UNIVERSITY OF LEEDS

International Conference on
Metabolic Syndromes

Cross-talk between Two Partners Regulates the Gating of the K_{ATP} Channel Pore

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Introduction

Potassium Channels

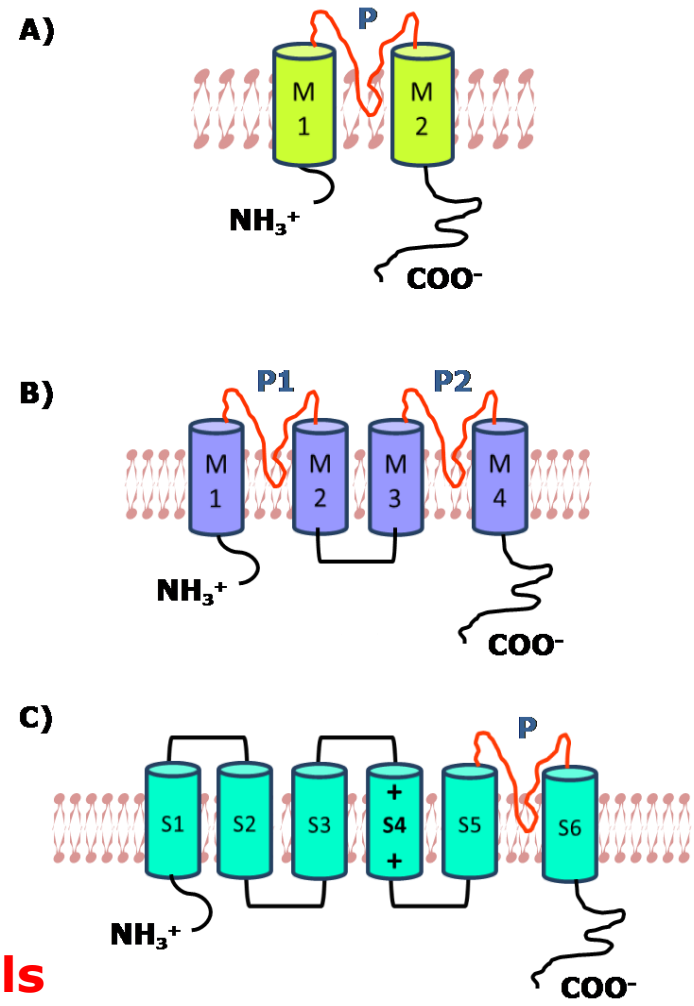
Role

- Shape action potentials
- Resting membrane potentials

Diversity

- Calcium activated Potassium Channels
- Voltage-gated Potassium Channels
- Twin pore Potassium Channels
- **Inwardly Rectifying Potassium Channels**

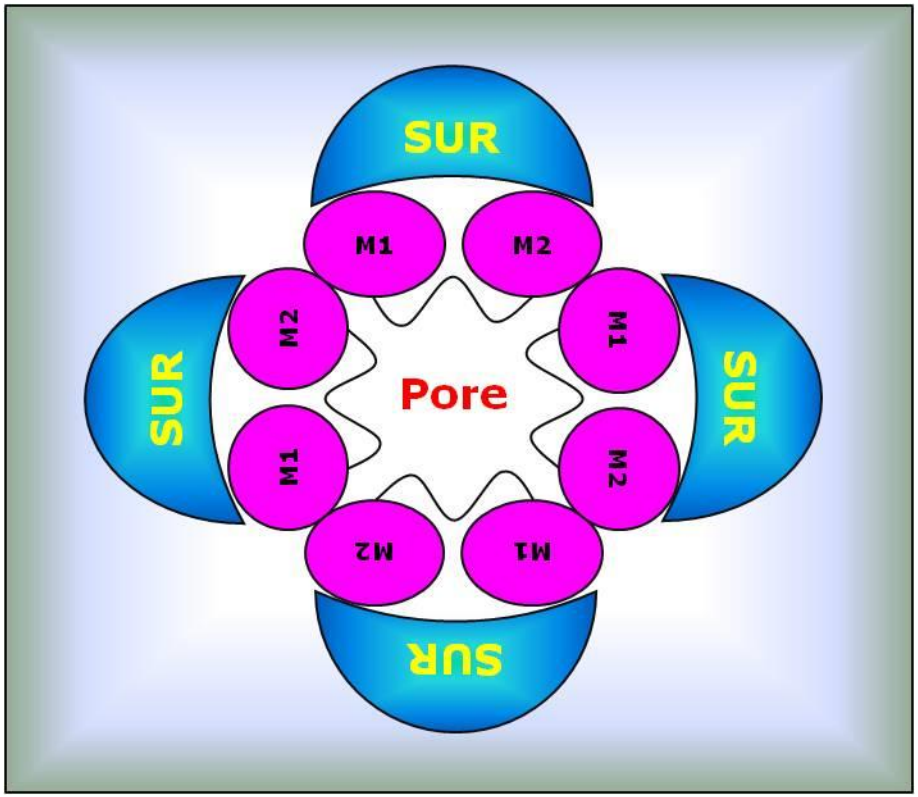
Structure



Hetero-Octameric $Kir6_4/SUR_4$ Complex

K_{ATP} channels are composed of:

- **4 pore-forming Kir6.x:** Kir6.1 or Kir6.2
- **4 regulatory SUR:** SUR1, SUR2A or SUR2B



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Why Understanding Allosteric Communications between K_{ATP} Channels Subunits is so Important

Various Tissues / Cells

Brain/Nerve Cells
Kir6.2 + SUR1

Mitochondria
Kir6.1 + SUR1

Regulation of insulin

Pancreatic β-Cells

Kir6.2 + SUR1

Kir6.1 + SUR2A

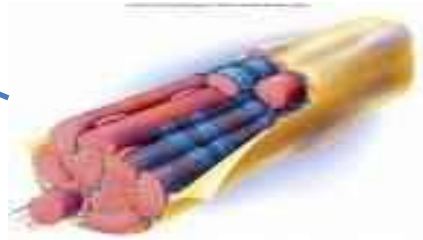
??

