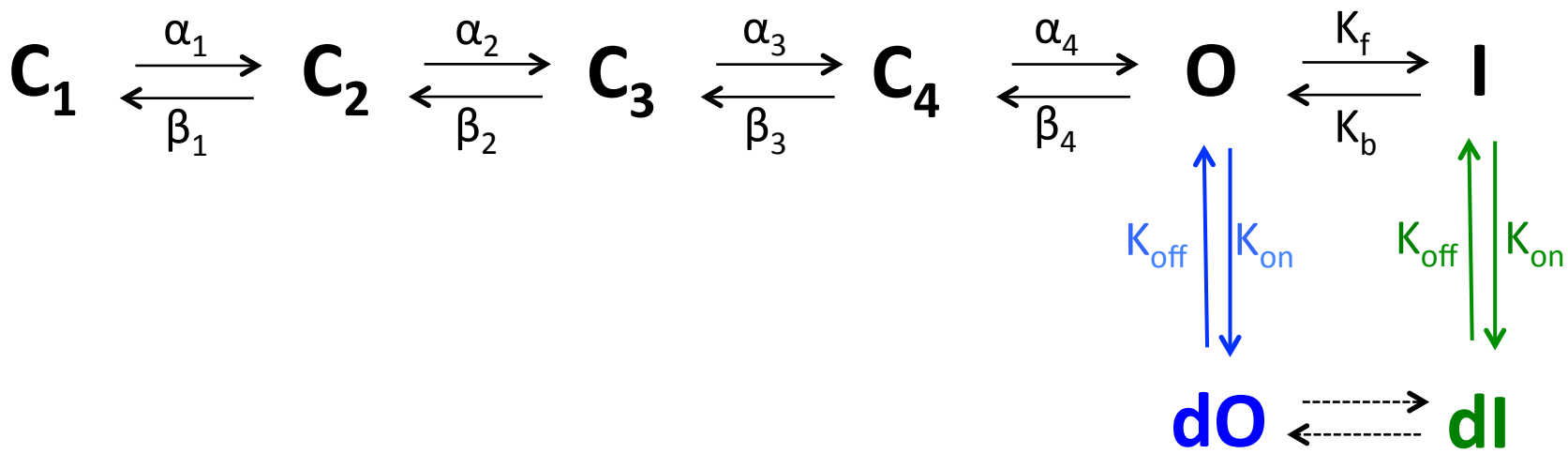

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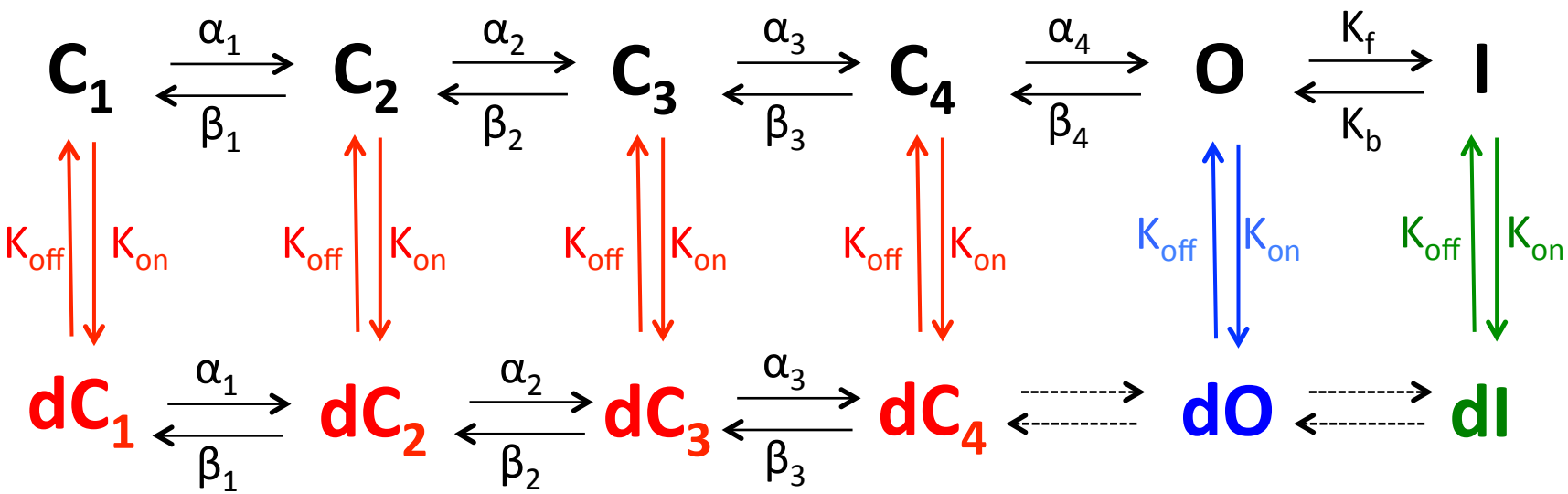
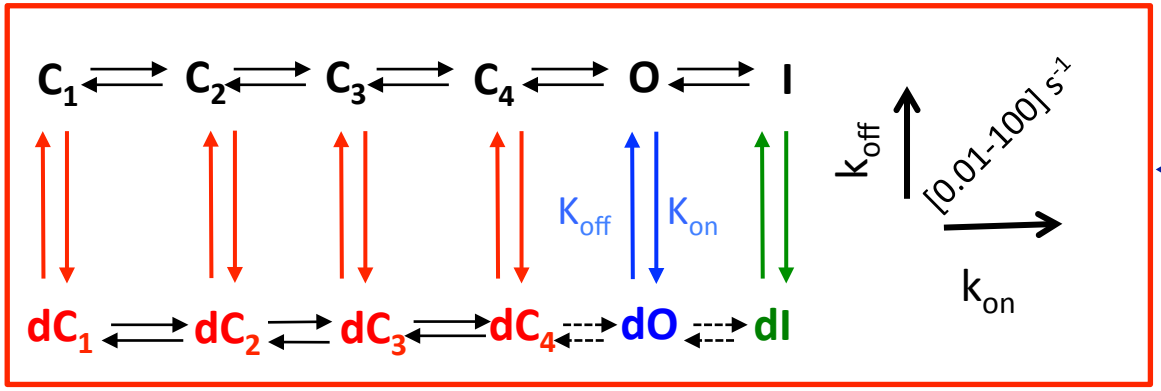


