

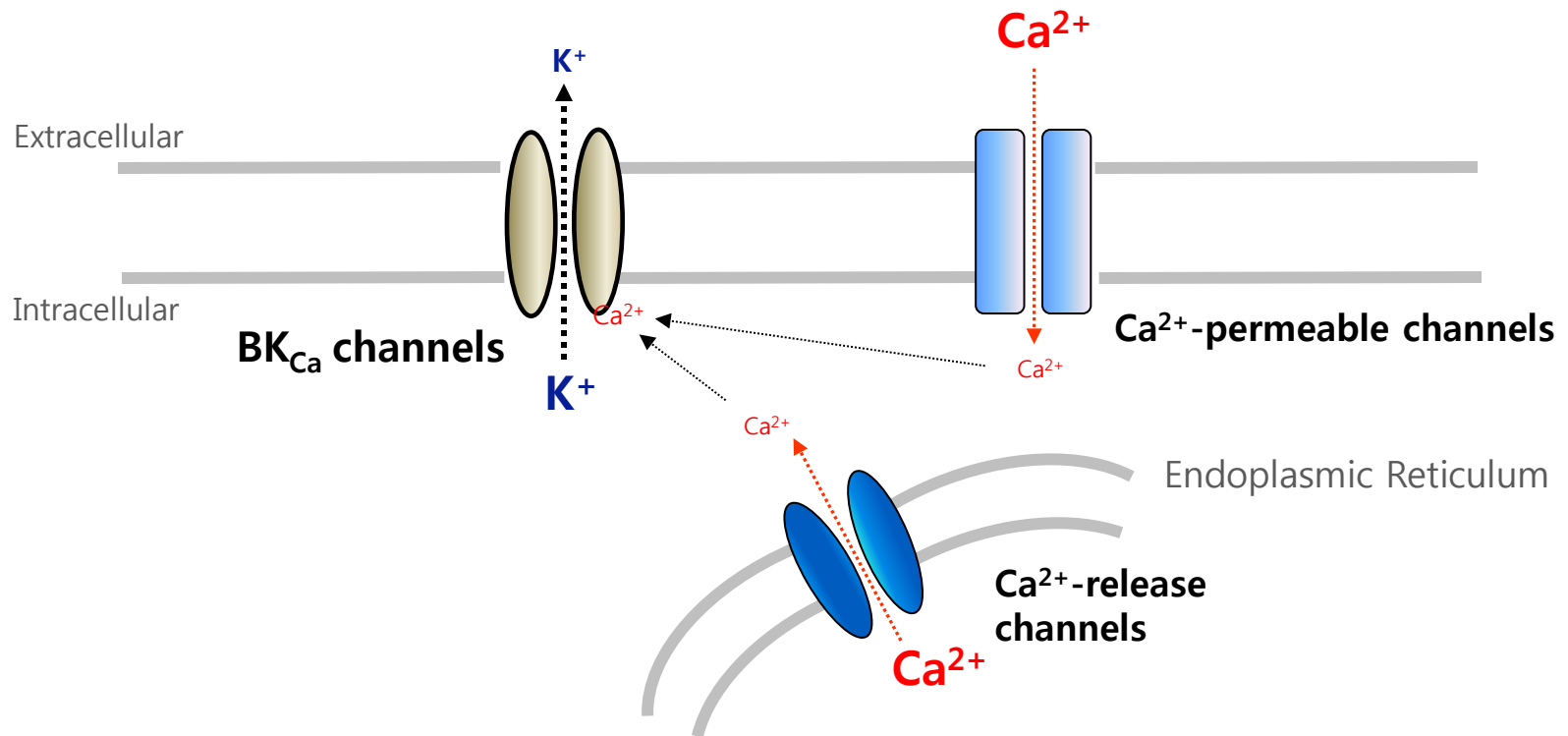


# Discovery of novel modulators for BK<sub>Ca</sub> channel using a new cell-based assay platform

Chul-Seung Park, Ph.D.  
School of Life Sciences  
GIST



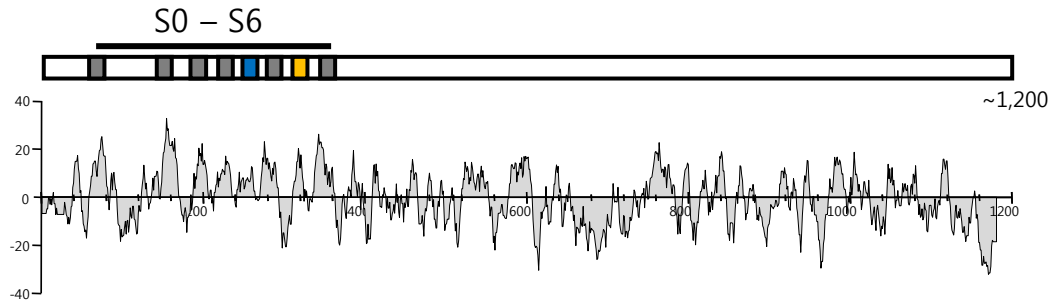
# Large-conductance calcium-activated potassium (BK<sub>Ca</sub> or Maxi-K) channels



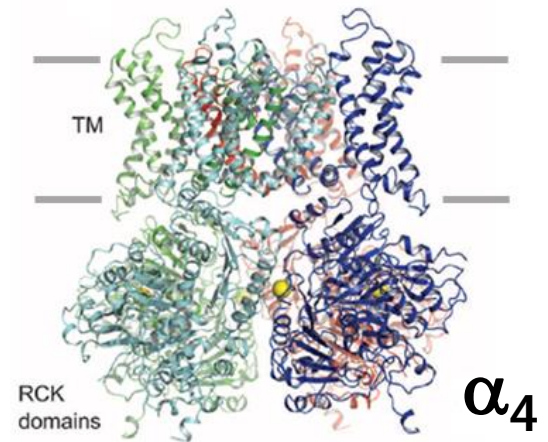
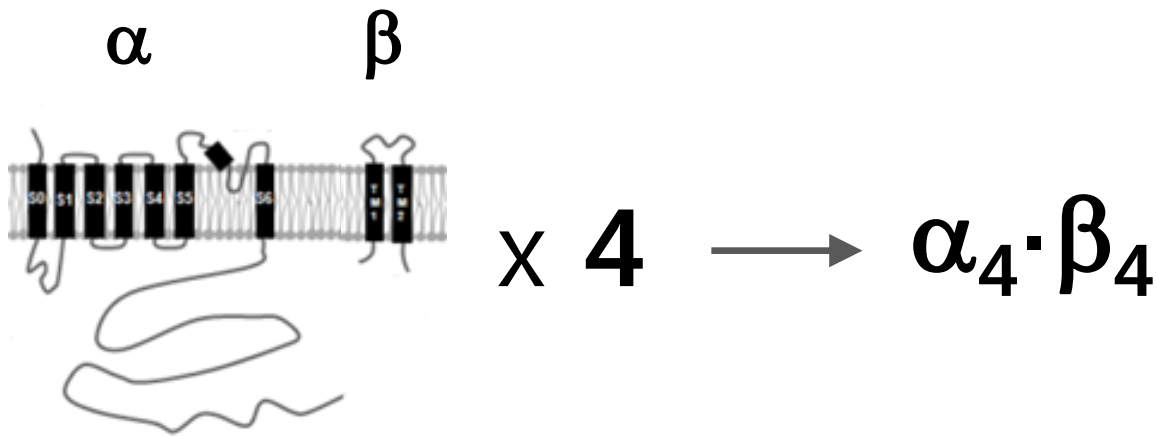
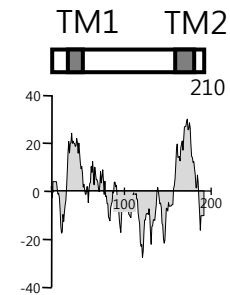
A class of K<sup>+</sup>-selective **channels** activated by **intracellular** Ca<sup>2+</sup> with **large** (or **big**) single-channel **conductances**

# BK<sub>Ca</sub> channels: subunits and assembly

**$\alpha$  subunit** (KCNMA1, Slowpoke1 (or Slo1))



**$\beta$  subunit** (KCNMB1 – 4,  $\beta$ 1 – 4)

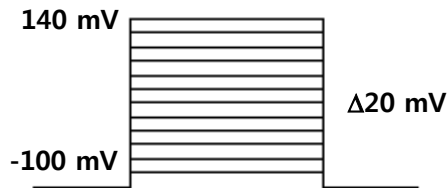
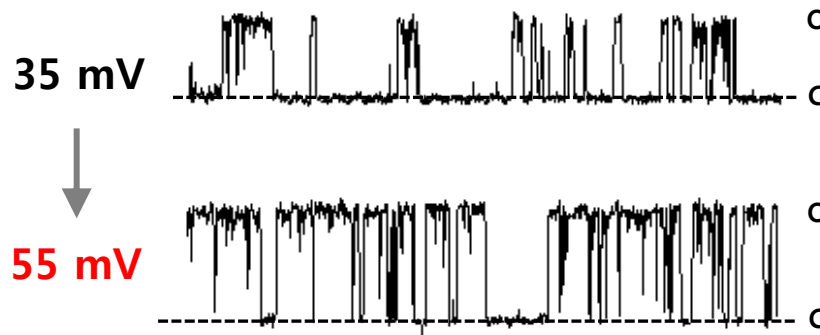