K_{ATP} Channels

Vascular Smooth Muscle
Kir 6.1/6.2 + SUR2B

vascular tone regulation

Cardiac
Kir6.2 + SUR2A



Skeletal Muscle
Kir6.2 + SUR2A

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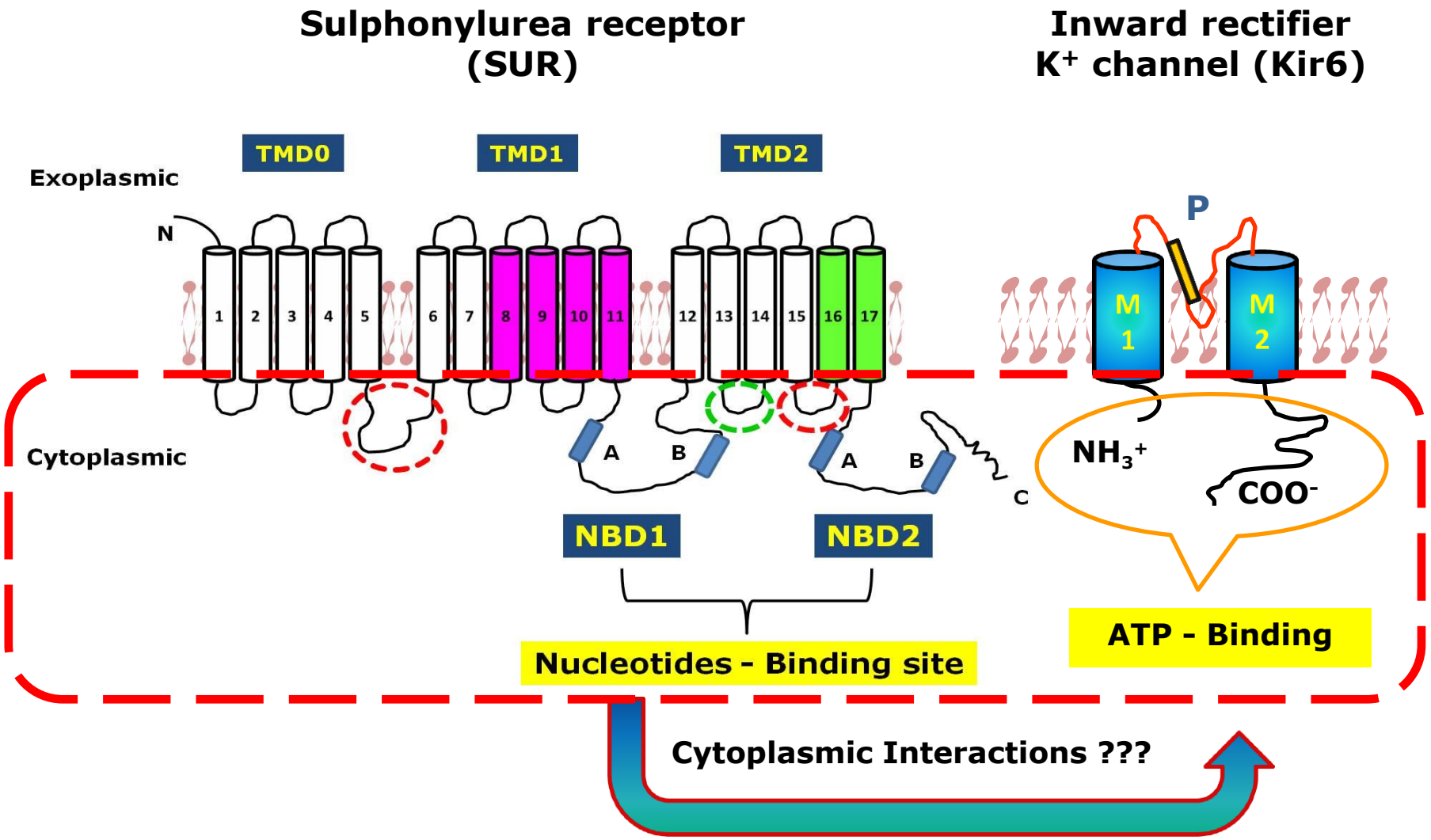

Agonists

Green pinacidil and cromakalim

Pink diazoxide

Antagonists

Red sulphonylureas (glibenclamide)

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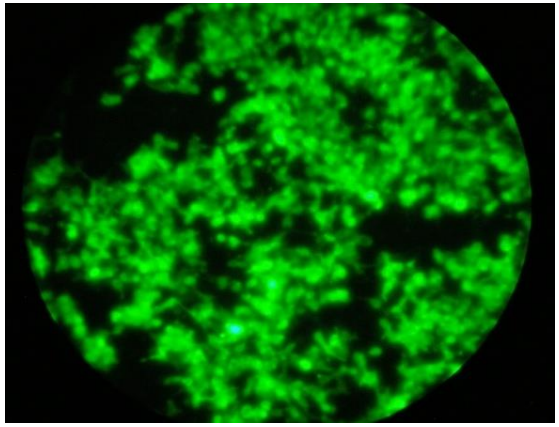
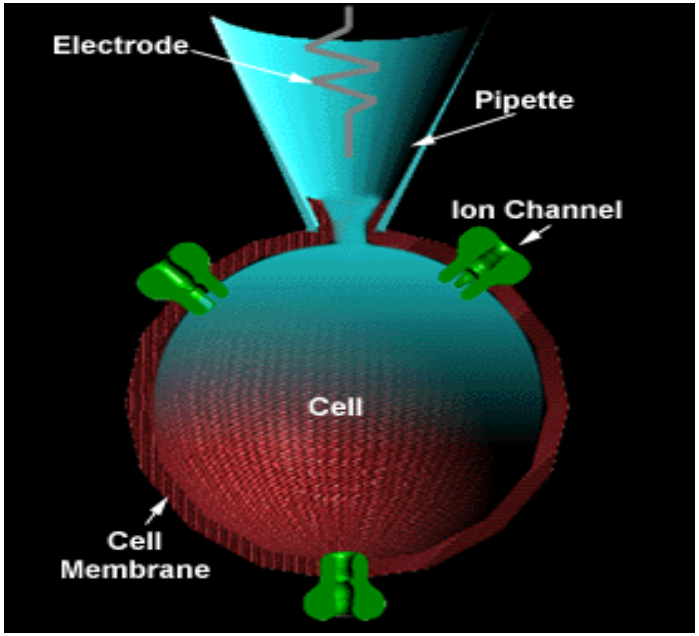
Electrophysiological Study

- **HEK293 Cells**
- **EGFP**
- **Transfection**

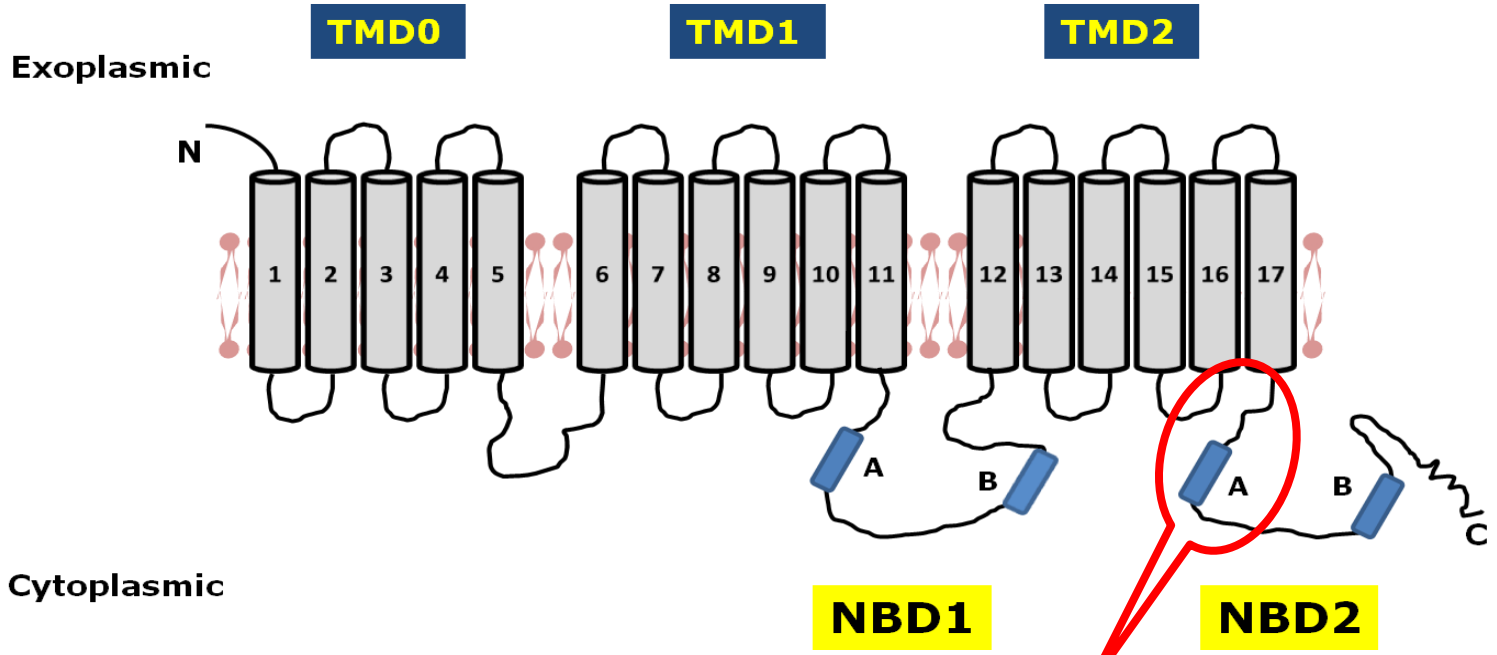


Kir6.2 WT-EGFP / SUR2A WT

Patch Clamp Configurations



Sulphonylurea receptor (SUR)