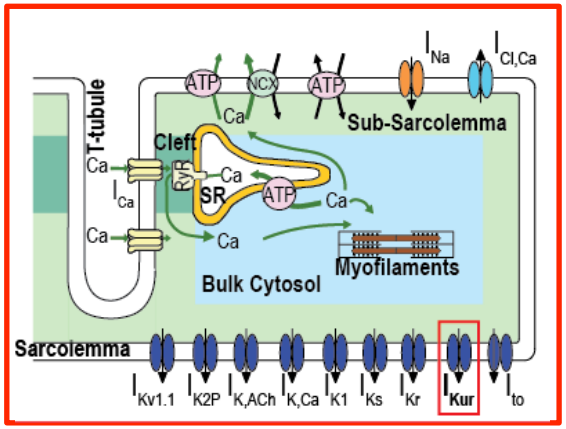
Goal: Fast atrial rate selectivity

I_{Kur} and Drug-Binding Schemes



Lee et al., *Prog Biophys Mol Biol* 2016

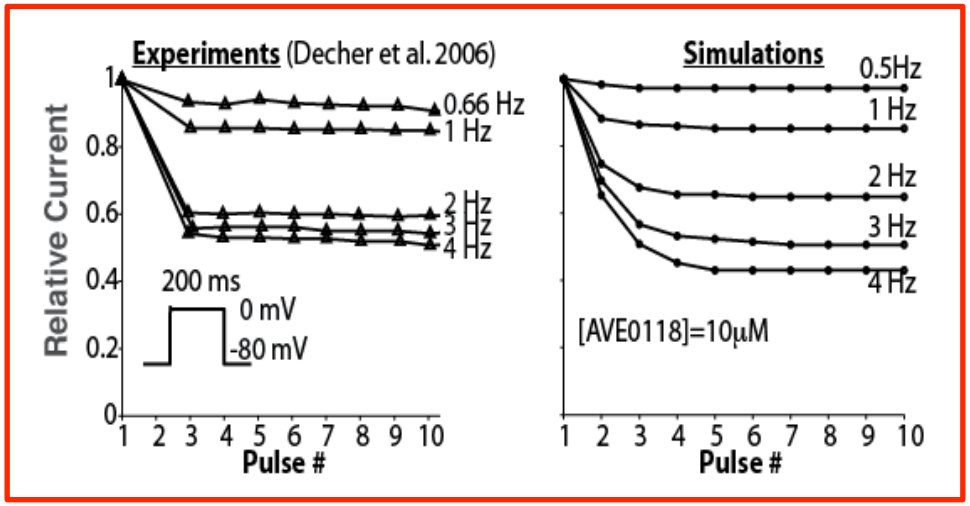
Atrial Cell Models



Grandi et al., *Circ Res* 2011
Morotti et al., *JMCC* 2016

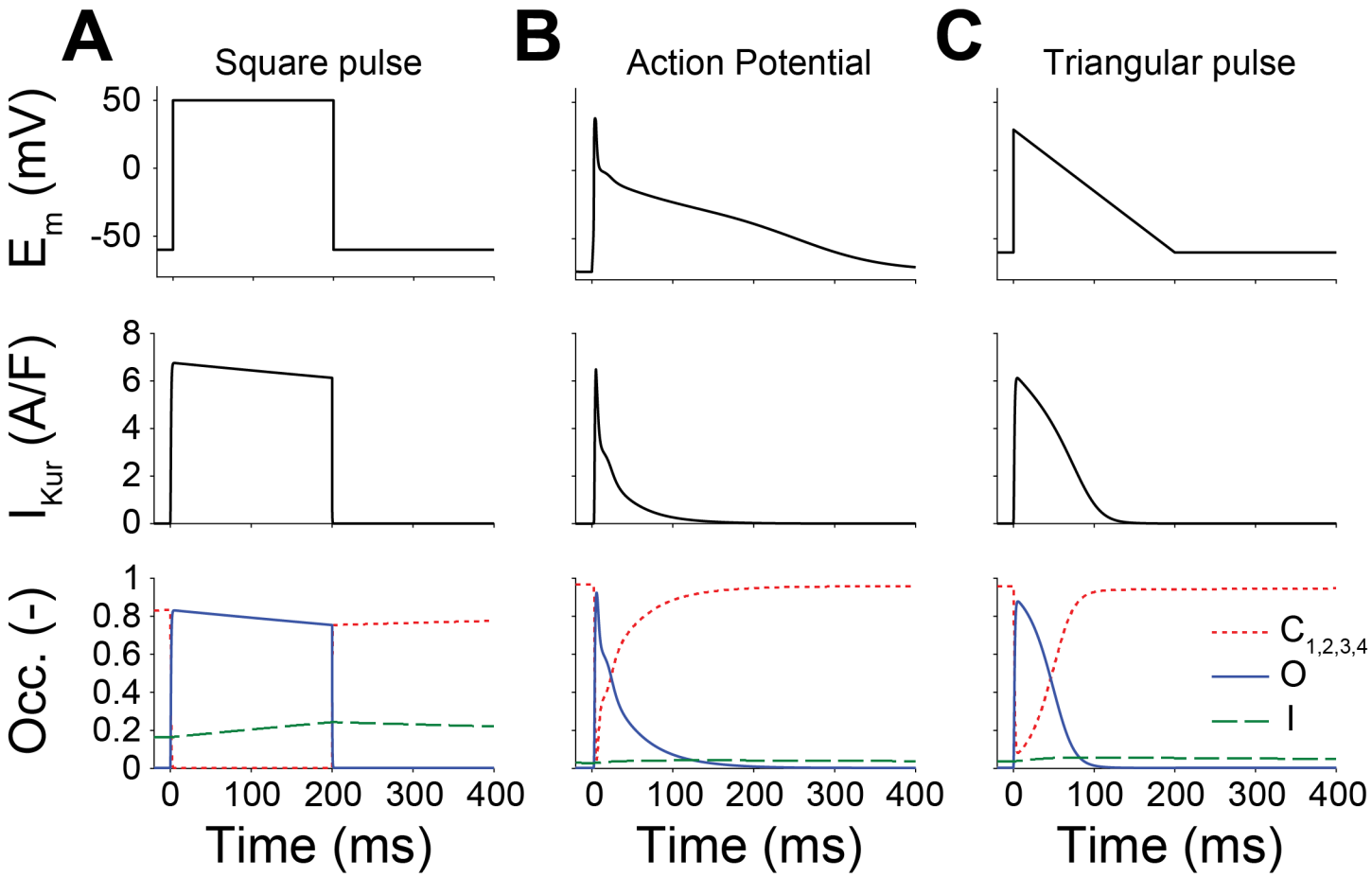
To determine drug properties causing **fast pacing rate selectivity** in:

- I_{Kur} inhibition
- Atrial EP properties (APD & ERP prolongation)



Experimental Validation (Heike Wulff)

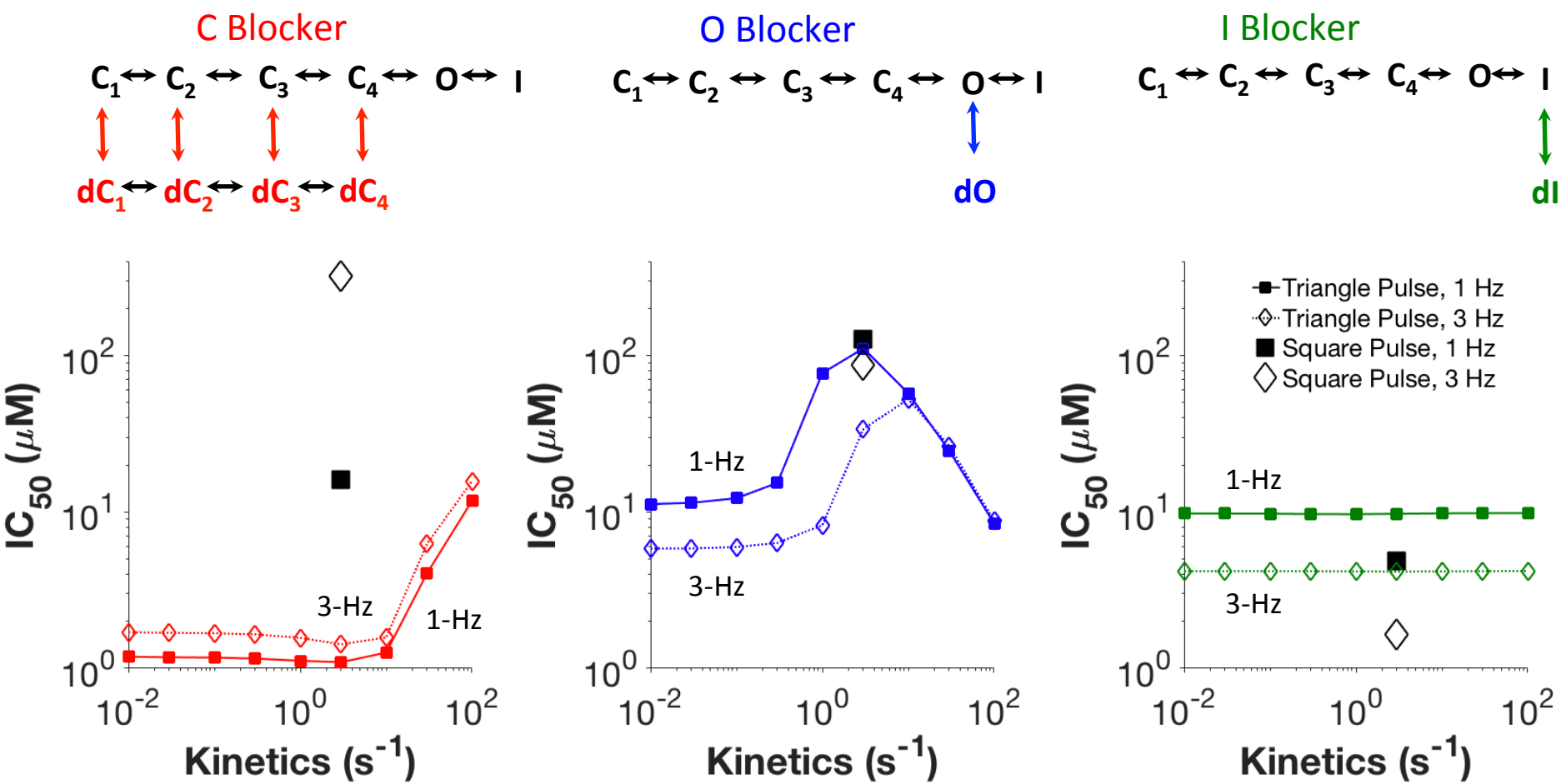
IC_{50} values heavily depend on the chosen voltage-clamp protocol



Ellinwood et al., *Chaos* 2017

*1Hz pacing

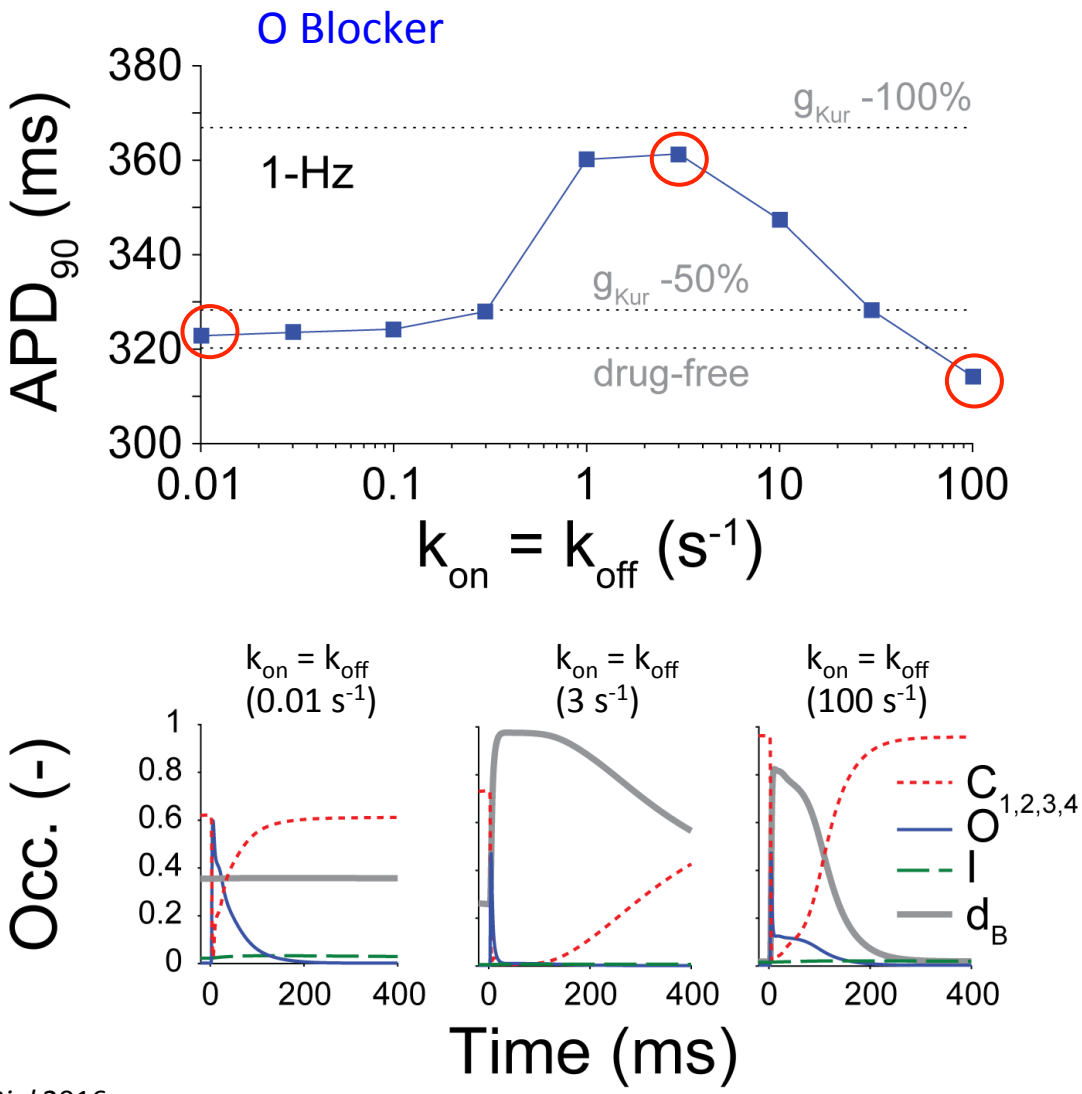
IC_{50} values vary given a drug's state specific affinity and binding kinetics



Ellinwood et al., *Chaos* 2017

*$K_{off} = K_{on}$ = Kinetics

APD_{90} depends on binding kinetics even when using a [drug] equal to the IC_{50} value