*Science* 329:182 (2010)

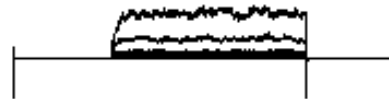
# Dual activation of BK<sub>Ca</sub> channels: voltage and Ca<sup>2+</sup>

2  $\mu\text{M}$  [Ca<sup>2+</sup>]<sub>i</sub>

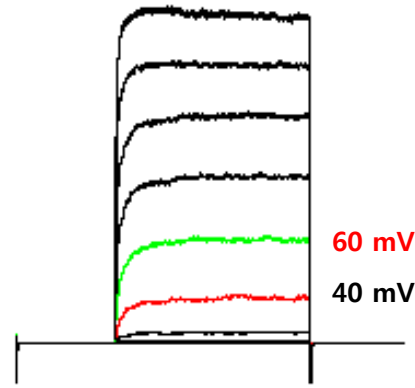
10  $\mu\text{M}$  [Ca<sup>2+</sup>]<sub>i</sub>



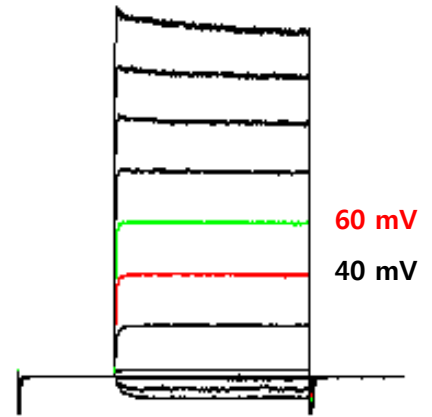
Un-injected



Ca<sup>2+</sup>-free



2  $\mu\text{M}$  [Ca<sup>2+</sup>]<sub>i</sub>



10  $\mu\text{M}$  [Ca<sup>2+</sup>]<sub>i</sub>

$$I_{\text{BK(Ca)}} = G_{(\text{V,Ca})} \times V$$

1 nA

100 ms

# Physiological functions of BK<sub>Ca</sub> channel

- **Nervous system**

- Controlling excitability of neurons and influencing neurotransmitter release; modulating synaptic efficacy

- **Cardiovascular system**

- Maintaining 'vasomotor tone'

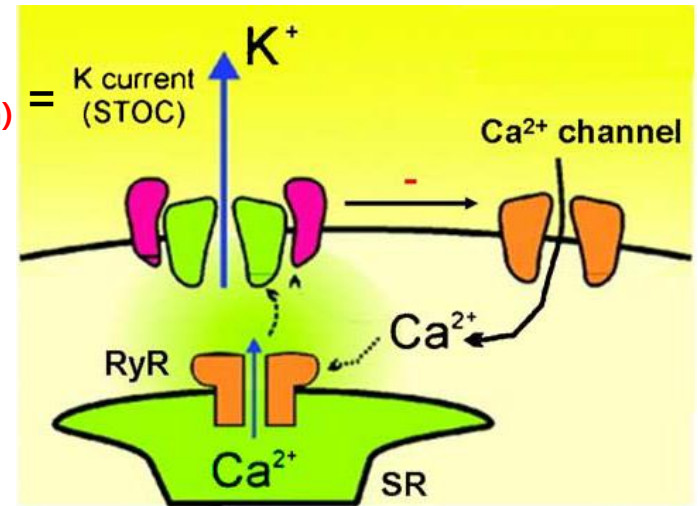
- **Urinary system**

- Relaxing urinary bladder smooth muscle

- **Other systems:** reproductive, gastrointestinal, immune, *etc.*

# BK<sub>Ca</sub> channel and micturition

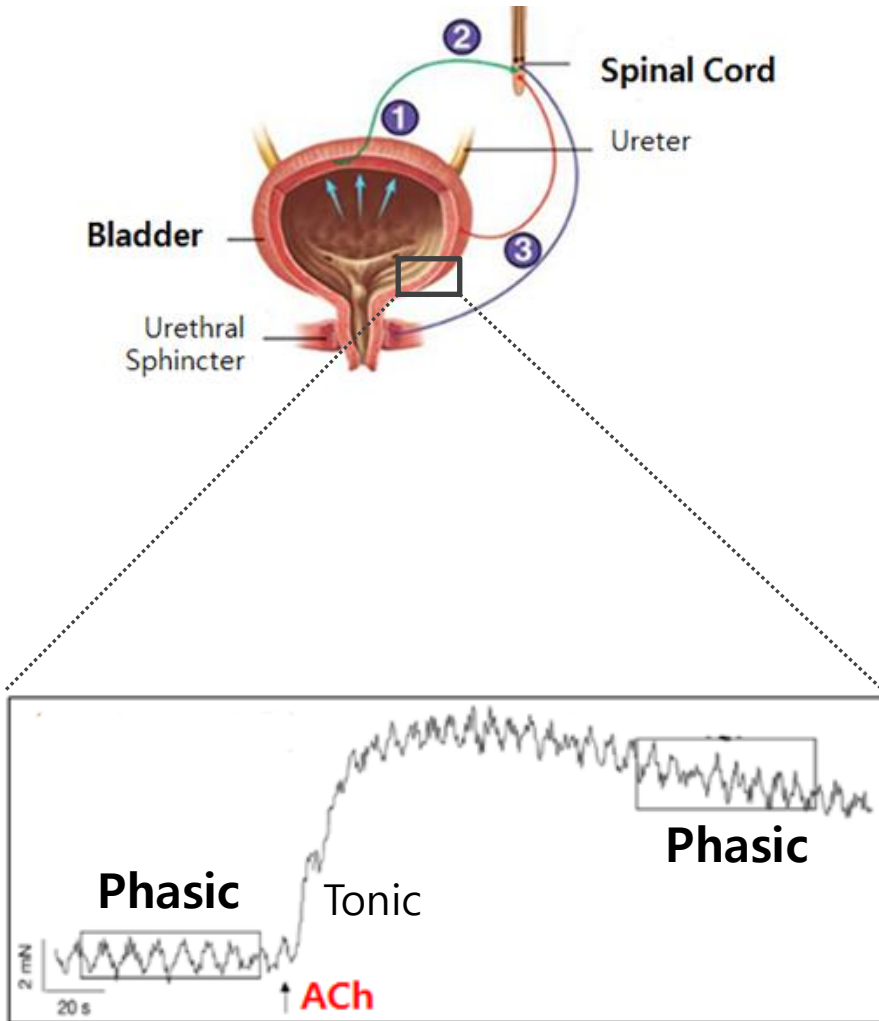
Physiological mechanism of  
'phasic contraction-relaxation'



*Pharmacol & Therapeutics*. 110:103 (2006)

**STOC** (spontaneous transient outward current)  
relaxing detrusor muscle during  
'filling phase' !

∴ Therapeutic potential of **BK<sub>Ca</sub> channel activators** against **overactive bladder** and/or **urinary incontinence!**



Two different contractions of  
bladder muscle

**However, we *still* do not have any BK<sub>Ca</sub> channel activators that are therapeutically used or proven to be clinically effective.**

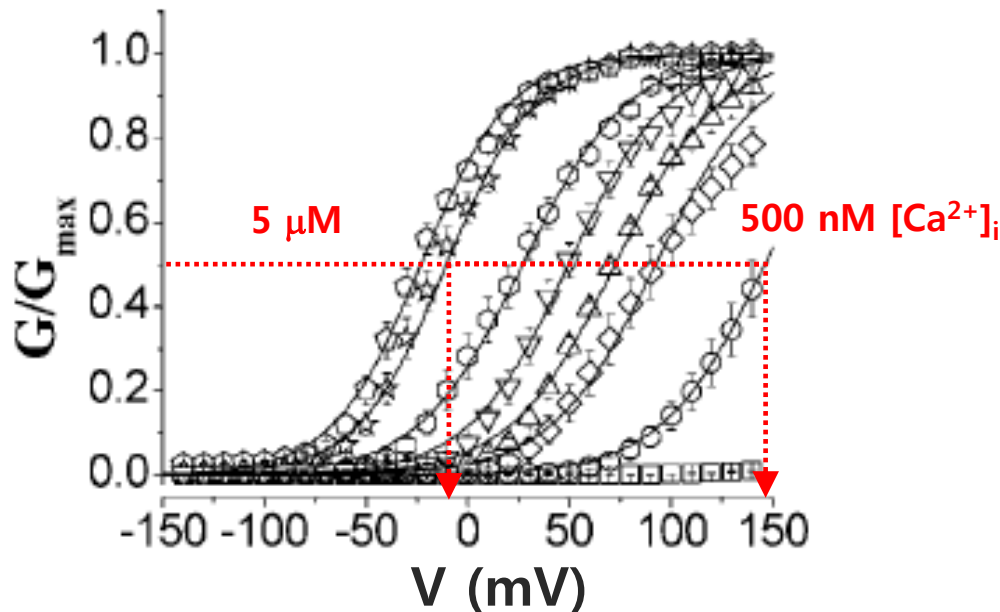
**← ∴ We have *not* been *able to* screen enough compounds.**

\*Sucampo Pharmaceuticals, Inc. announced it has received approval of an sNDA (supplemental New Drug Application) for Rescula® (unoprostone isopropyl ophthalmic solution) 0.15% for the lowering of intraocular pressure (IOP) in patients with open-angle glaucoma or ocular hypertension from the U.S. Food and Drug Administration (FDA). ..... Rescula is a BK (Big Potassium) channel activator, which is different from other IOP-lowering agents.

