USING FLUORESCENCE MICROSCOPY TO IDENTIFY A POTENTIAL NEW TREATMENT FOR HEART FAILURE

Dr. Ryan Himes
Assistant Professor of Biological Sciences, Olivet Nazarene University

BIOCHEMISTRY
including biophysical chemistry & molecular biology

Article

pubs.acs.org/biochemistry

L30A Mutation of Phospholemman Mimics Effects of Cardiac Glycosides in Isolated Cardiomyocytes

Ryan D. Himes,[†] Nikolai Smolin,[†] Andreas Kukol,[‡] Julie Bossuyt,[§] Donald M. Bers,[§] and Seth L. Robia*[,†]

[†]Department of Cell and Molecular Physiology, Loyola University Chicago, Maywood, Illinois 60153, United States

[‡]School of Life and Medical Sciences, University of Hertfordshire, Hatfield, U.K.

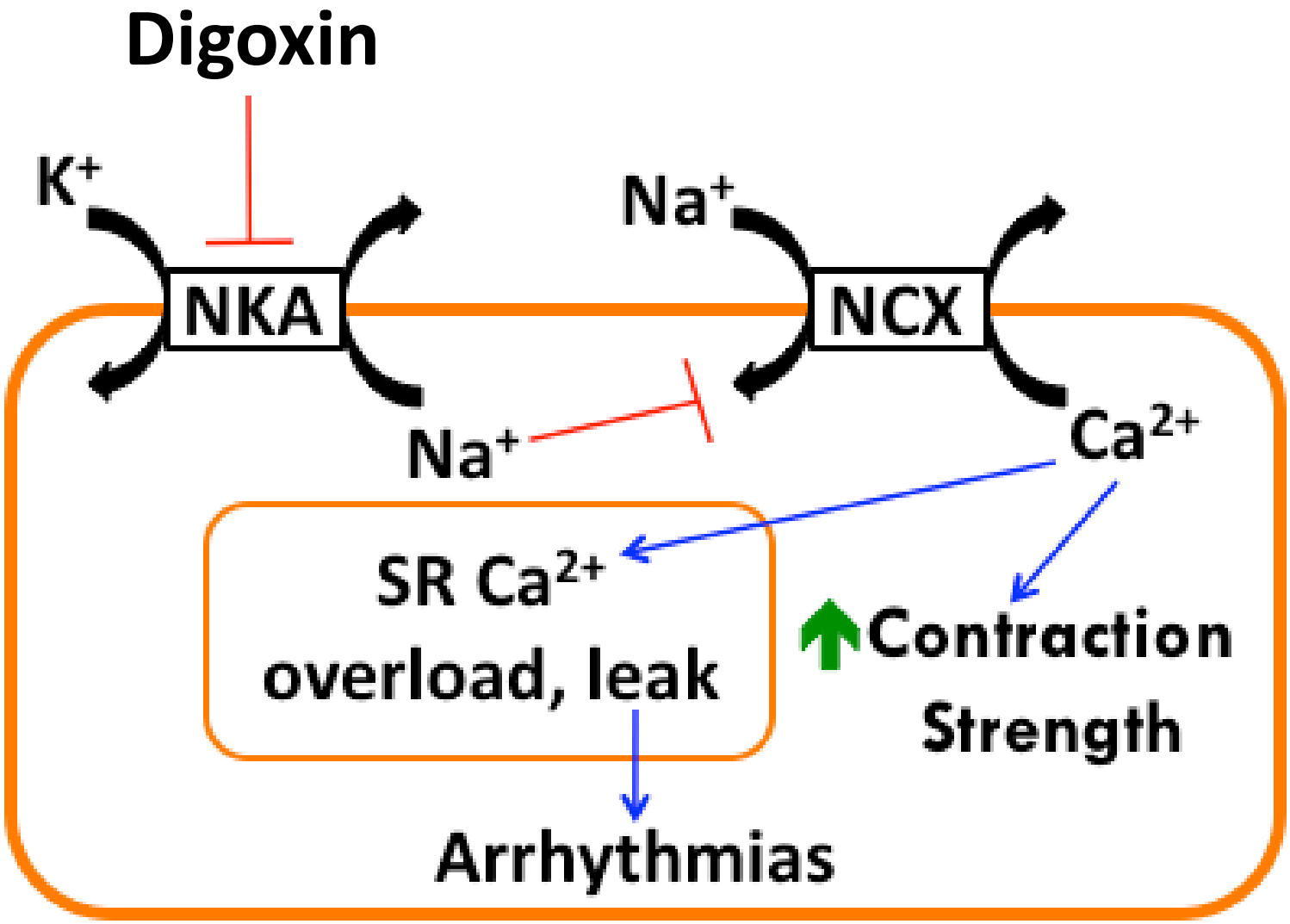
[§]Department of Pharmacology, The University of California, Davis, California 95616, United States

Why Study Heart Failure?

- Heart failure = cardiac output that is insufficient to meet the demands of the body.
- Affects 6.5 million US adults (~3% of pop.)
- 1 in 8 deaths has heart failure mentioned on death certificate.[1]
- Digoxin causes an increase in cardiac contraction strength, but has a narrow therapeutic window.[2]

1. AHA 2017 Statistical Update, *Circulation*, 135:e378-e384.

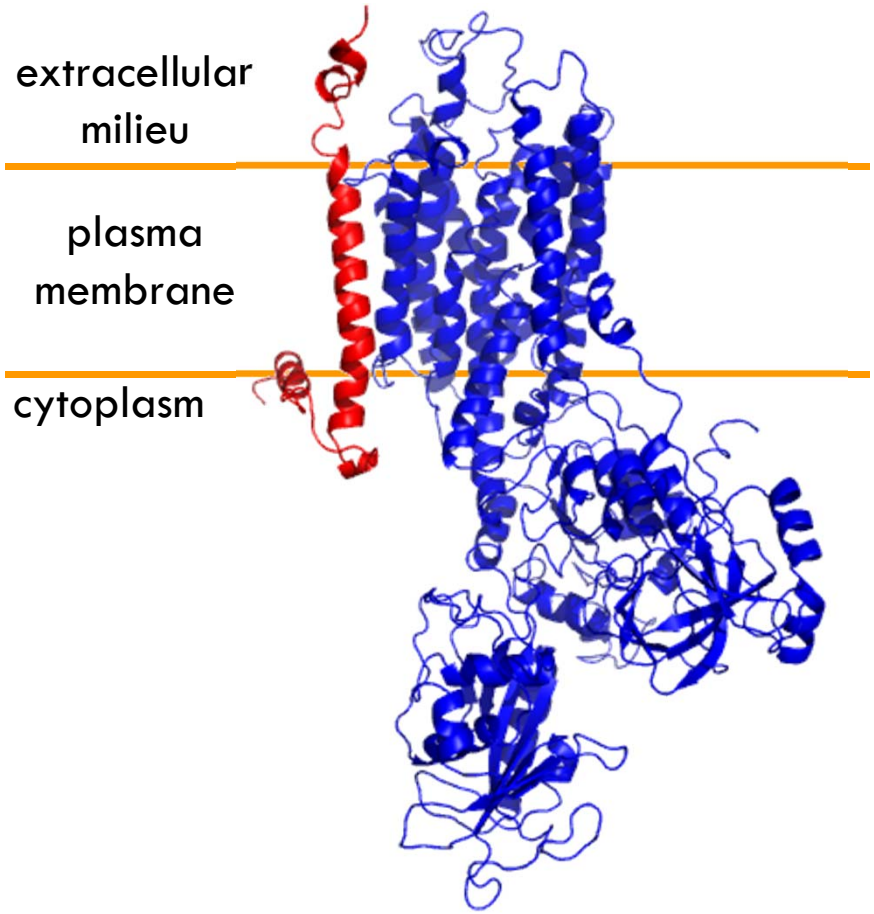
2. Bers, DM. *EC Coupling and Cardiac Contractile Force*, 2001.


Digoxin
K+
NKA
Na+
NCX
Na+
Ca2+
SR Ca2+
overload, leak
Contraction
Strength
Arrhythmias

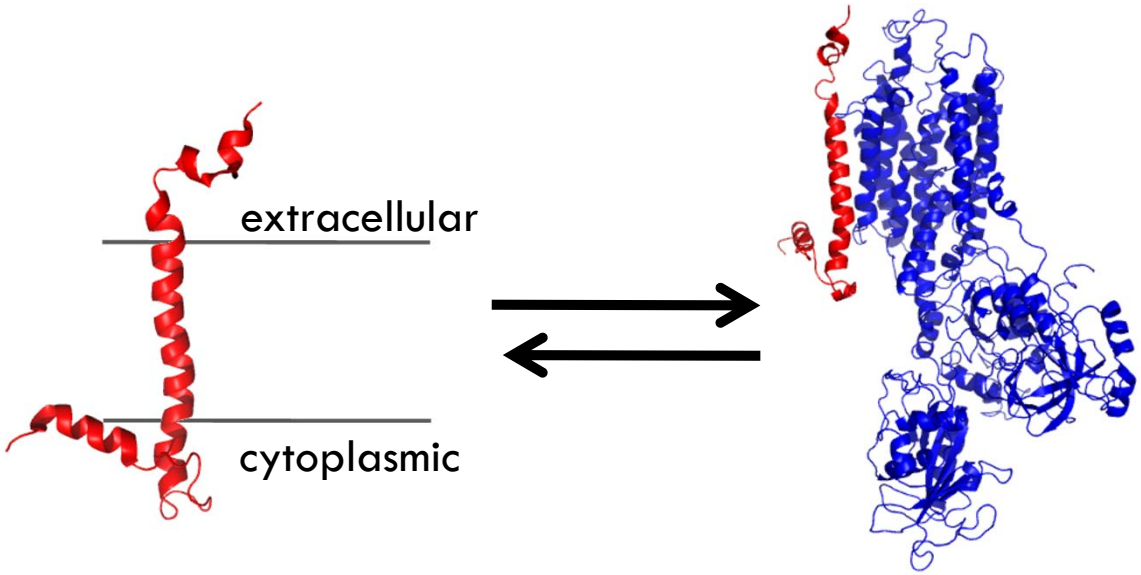
Central Question

Can cardiac contraction strength be improved by mutating phospholemman (PLM)?