Ellinwood et al., *Chaos* 2017
Lee et al., *Prog Biophys Mol Biol* 2016

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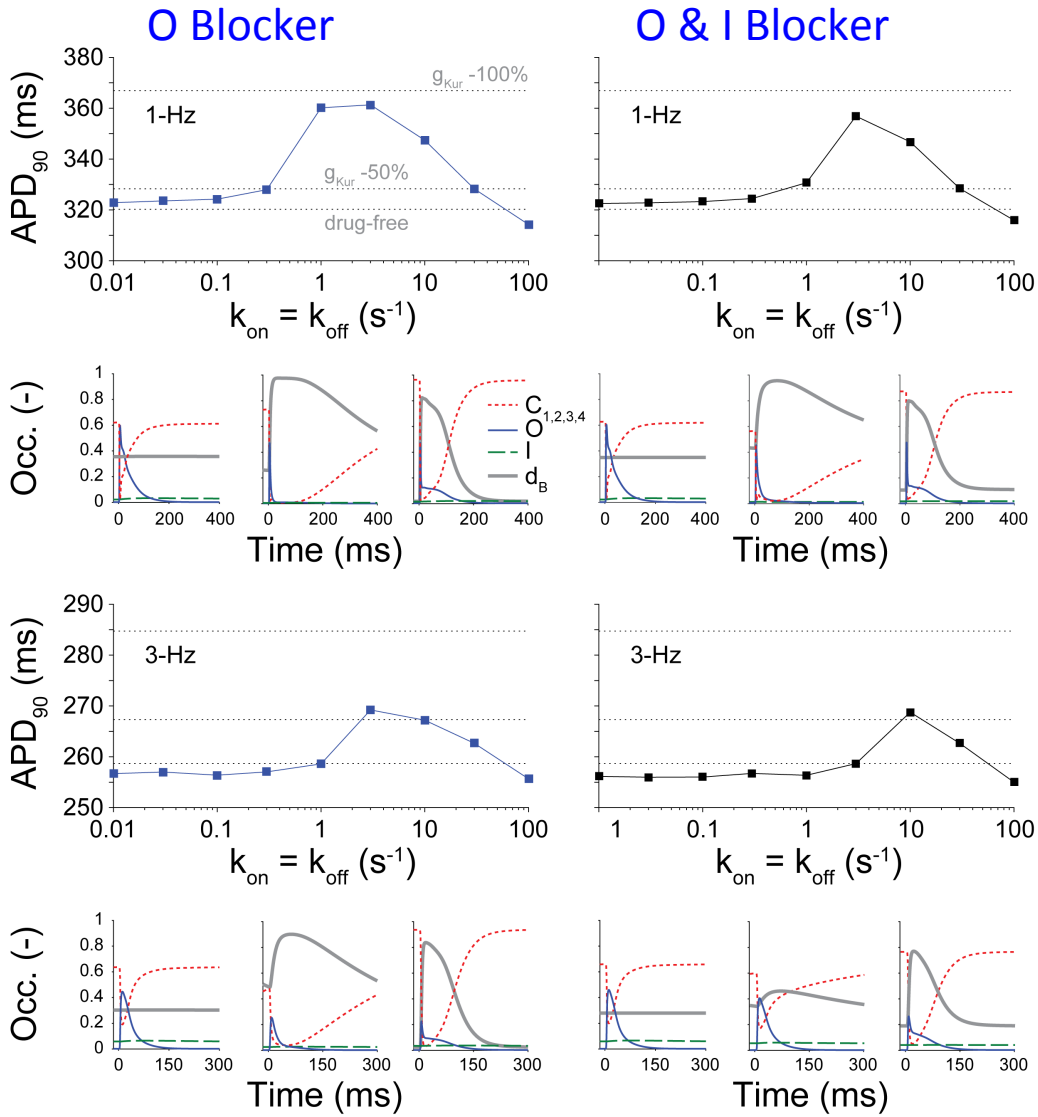
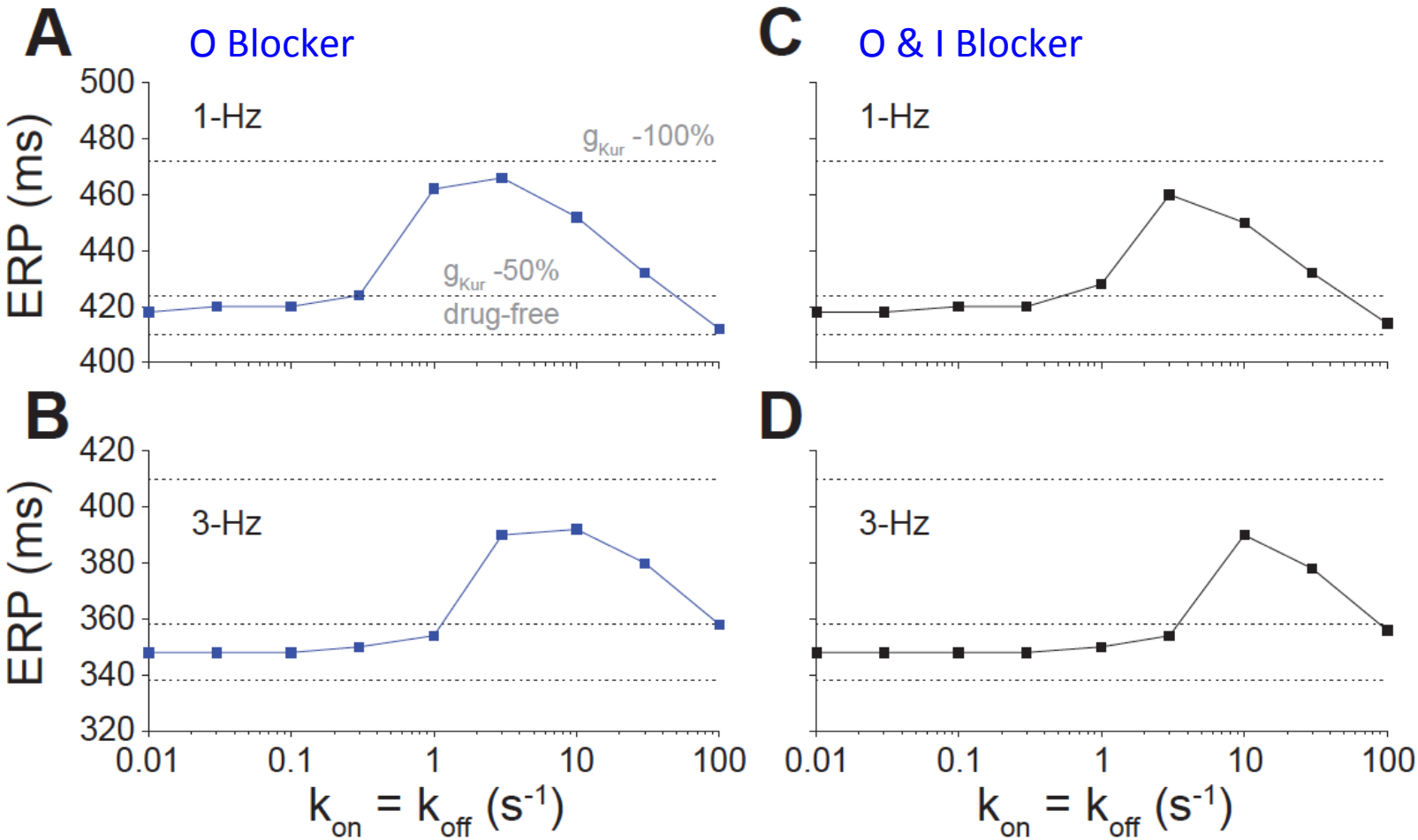


Ellinwood et al., *Chaos* 2017

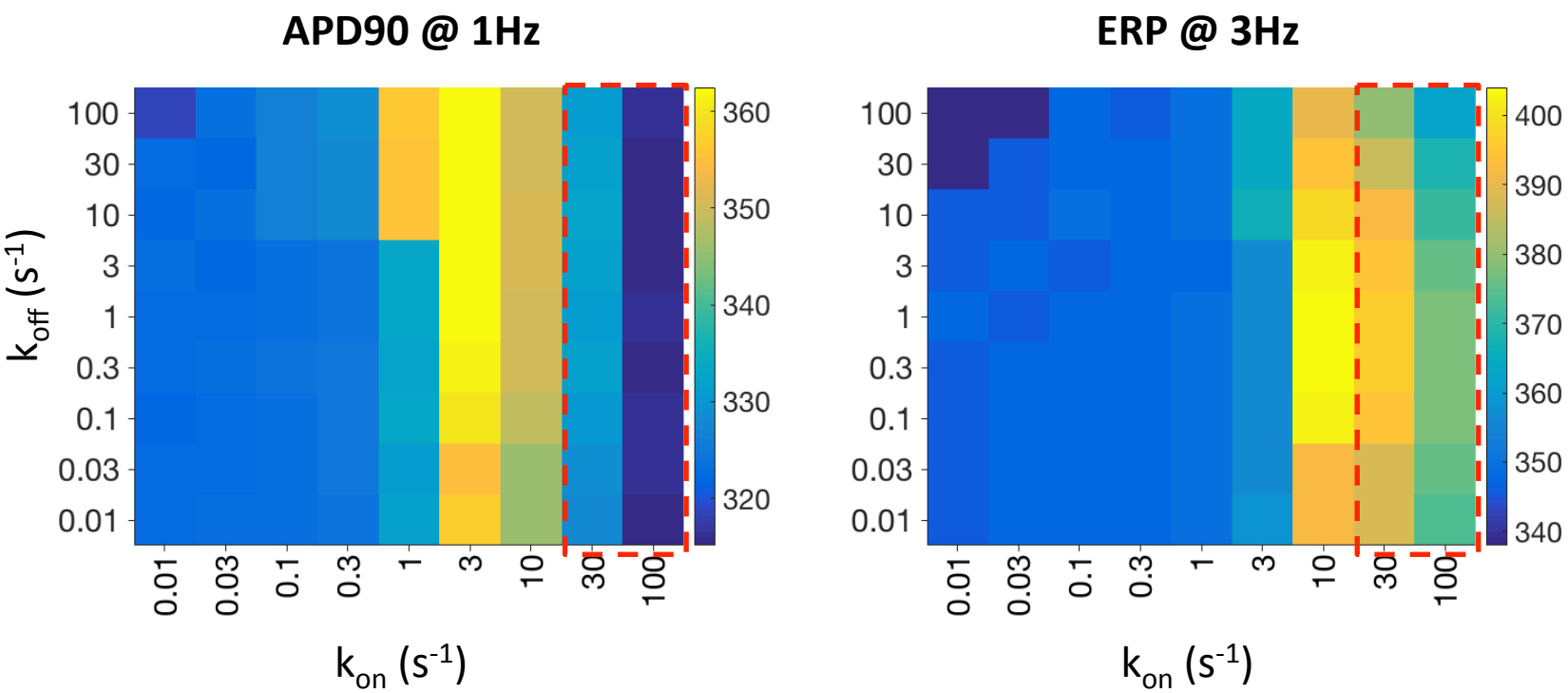
ERP depends on binding kinetics even when using a [drug] equal to the IC_{50} value