- News from AOA (American Optometric Association), 2013.1.17

# Difficulties of assaying BK<sub>Ca</sub> channels in intact cells using non-electrophysiological techniques

- Resting cytosolic [Ca<sup>2+</sup>] : ~**100 nM**
- $K_d^{\text{app}}$  of BK<sub>Ca</sub> channel for intracellular Ca<sup>2+</sup> : >**1  $\mu\text{M}$**



$\therefore$  Need to shift  $G$ - $V$  curve *significantly* to **the left** in order to make BK<sub>Ca</sub> channels active under physiological  $V_m$ !

How?

~~$[\text{Ca}^{2+}]_i$~~   $\uparrow$  !

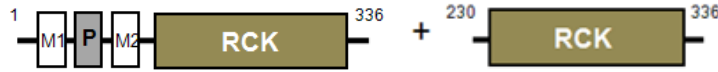
***Then, any other ways?***

Not very reliable for CBA!



# Ca<sup>2+</sup>-dependent gating of Ca<sup>2+</sup>-activated K<sup>+</sup> channels: 'gating ring' of RCK octamers

**MthK** (Ca<sup>2+</sup>-activated)



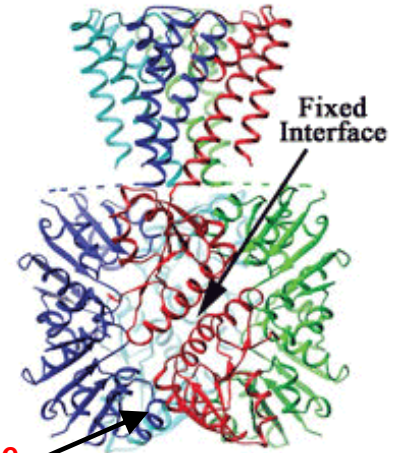
**BK<sub>Ca</sub>** (Ca<sup>2+</sup> and voltage-activated)



4 x (RCK1:RCK2) ?

Kim, *et al.*, *J Biol Chem.*  
281:38573 (2006)

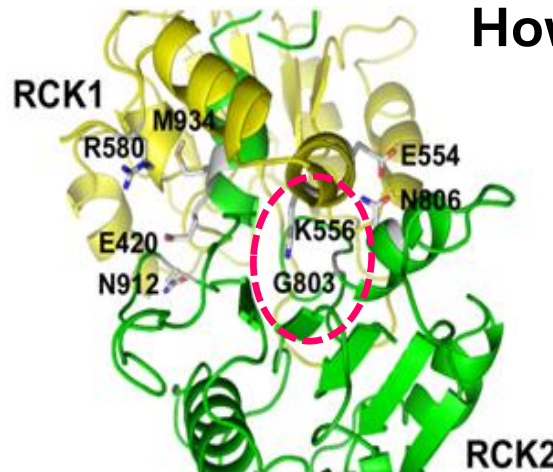
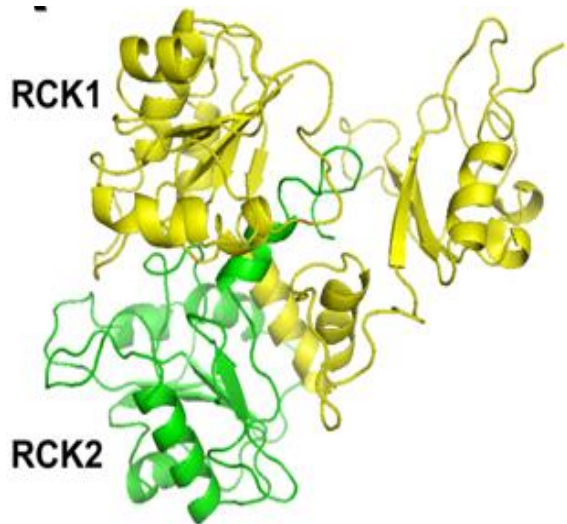
4 x (RCK)<sub>2</sub>  
(‘Gating ring’)



**Flexible interface**

*Neuron* 29:593 (2001);  
*Nature* 417:515 (2002)

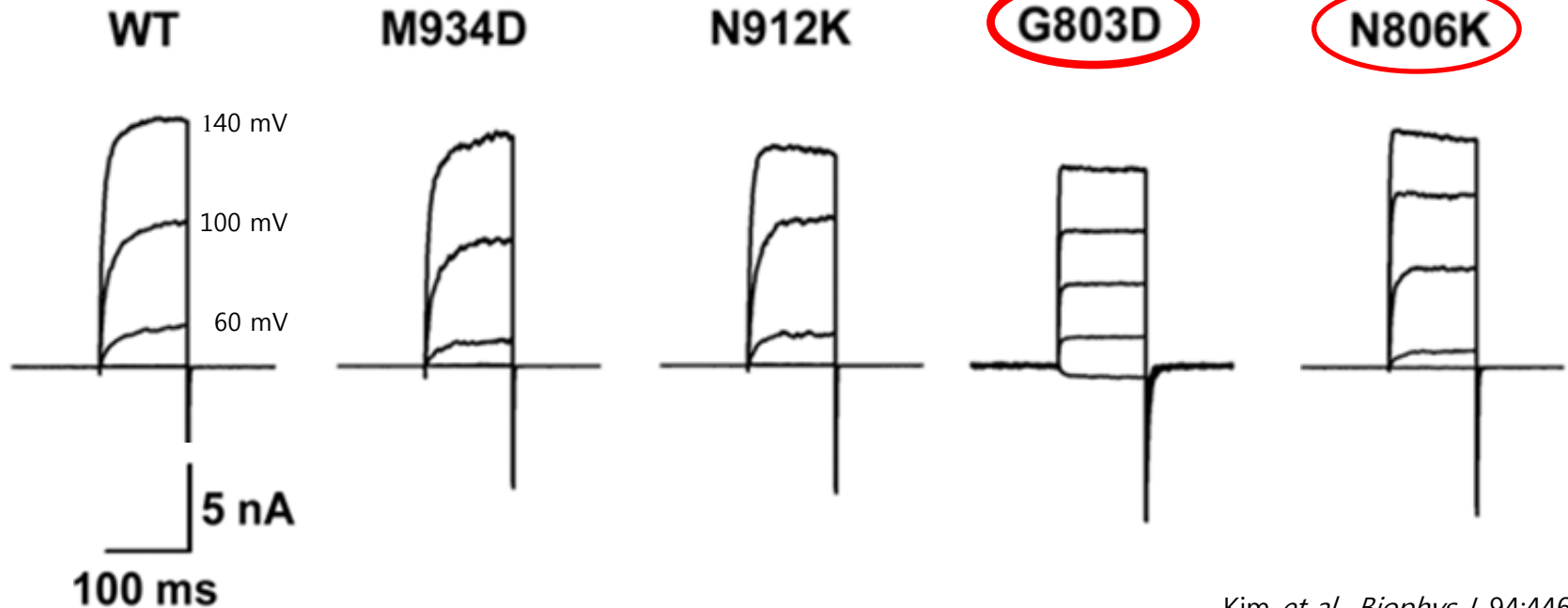
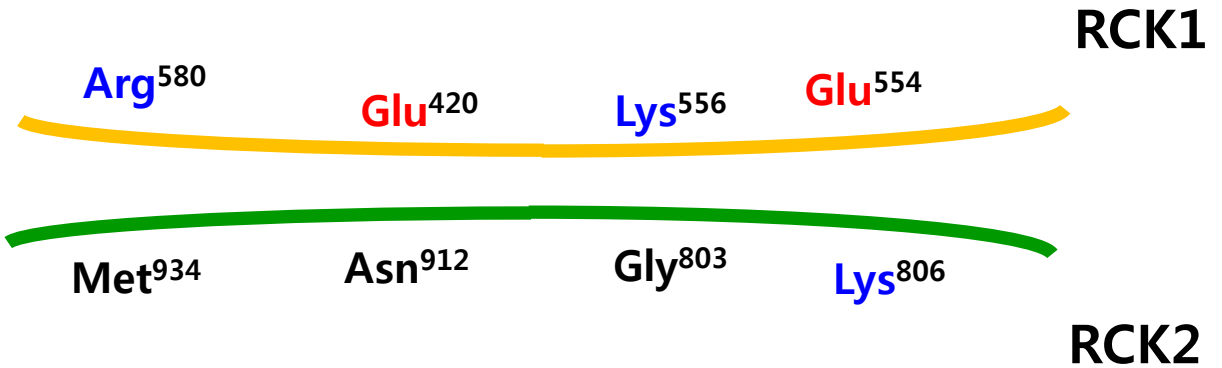
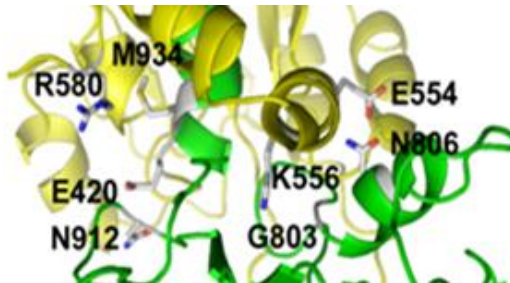
## A structural model of ‘flexible interface’ between RCK1 and RCK2