PLM-NKA



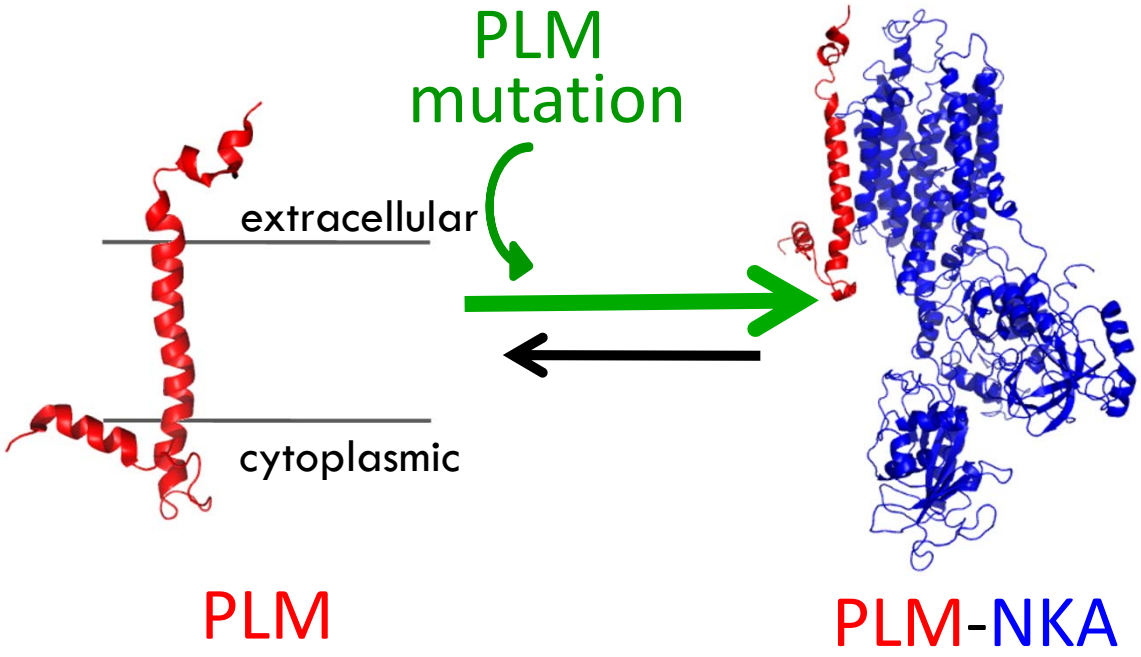
Central Hypothesis



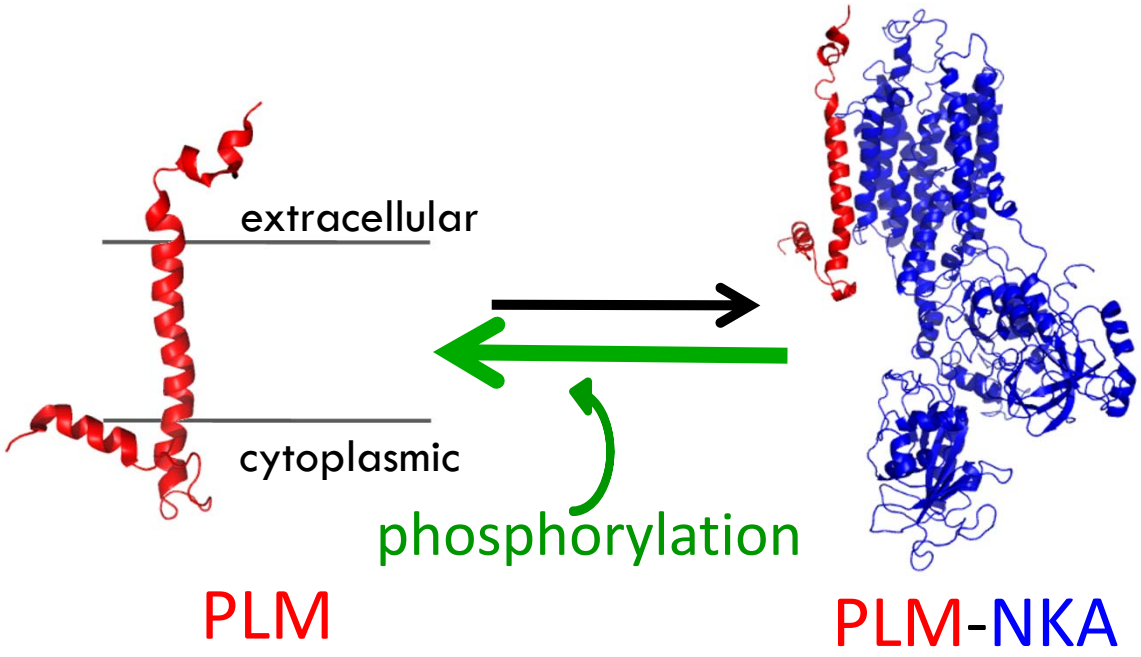
PLM

PLM-NKA

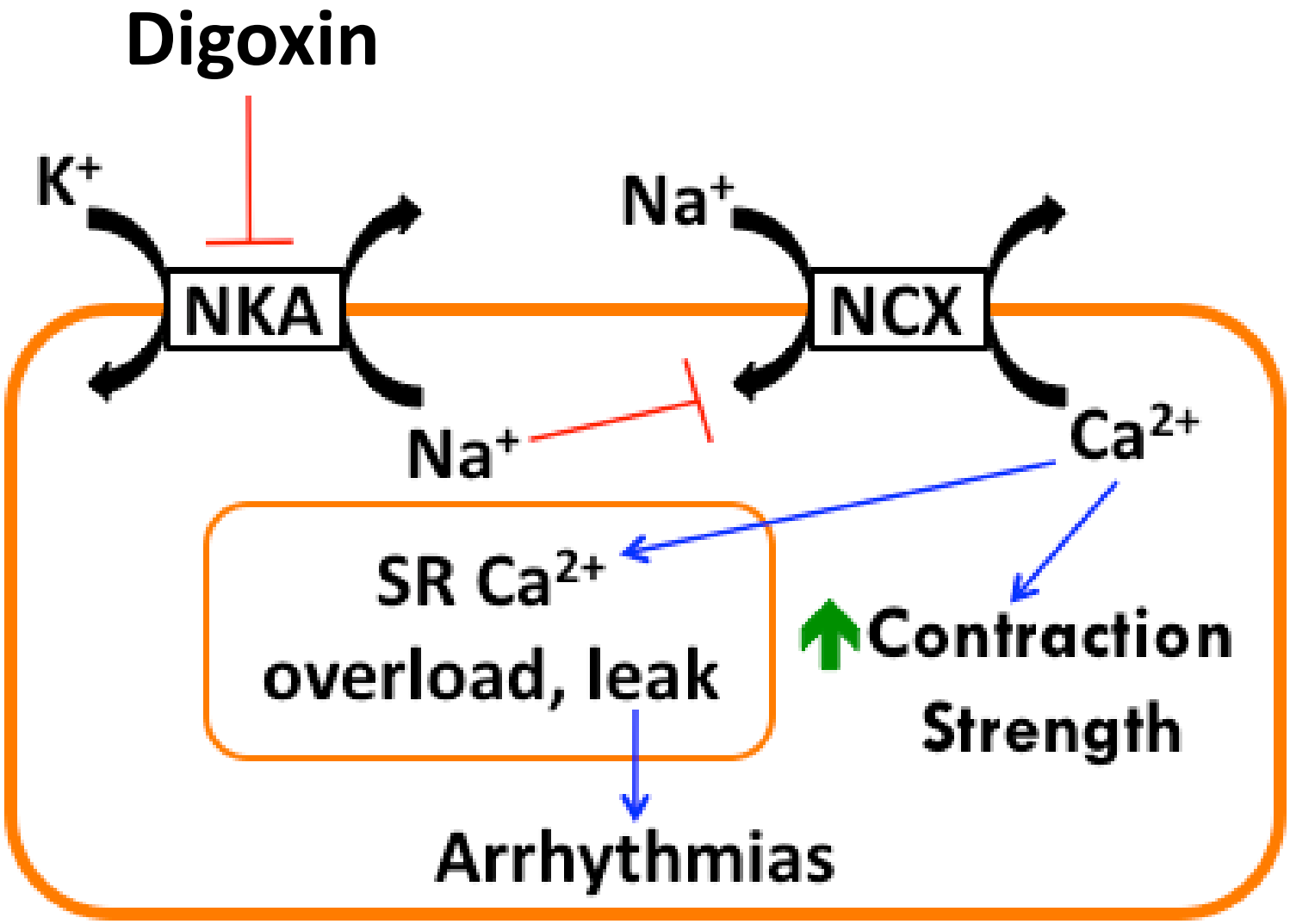
Central Hypothesis

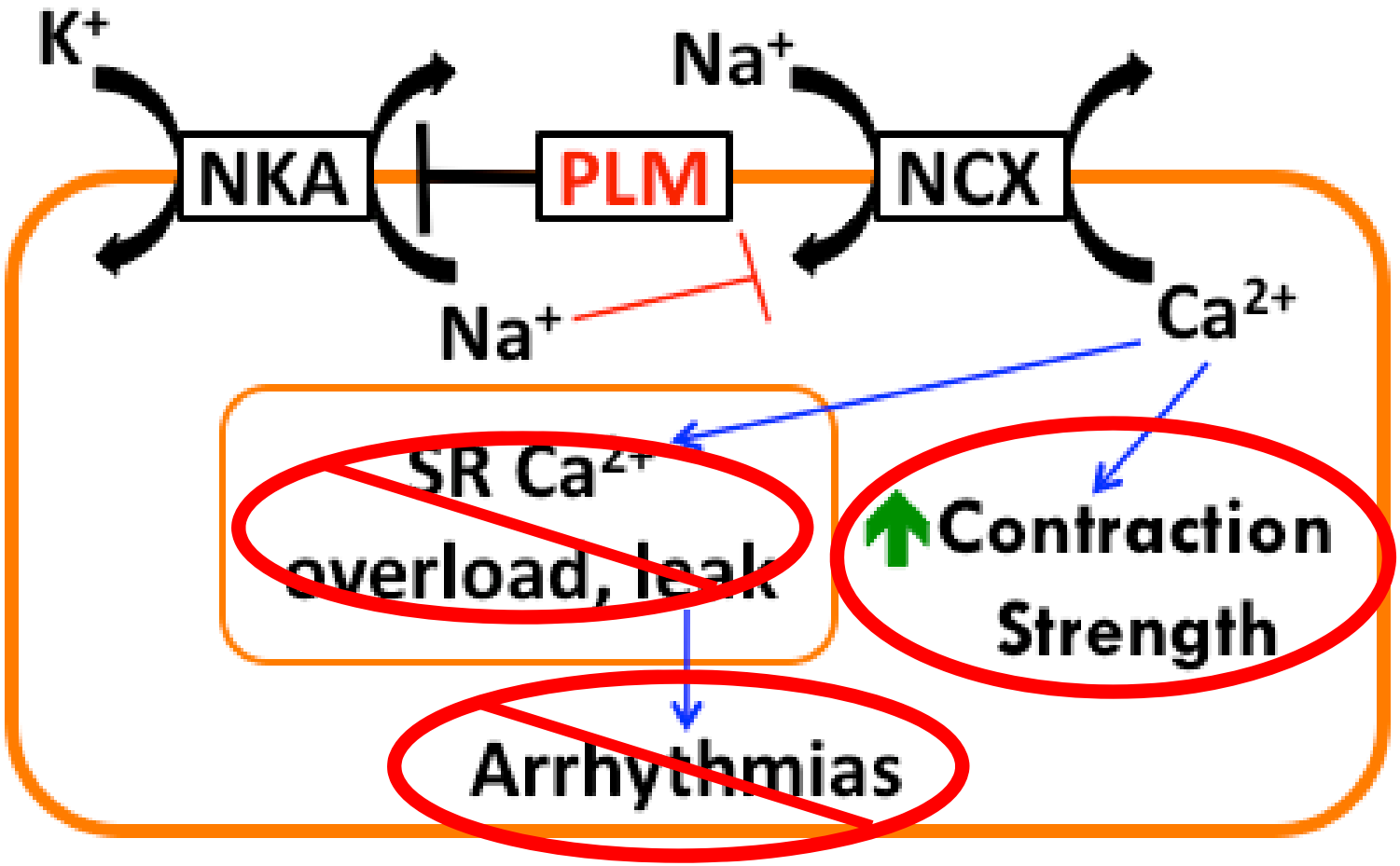


Central Hypothesis

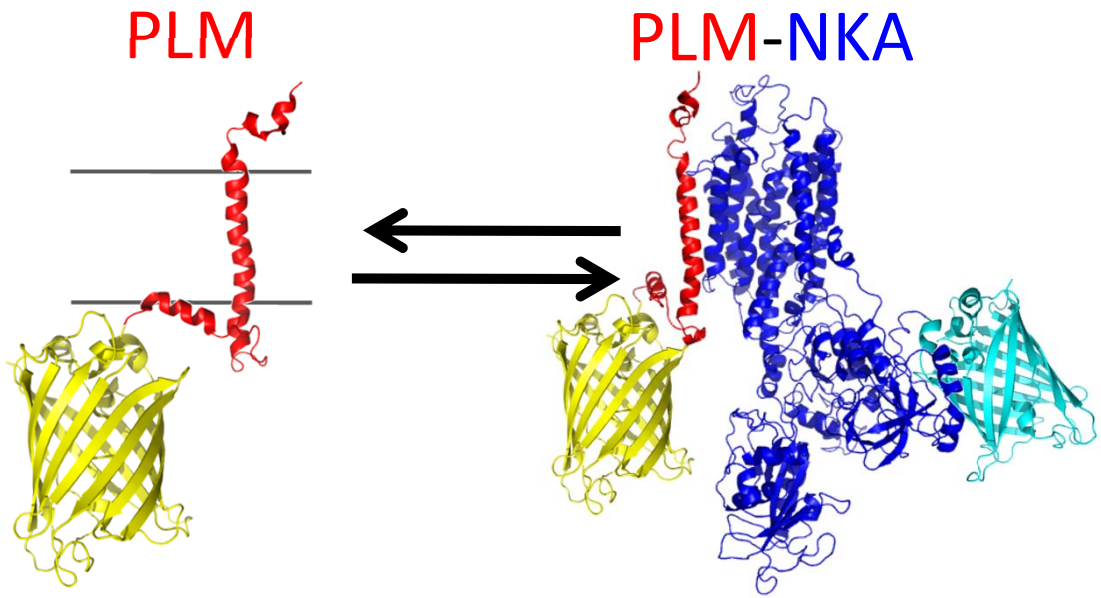