rSUR2A-CTE

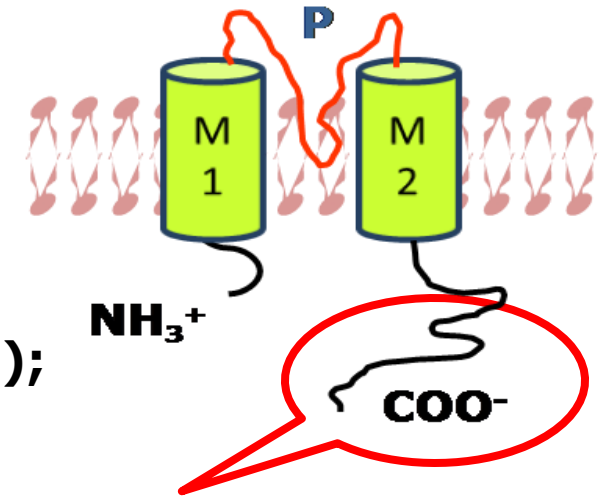
- Minimal interacting fragment
- 65 amino acids
- **E1318, K1322 and Q1336**

Rainbow et al., 2004, The Biochemical journal *379*, 173-181

Previous Work

- **Proximal C-terminal** of SUR2A is a crucial link between ligand binding to SUR2A and Kir6.2 **activation**

Kir6.2

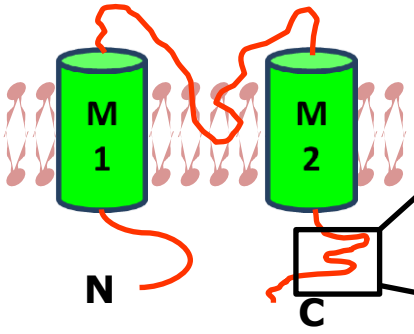


- **Kir6.2/2.1 Chimaeras and rSUR2A-CTC (C6);**

Interaction domain on Kir6.2 is located within the 75 amino acids of the C-terminal tail beyond residue 315

Dupuis, J.P., 2008, The Journal of physiology *586*, 3075-3085

Identification of Critical Conserved Residues for Co-ip



D323K

K338E

RKR369-371AAA

K377A

Kir6.2
(315-390 aa)

```
Kir6.2    QDLEIIVILEGVVETTGITTQARTSYLADEILWGQRFVPIVAEEDGRYSVDYSKFGNTVK 338
Kir6.1    QDLEVIVILEGVVETTGITTQARTSYIAEEIQWGHRFVSIVTEEEGVYSVDYSKFGNTVR 347
Kir2.1    ADFEIVVILEGMVEATAMTTQCRSSYLANEILWGHRYEPVLFEEKHCYKVDYSRFHKTYE 349
```

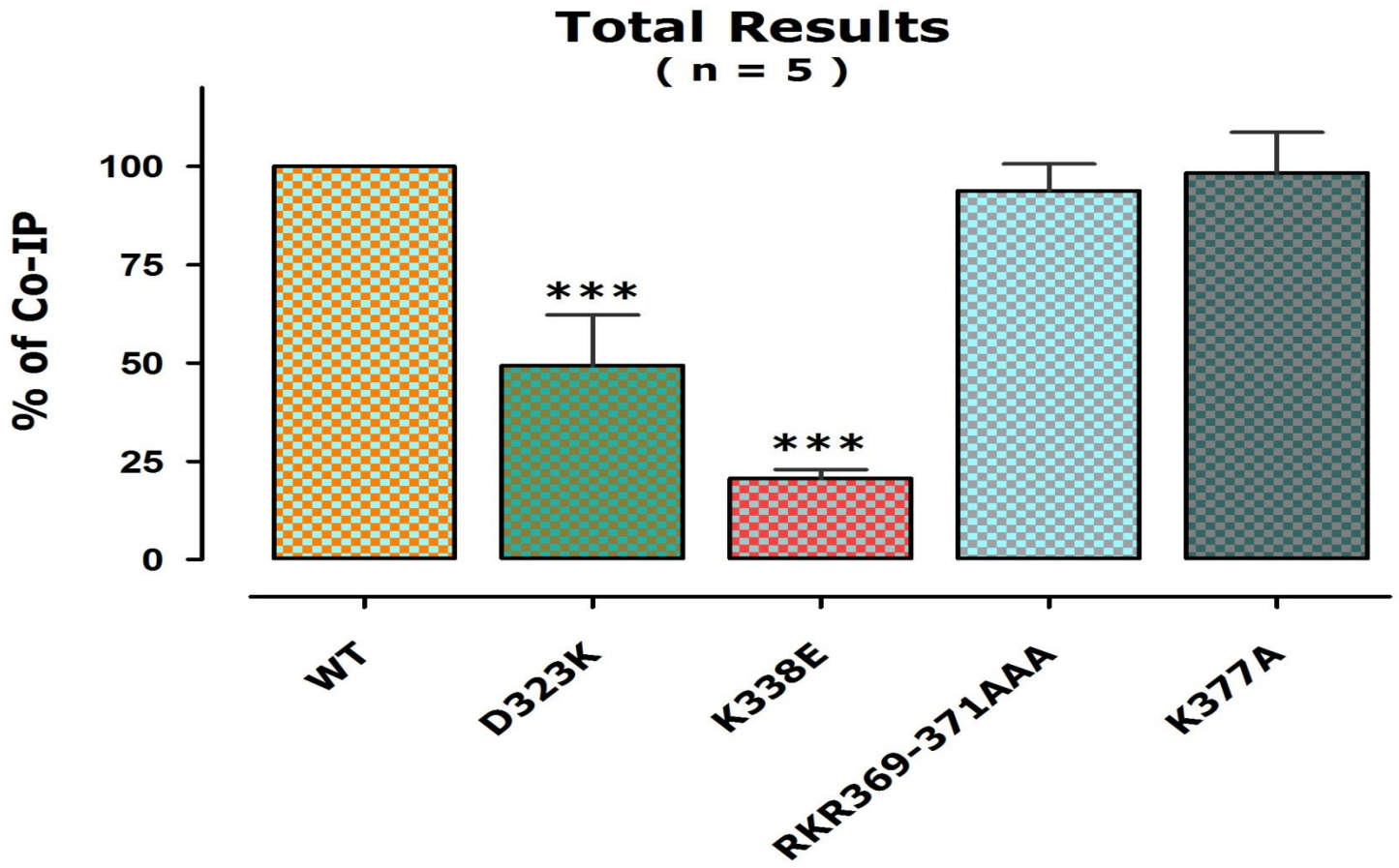
✂ Δ36

```
Kir6.2    VP-TPLCTARQLDEDRSLLDALTLAS---SRGPLRKR-------------------SVA 374
Kir6.1    VA-APRCSARELDEKPSILIQTLQKSELSHQNSLRKRNSMRRNNSMRRSNSIRRNNSSLM 406
Kir2.1    VPNTPLCSARDLAEKKYILSNANSFCYENEVALTSKEEEDSENGVPESTSTDSPPGIDLH 409
```

