KING'S College LONDON

Importance of AOPs in Next Generation Risk Assessment

The case study of cardiotoxicity

Dr Luigi Margiotta-Casaluci

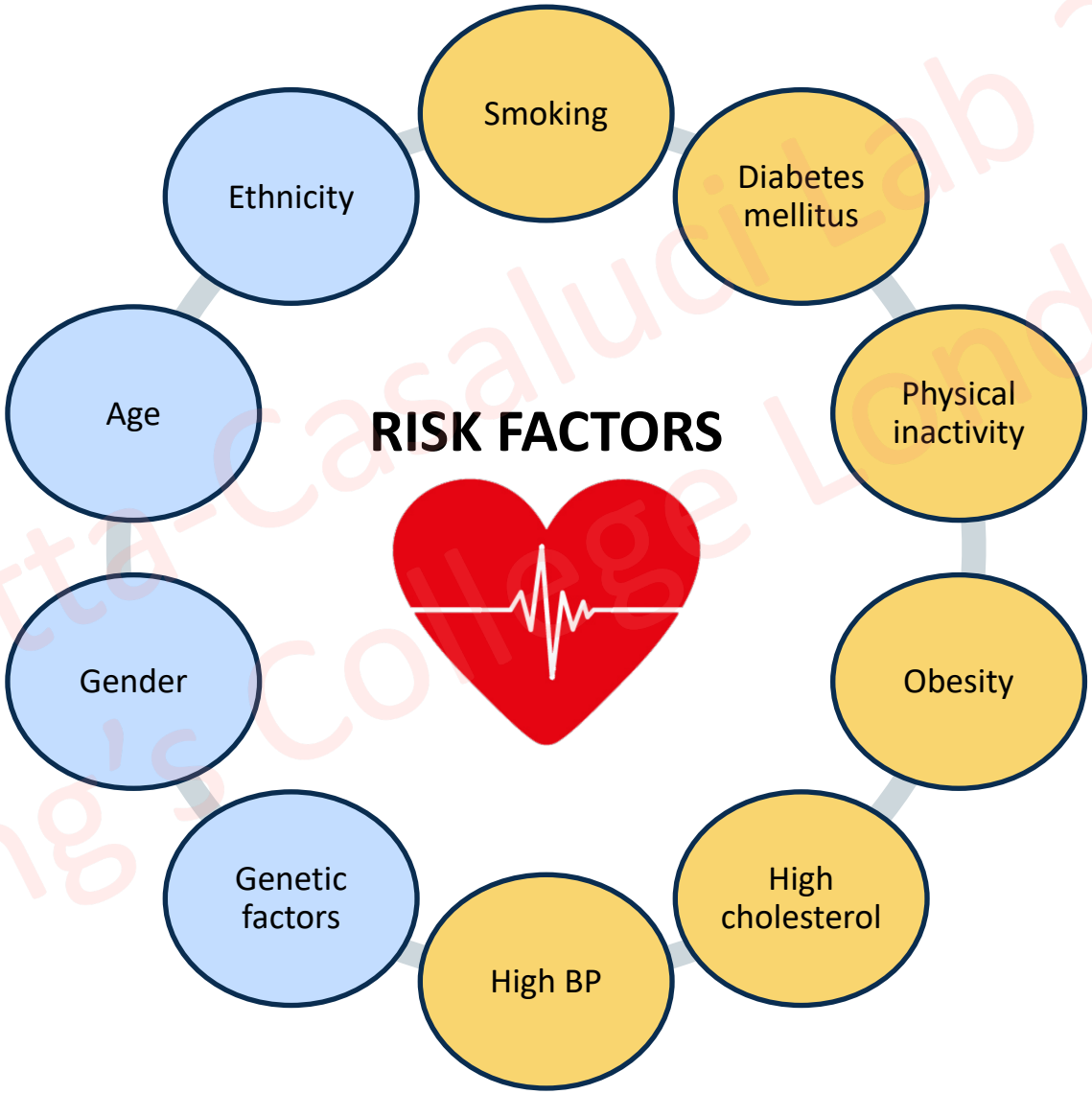
24 February 2022, London, UK

NC3Rs-BTS-HSE CRD Workshop

Increasing confidence in New Approach Methodologies for regulatory decision-making

NC 3Rs

Cardiovascular diseases are the leading cause of death globally



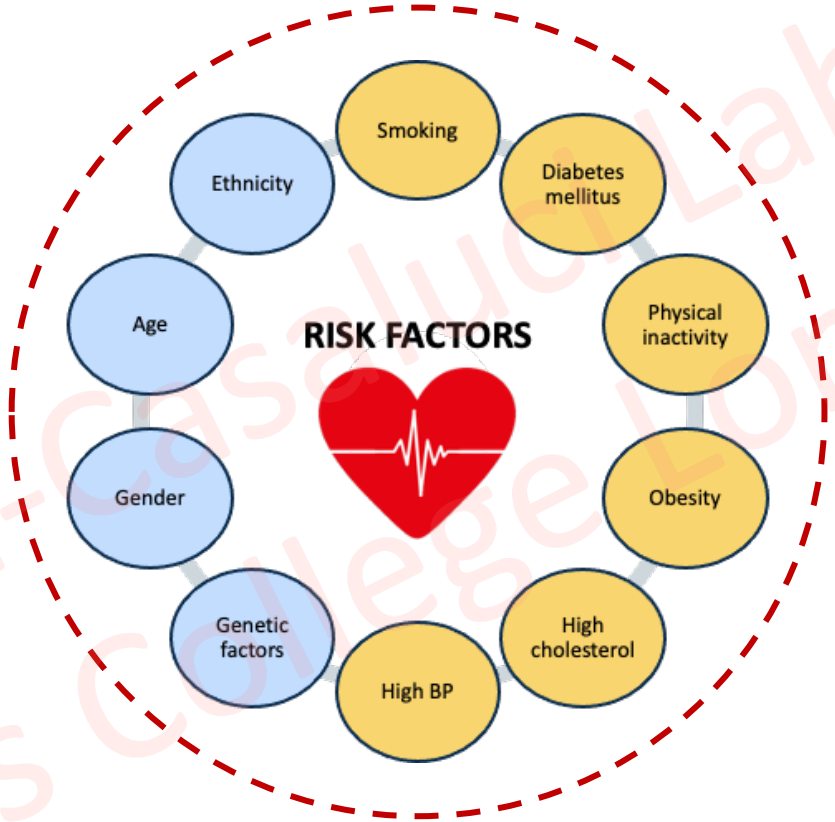
Chemicals represent additional modifiable factors affecting CVD risk

Intended
cardiovascular effects

PHARMACEUTICAL COMPOUNDS

Unintended
cardiovascular effects

Cost-benefit assessment scenario
E.g. Is the side effect tolerable?

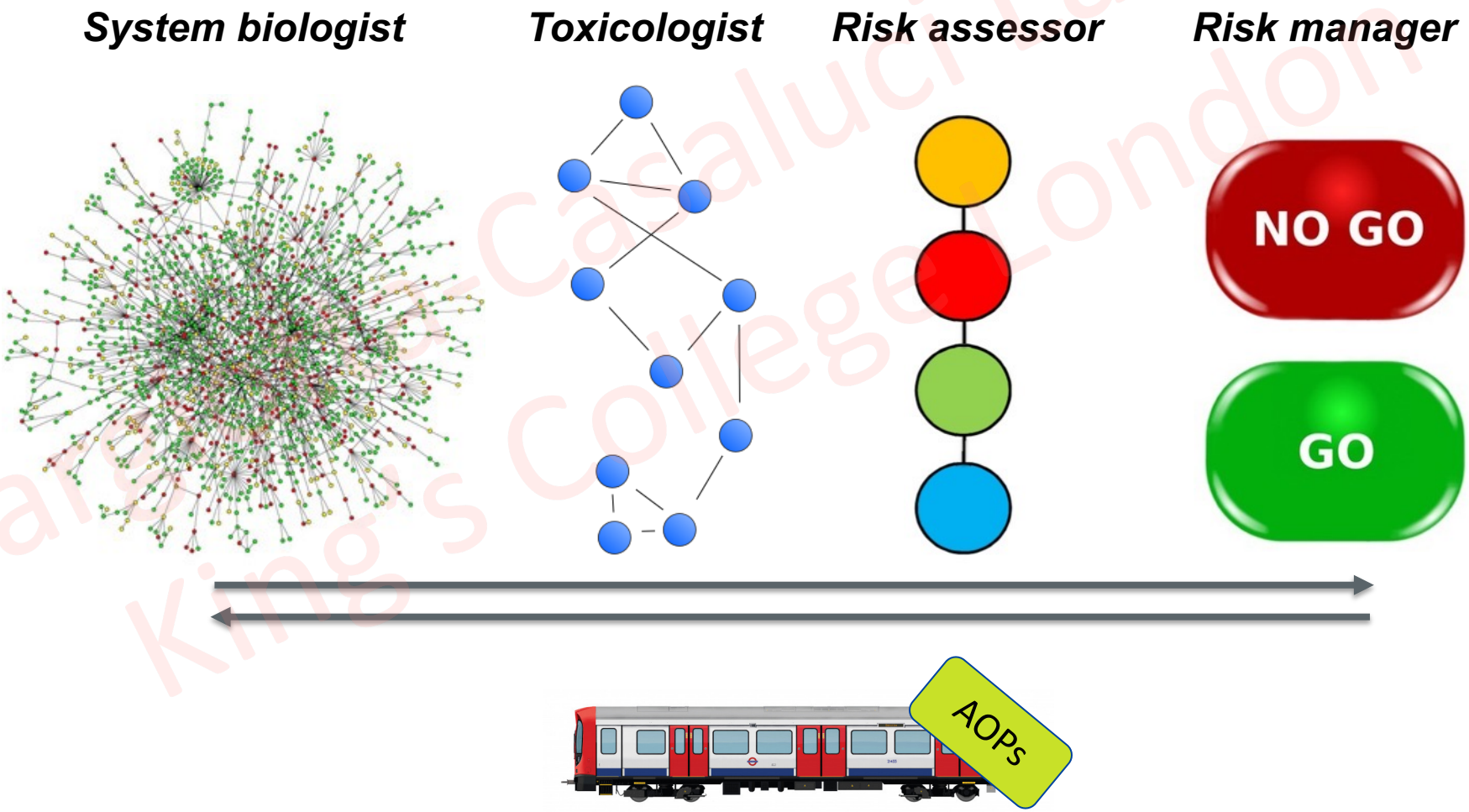


NON-PHARMACEUTICAL CHEMICALS

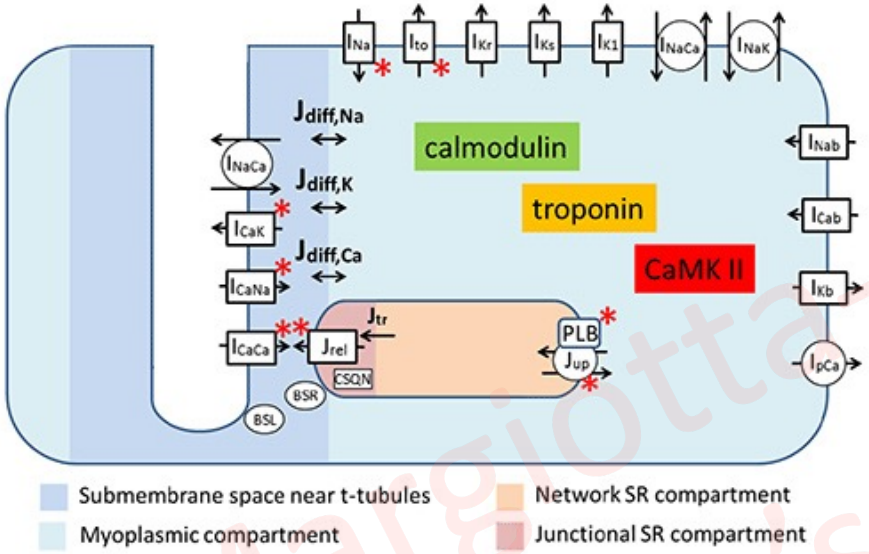
Unintended
cardiovascular effects

Cost-benefit assessment scenario
Never tolerable

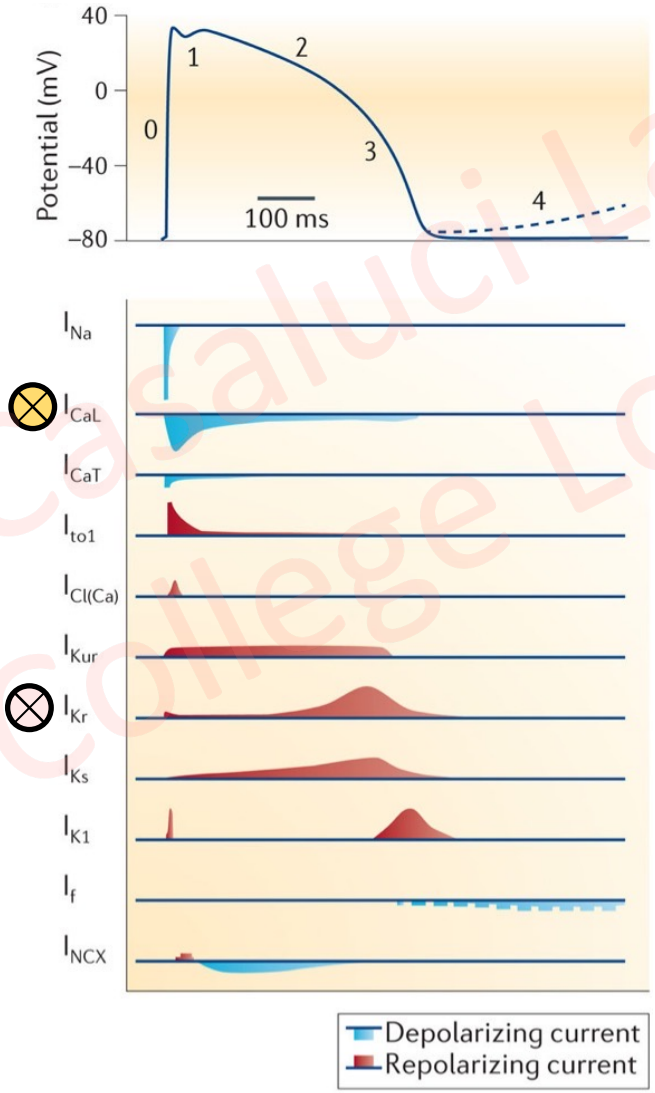
How can (apparently simple) AOPs help us to navigate highly complex toxicological scenarios?