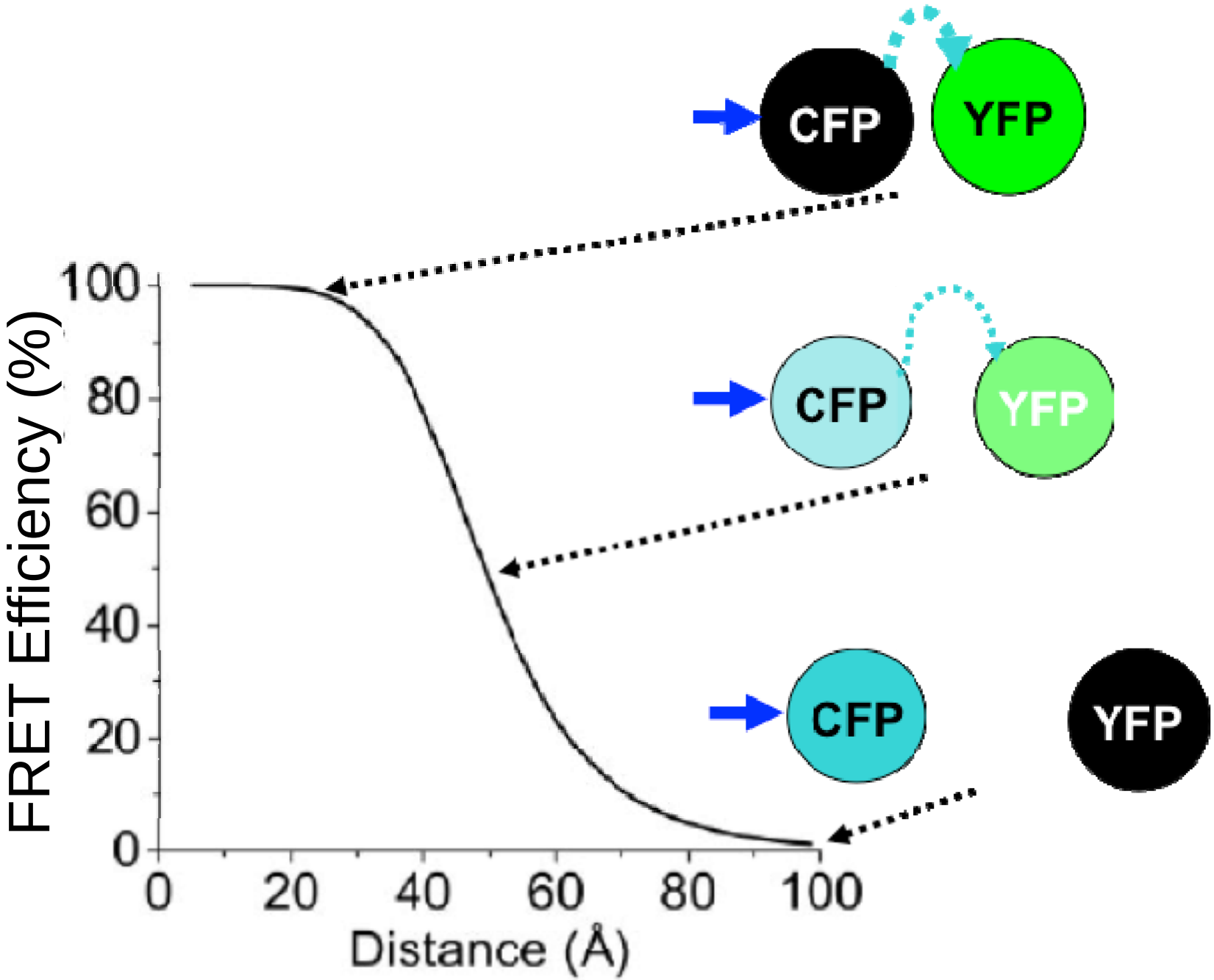
Song, Q. et al. (2011). JBC, 286(11), 9120–9126.


Digoxin
K+
NKA
Na+
NCX
Na+
Ca2+
SR Ca2+
overload, leak
Contraction
Strength
Arrhythmias

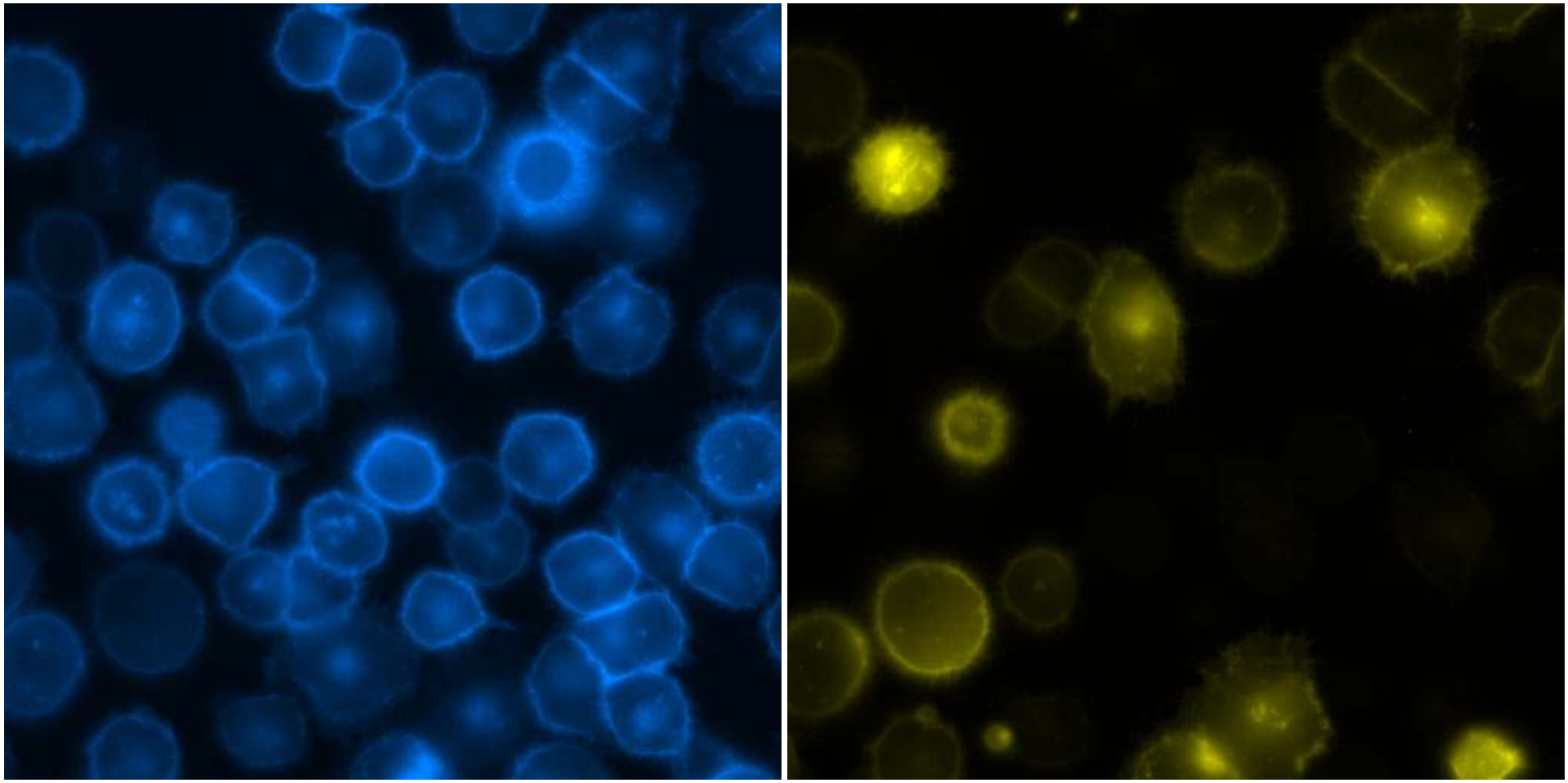

K+
Na+
NKA
PLM
NCX
Na+
Ca2+
SR Ca2+
overload, leak
Contraction
Strength
Arrhythmias