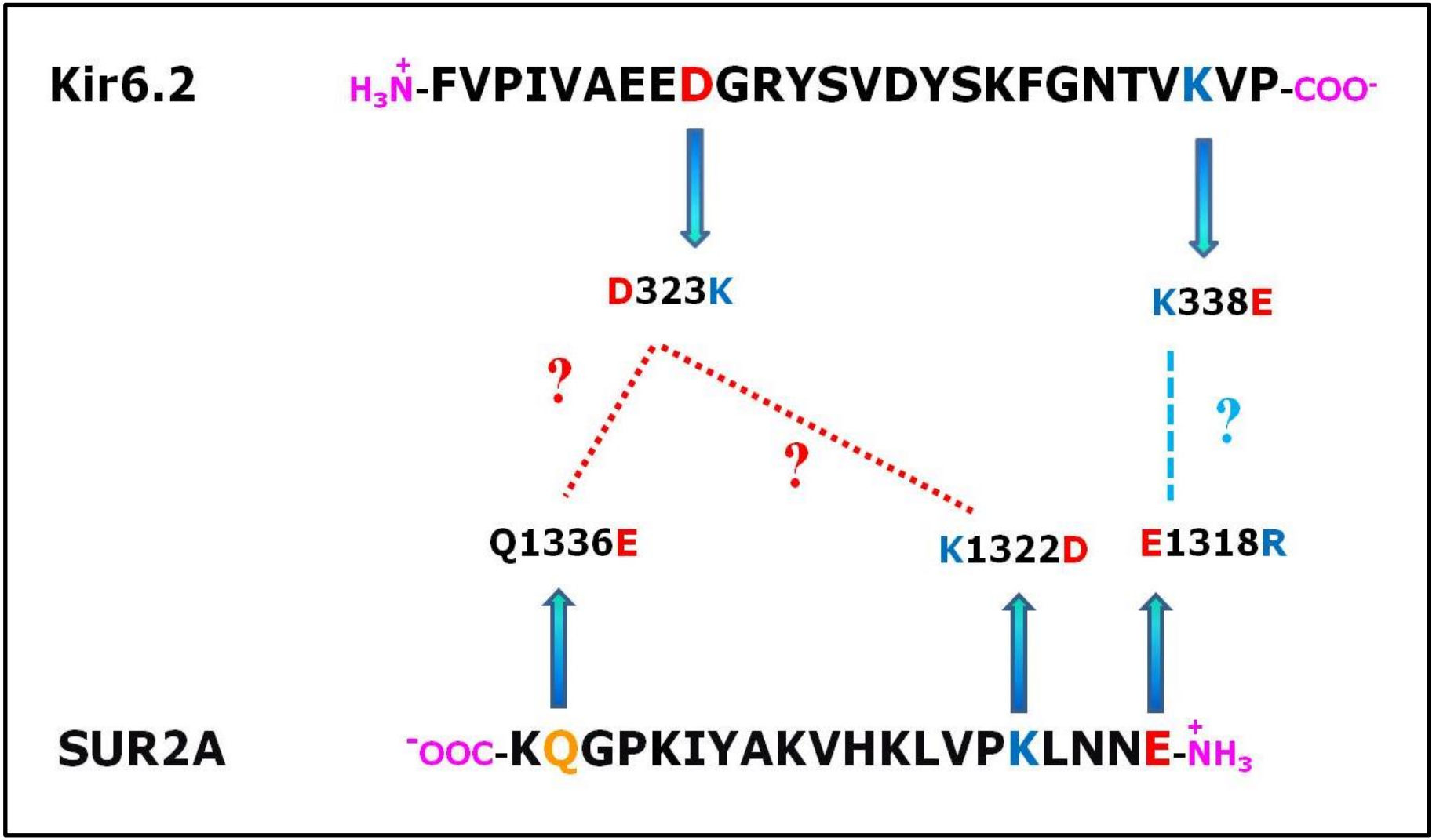
```
Kir6.2    VAKAKPKFSISPDSLS-- 390
Kir6.1    VPKVQFMTPEGNQCPSES 424
Kir2.1    NQASVPLEPRPLRRESEI 427
```

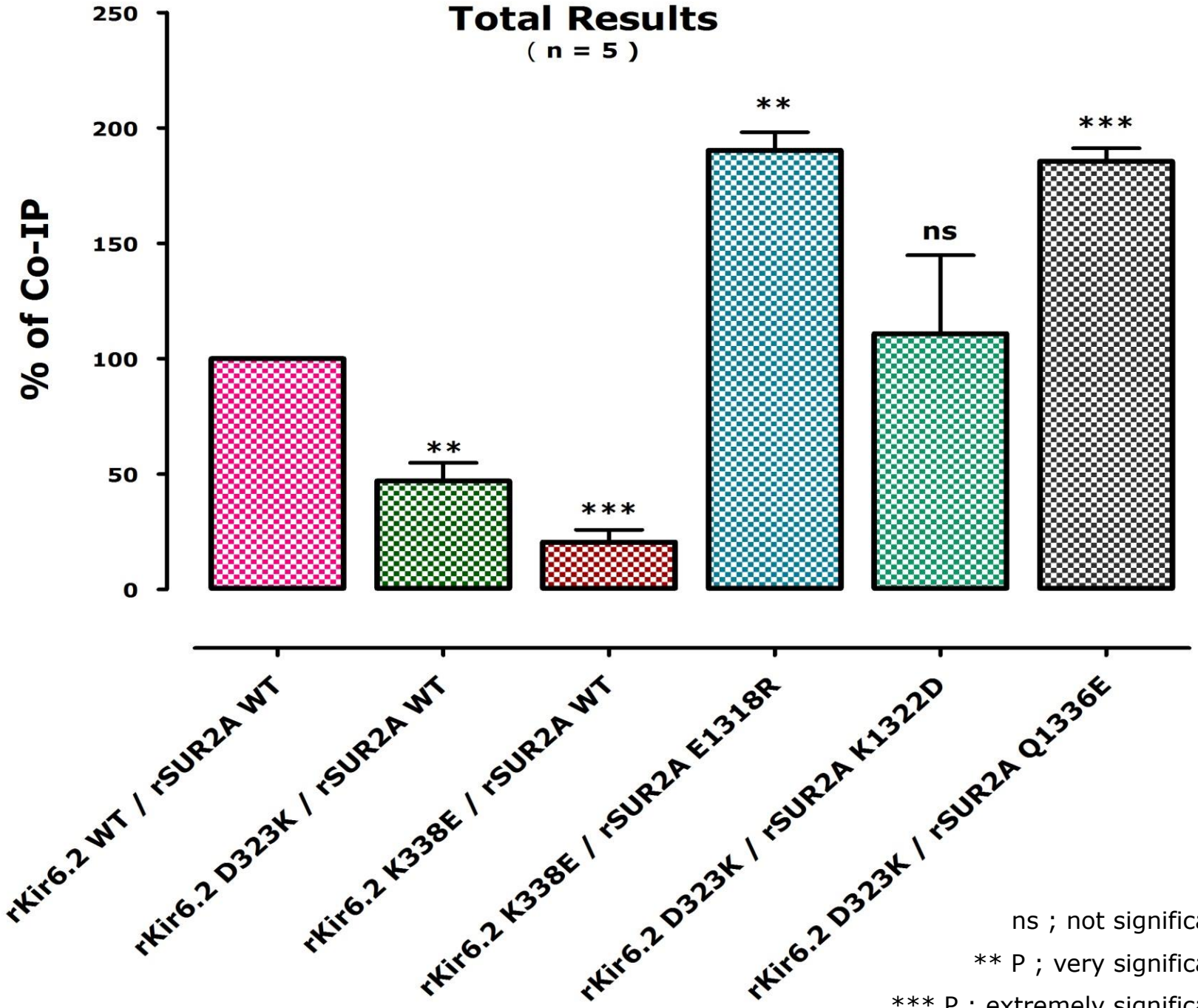
✂Δ36 Tucker *et al*, 1997, Nature 387: 179-183