Ellinwood et al., *Chaos* 2017

Fast on rates allow for fast pacing rate selectivity

O & I Blocker ($K_O = K_I$)

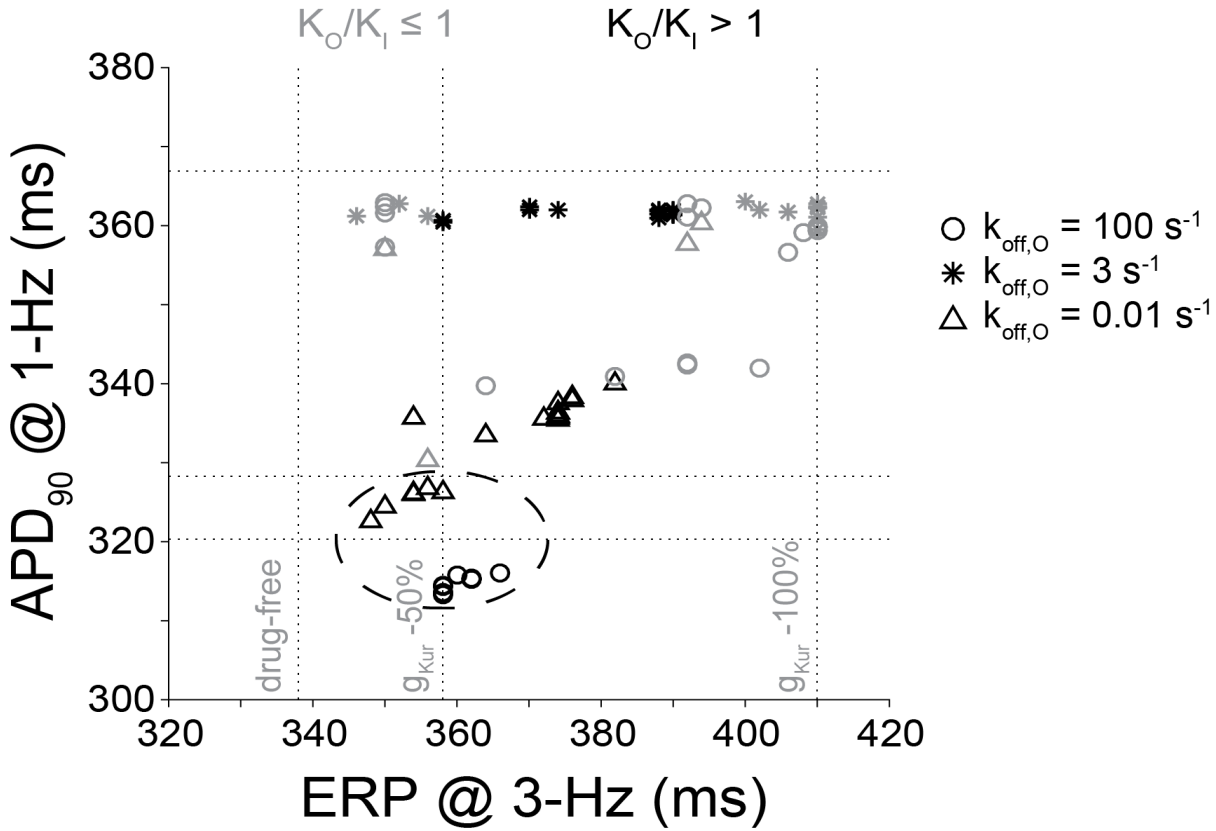


*Best-performing drugs cause ERP prolongation at 3-Hz pacing and limited to no APD prolongation at 1-Hz pacing

Ellinwood et al., *Chaos* 2017

Inactivated state binding and intermediate off rates cause cardiotoxicity

O & I Blocker ($K_O \neq K_I$)



*Best-performing drugs cause ERP prolongation at 3-Hz pacing and limited to no APD prolongation at 1-Hz pacing

Ellinwood et al., *Chaos* 2017

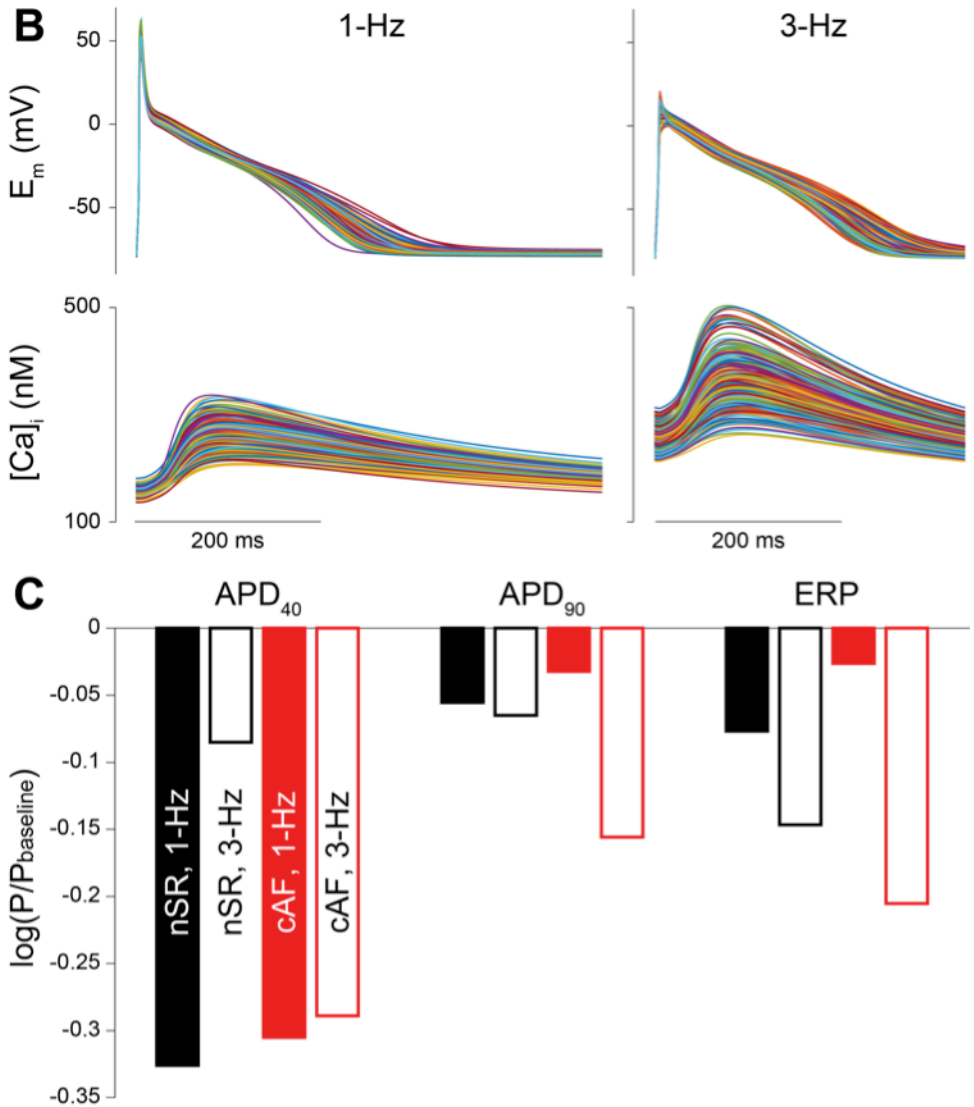
Summary (ii)

- Potency of drugs is highly affected by both state-specific affinity and drug-binding kinetics
- The effects of a particular state-specific I_{Kur} inhibitor on atrial electrophysiology is determined by the relationship between kinetics of channel activation and deactivation during an AP and drug-binding kinetics
- We identified a parameter space in which drugs display anti-AF properties and limited changes in nSR

Given reports that I_{Kur} is diminished in cAF, does I_{Kur} block matter in cAF conditions?

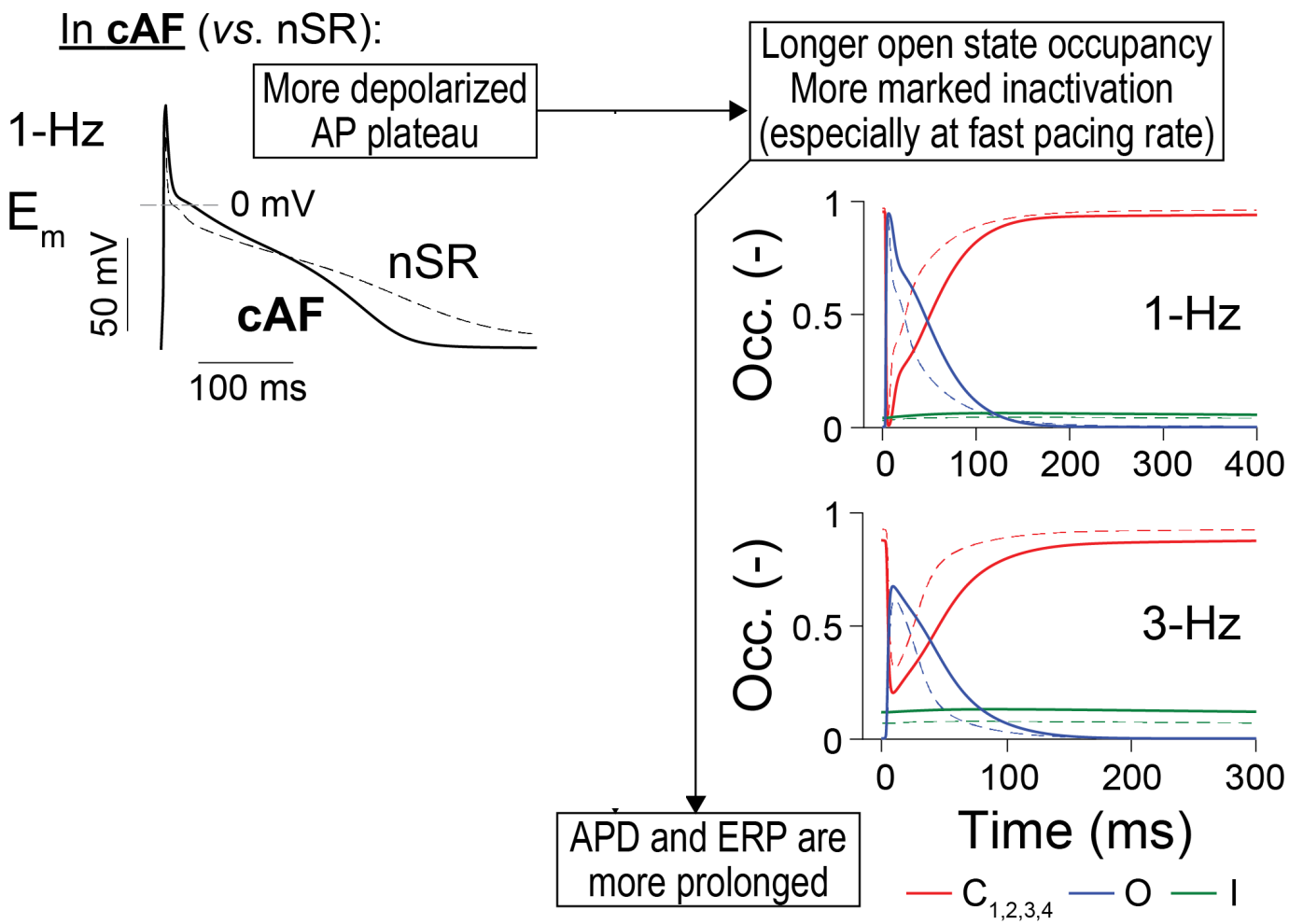
I_{Kur} plays a role in APD and ERP in cAF