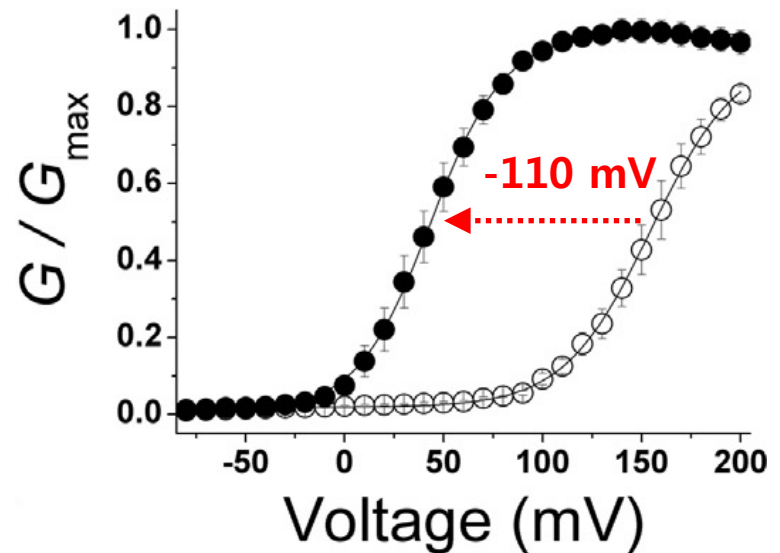
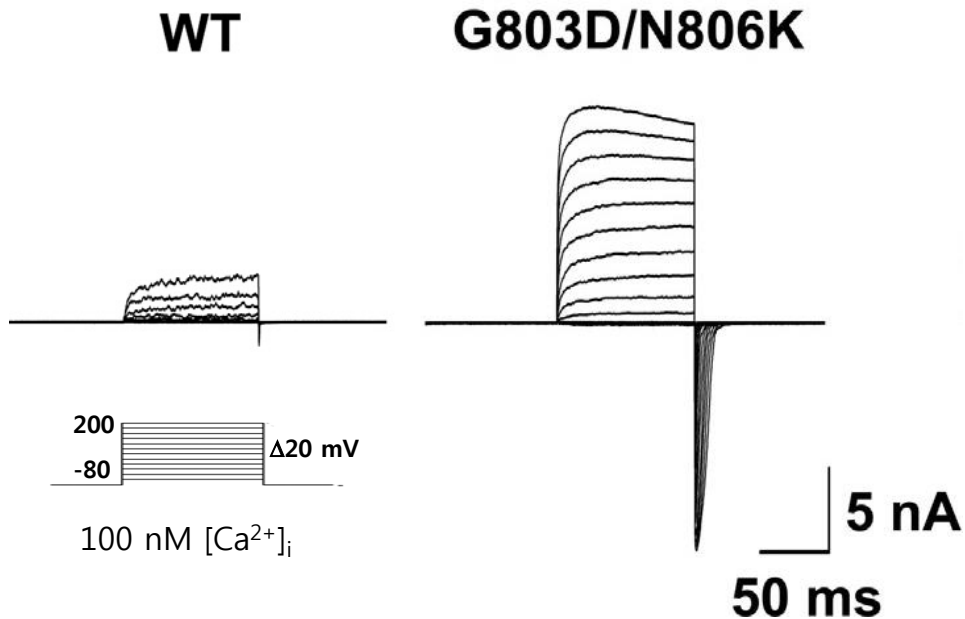
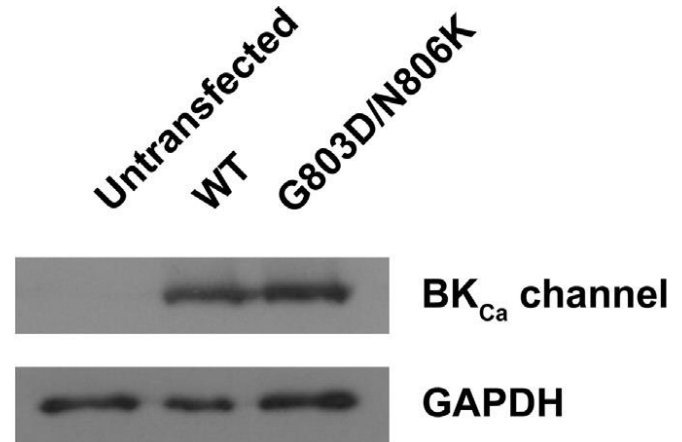
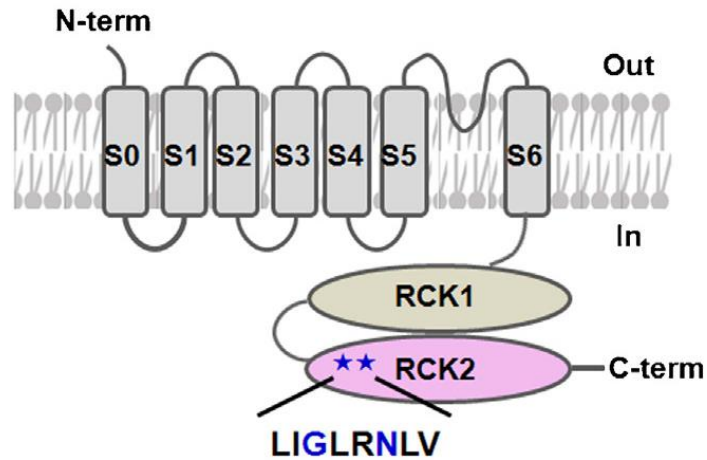
How to test the structural model?

+ **K**<sup>556</sup>  
.....  
- **D**<sup>803</sup>

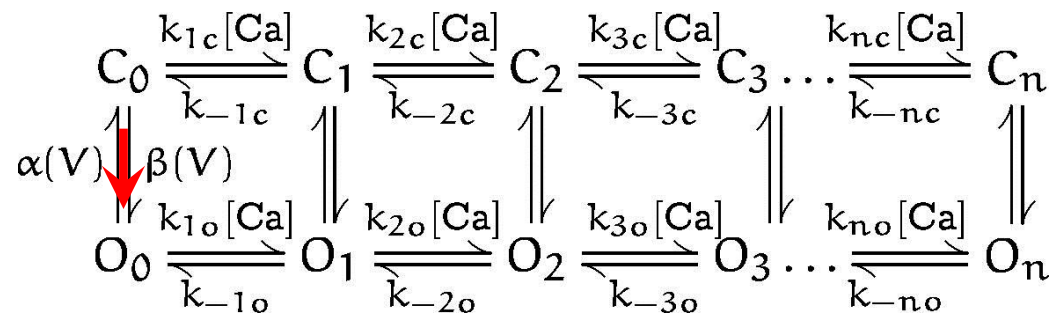
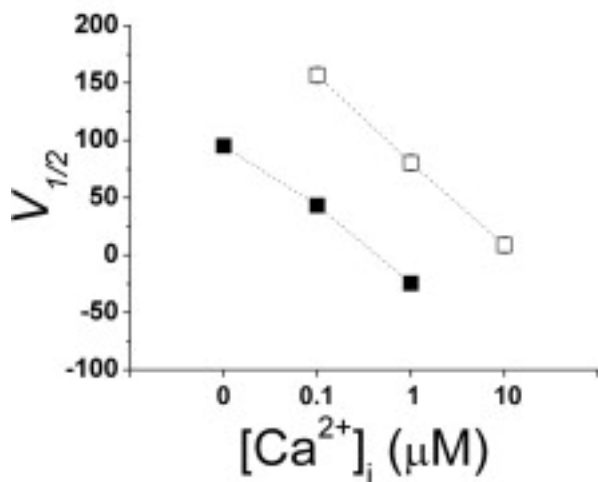
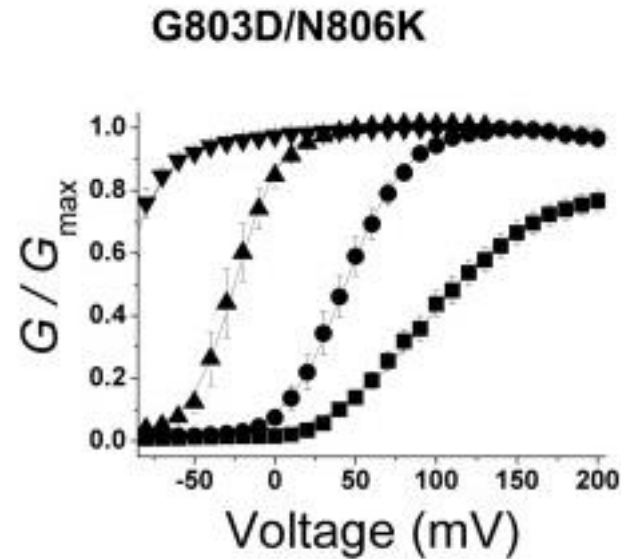
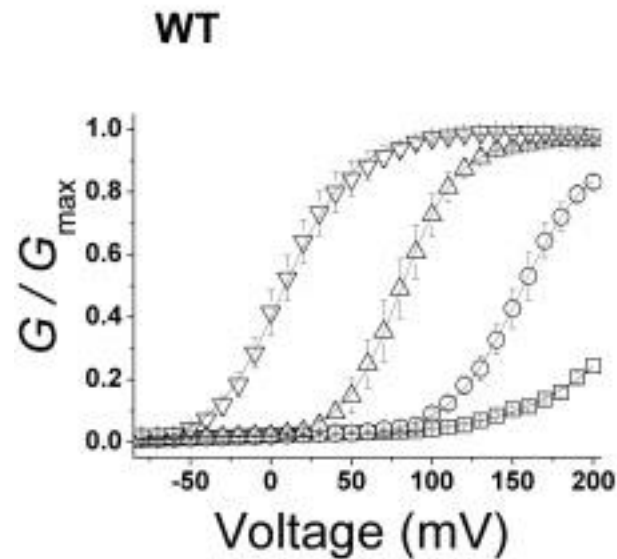
# Effects of 'engineered' charge-pairs on channel activation