Approach for Screening Mutants: Measuring Binding Affinity with FRET

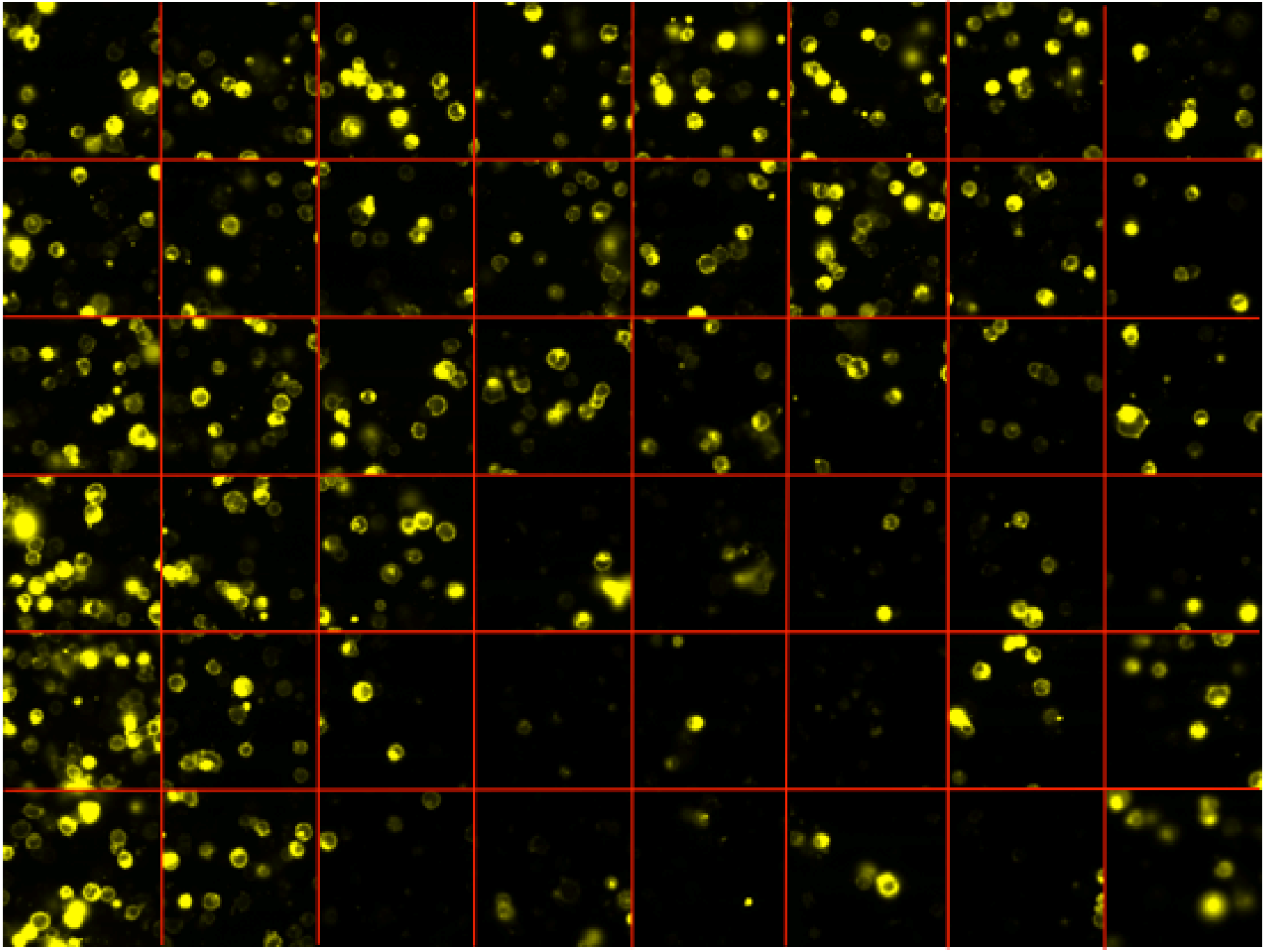



FRET Efficiency (%)
100
80
60
40
20
0
0
20
40
60
80
100
Distance (Å)
CFP
YFP
CFP
YFP
CFP
YFP

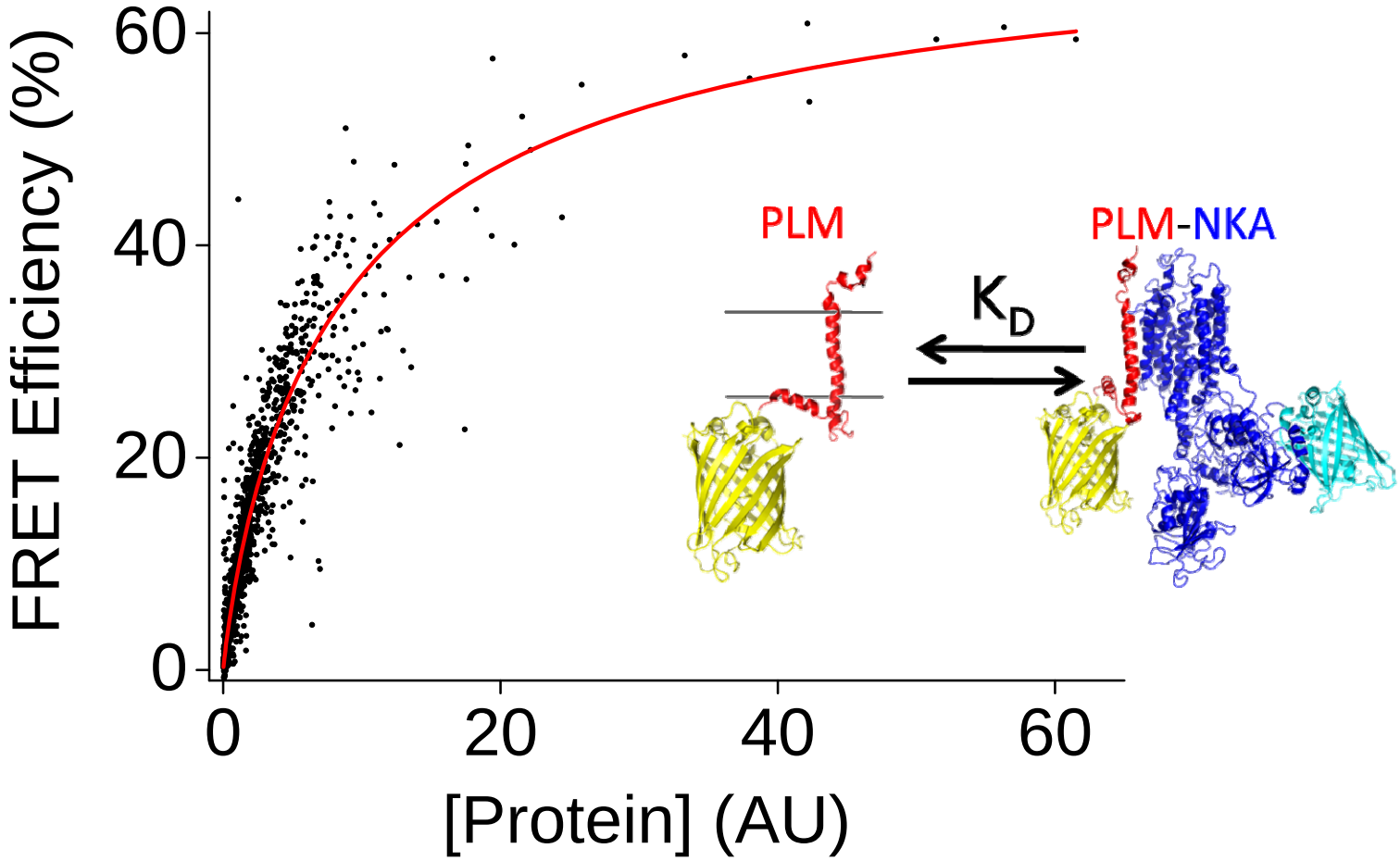
HEK cells co-transfected with **CFP**-NKA and PLM-**YFP**



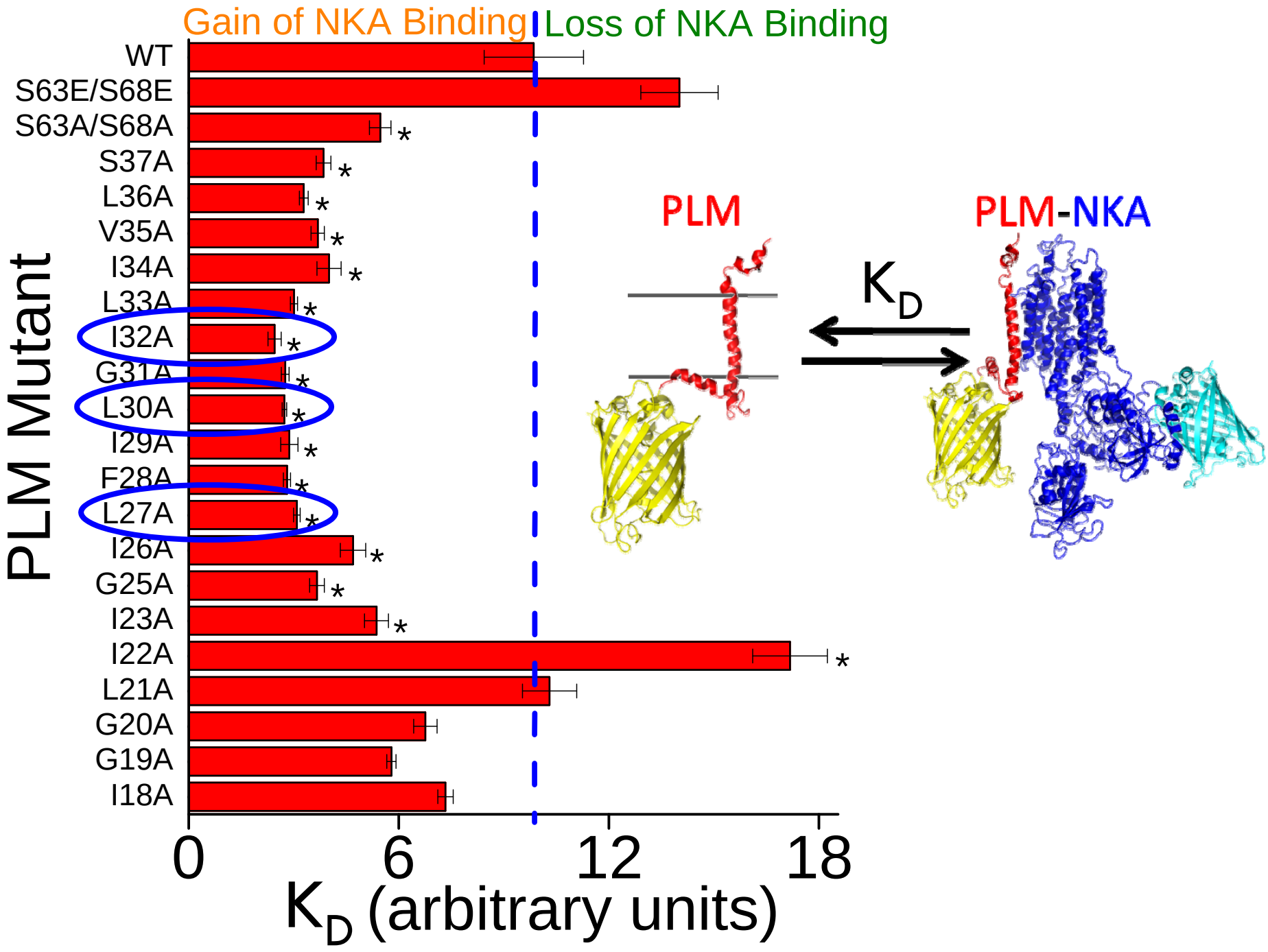
Cell line courtesy of Dr. Julie Bossuyt (UC Davis)