- Randomly varied 19 maximum conductances/transport rates in baseline nSR and cAF myocyte models
- Multivariable linear regression analysis (Sobie)
- Outputs: **APD_{40}**, **APD_{90}**, and **ERP**, **CaT amplitude**, **diastolic Ca concentration**, and **CaT time to 50% decay**



Ellinwood et al., *Front Pharmacol* 2017

APD and ERP are more sensitive to I_{Kur} changes in cAF vs. nSR



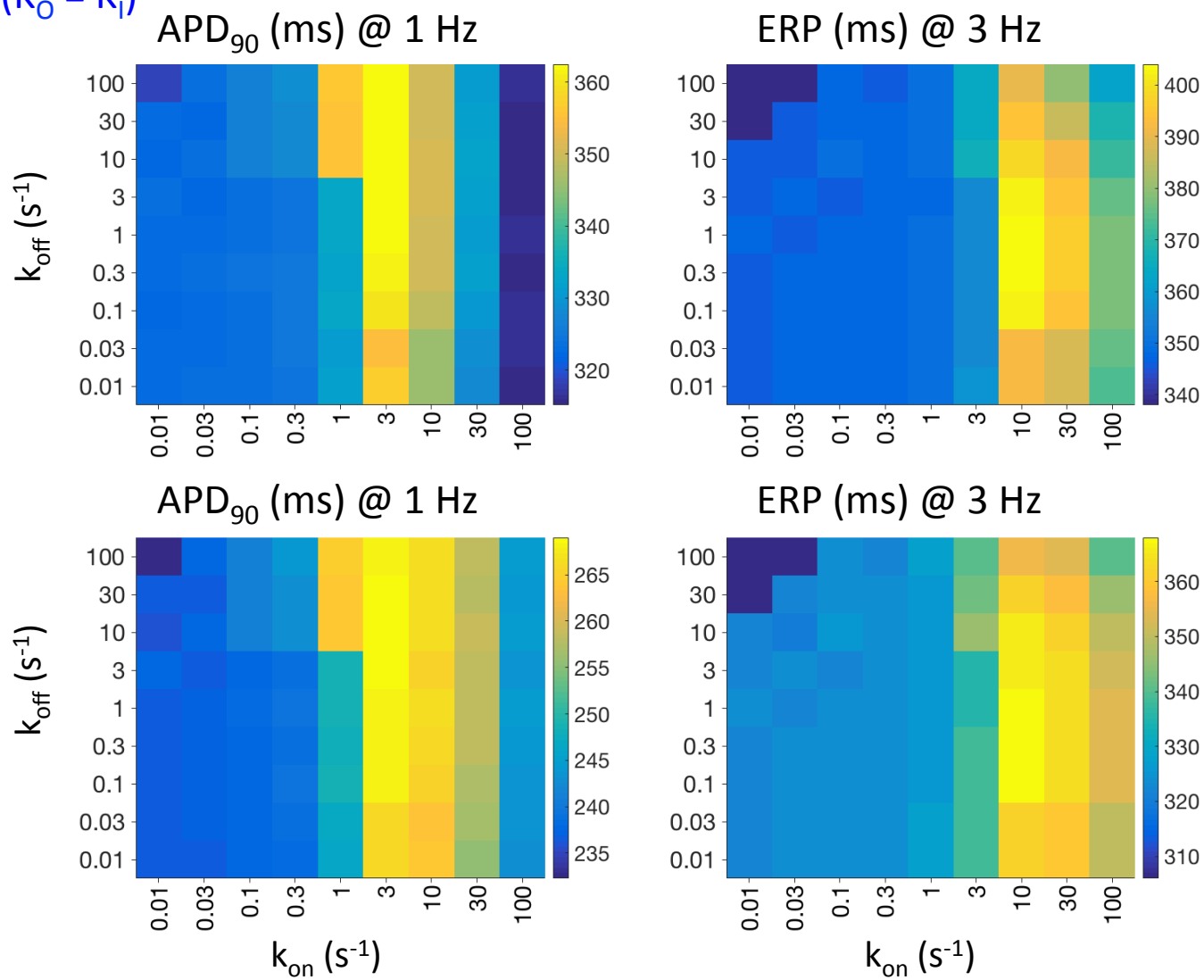
Ellinwood et al., *Front Pharmacol* 2017

Efficacy and safety of I_{Kur} inhibitors in cAF

O & I Blocker ($K_O = K_I$)

nSR

cAF



Ellinwood et al., *Front Pharmacol* 2017

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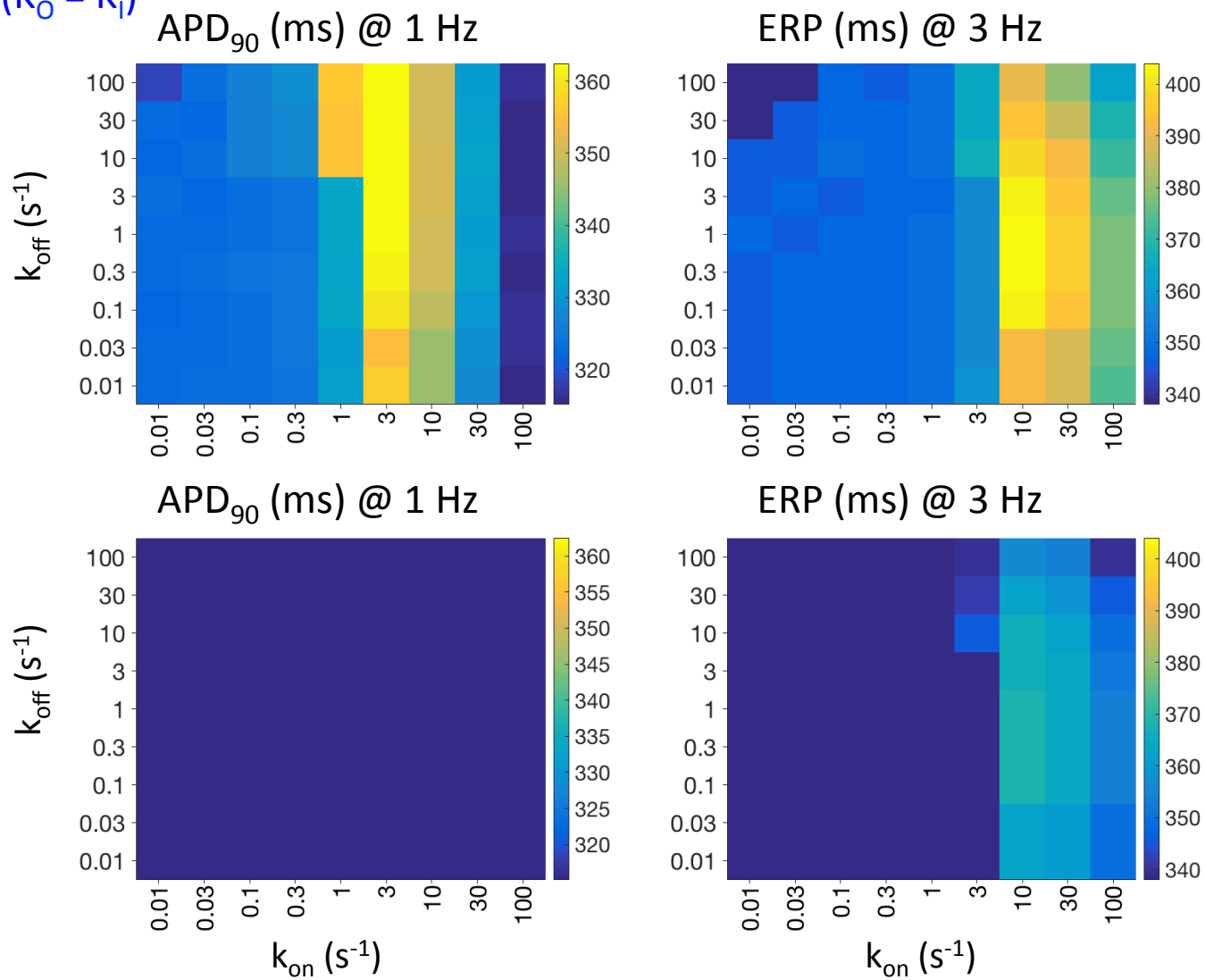