Identification of **Kir6.2 D323 and K338** as Residues Involved in Interaction with MBP-rSUR2A-CT-C



Do the Swap Mutants of Kir6.2 and SUR2A Restore the Interaction and the Function?





ns ; not significant

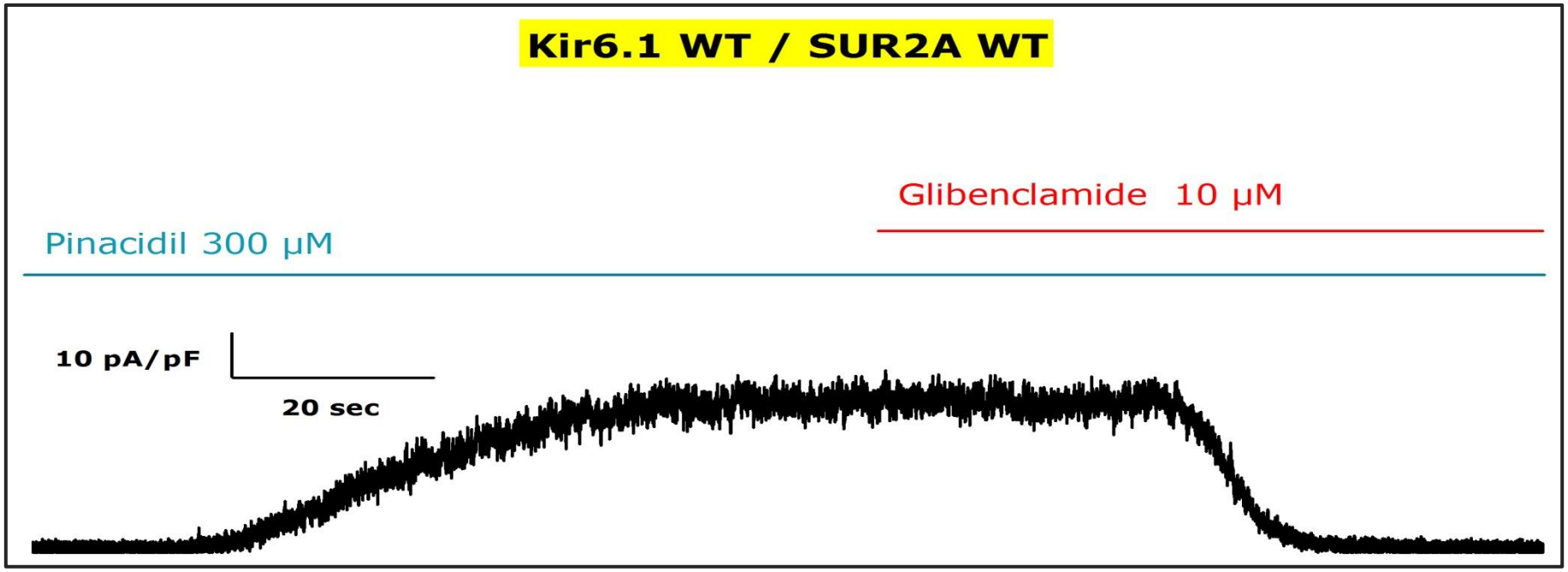
** P ; very significant

*** P ; extremely significant

Are Kir6.1 E332 and R347 Involved in Allosteric Communications?

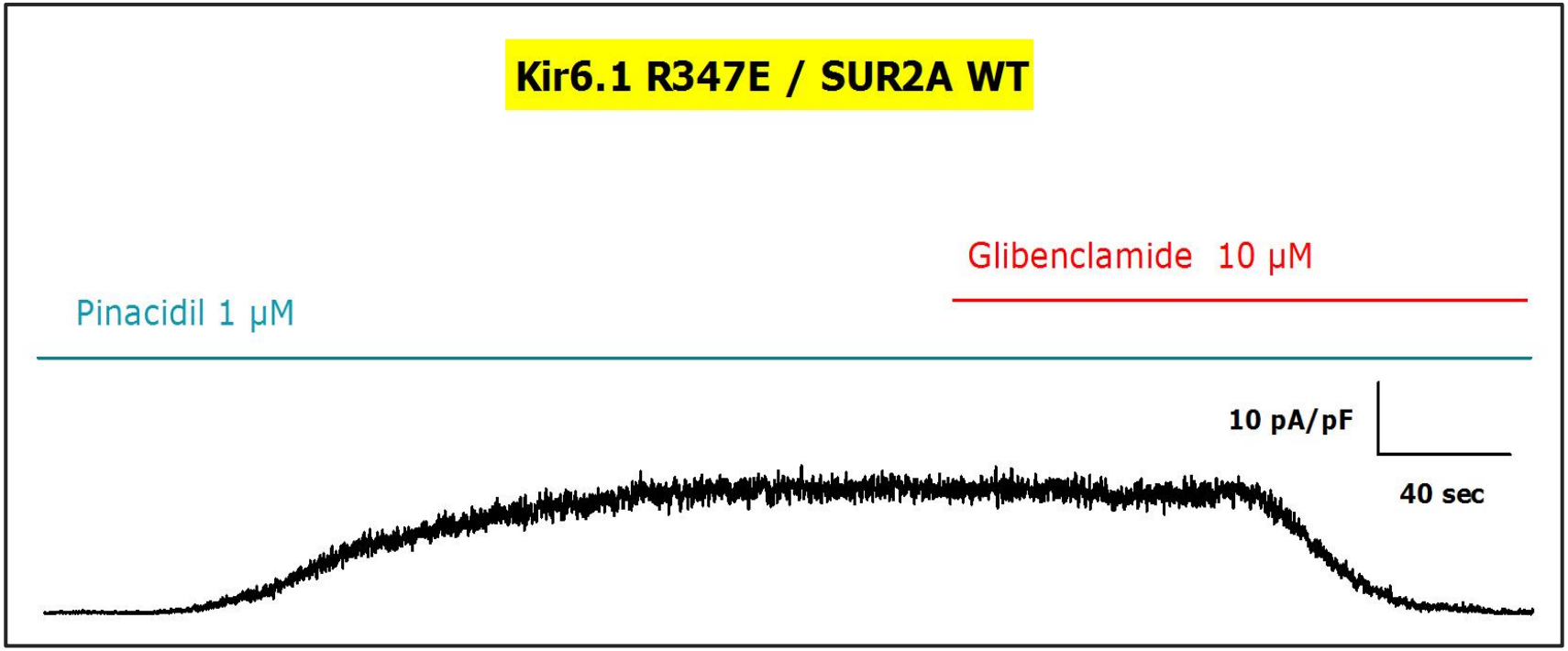
```
Kir6.2    QDLEIIVILEGVVETTGITTQARTSYLADEILWGQRFVPIVAEEDGRYSVDYSKFGNTVK 338
Kir6.1    QDLEVIVILEGVVETTGITTQARTSYIAEEIQWGHRFVSIVTEEEGVYSVDYSKFGNTVR 347
```