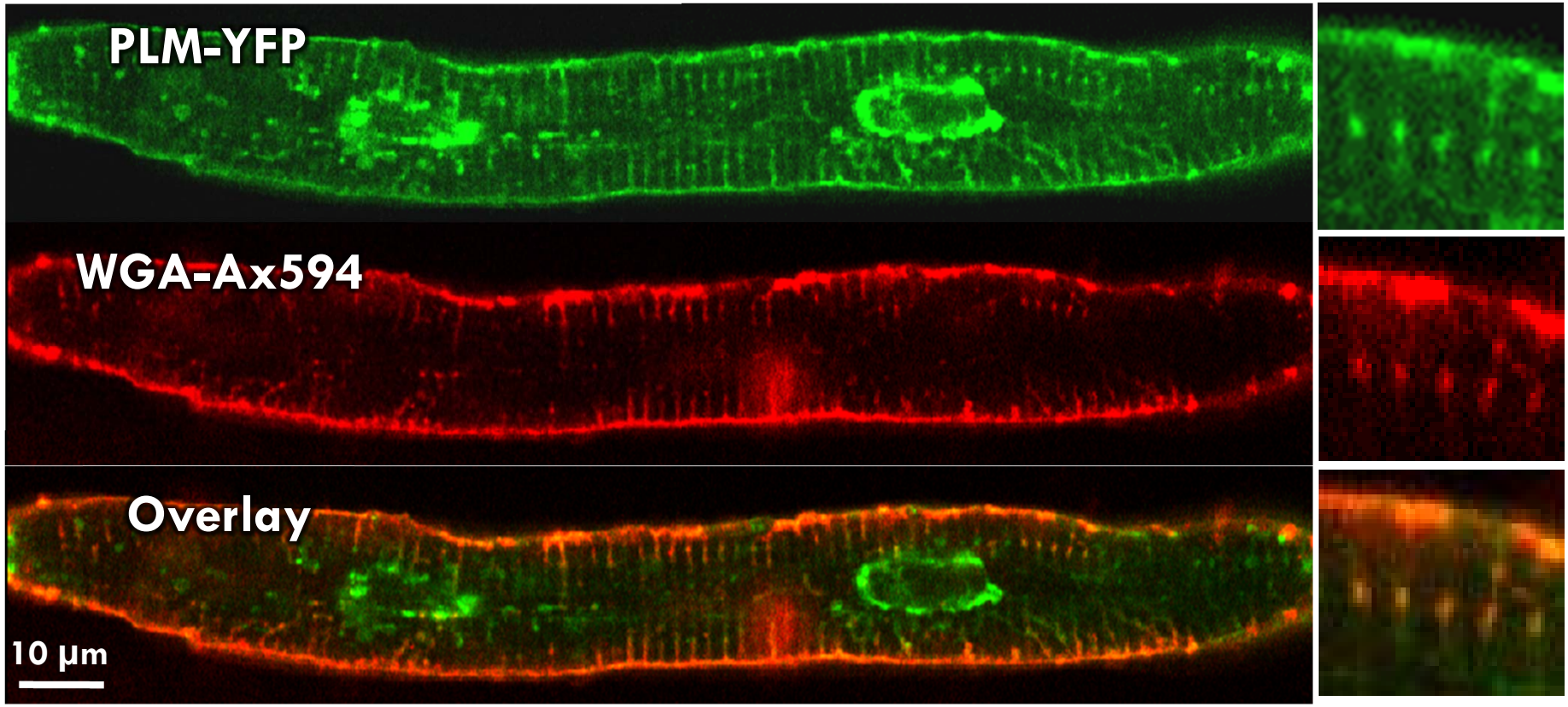


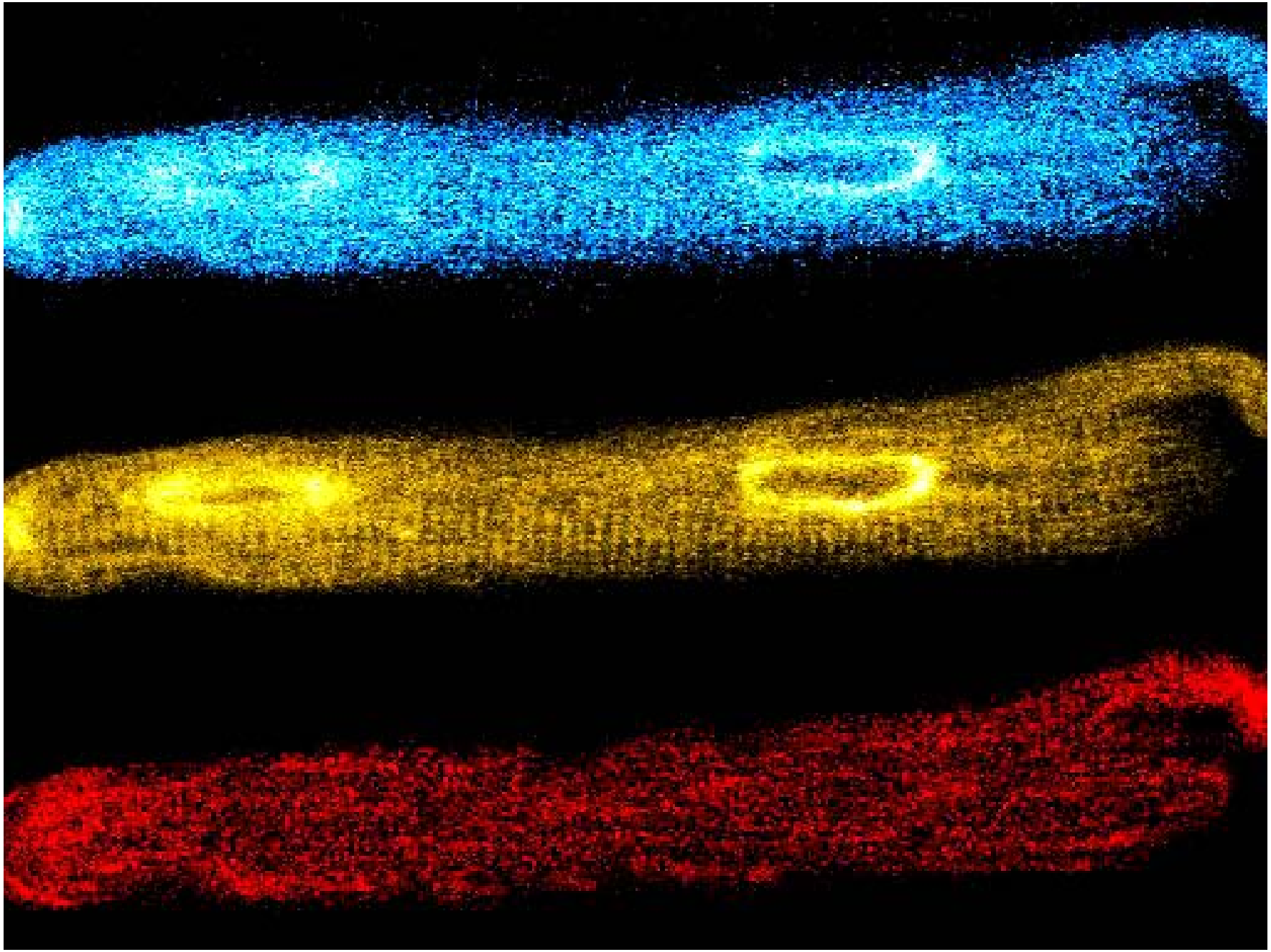
Measuring Binding Affinity with FRET



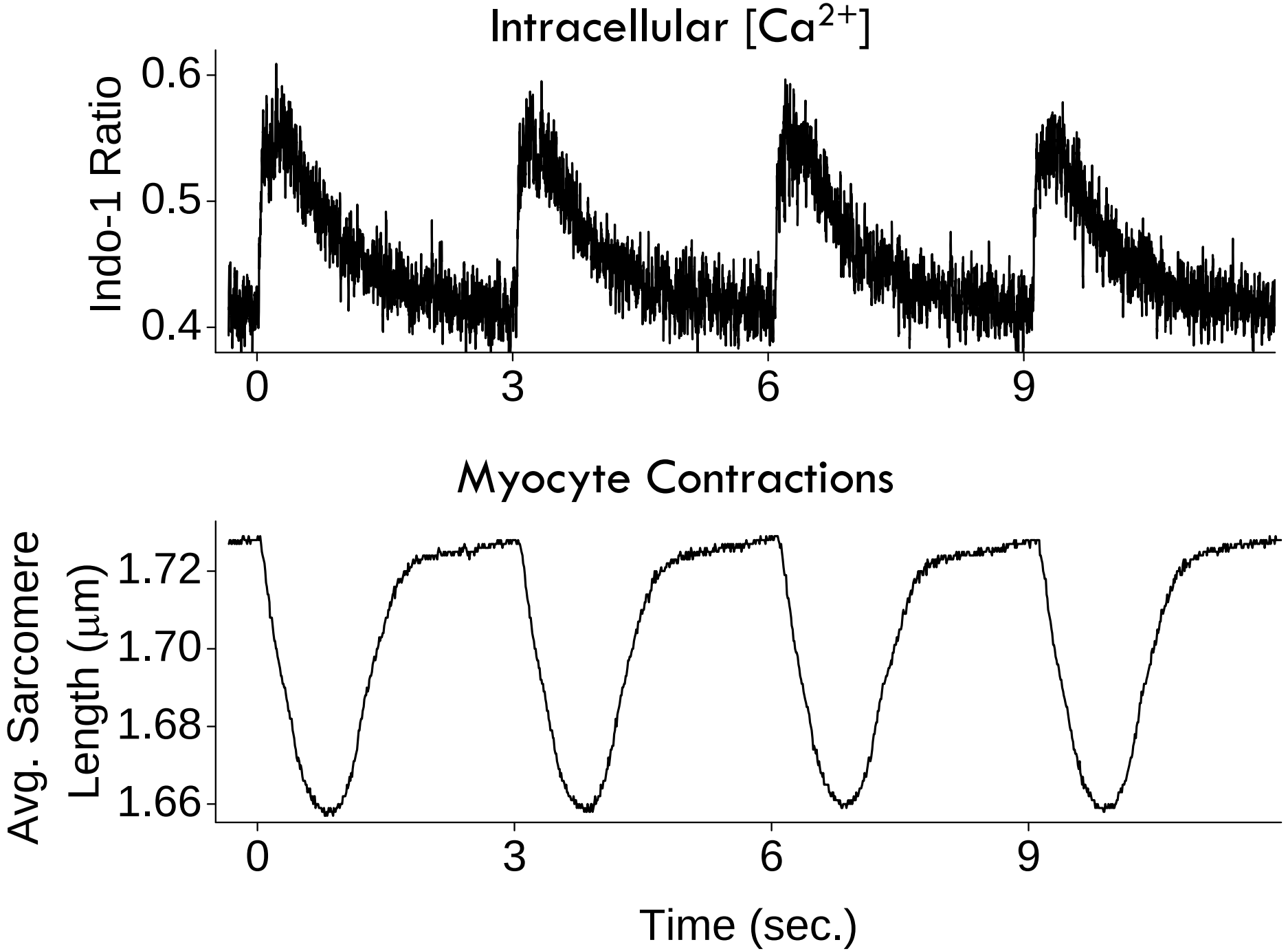
$$FRET\ Eff.\ (\%) = FRET_{max} * [Protein]^n / (K_D^n + [Protein]^n)$$