Ellinwood et al., *Front Pharmacol* 2017

Efficacy and safety of I_{Kur} inhibitors in cAF

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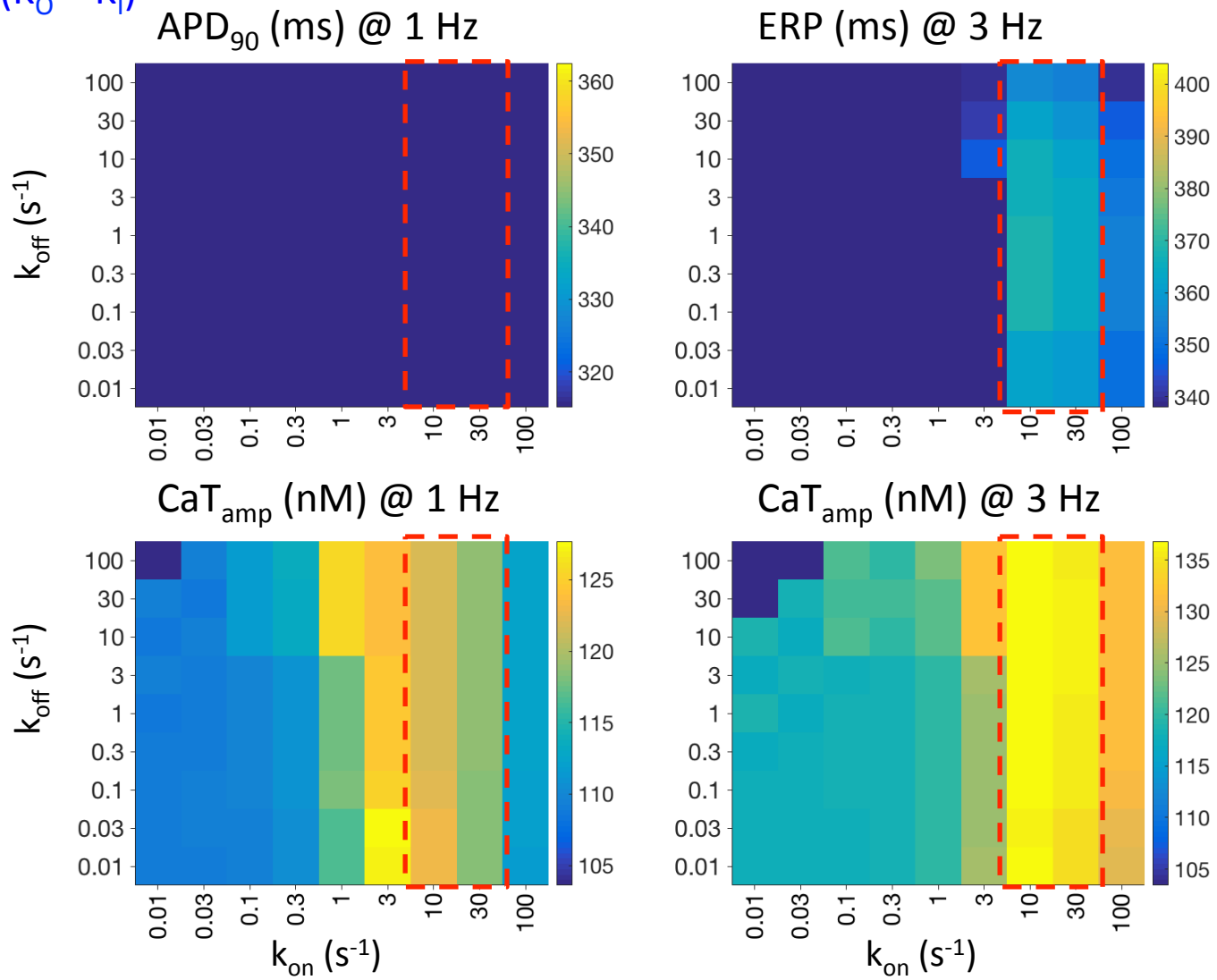
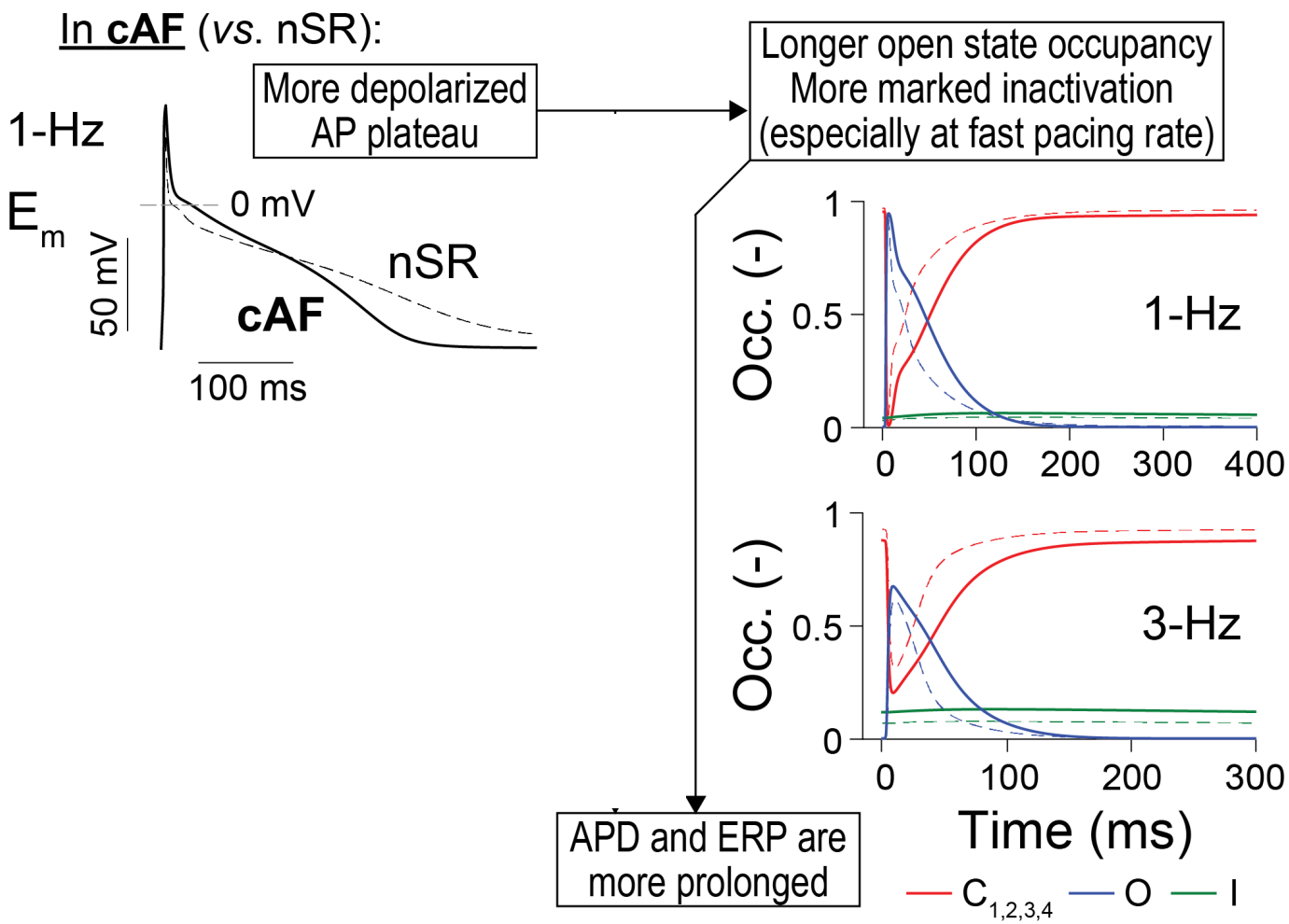


Ellinwood et al., *Front Pharmacol* 2017

Efficacy and safety of I_{Kur} inhibitors in cAF vs. nSR



Ellinwood et al., *Front Pharmacol* 2017

Summary (iii)

- I_{Kur} is a promising target in cAF conditions, as despite being downregulated it impacts atrial EP parameters more than in nSR

- I_{Kur} inhibitors are more potent in cAF vs. nSR, and pose less safety concerns

- But is the extent of ERP prolongation in cAF sufficient to prevent arrhythmia?

AF-selective pharmacotherapy

Atrium-selective (*vs.* ventricle)

* **Atrium-specific K channel expression** (I_{Kur}, $I_{K,Ca}$, $I_{K,ACh}$, I_{TASK})

* **Atrio-ventricular differences in AP properties** (depolarized RMP, negative plateau, faster phase-1 and slower phase-3 repolarization)

* **Atrio-ventricular differences in I_{Na}**

High rate-selective (*vs.* nSR)

* **State-dependent block** (open vs. inactivated)

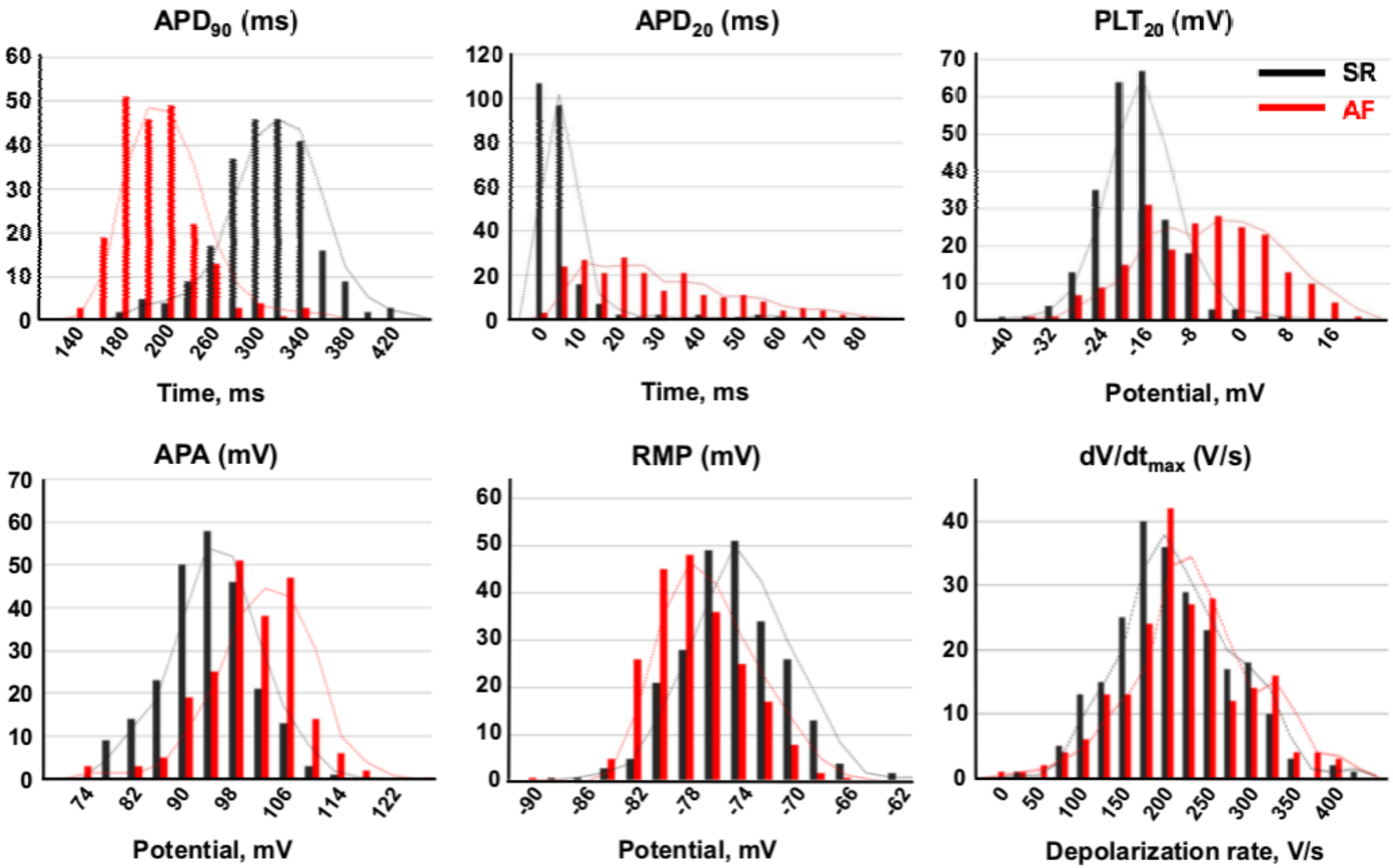
Haibo Ni

Stefano Morotti

- To test (**synergistic**) anti-AF effects of **<u>combined block</u>** of atrial-selective currents
- I_{Kur}, $I_{K,Ca}$, and $I_{K2P(TASK)}$