- Kir 6.1 E332K
- Kir 6.1 R347E

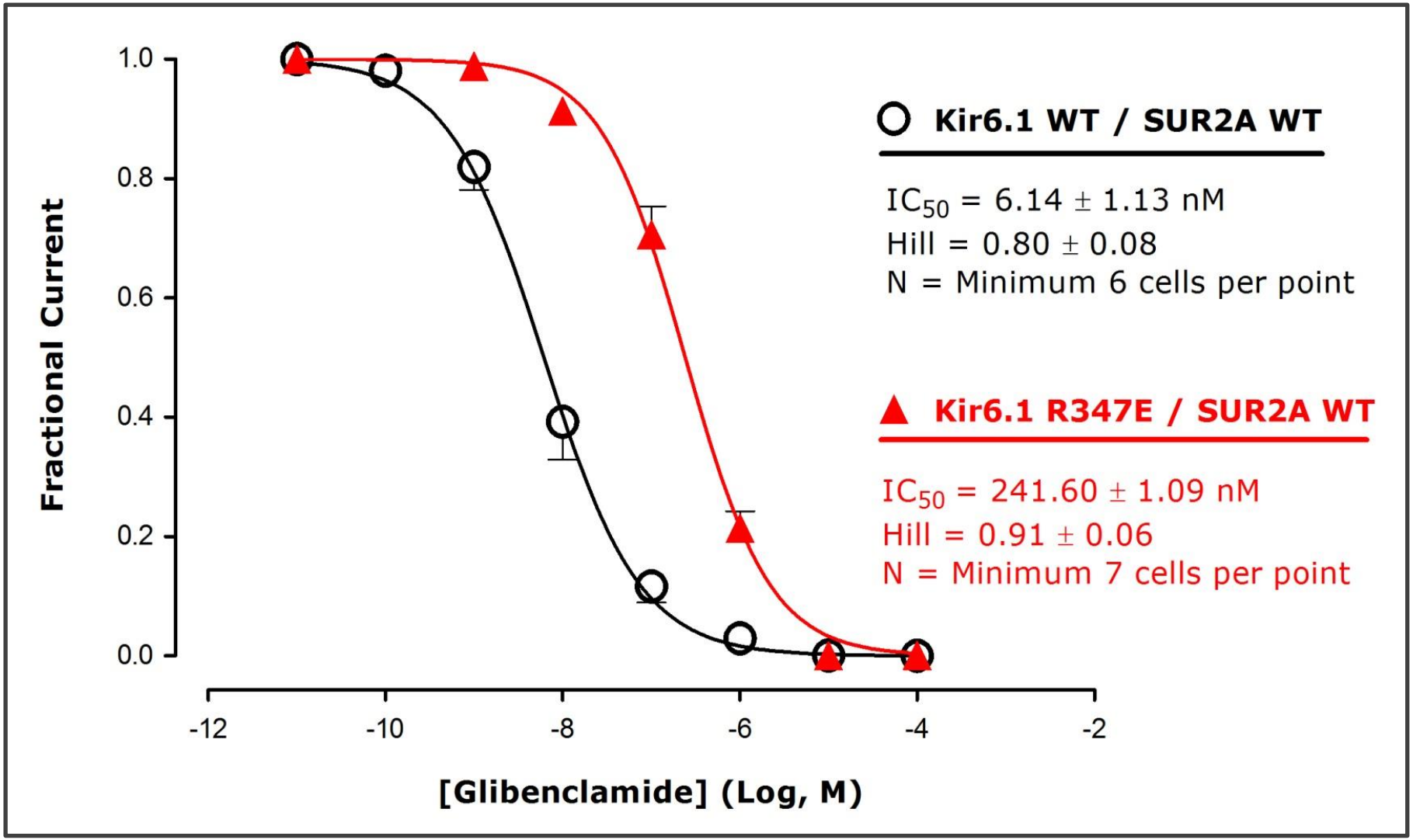


Pharmacological Response and Functional Characterization of Kir6.1 R347

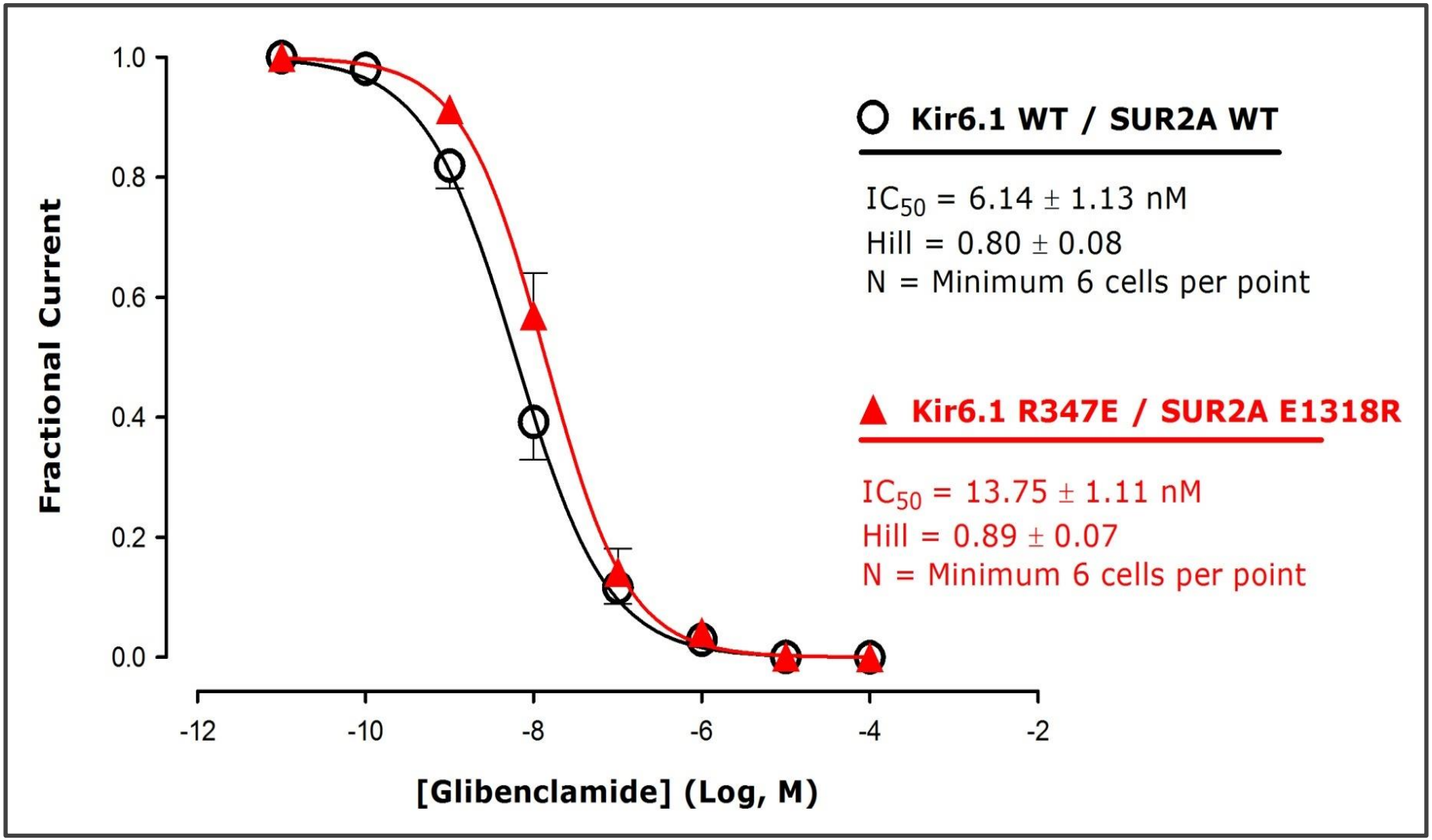
```
Kir6.2    QDLEIIVILEGVVETTGITTQARTSYLADEILWGQRFVPIVAEEDGRYSVDYSKFGNTVK 338
Kir6.1    QDLEVIVILEGVVETTGITTQARTSYIAEEIQWGHRFVSIVTEEEGVYSVDYSKFGNTVR 347
```