Gain of NKA Binding
Loss of NKA Binding
PLM Mutant
WT
S63E/S68E
S63A/S68A
S37A
L36A
V35A
I34A
L33A
I32A
G31A
L30A
I29A
F28A
L27A
I26A
G25A
I23A
I22A
L21A
G20A
G19A
I18A
*
0
6
12
18
K_D (arbitrary units)
PLM
PLM-NKA
K_D

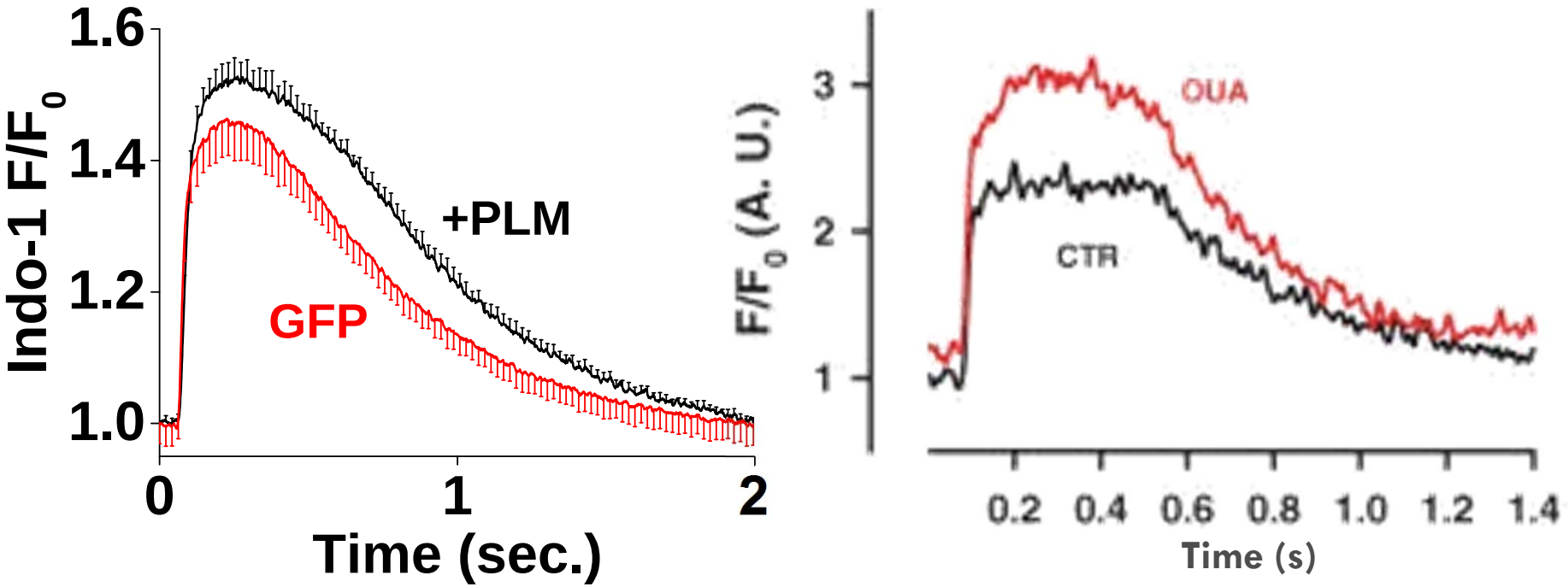

PLM-YFP
WGA-Ax594
Overlay
10 µm



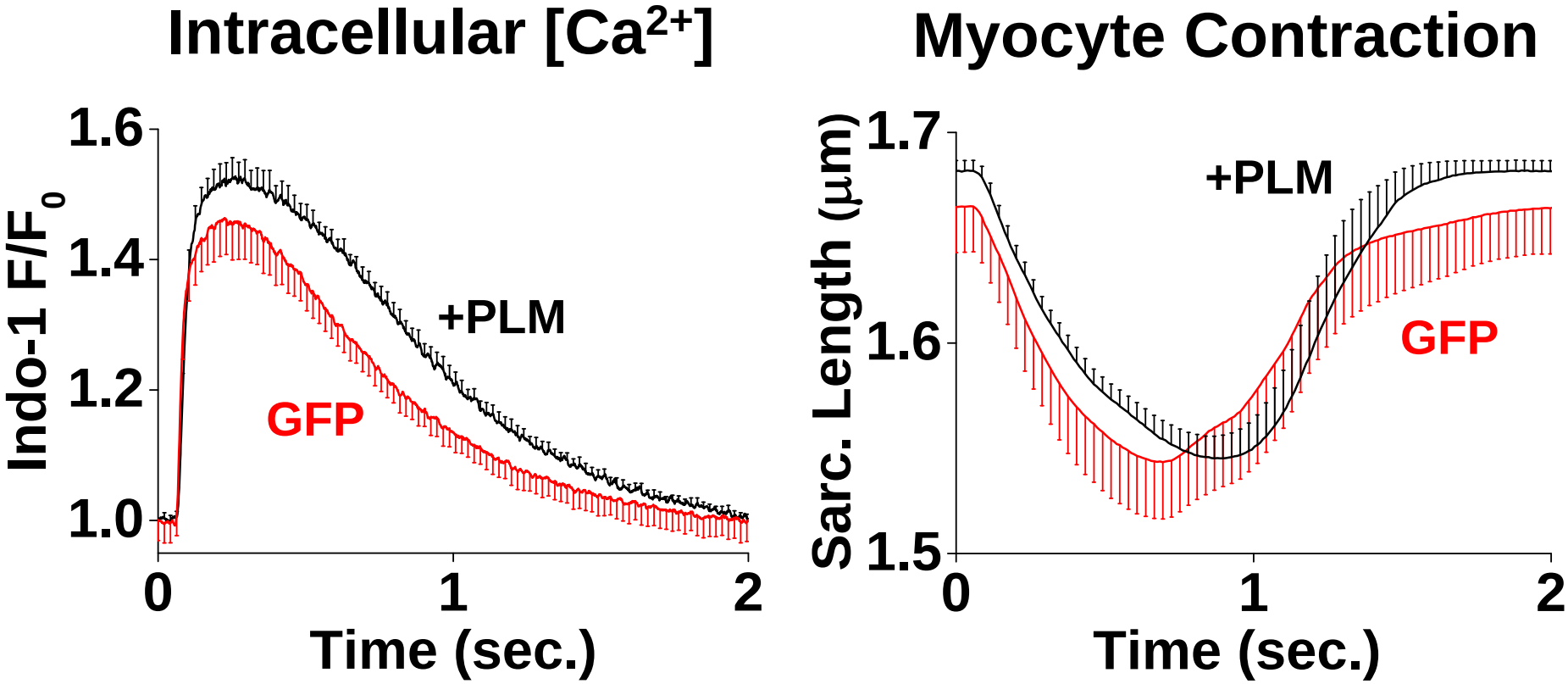
Video courtesy of Dan Blackwell

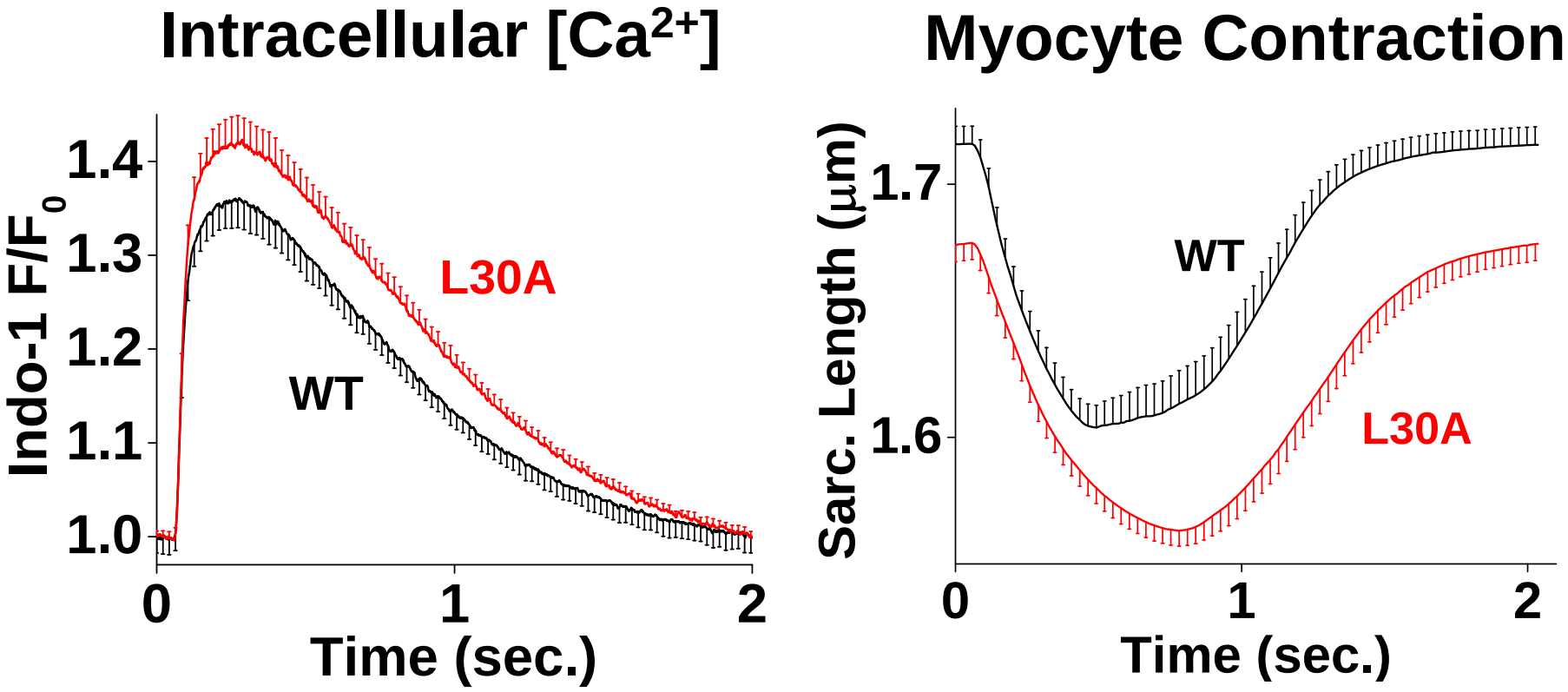

Intracellular [Ca2+]
Indo-1 Ratio
0.6
0.5
0.4
0
3
6
9
Myocyte Contractions
Avg. Sarcomere Length (µm)
1.72
1.70
1.68
1.66
0
3
6
9
Time (sec.)