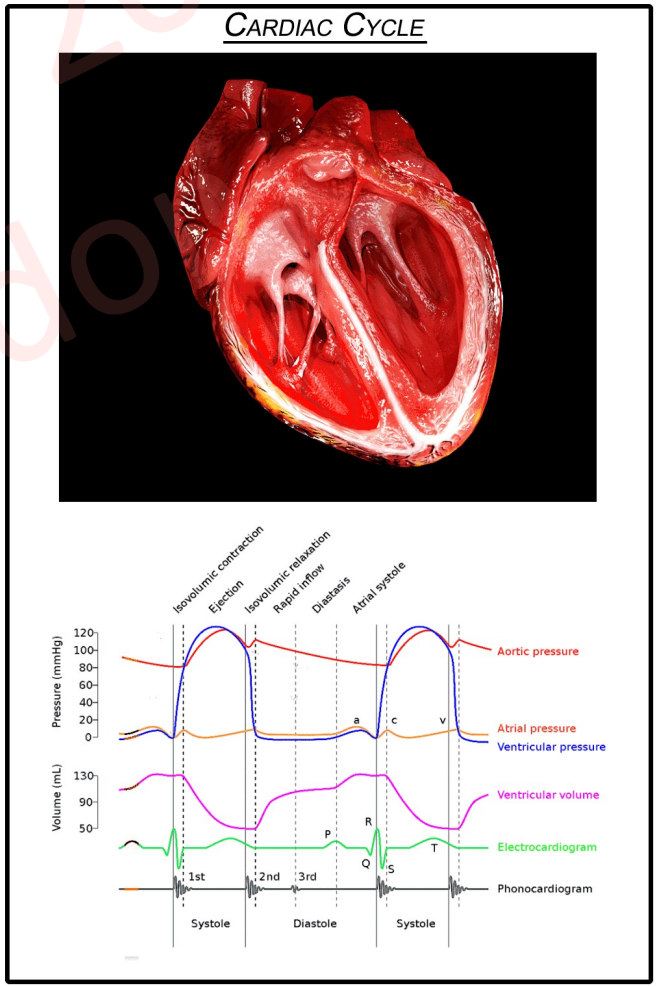
Mapping cardiotoxicity pathways - Going beyond hERG



O'Hara et al. 2011. PLoS Comput Biol 7(5): e1002061



Gintant et al. 2016
Nature Reviews Drug Discovery 15, 457–471

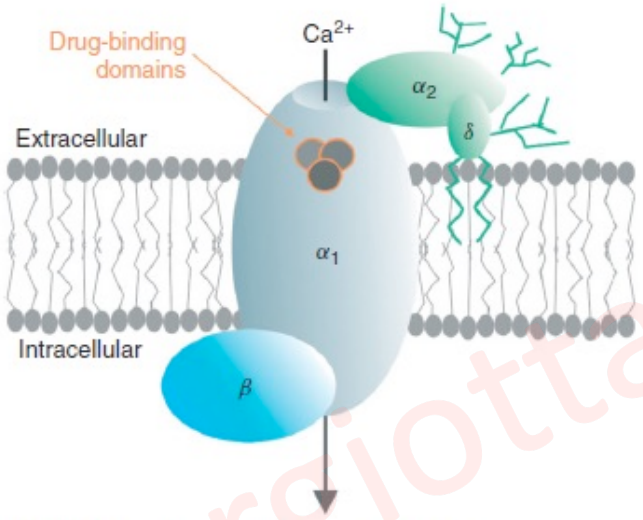


AOP development – MIE and AO

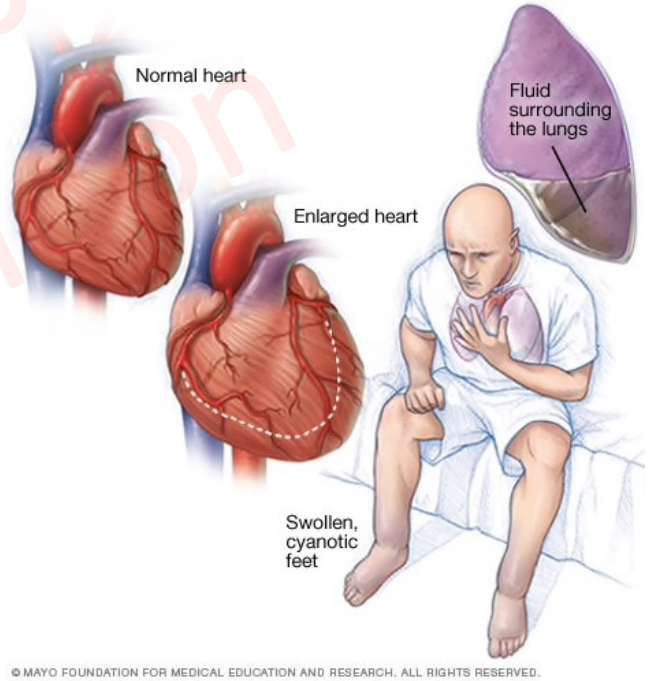
L-type calcium channels (LTCCs) blockade

?

Heart failure



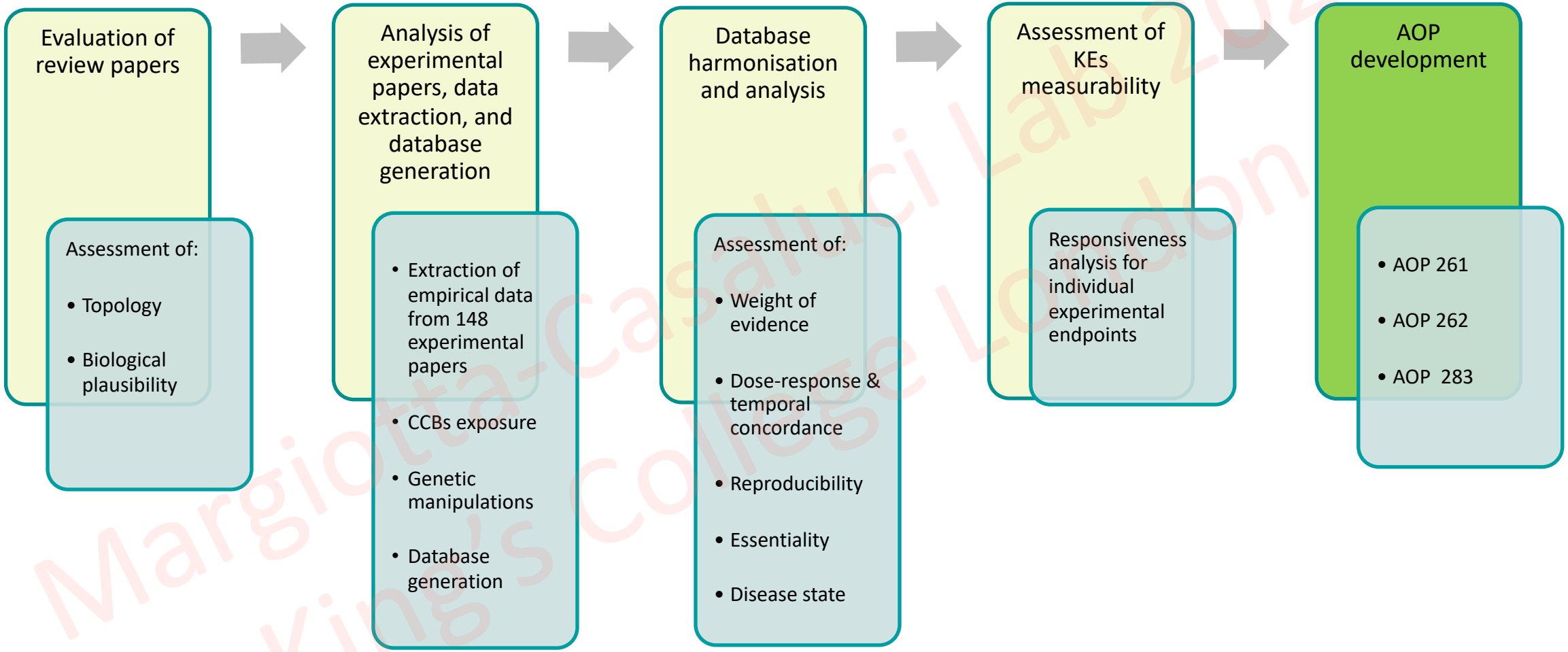
KE1 → KE2 → KE_n



Striessnig et al. 2014;
Wiley Interdiscip Rev Membr Transp Signal. 3(2): 15–38.

- 920,000 adults are living with heart failure in the UK (*26 million people worldwide*)
- 200,000 new diagnoses of heart failure every year
- Estimated cost - £2bn per year in England (2% of the total NHS budget) (NICE, 2018)

AOP development workflow



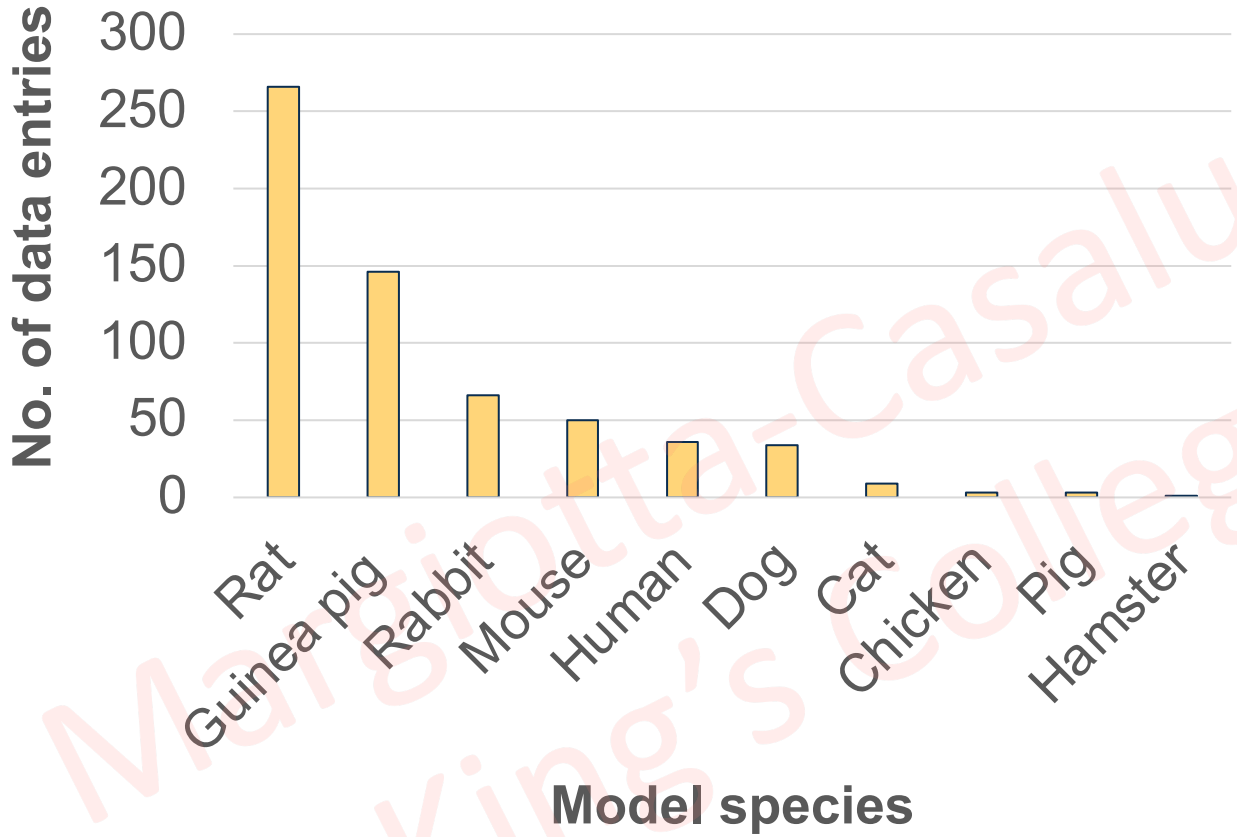
AOP 261 - L-type calcium channel blockade leading to the disruption of cardiac electrophysiology
AOP 262 - L-type calcium channel blockade leading to heart failure via decrease in cardiac contractility
AOP 283 - L-type calcium channel blockade leading to hypotension