# G803D/N806K double mutant: a 'hyperactive' BK<sub>Ca</sub> channel



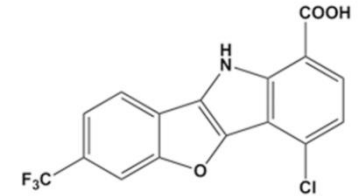
# A parallel shift in $V_{1/2}$ vs. $[Ca^{2+}]_i$ curve: changes in *intrinsic* open-closed equilibrium



Voltage-dependent allosteric model of  $BK_{Ca}$  channel



# Potential of G803D/N806K mutant by a BK<sub>Ca</sub> activator



4-chloro-7-(trifluoromethyl)-  
10H-benzofuro[3,2-b]indole  
-1-carboxylic acid (CTBIC)

Ha *et al. Mol Pharmacol.* 69:1007  
(2006); Lee *et al. Mol Pharmacol.*  
82:143 (2012)

WT

Vehicle

10  $\mu$ M CTBIC

G803D/N806K

Vehicle

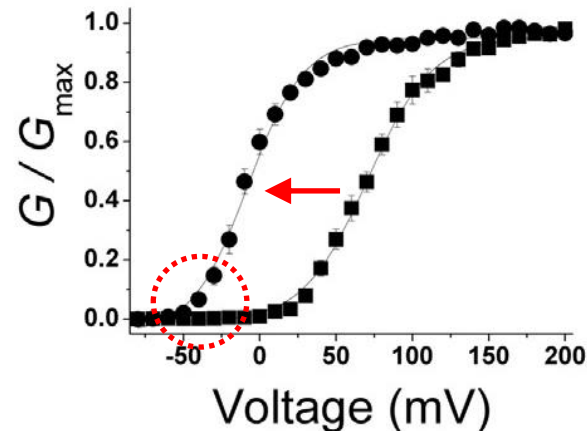
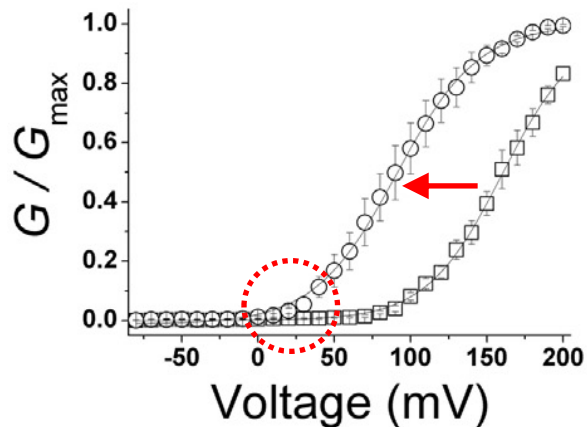
10  $\mu$ M CTBIC