Grandi and Maleckar, *Pharmacol Therap* 2016
Ellinwood et al., *Chaos* 2017 & *Front Pharmacol* 2017

Population of human atrial models calibrated with Ravens database

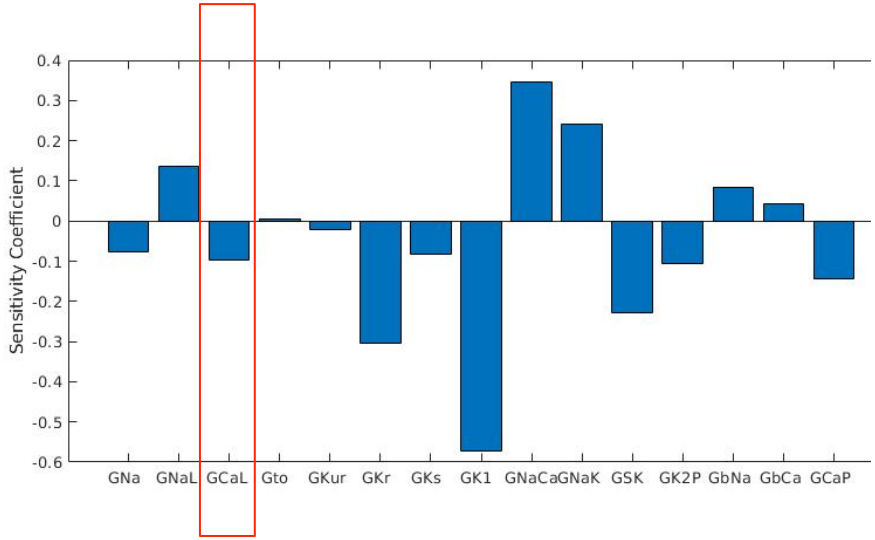


Ravens et al., Med Biol Eng Comput. *2015*

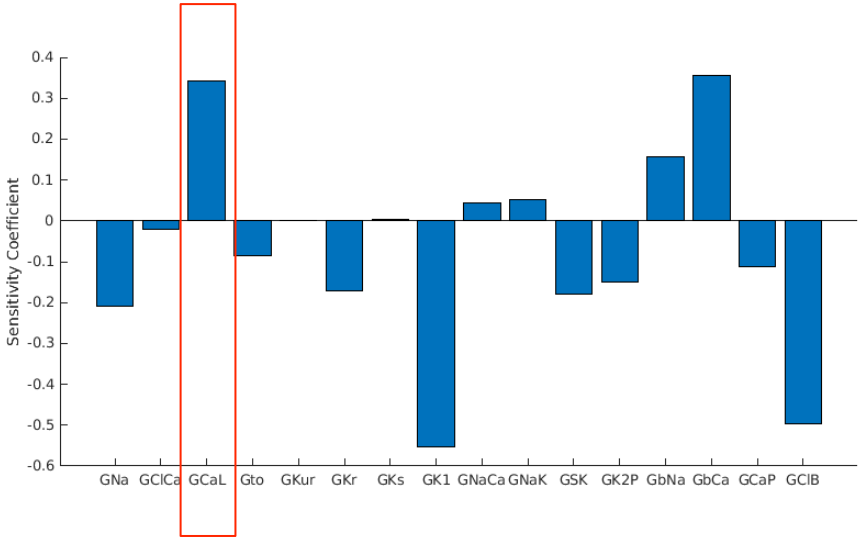
Sensitivity analysis reveals differences in notch and dome vs. triangular AP models

- APD_{90}

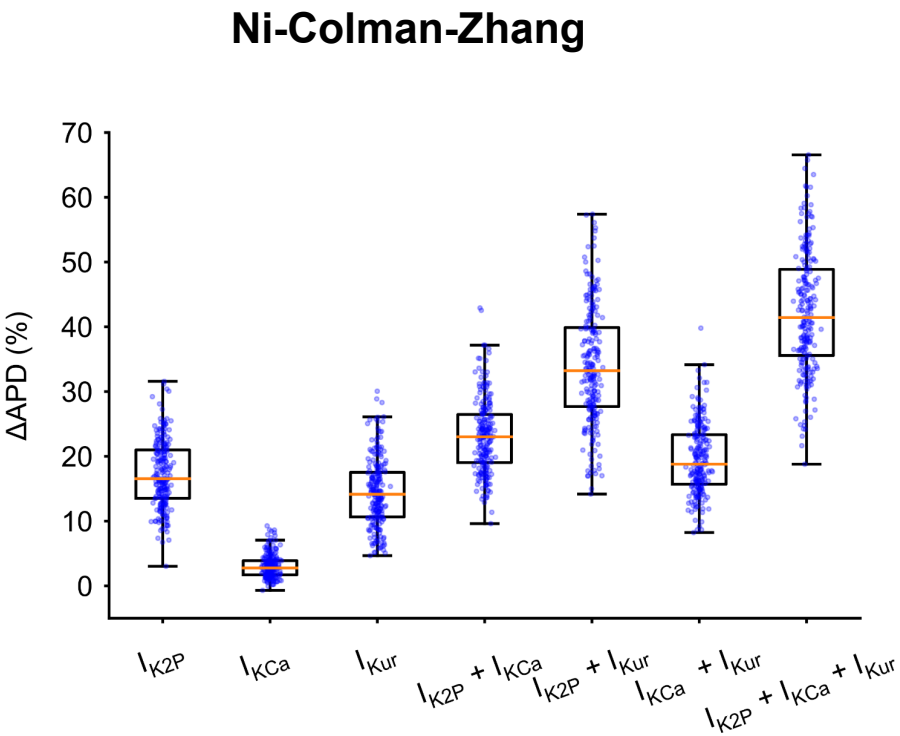
Ni-Colman-Zhang model

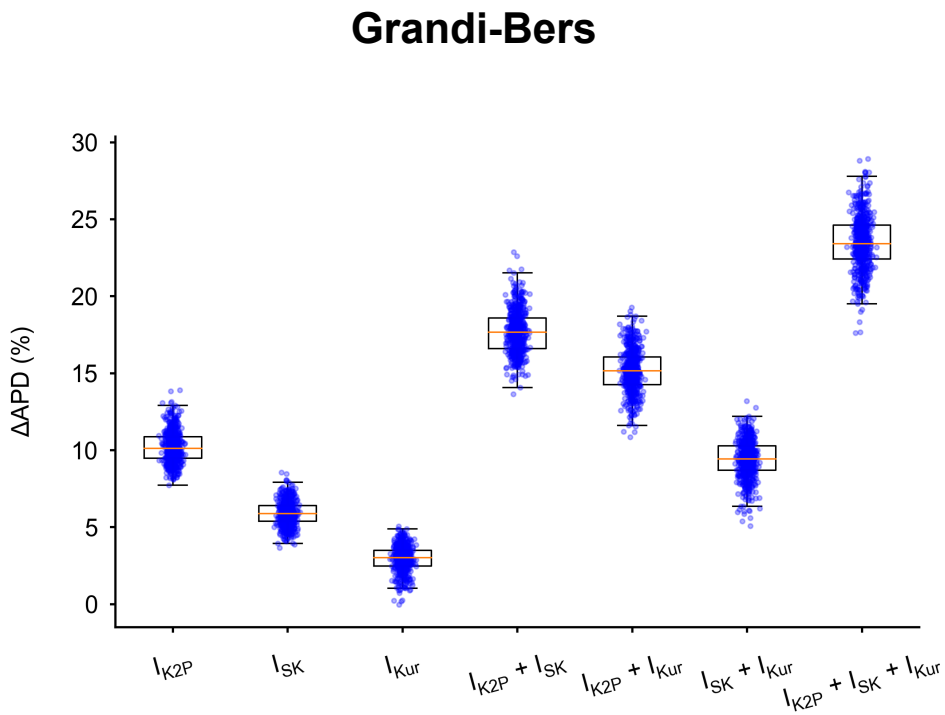


Grandi-Bers model



Single cell APD prolongation: both AP models suggest synergistic APD prolongation





Single cell simulations - APD: both AP models suggest synergistic APD prolongation

- Synergy in APD prolongation
- AB > A + B
- Two way ANOVA (interaction term)
- Linear contrast

1 Hz

AF model index		0	1	2	3	4	5	6	7	8	9	10	11
Significance level of synergy	$I_{K2P} + I_{KCa}$	***	*	***	***	***	***	**		***	*	***	***
	$I_{K2P} + I_{Kur}$								*		**		**
	$I_{KCa} + I_{Kur}$	#	*	*	**	**	**			*		**	*
	$I_{K2P} + I_{KCa} + I_{Kur}$			*	*	***	***			#		**	
Average increase in APD prolongation due to synergy: $\Delta APD\downarrow Combined\ Block - \sum\uparrow \Delta APD\downarrow Individual$		2.5%	3.7%	5.9%	7.1%	10.3 %	10.9 %	2.2%	-2.8 %	5.9%	0.2%	10.1 %	4.2%

Synergy # P < 0.1 * P < 0.05 ** P < 0.01 *** P < 0.001 **Competition** * P < 0.05 ** P < 0.01

Single cell simulations - APD: both AP models suggest synergistic APD prolongation