Literature review and data extraction

CCBs exposure studies

Drug	Class	Total no. of data points	Affinity to LTCC (1C) (Lowest Ki, nM)*	Species	Data source
Nifedipine	Dihydropyridine CCB	345	0.5	Rat	ChEMBL
Amlodipine	Dihydropyridine CCB	114	20	Rat	ChEMBL
Felodipine	Dihydropyridine CCB	14	0.053	Rat	ChEMBL
Nisoldipine	Dihydropyridine CCB	2	0.476	Rat	ChEMBL
Nimodipine	Dihydropyridine CCB	2	0.156	Rat	ChEMBL
Nitrendipine	Dihydropyridine CCB	1	0.246	Rat	ChEMBL
Diltiazem	Benzothiazepine CCB	123	16 nM	Rat	ChEMBL
Verapamil	Phenylalkylamine CCB	272	12 nM	Rat	ChEMBL
Fendiline	Phenylalkylamine/non-selective CCB	17	17000 (**IC50, Ki n/a)	Rat	ChEMBL
Mibefradil	Non selective CCB	44	156 nM (**IC50, Ki n/a)	Human	ChEMBL

Most common model species in our database & endpoint classification

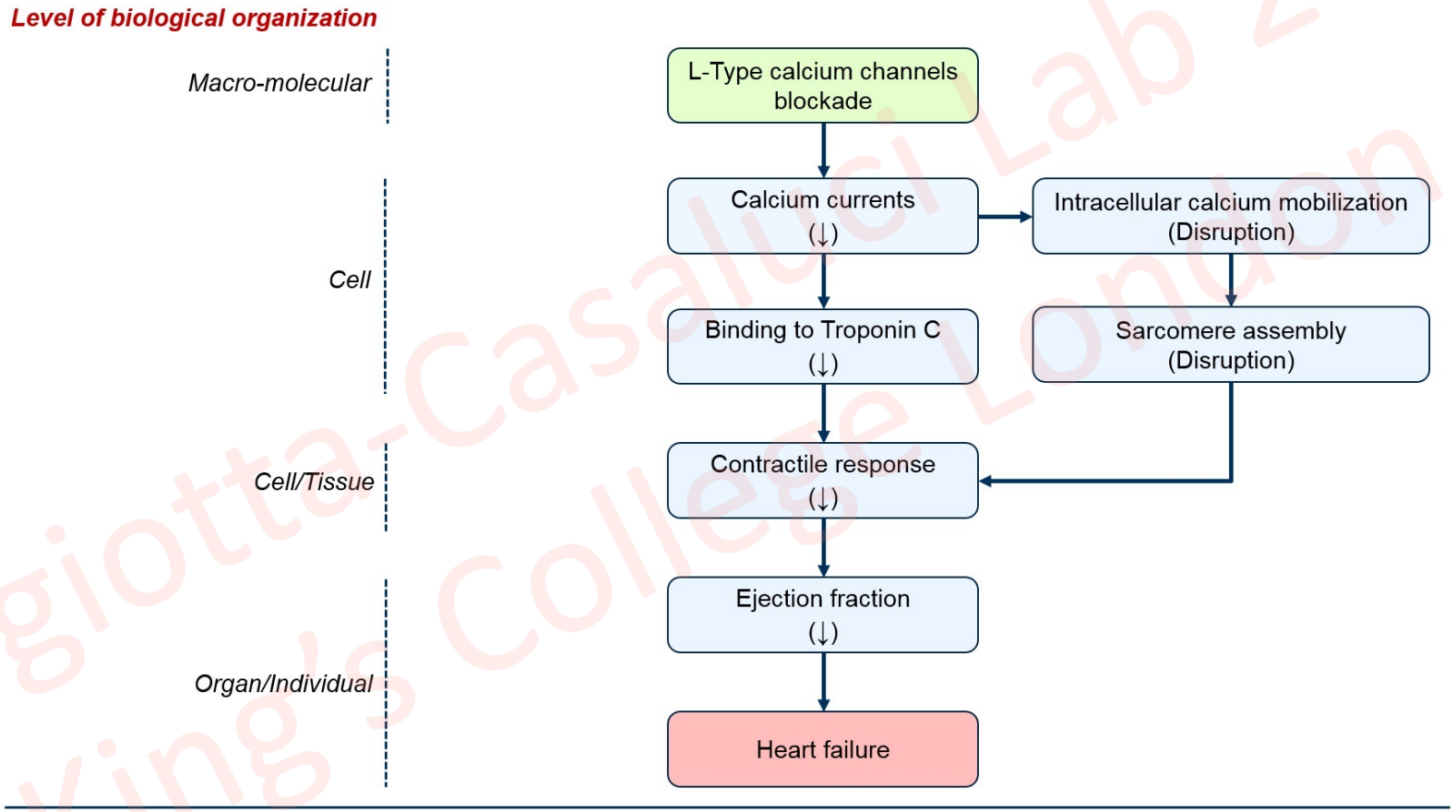


Assay type	No. of data entries
In vivo	81
Ex vivo	153
In vitro	373

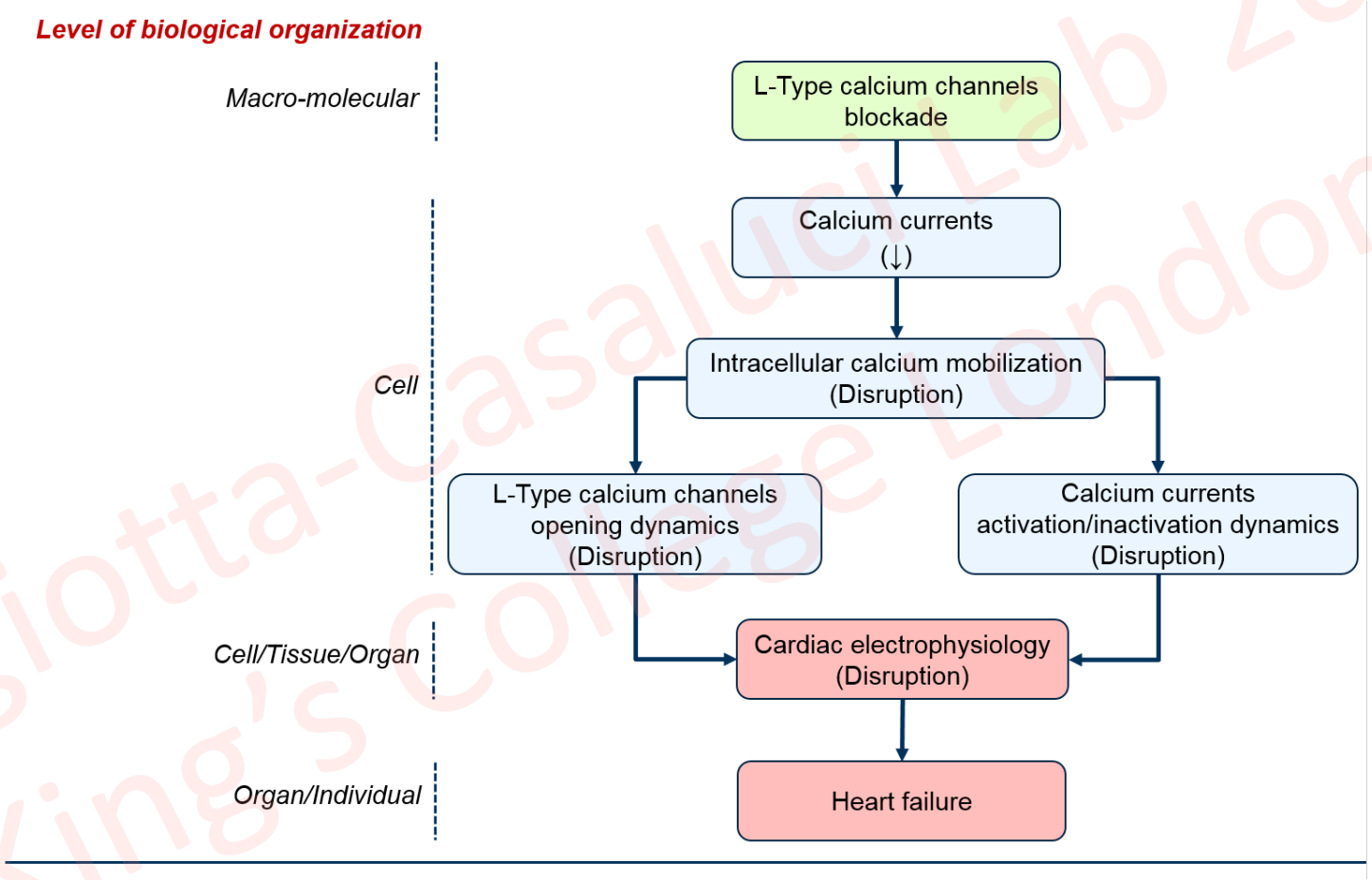
Example of extracted data

Reference, Species, Model info, Drug, Details of genetic manipulation, Effect/No Effect concentration, Dose-response concordance, Quantification method, Exposure duration