5 nA

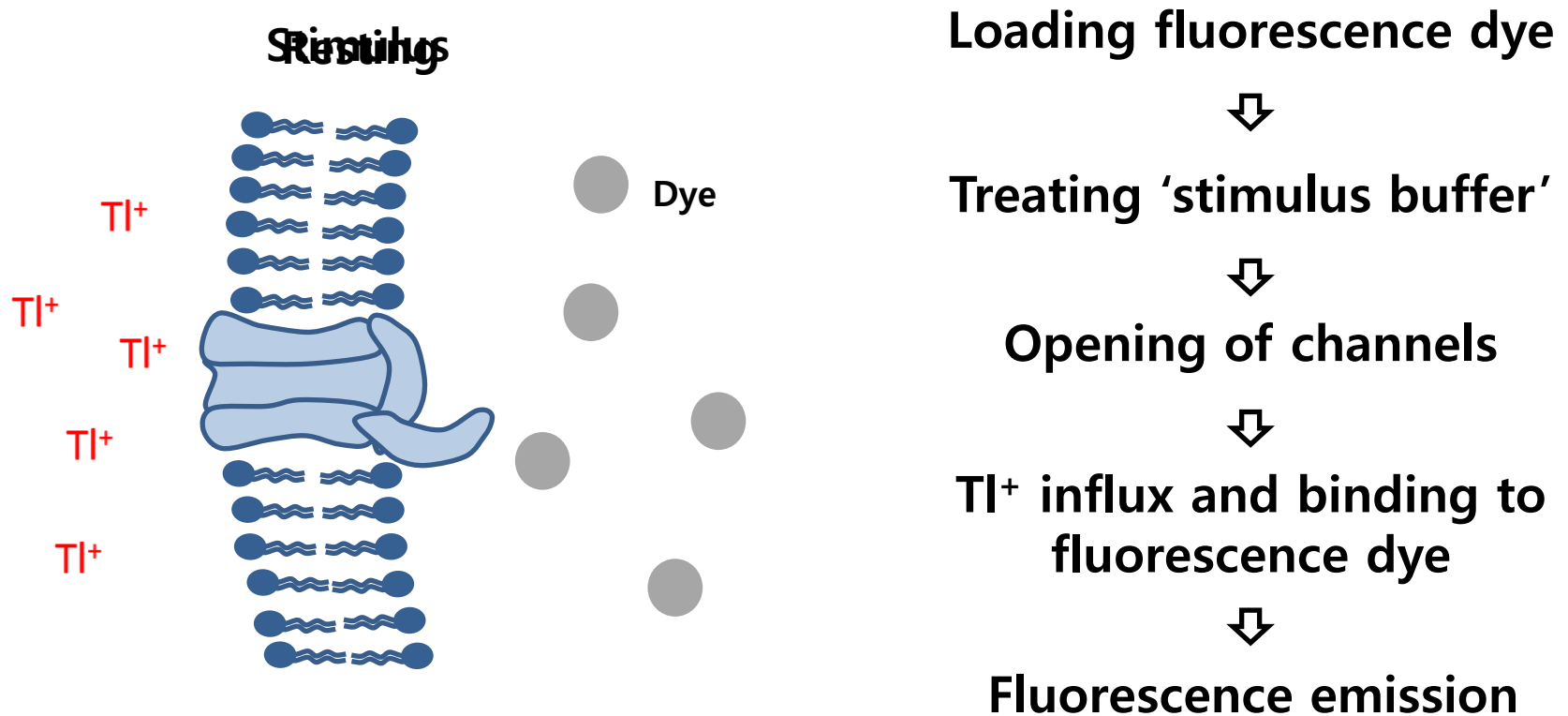
50 ms

5 nA

50 ms

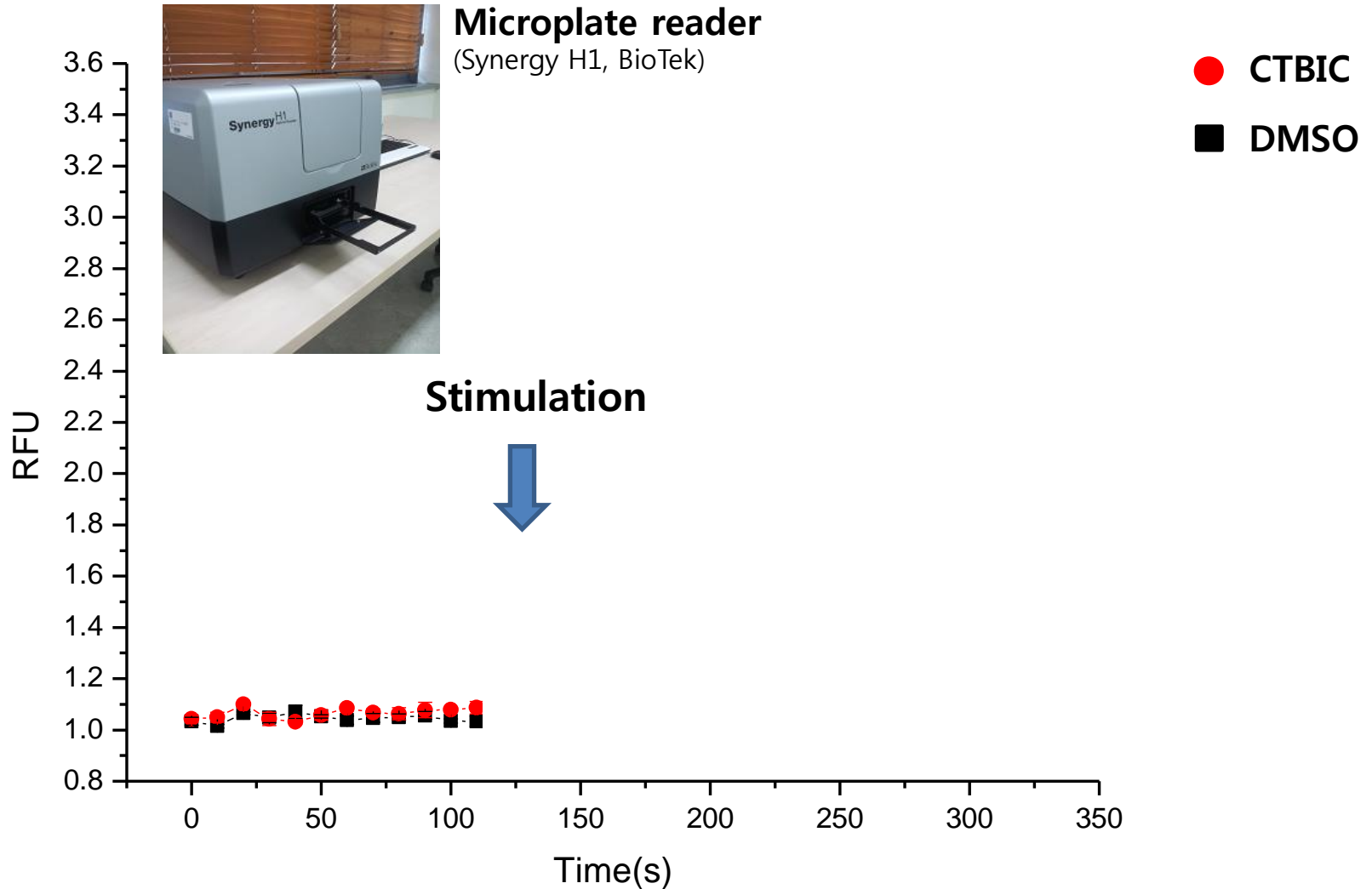


# A cell-based platform for BK<sub>Ca</sub> channel using FluxOR™ assay

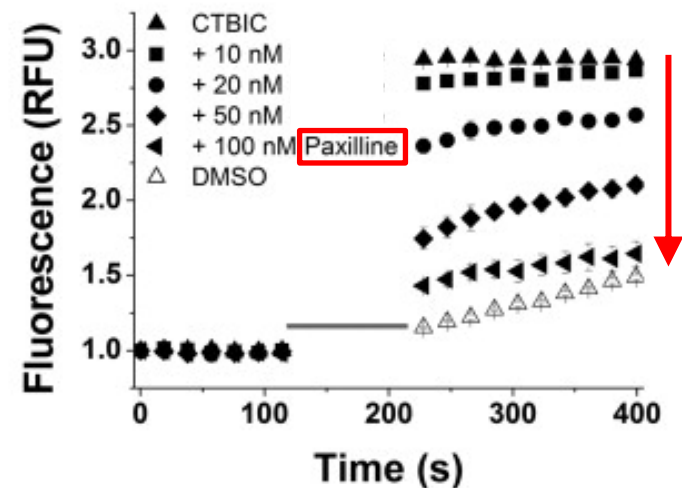
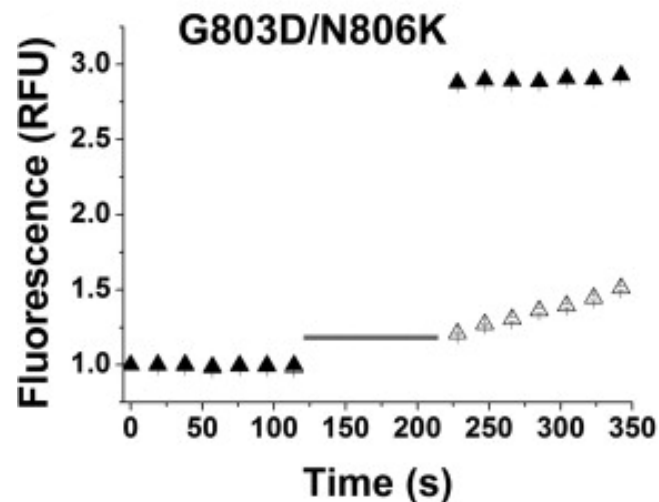
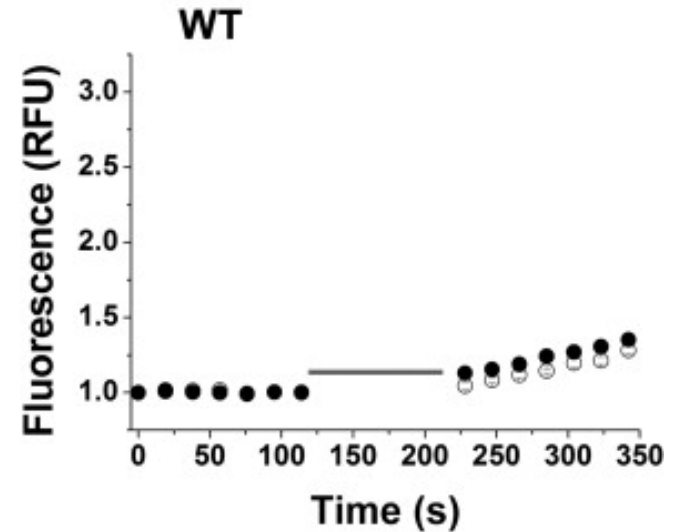
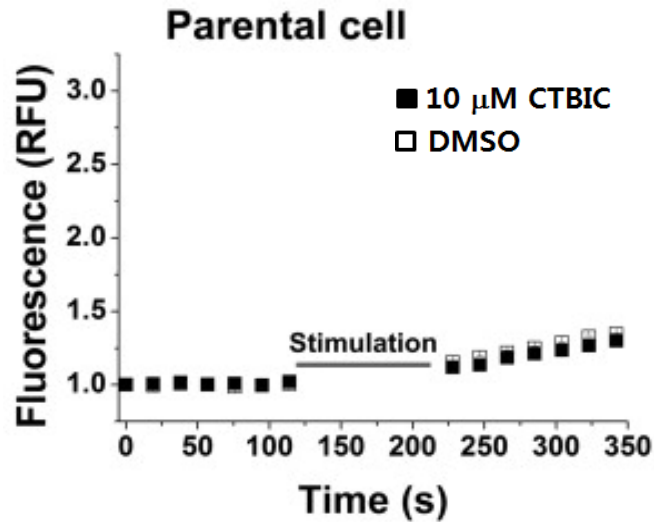


**FluxOR™ assay for voltage-gated K<sup>+</sup> channels** (Life Technologies Co.)

# Robust fluorescence signals obtained from G803D/N806K mutant channel: FluxOR™ assay



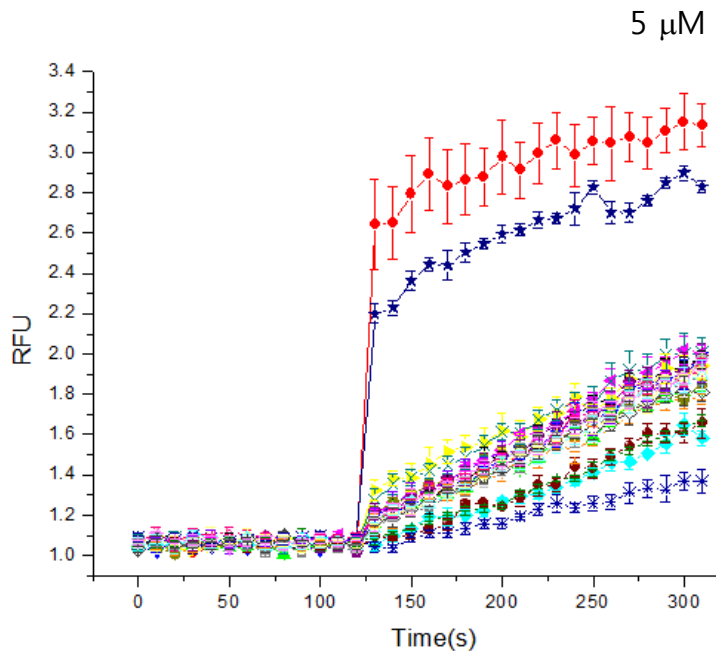
# The hyperactive BK<sub>Ca</sub> channel activated by CTBIC at resting [Ca<sup>2+</sup>]<sub>i</sub>





# Screening for new BK<sub>Ca</sub> channel activators: natural compound library from KRICT\*

\*KRICT (Korea Research Institute of Chemical Technology)