- NCZ model: Synergy in APD prolongation seen in all models, contributing to 13% to 32% prolongation compared to additive effects
- Similar results demonstrated in GB model

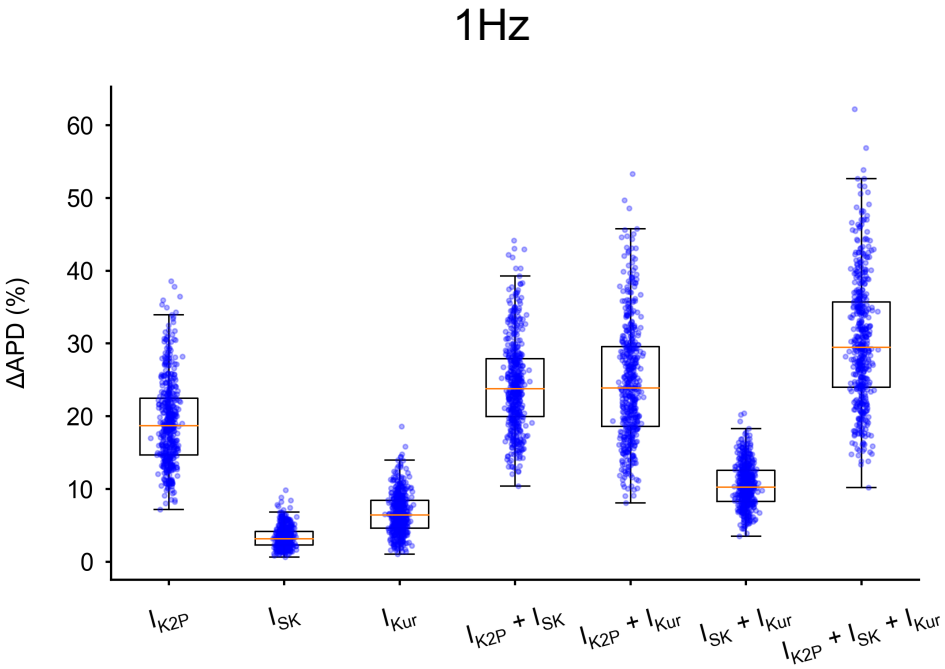
3 Hz

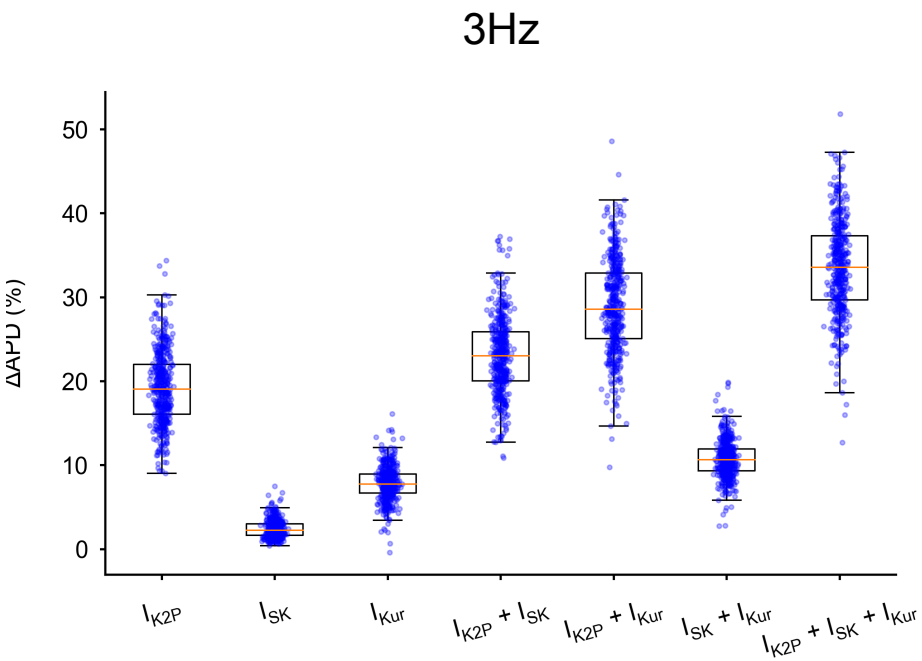
AF model index		0	1	2	3	4	5	6	7	8	9	10	11
Significance level of synergy	$I_{K2P} + I_{KCa}$	**	**	***	***	***	***	***	*	***	**	***	***
	$I_{K2P} + I_{Kur}$	**	***	**	***	**	***	*	**	*	**	#	
	$I_{KCa} + I_{Kur}$	*	**	**	***	***	***	*	#	**	*	***	***
	$I_{K2P} + I_{KCa} + I_{Kur}$	***	***	***	***	***	***	***	***	***	***	***	***
Average increase in APD prolongation due to synergy: $\frac{\Delta APD_{Combined\ Block} - \sum \Delta APD_{Individual\ Block}}{\sum \Delta APD_{Individual\ Block}}$		24.8%	16.8%	29.0%	20.7%	31.7%	26.2%	14.8%	13.9%	18.6%	16.1%	24.6%	19.6%

Synergy # P < 0.1 * P < 0.05 ** P < 0.01 *** P < 0.001 **Competition** * P < 0.05 ** P < 0.01

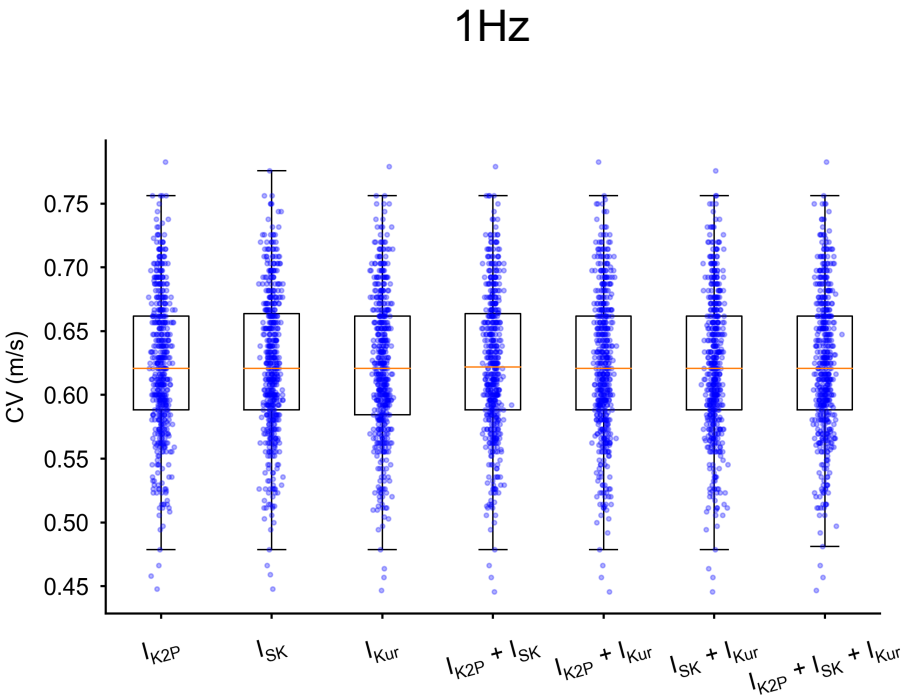
1D simulations - APD: synergistic APD prolongation

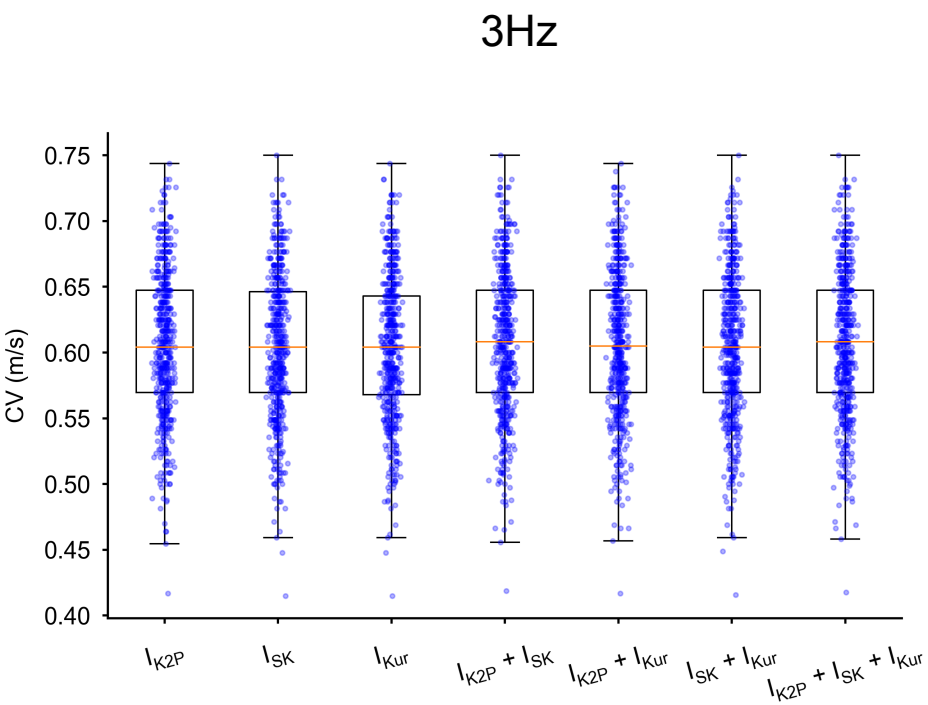
S1 pacing 1D strand model, 150 nodes





1D simulations – conduction velocity: no effects





Summary (iv)

- Depending on AF-remodeling induced modulations on atrial-predominant K currents (I_{Kur}, I_{K2P} and I_{KCa}), their combined block can synergistically prolong APD at 1 Hz
- At 3 Hz, simultaneous block of atrial-predominant K currents exerts significant synergistic APD prolongation in AF-remodeled atrial cells, suggesting that the ***synergy in APD prolongation is rate-selective***
- Combined block of atrial-selective currents may be a valuable strategy for the pharmacological management of AF

Acknowledgments

UC DAVIS

Grandi Lab
Dr. Stefano Morotti
Dr. Haibo Ni
Dr. Nick Ellinwood
Lin-Lin Liu

Clancy Lab
Vorobyov Lab
Lewis Lab
Dr. Nip Chiamvimonvat

UC San Diego

Dr. Andrew McCulloch

simula

Dr. Andy Edwards

UNIVERSITÄT DUISBURG ESSEN

Dr. Dobromir Dobrev

BILL BERTKEN SUDDEN DEATH PREVENTION

National Heart Lung and Blood Institute

American Heart Association

Heart Rhythm Society