AOP 261: Disruption of cardiac contractility

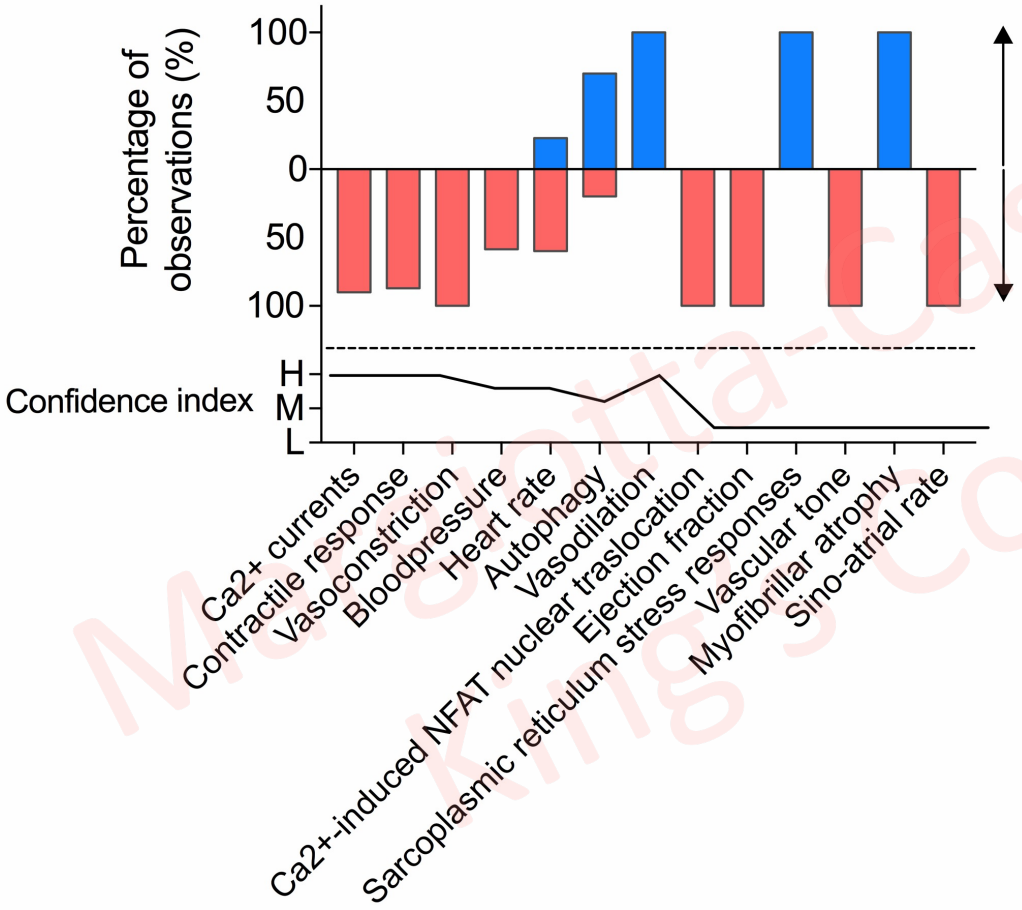


AOP 262: Disruption of cardiac electrophysiology

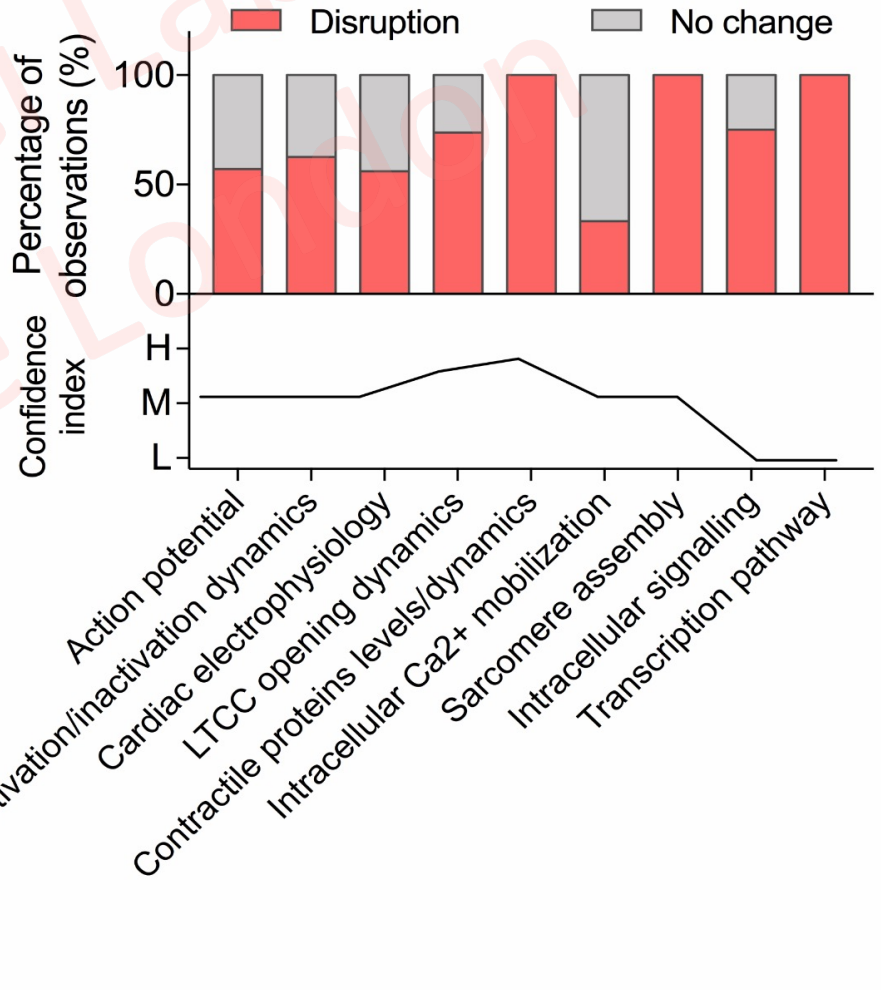


Effect direction and confidence assessment of KEs

Bidirectional KEs

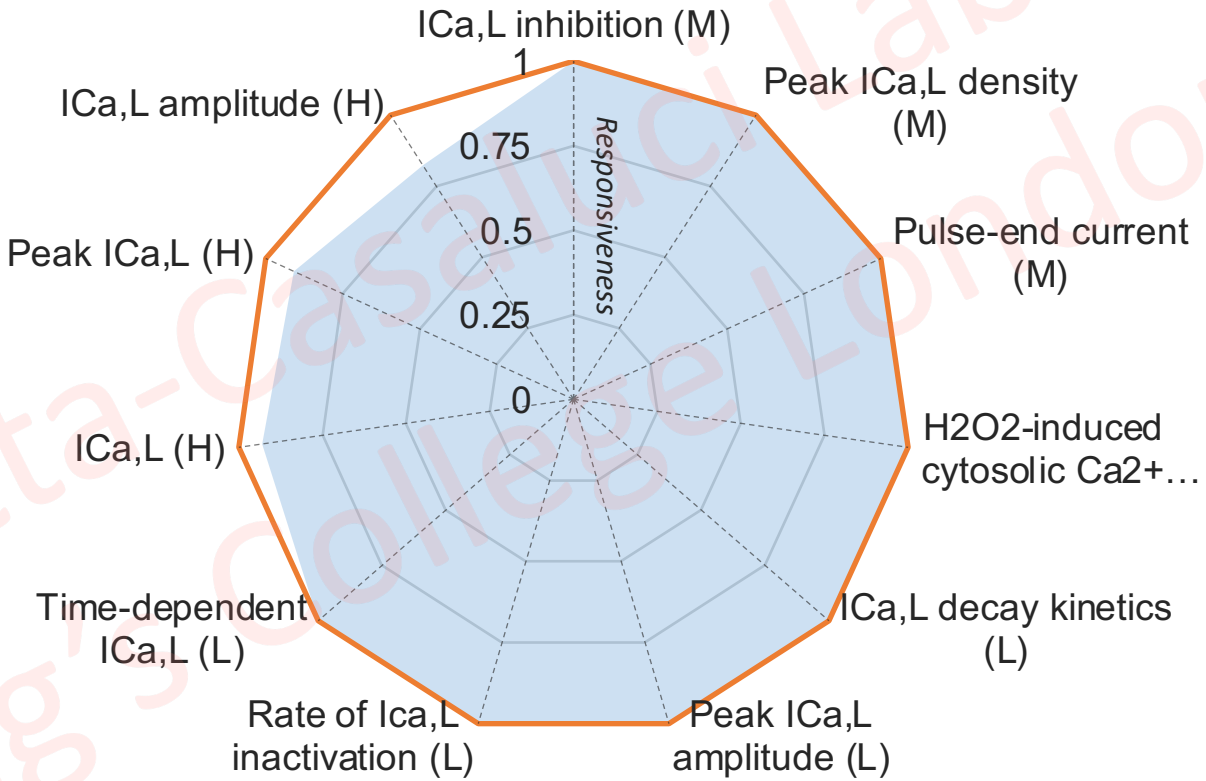


Non-Bidirectional KEs



Responsiveness analysis of experimental parameters

KE: Calcium current, Decrease

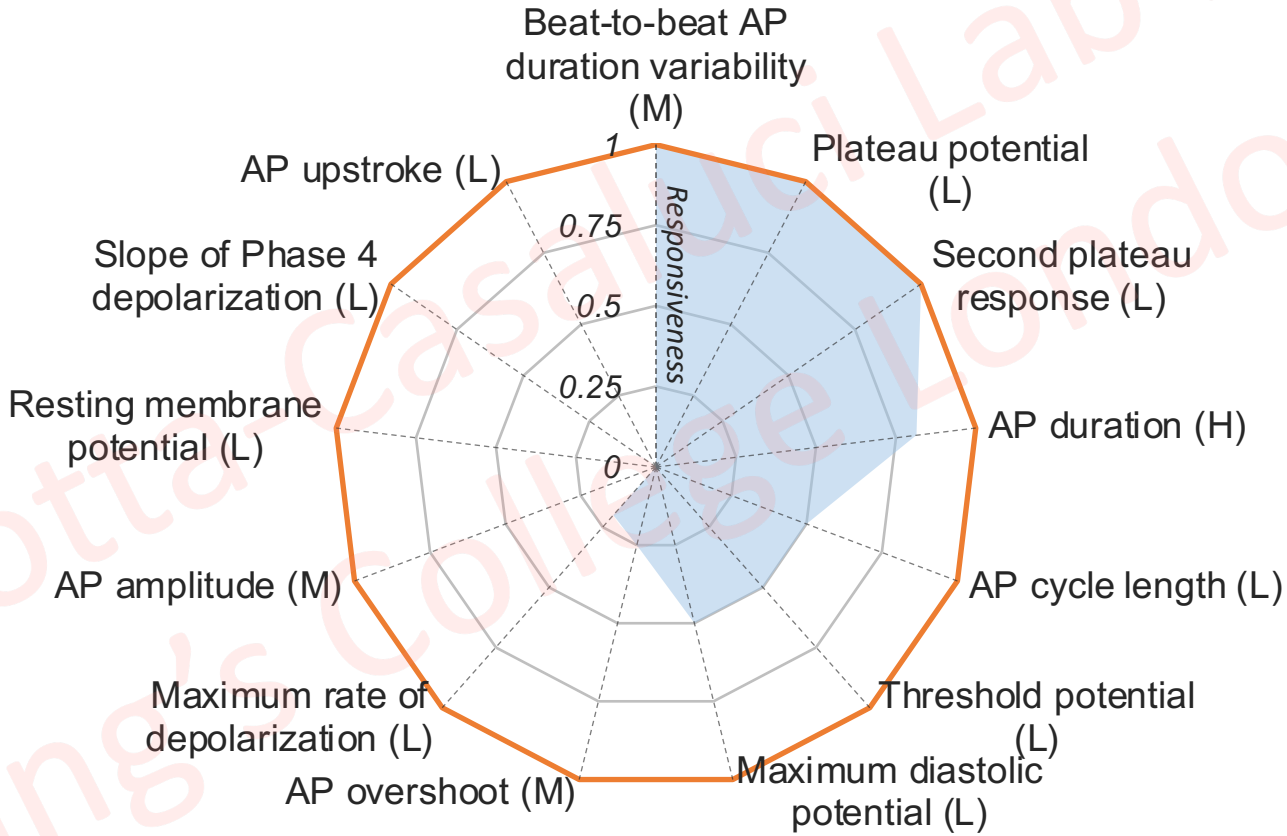


Total no. of data points = 91

Nifedipine: 37
Verapamil: 25
Diltiazem: 17
Fendiline: 6
Felodipine: 3
Amlodipine: 2
Semotiadil: 1

Responsiveness analysis of experimental parameters

KE: *Action potential, Disruption*



Total no. of data points = 65
Nifedipine: 43
Amlodipine: 12
Verapamil: 10

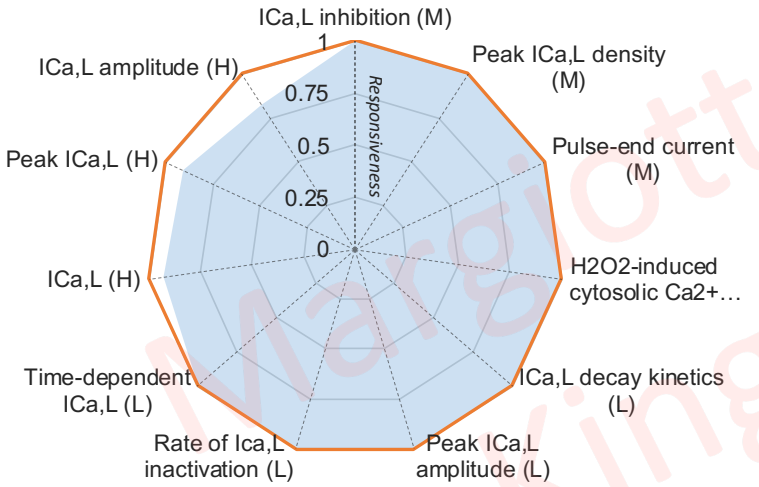
A more informative visualisation of AOPs