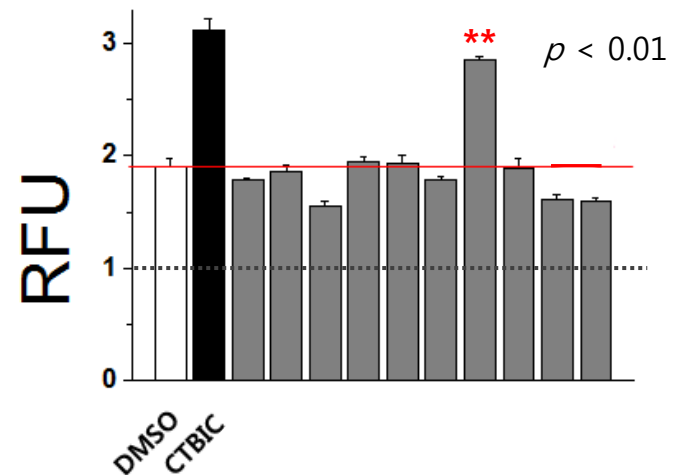
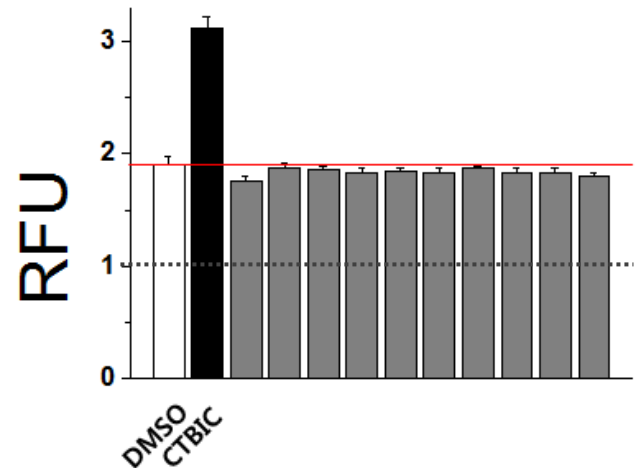
**794** KRICT compounds



**23** activator candidates



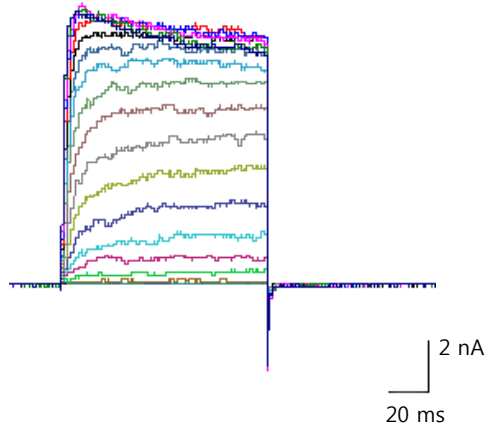
**Top 6** selected!



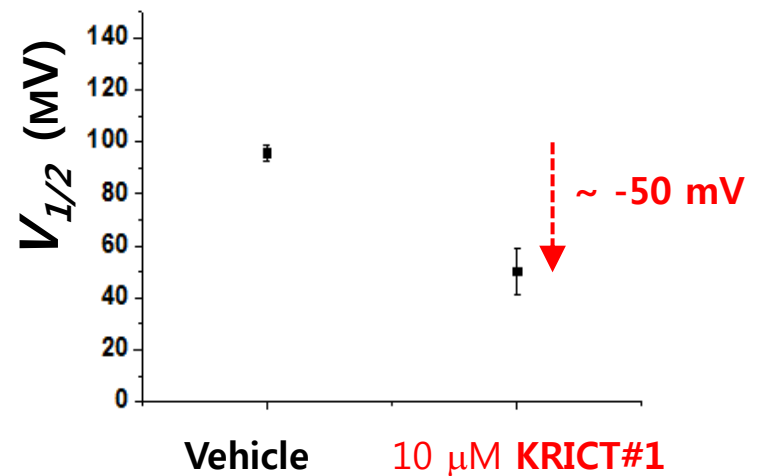
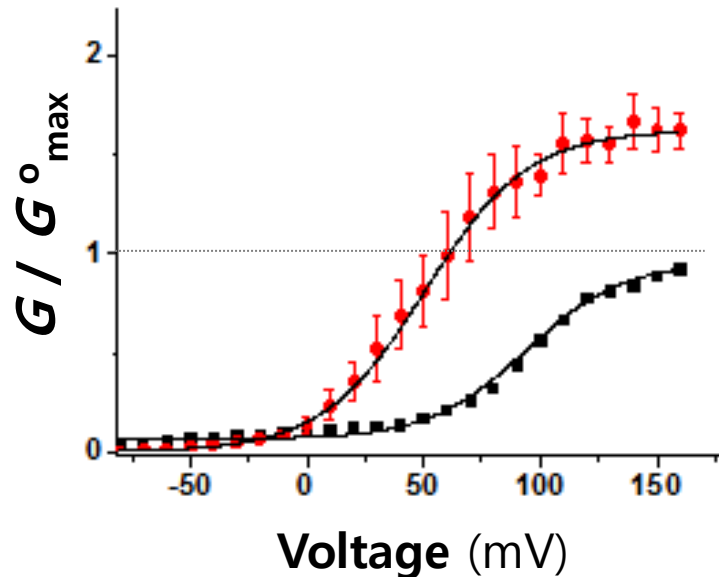
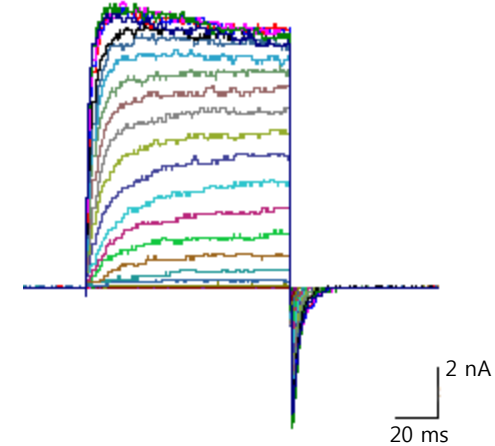
(Unpublished observations)

# Electrophysiological validation of an activator candidate for BK<sub>Ca</sub> channel

Vehicle

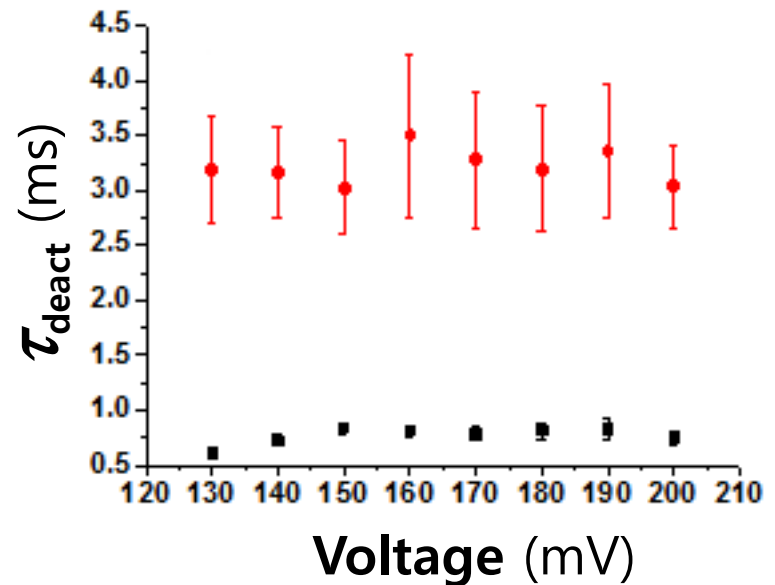
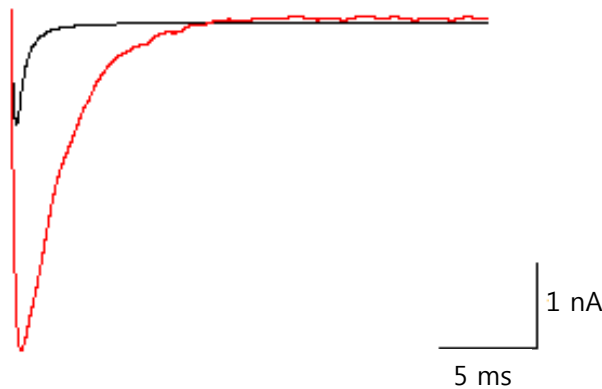
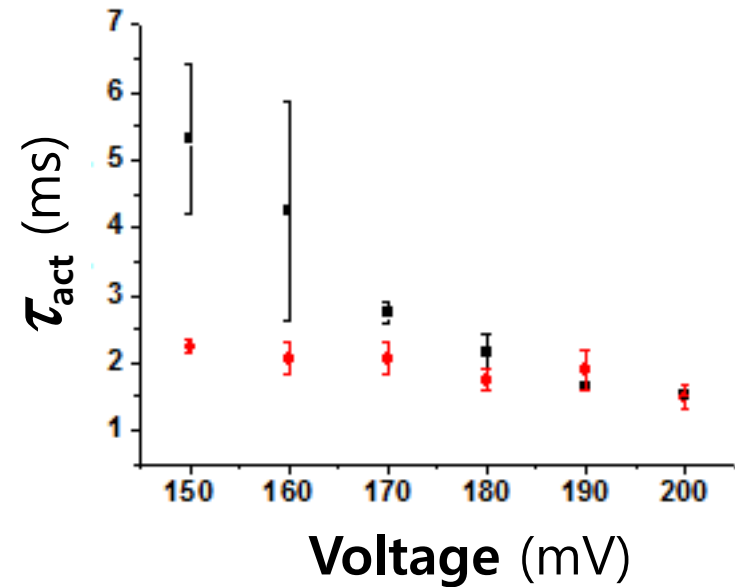
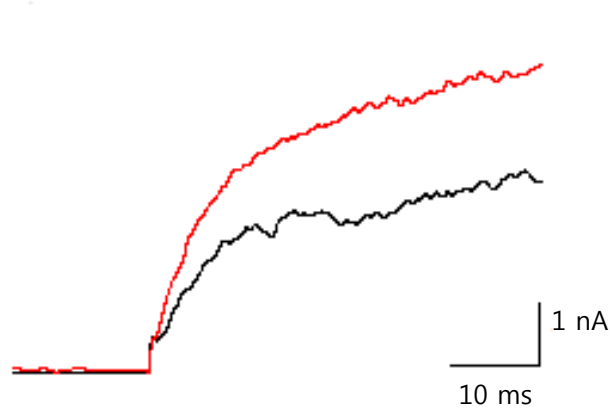