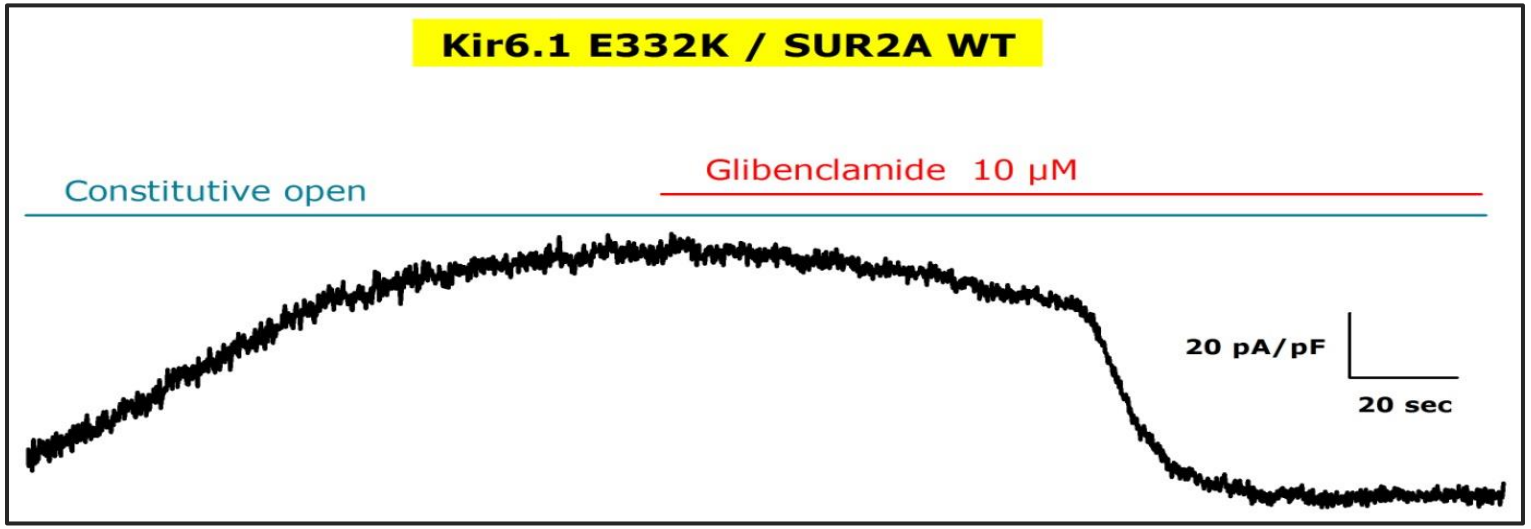
Kir6.1 R347E Mutant Disrupt the Function