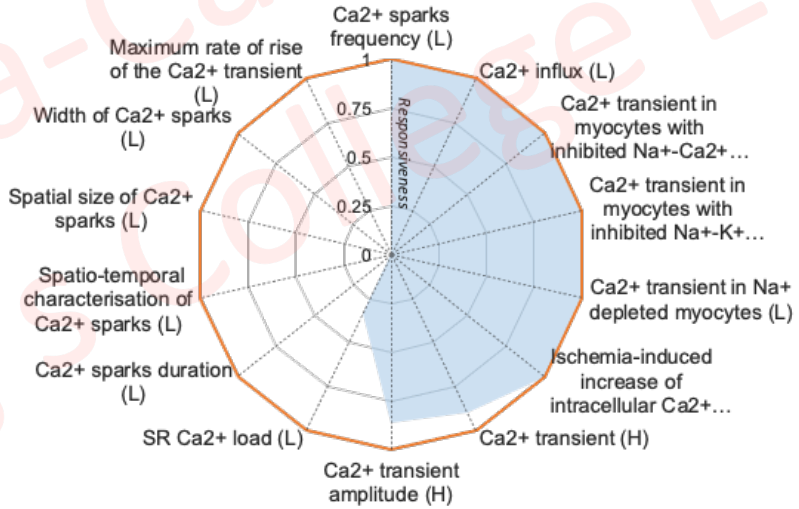
L-type calcium channel blockade

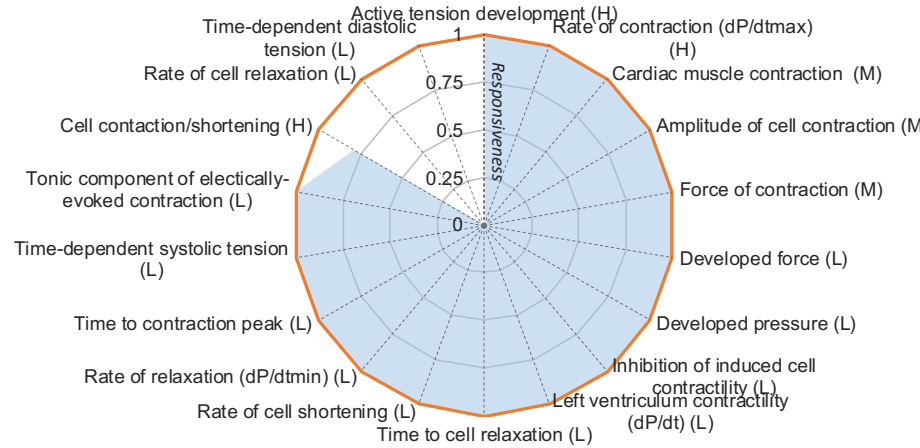
Calcium currents decrease

Disruption of intra-cellular calcium mobilization

Decrease in cardiac contractility







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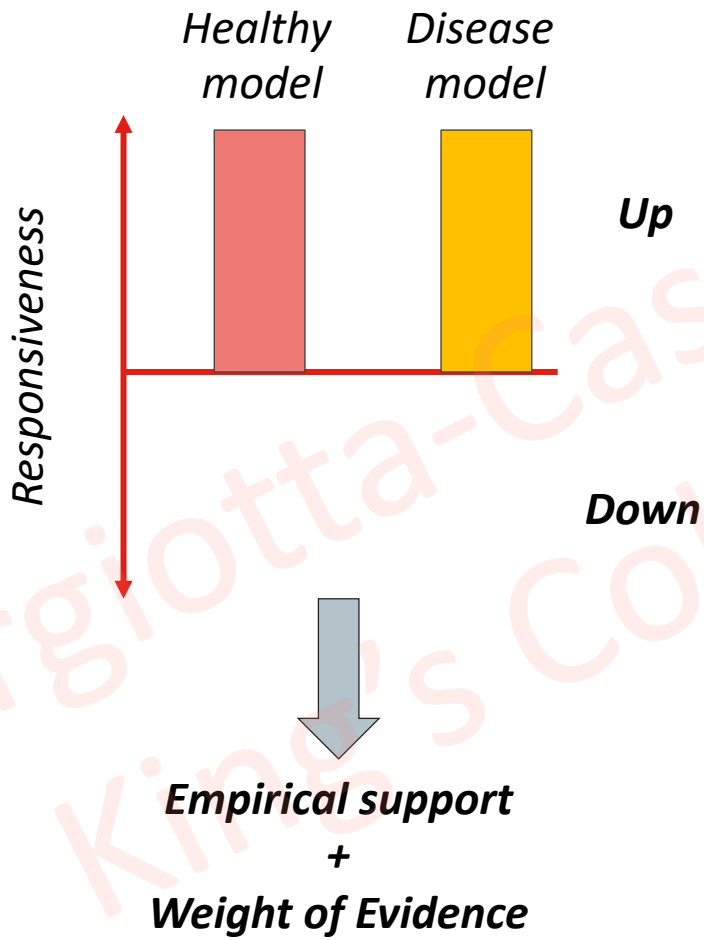
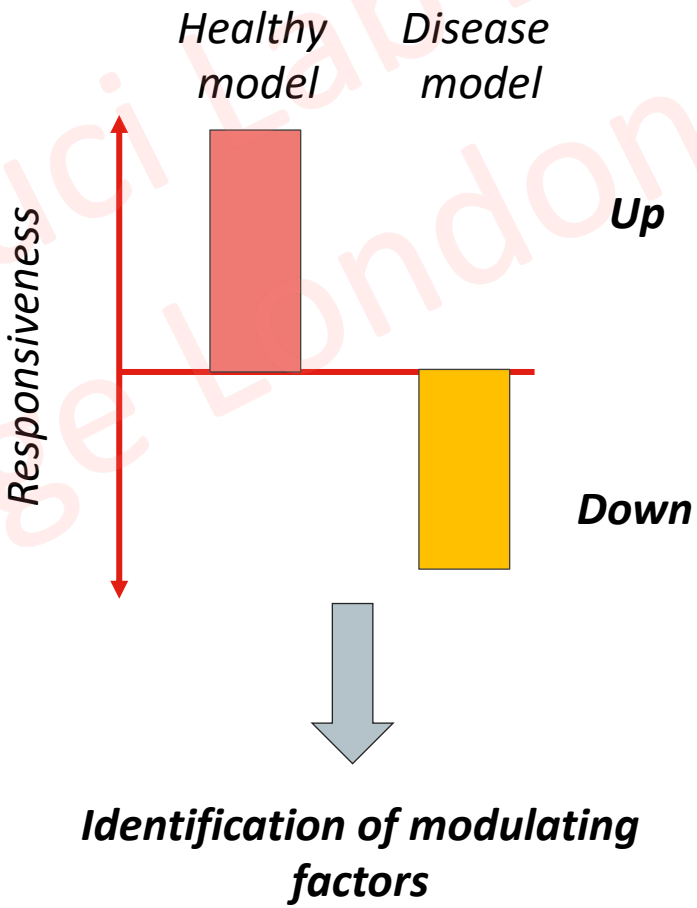
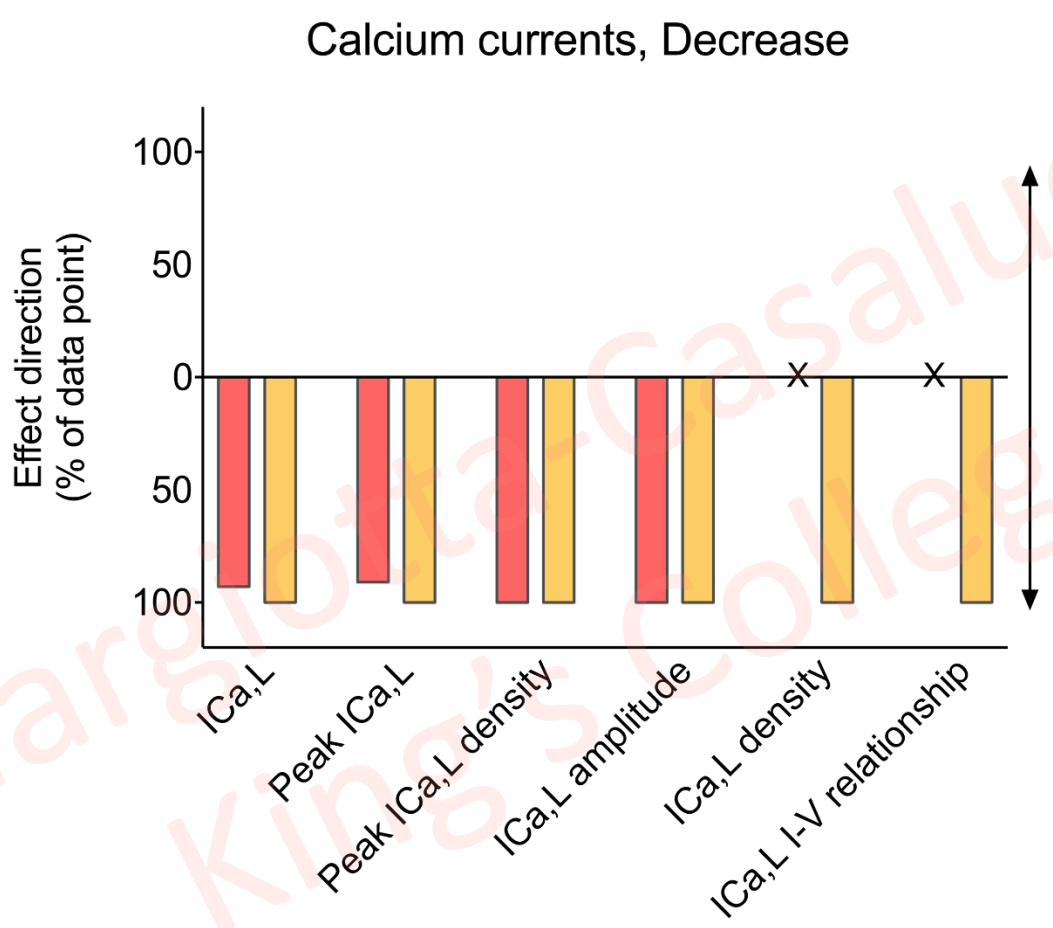
Influence of disease state on the KEs





+300 data points

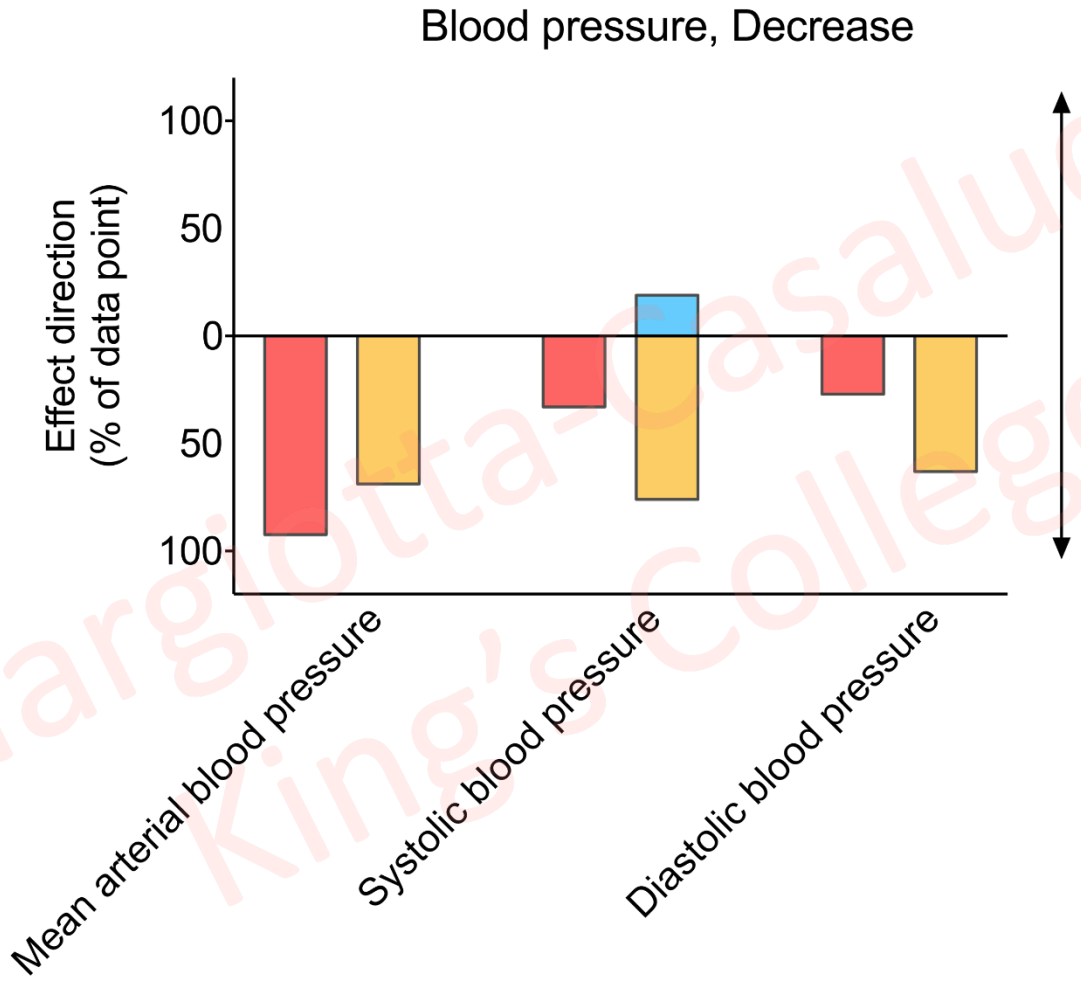
Influence of disease state on the KEs



Disease type

- *Arrhythmia*
- *Brugada syndrome phenotype*
- *Cerebral ischemia*
- *Coronary artery ligation*
- *Endotoxemia*
- *Long QT syndrome type 4*
- *Myocardial infarction*
- *Multiple organ dysfunction syndrome*
- *Tachycardia*
- *Terminally failing human hearts*
- *Tetralogy of Fallot*