10  $\mu$ M KRICT#1



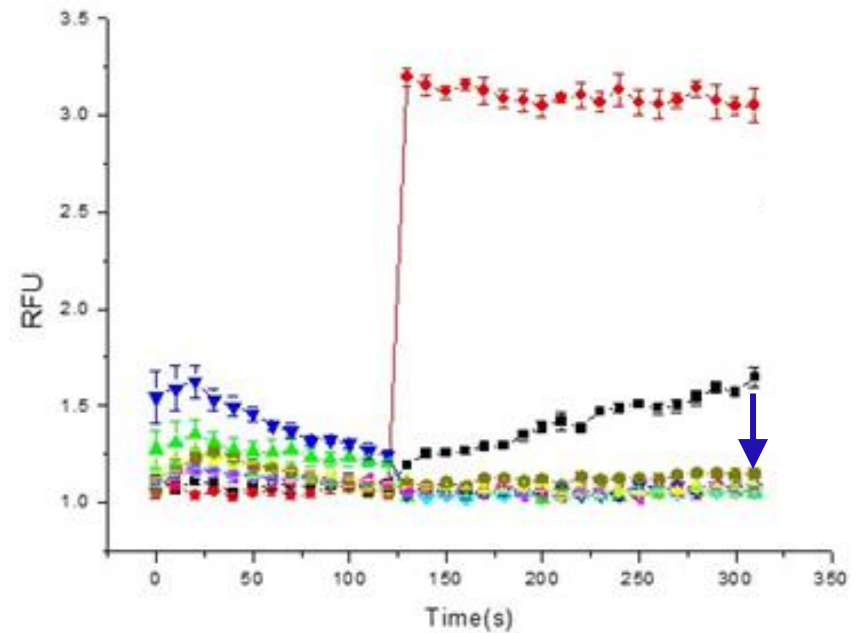
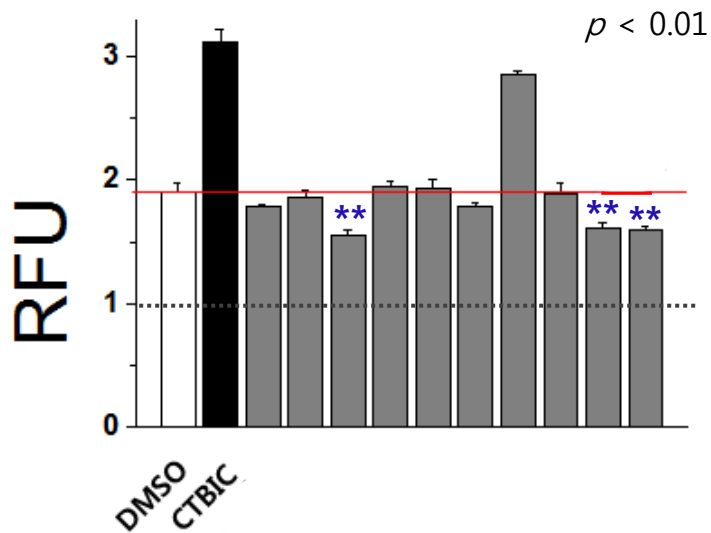
(Unpublished observations)

# Effects of KRICT#1 on gating kinetics of BK<sub>Ca</sub> channel



(Unpublished observations)

# Can we *also* screen the inhibitors for BK<sub>Ca</sub> channel?



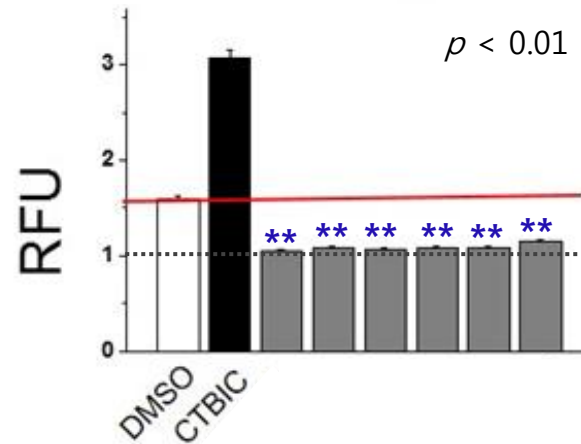
794 KRICT compounds



65 inhibitor candidates



Top 6 selected!



✓ Inhibitor activities *still* need to be confirmed!



# Structure-activity relationship (SAR) studies for the natural derivatives of KRICT#1 using cell-based assay

KRICT#1



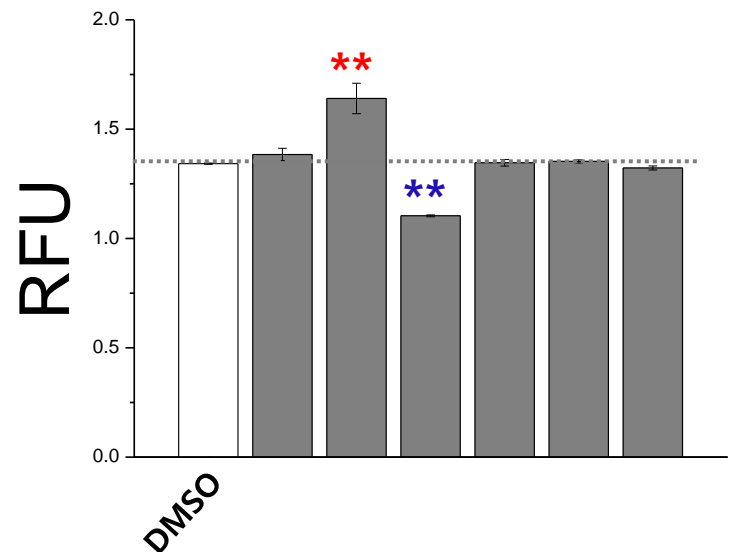
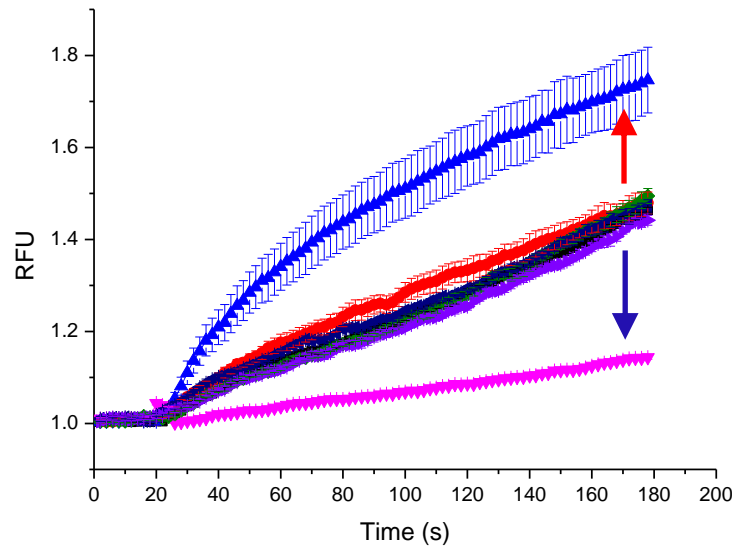
19 natural derivatives



SAR

1 activator and 4 inhibitors candidates

FlexStation 3™  
(Molecular Devices)



(Unpublished observations)

# Summary

- **A double mutation** allows BK<sub>Ca</sub> channel to be activated under physiological voltage ranges at resting [Ca<sup>2+</sup>]<sub>i</sub>.
- Using the 'hyperactive' channel mutant, a **cell-based assay** was established for BK<sub>Ca</sub> channel.
- A **fluorogenic assay platform** originally developed for K<sub>v</sub> channel was successfully applied for BK<sub>Ca</sub> channel cell-based assay.
- Utility of the **new BK<sub>Ca</sub> channel assay platform** was **validated** by obtaining novel natural compounds altering the channel activity.
- BK<sub>Ca</sub> channels can now be subjected to library screenings with **reasonably high throughputs**.
- We are currently searching chemical libraries (*e.g.*, Prestwich®) to obtain **new sets of BK<sub>Ca</sub> channel modulators**.

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Yoon, Yi-seul

Choi, Ja Hyun

Jeon, Seungje

Lee, Sojung



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