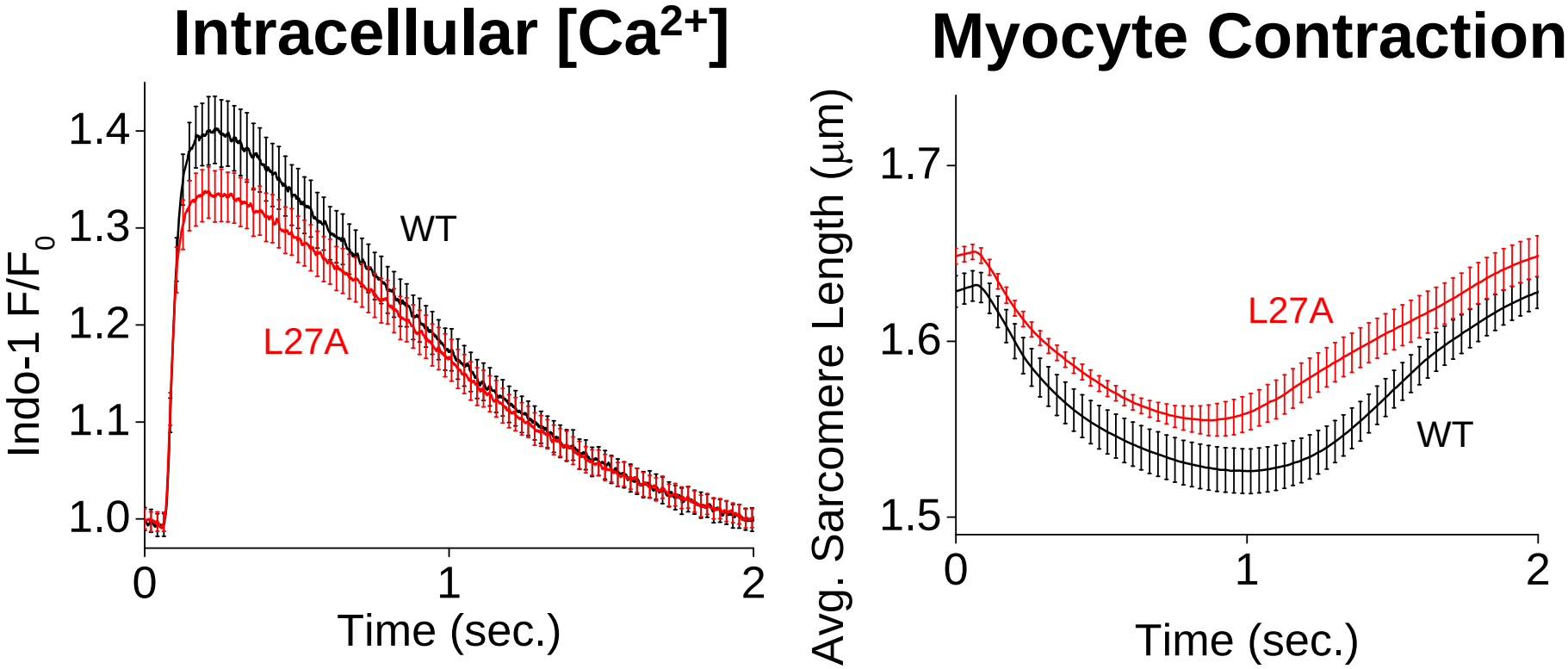
Intracellular [Ca^{2+}]



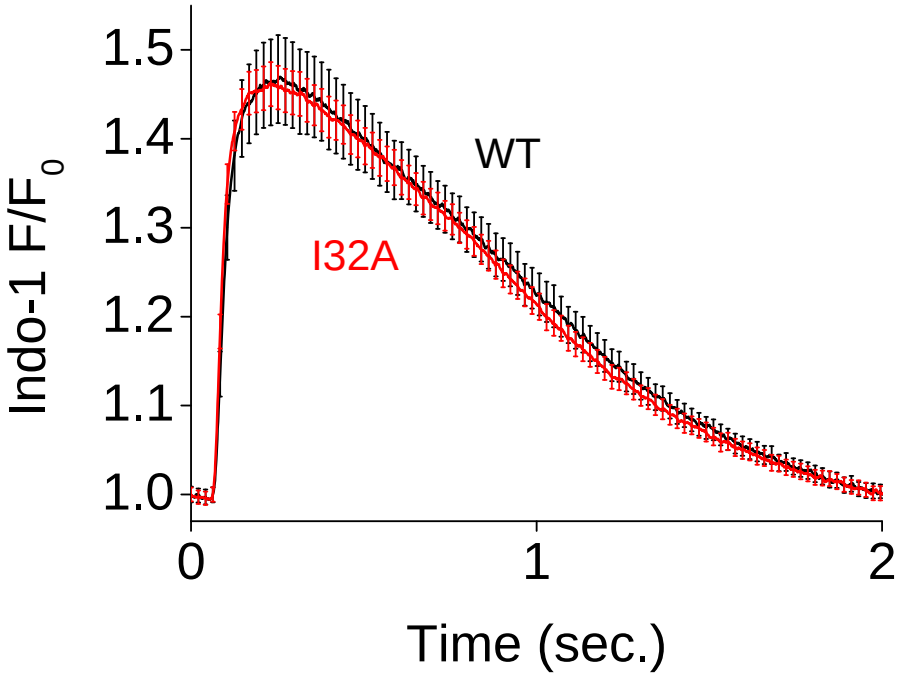
Altamirano et al., 2006. *J. Phys.* 575(3):850.


Intracellular [Ca2+]
Indo-1 F/F0
1.6
1.4
1.2
1.0
+PLM
GFP
0
1
2
Time (sec.)
Myocyte Contraction
Sarc. Length (μm)
1.7
1.6
1.5
+PLM
GFP
0
1
2
Time (sec.)

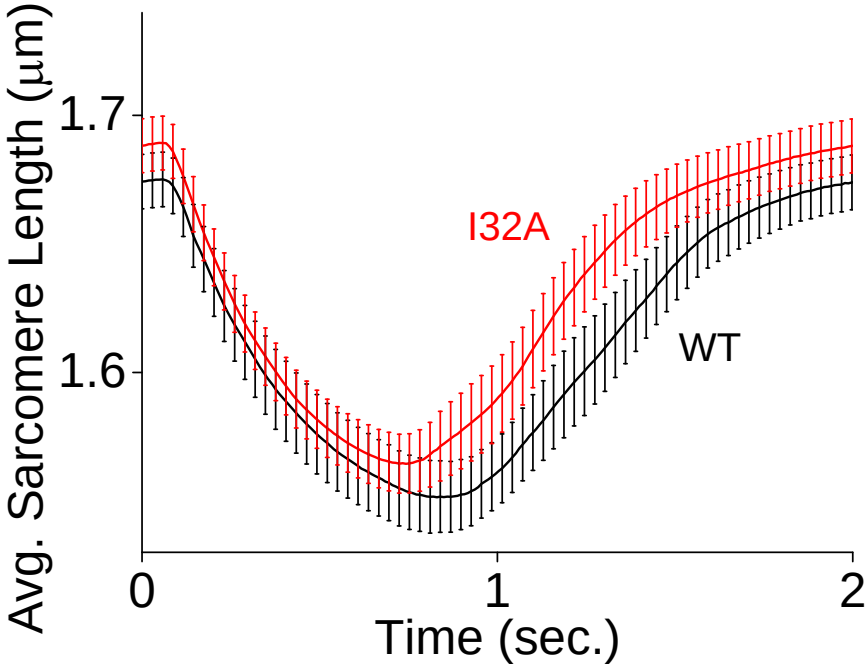

Intracellular [Ca2+]
Indo-1 F/F0
1.4
1.3
1.2
1.1
1.0
L30A
WT
0
1
2
Time (sec.)
Myocyte Contraction
Sarc. Length (μm)
1.7
1.6
WT
L30A
0
1
2
Time (sec.)


Intracellular [Ca2+]
Indo-1 F/F0
1.4
1.3
1.2
1.1
1.0
WT
L27A
0
1
2
Time (sec.)
Myocyte Contraction
Avg. Sarcomere Length (μm)
1.7
1.6
1.5
L27A
WT
0
1
2
Time (sec.)

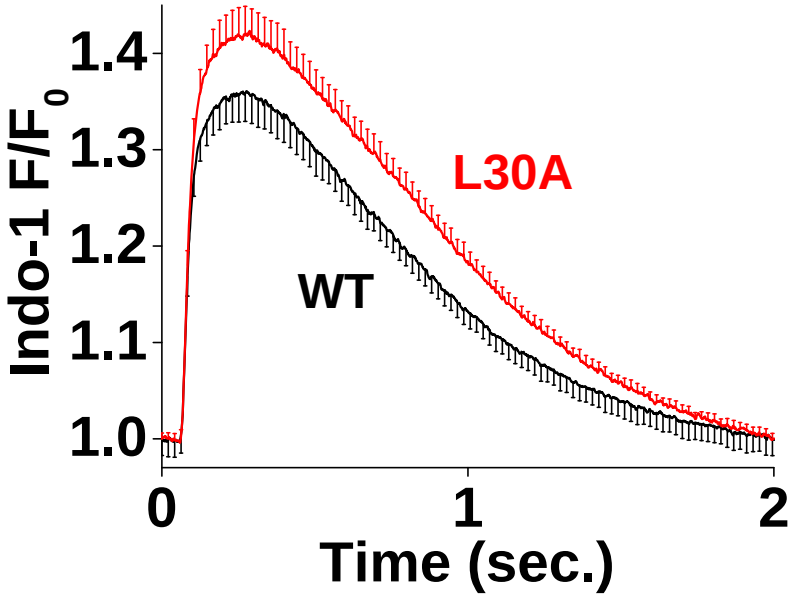
Intracellular [Ca^{2+}]



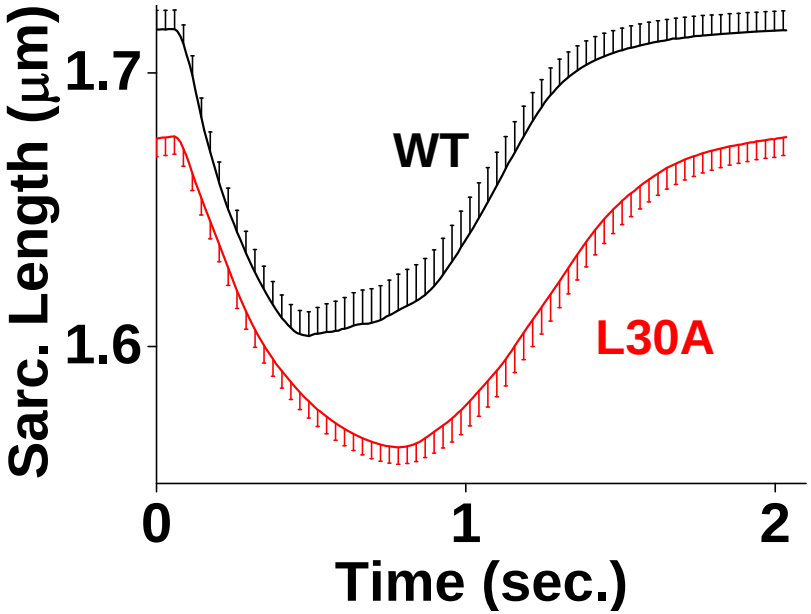
Myocyte Contraction



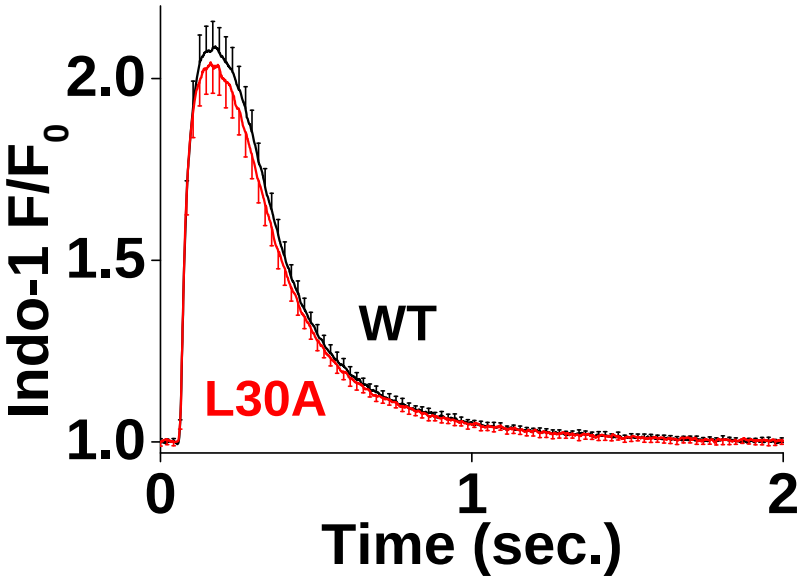
Intracellular [Ca^{2+}]