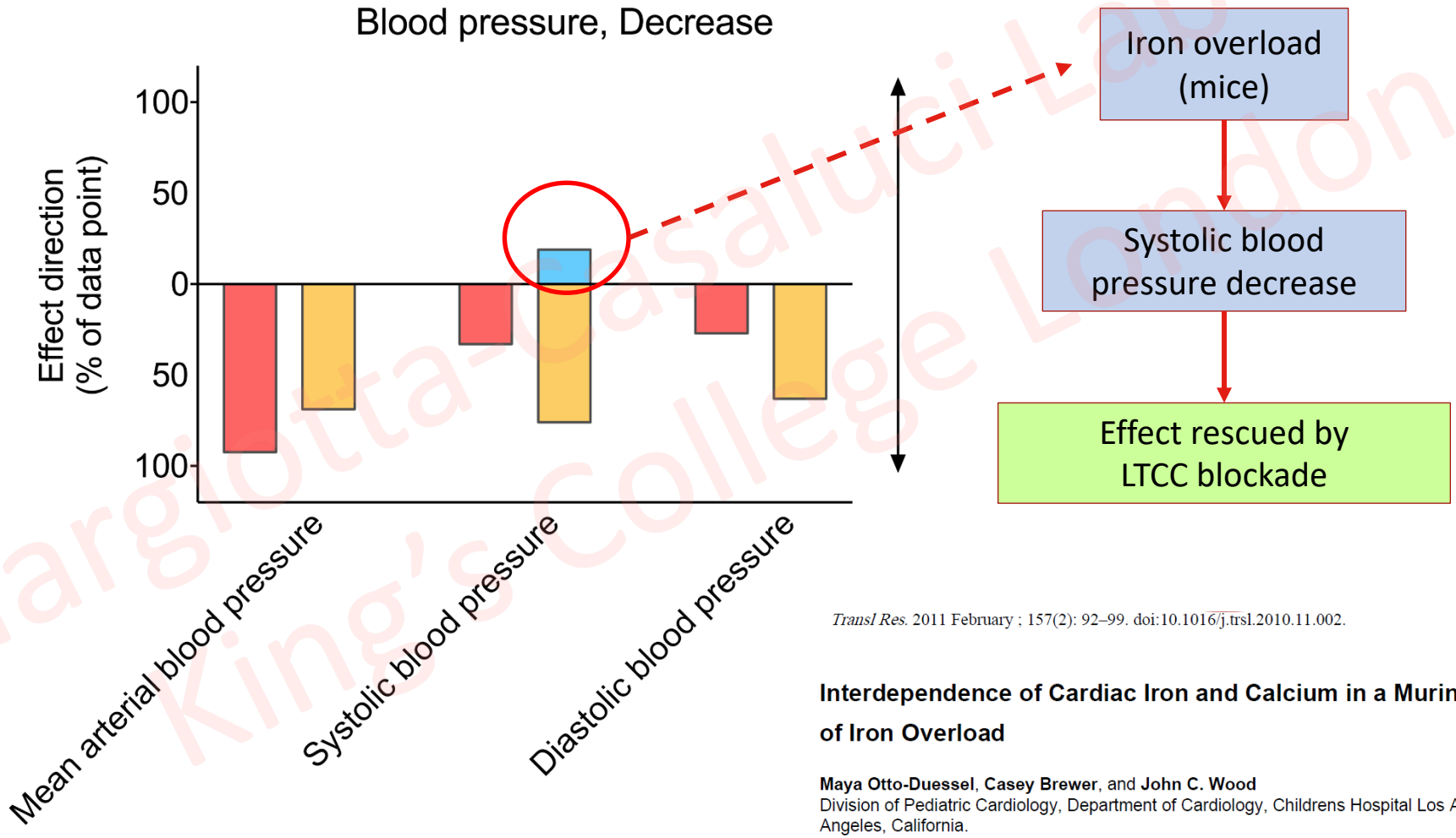
Influence of disease state on the KEs



Disease type

- *Alcohol dependence*
- *Balloon injury of the carotid artery*
- *Chronic atrioventricular block*
- *Heart failure*
- *High salt diet*
- *Hypertension*
- *Iron overload*
- *Ischemia*
- *Myocardial infarction*
- *Rapid atrial pacing*
- *Patients undergoing coronary angiography with or without percutaneous coronary interventions*
- *SHR hydronephrotic model*

Influence of disease state on the KEs

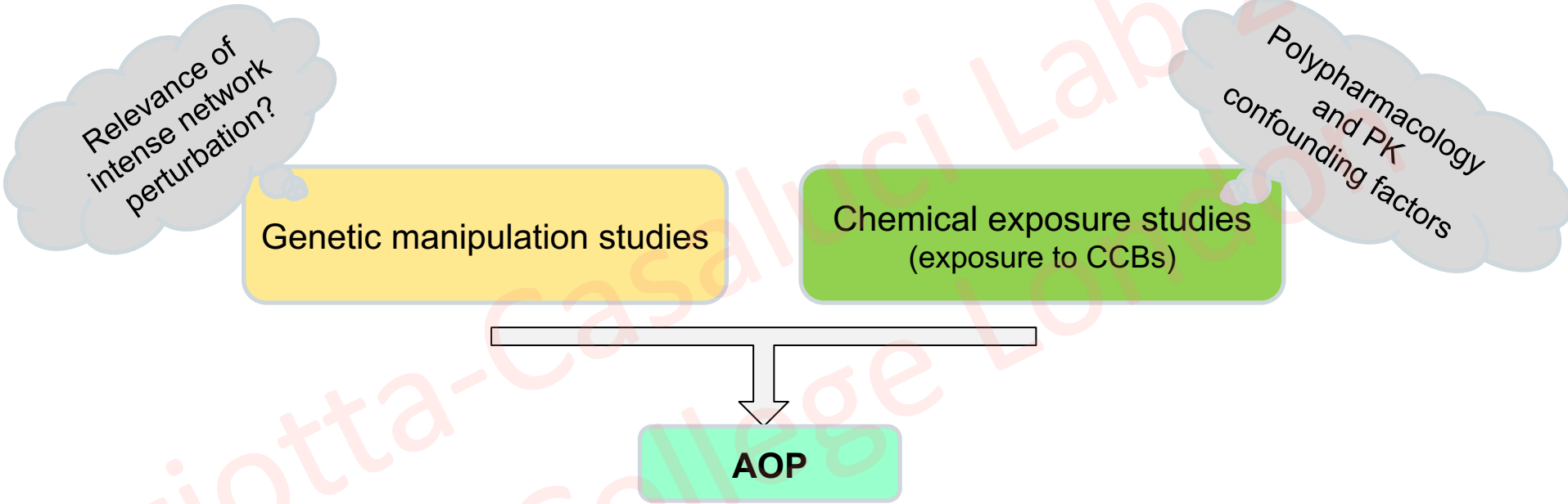


Transl Res. 2011 February ; 157(2): 92–99. doi:10.1016/j.trsl.2010.11.002.

Interdependence of Cardiac Iron and Calcium in a Murine Model of Iron Overload

Maya Otto-Duessel, **Casey Brewer**, and **John C. Wood**
Division of Pediatric Cardiology, Department of Cardiology, Childrens Hospital Los Angeles, Los Angeles, California.

Genetic manipulation studies and essentiality assessment



CCB-induced effects + 146 data points describing the effects of genetic manipulations of various component of the pathway:

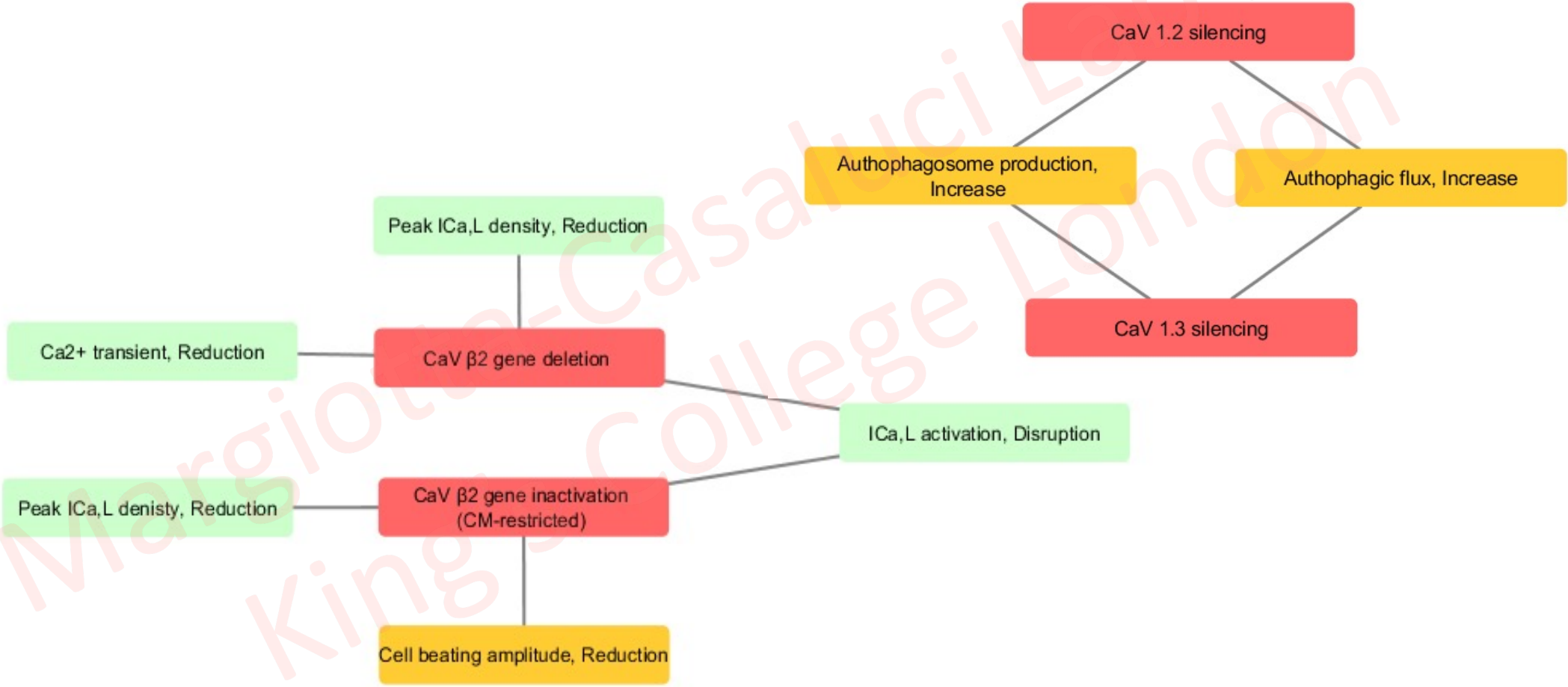
- *Cav1.2, Cav1.3*
- *Calmodulin*
- *Calmodulin kinase II*
- *Cardiac Troponin T*

Cav1.2 variants/mutations and disease

Target	Relationship type	Disease
CACNA1C	contributes to	Attention deficit hyperactivity disorder
	contributes to	Autism spectrum disorder
	contributes to	Bipolar disorder
	has phenotype	Brugada syndrome
	is marker for	Brugada syndrome 3
	is marker for	Hypertension
	is marker for	Hypoglycaemia
	likely_pathogenic_for_condition	Idiopathic ventricular fibrillation, non Brugada type
	contributes to	Malignant exocrine pancreas neoplasm
	contributes to	Schizophrenia
	is marker for	Timothy syndrome
	contributes to	Unipolar depression

Consequences of genetic manipulation of Cav1.2

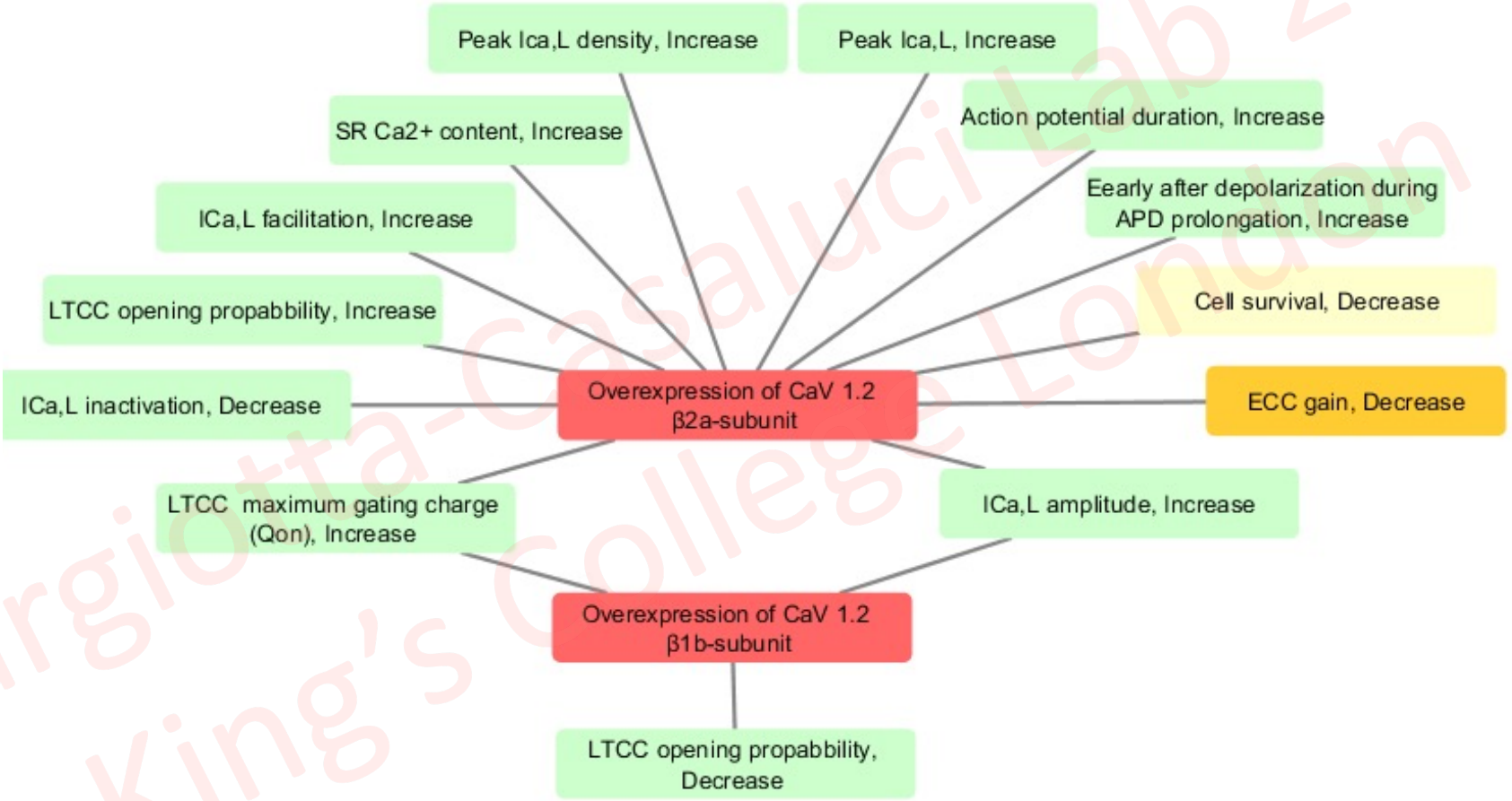
Silencing/Deletion/Inactivation



This data confirms the KE identified during the mining of empirical evidence

Consequences of genetic manipulation of Cav1.2

Overexpression of beta-subunits



All Beta subunits increased the native cardiac whole-cell L-type Ca2+ channel current density, but produced distinctive effects on channel inactivation kinetics.

Order of potency: β2a ≈ β4 > β1b > β3

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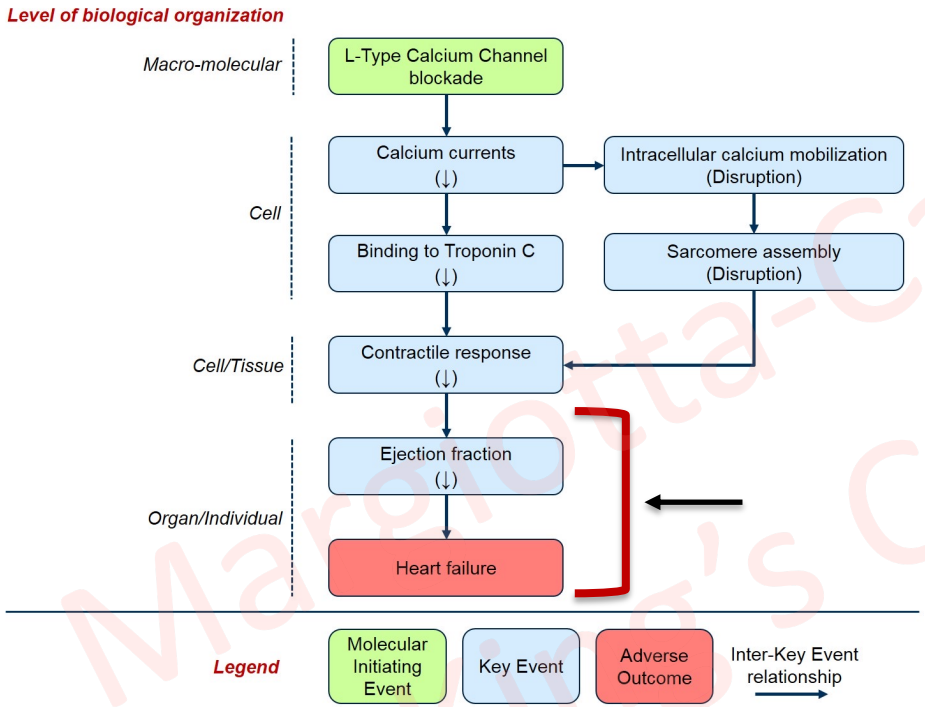
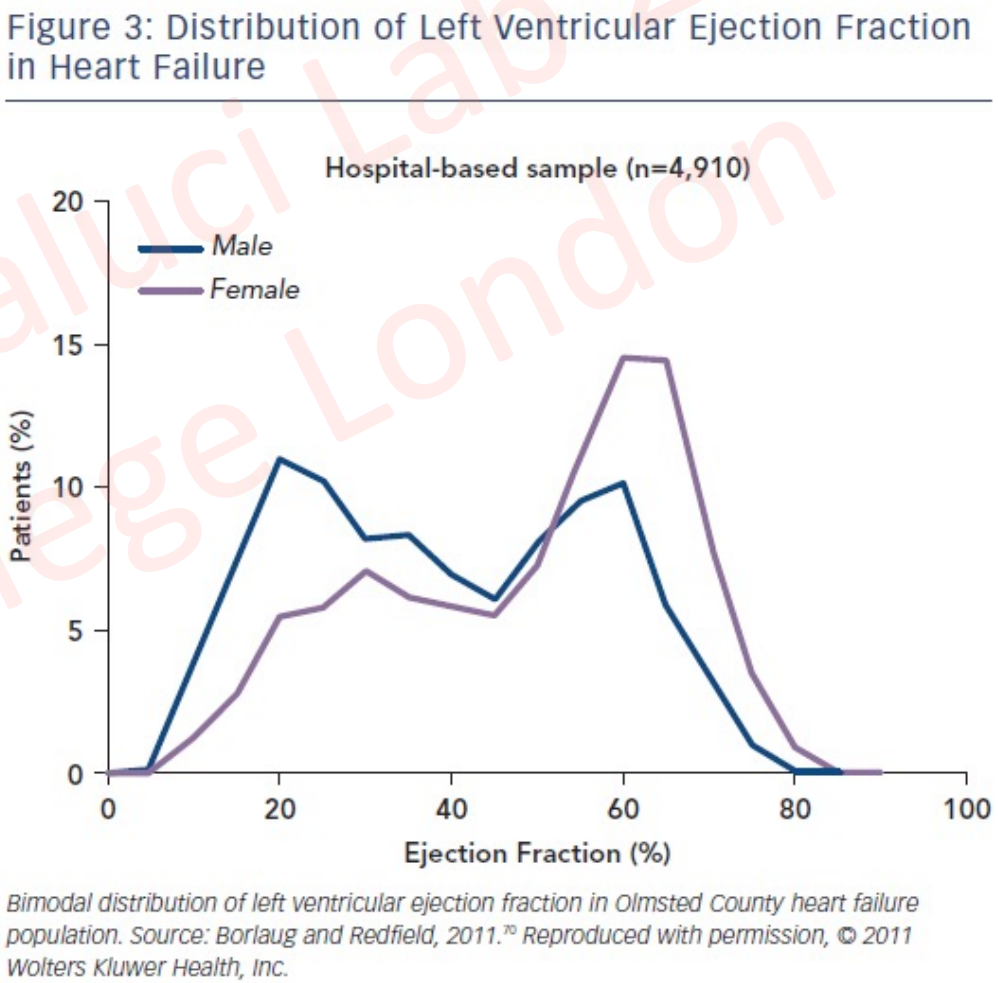
Ejection faction → Heart failure

AOP 261: Disruption of cardiac contractility

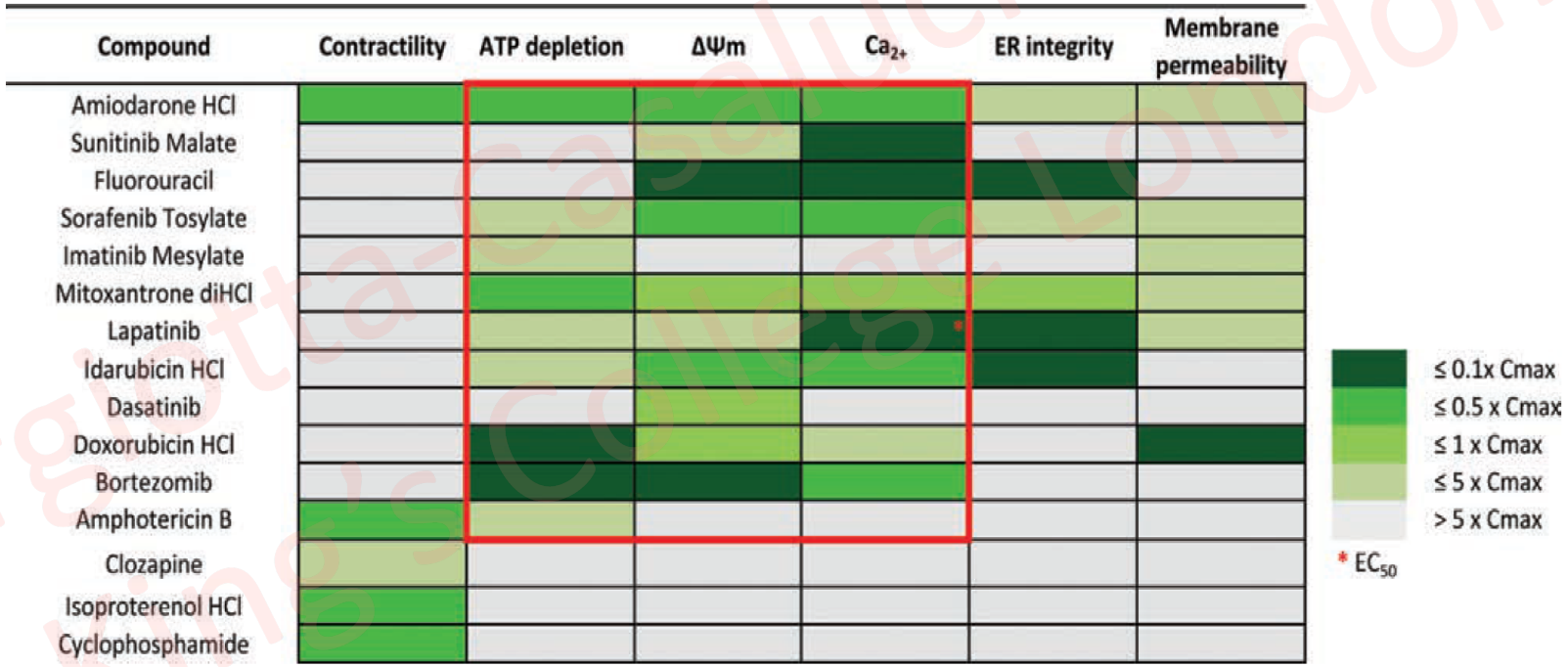




Savarese and Lund (2017) Cardiac Failure Review 2017;3(1):7–11.

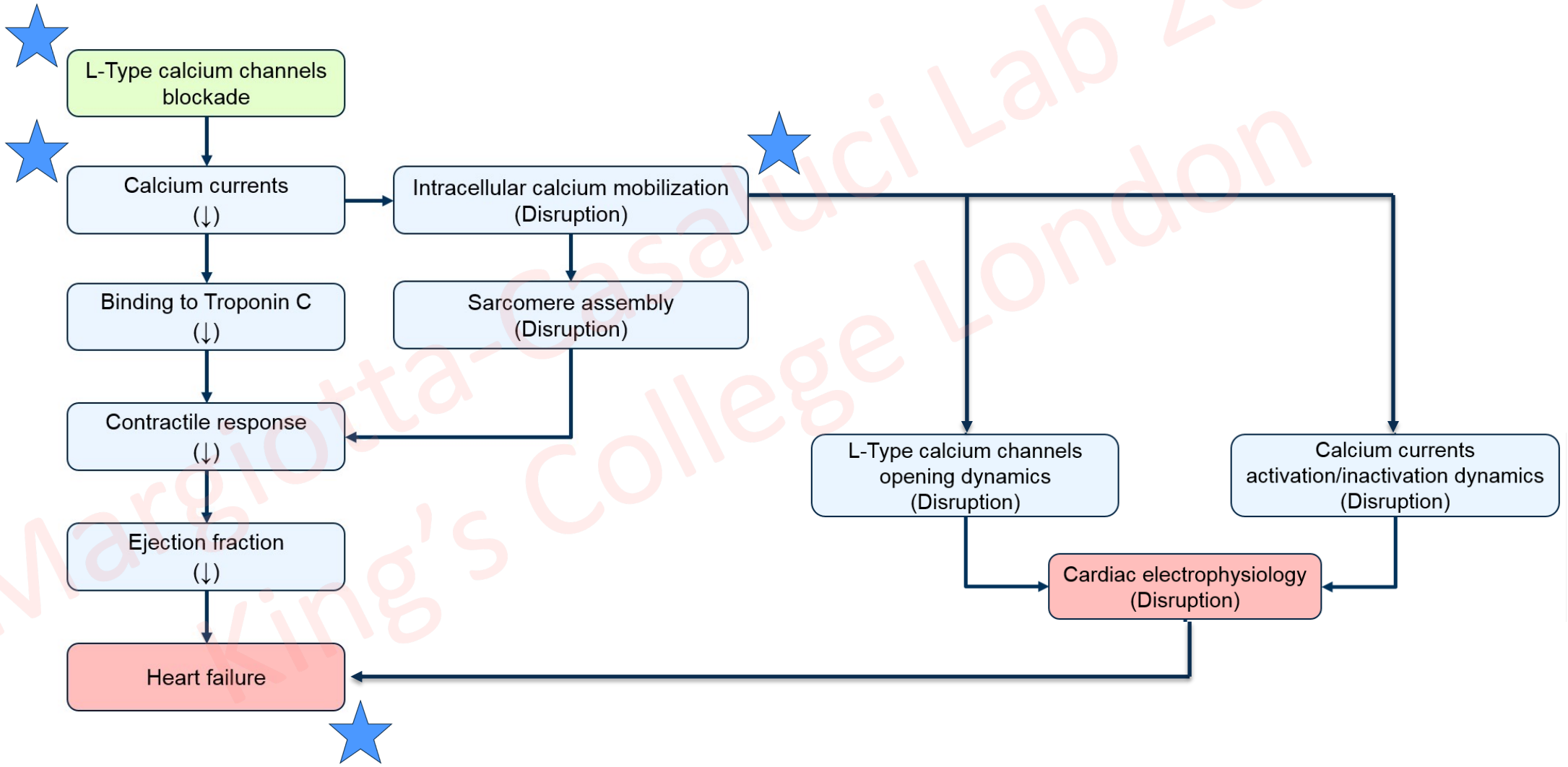
In vitro phenotypic profiling of structural cardiotoxins