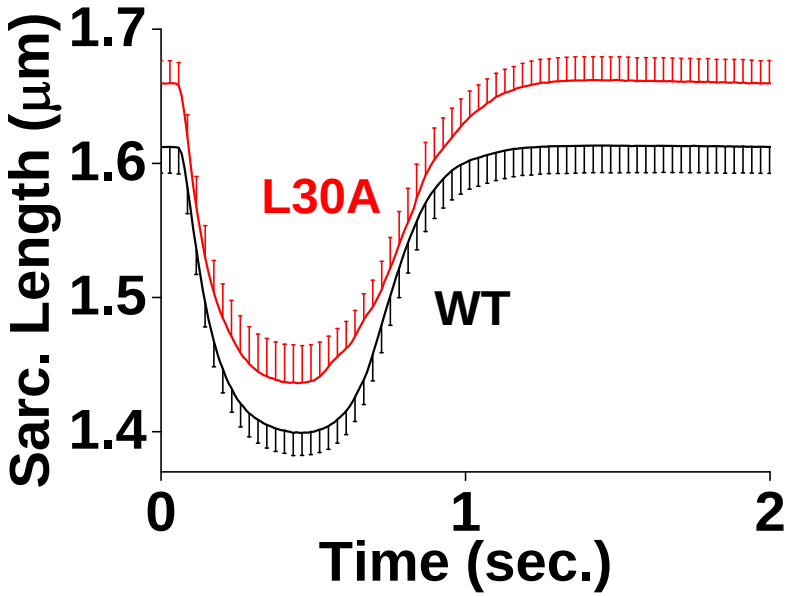


Myocyte Contraction



+ adrenaline

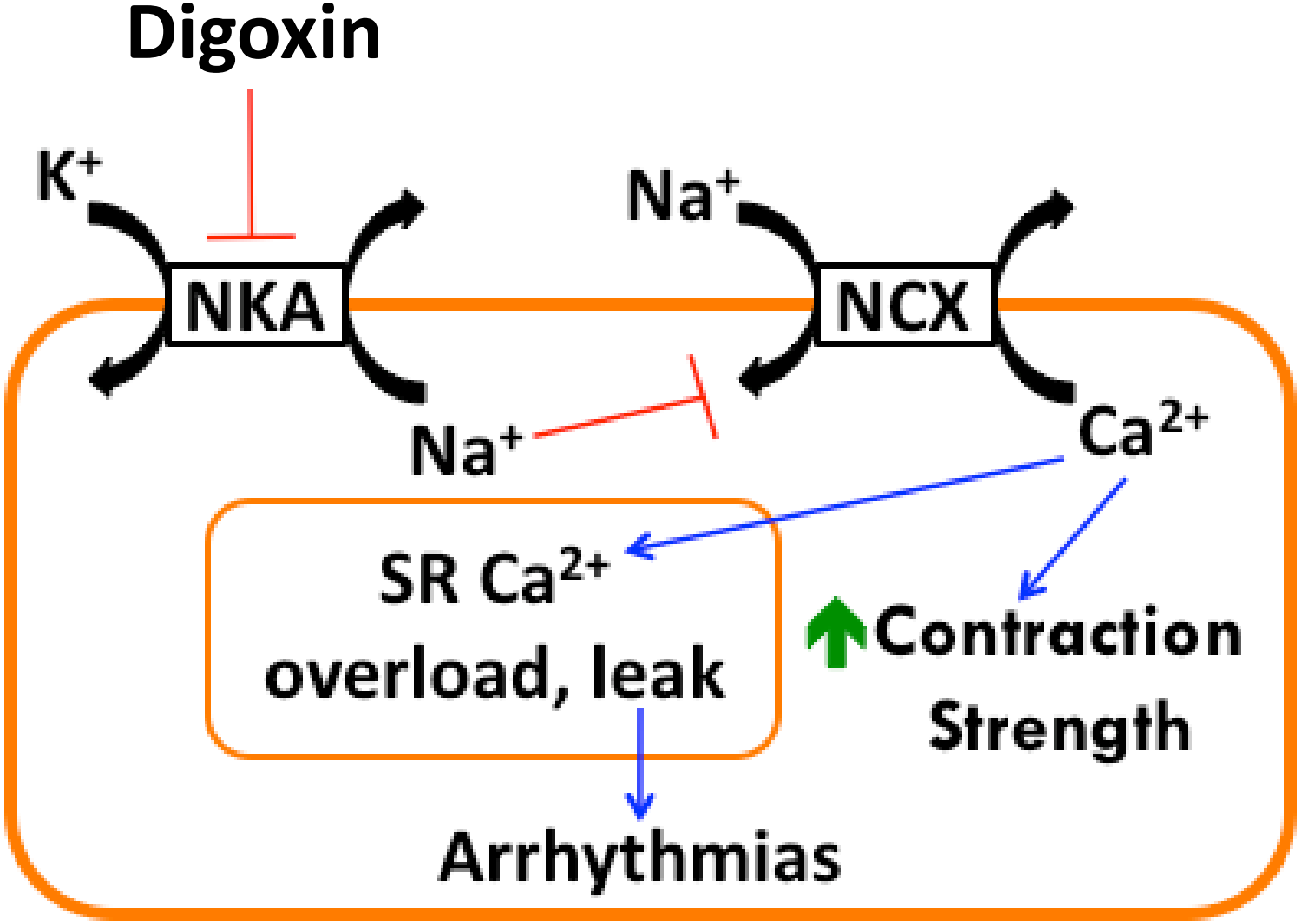




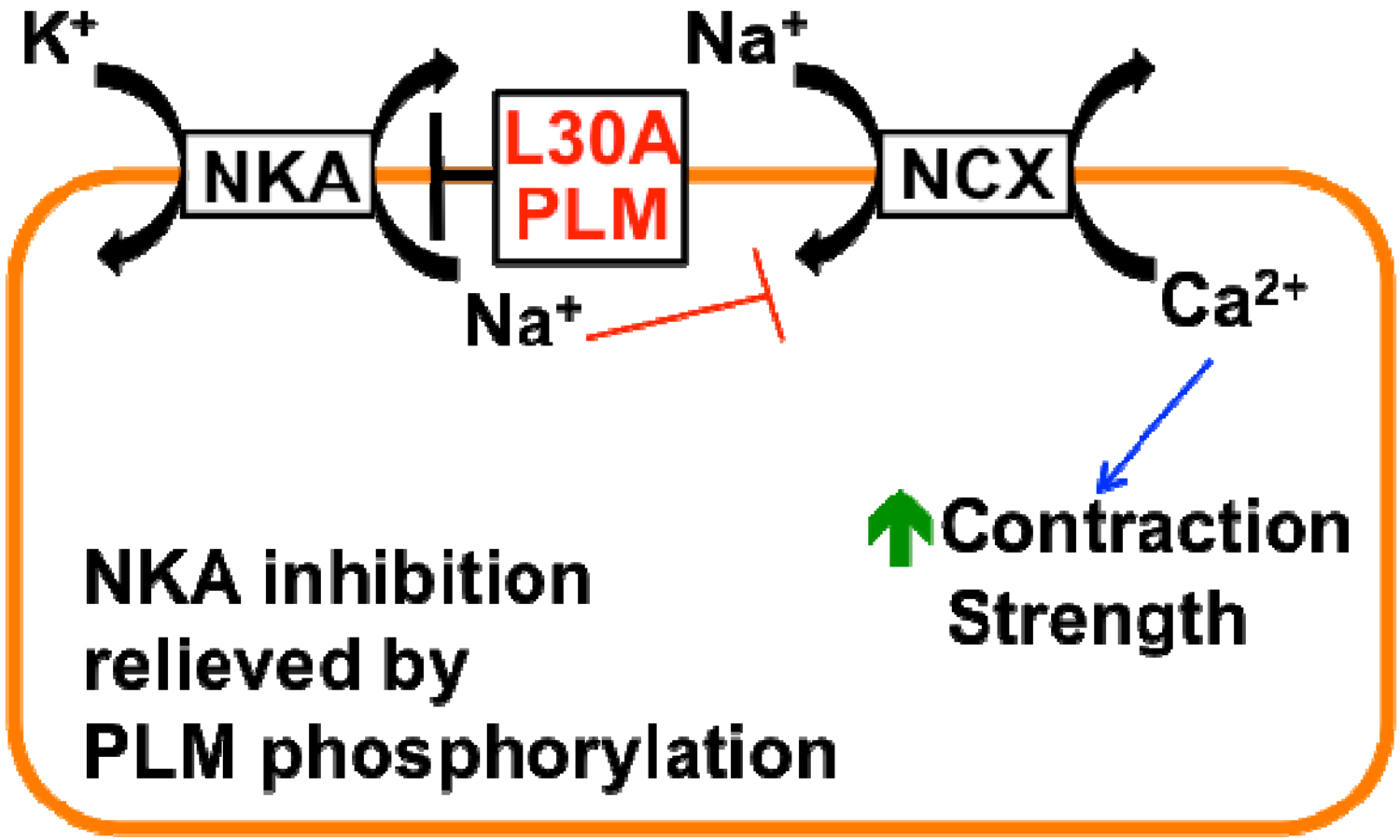
Conclusions

- L30A PLM caused increased binding to NKA.
- L30A PLM mimics the effect of digoxin on isolated cardiomyocytes.
- Adrenaline relieved this effect.
- L30A PLM could be a useful therapeutic for heart failure.

Pharmacological NKA Inhibition



Physiological NKA Inhibition



Acknowledgements

Seth Robia, Ph.D., Advisor

Nikolai Smolin, Ph.D.

Collaborators:

Don Bers, Ph.D. (UC Davis)

Julie Bossuyt, Ph.D. (UC Davis)

Andreas Kukol, Ph.D. (U. of Hertfordshire)

Academic Research Collaborations Program

In addition to providing direct-to-consumer genetic testing through the 23andMe® Service, 23andMe endeavors to advance biomedical science through genetic research. Some of those discoveries have and will come from our own scientists, but others will come from **collaborations with academic researchers external to 23andMe** through the 23andMe Academic Research Collaboration Program.