- Human embryonic stem cell–derived cardiomyocytes
- H9c2 cell line
- Canine cardiomyocytes



Pointon et al. (2013) Toxicological sciences 132(2), 317–326

LTCC-blockade-mediated AOP network



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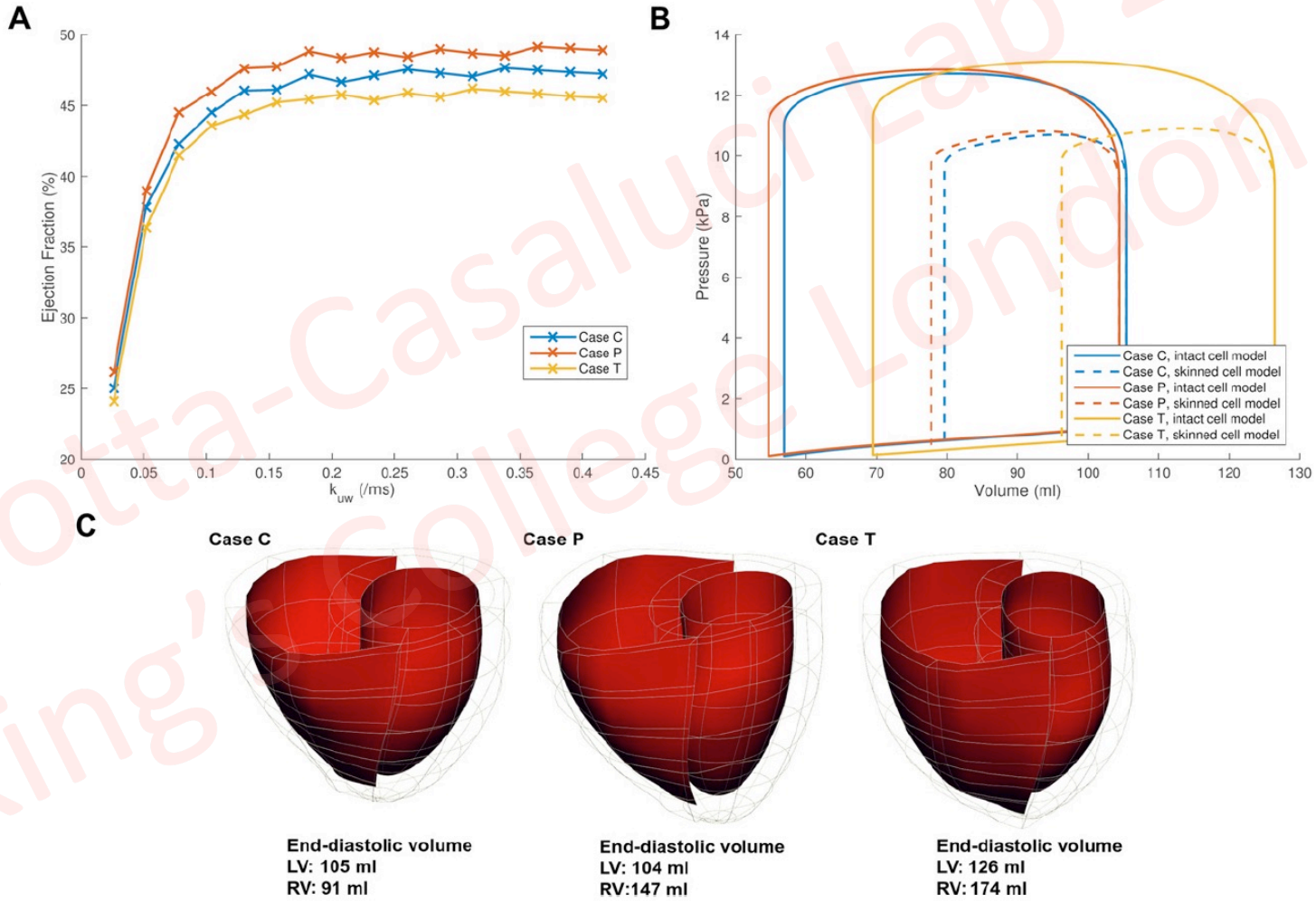
Moving towards the development of a quantitative AOP network

In silico modelling of cardiac contractility



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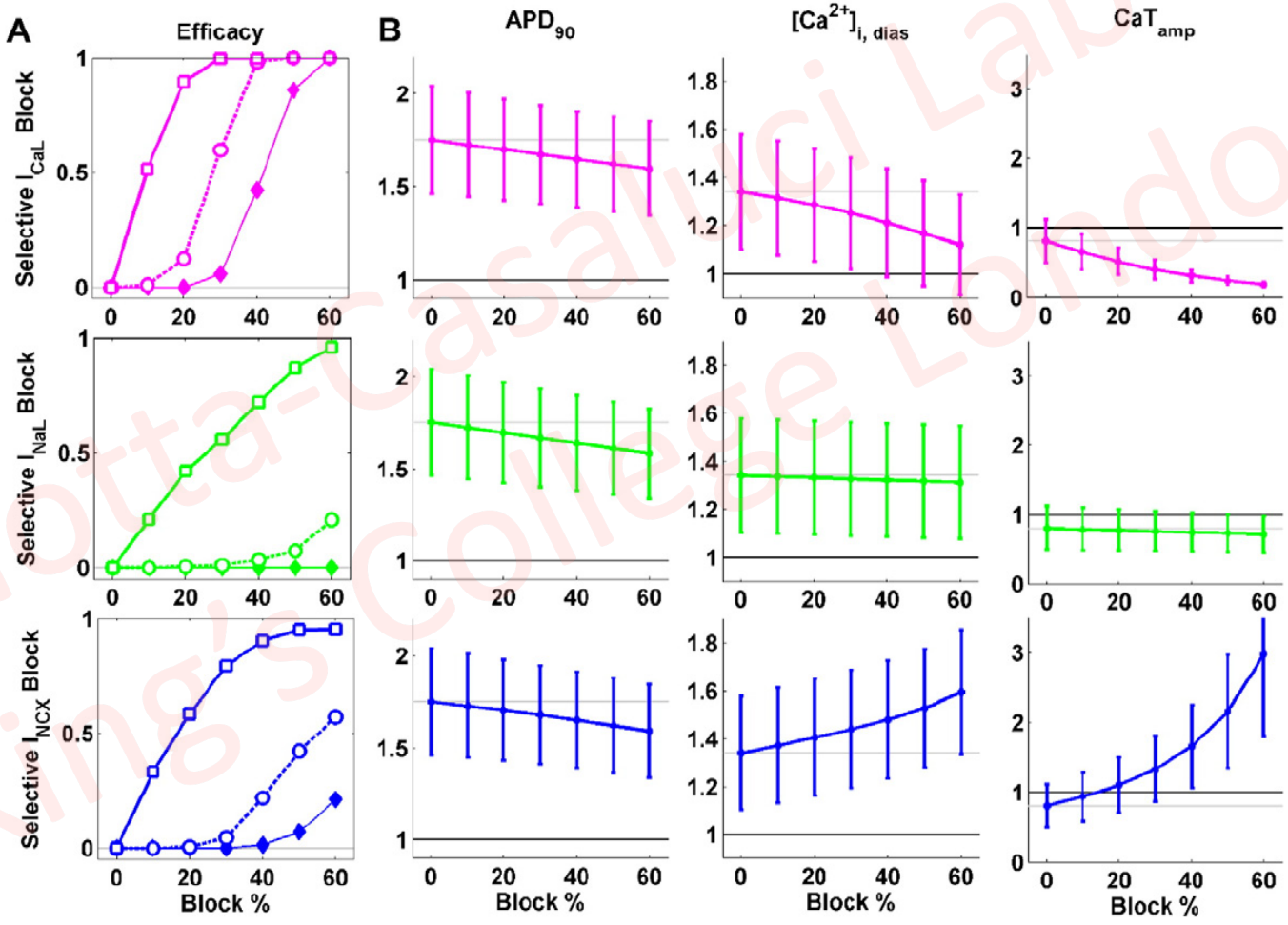
Land et al. (2017) Journal of Molecular & Cellular Cardiology 106: 68–83

Moving towards the development of a quantitative AOP network

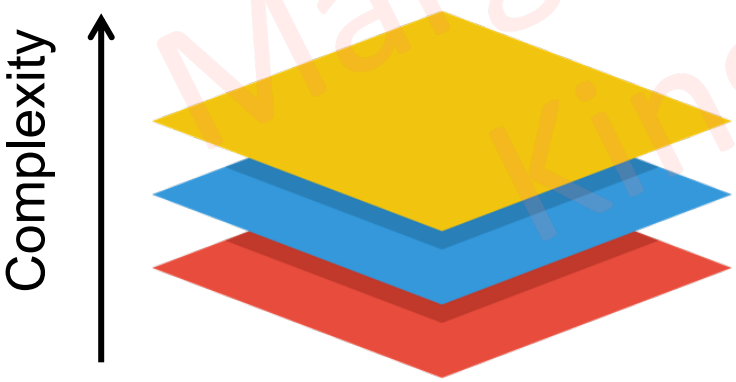
In silico modelling of cardiac electrophysiology



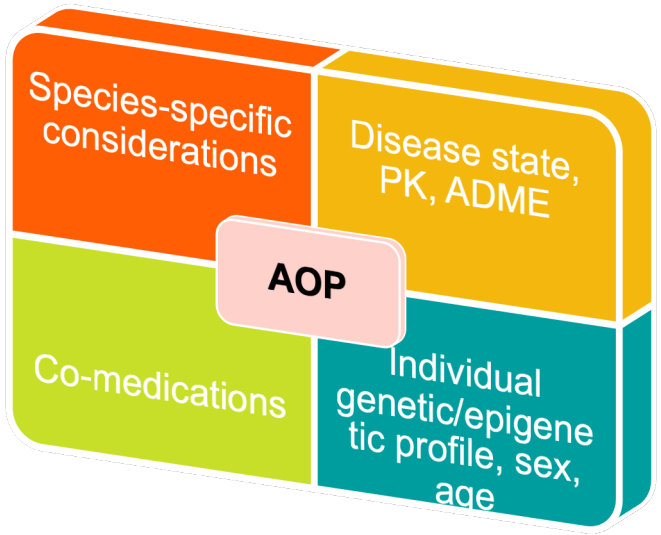
Passini et al. 2016 Journal of Molecular and Cellular Cardiology 96: 72–81

A few take home messages

- ✓ Data-driven AOP development can enhance the usability of AOPs across sub-disciplines
- ✓ The incorporation of quantitative information and KEs measurability considerations is essential *(even if the first version of our AOP is only qualitative)* . This can pave the way for the implementation of additional tailored & context-specific levels of complexity, if needed
- ✓ Network biology considerations can increase the relevance of AOPs for real-life scenarios



Quantitative AOP network
(multi-channel perturbation)
Quantitative AOP
(*in silico* modelling of electro-mechanical coupling)
AOP for cardiotoxicity
(LTCCs-blockade)



Acknowledgments

Get in touch

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Ines Moreira
(Brunel University London)

Dr Helen Prior
(NC3Rs)

National Centre for the Replacement Refinement & Reduction of Animals in Research

Brunel University London

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Dr Brigitte Landesmann
(European Commission)

Dr Fiona Sewell
(NC3Rs)

Dr Matthew Winter
(University of Exeter)