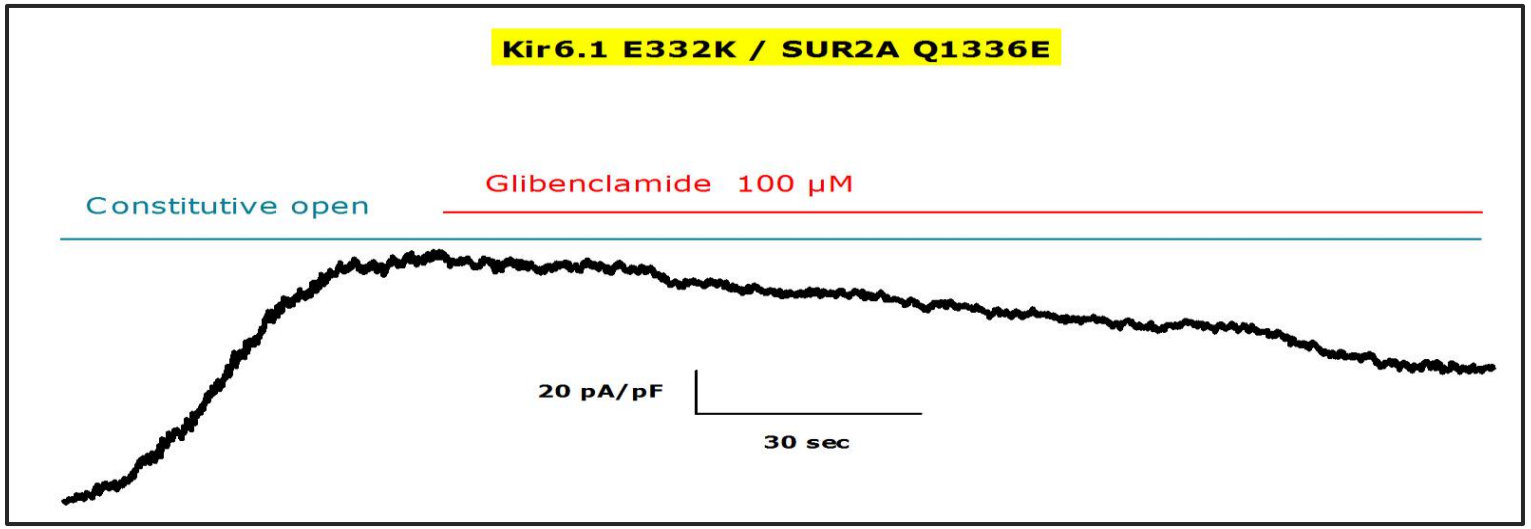


Inter-subunit Salt Bridge Communicate Glibenclamide Sensitivity

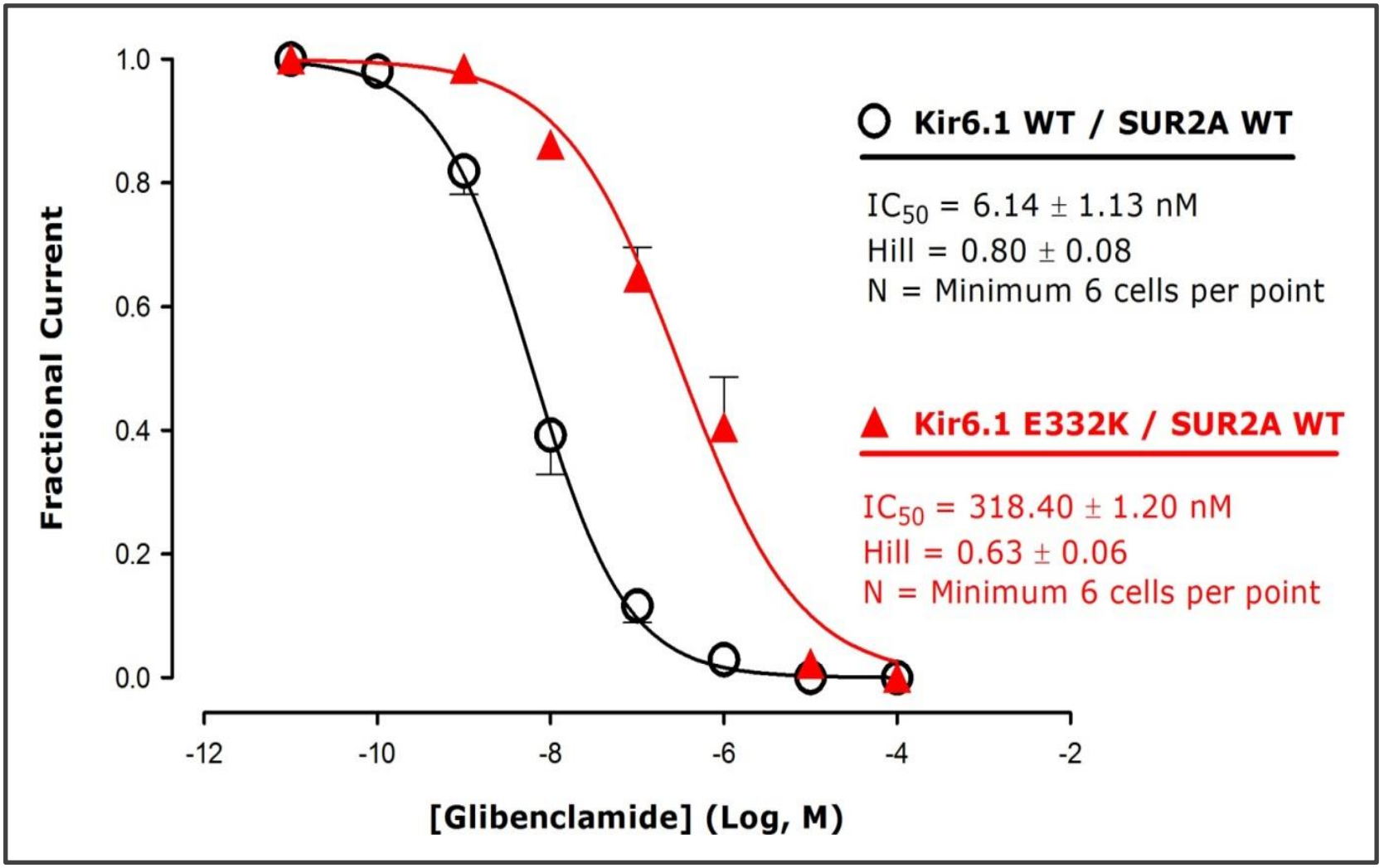


Kir6.1 E332K Mutant Disrupt the Function Causing Constitutive Open Channel

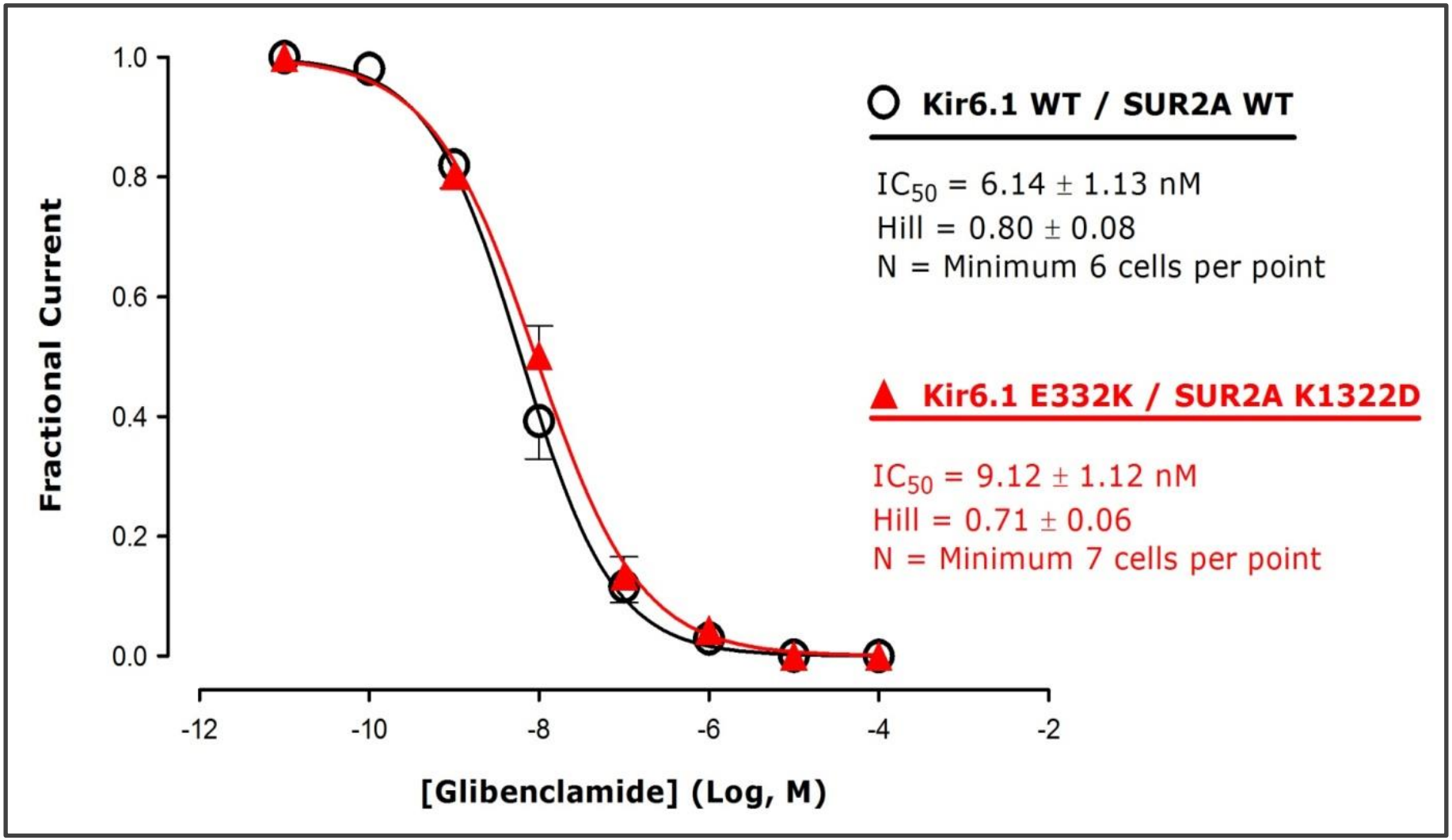




Kir6.1 E332K Mutant Disrupt the Function



Inter-subunit Salt Bridge Communicate Glibenclamide Sensitivity



Pharmacological Response and Functional Characterization of Kir6.1 / SUR2A

A)

Subunits		Pinacidil (μM)				
Kir6.1	SUR2A	EC_{50}	SE	Hill	SE	n (min cells/point)
WT	WT	43.90	1.28	1.03	0.23	6.00
E332K	WT	Constitutive				6.00
R347E	WT	0.71	1.21	0.94	0.20	6.00
E332K	Q1336E	Constitutive				12.00
R347E	E1318R	23.50	1.26	1.09	0.20	6.00
E332K	K1322D + Q1336E	Constitutive				12.00
E332K	K1322D	Constitutive				6.00

B)

Subunits		Glibenclamide (nM)				
Kir6.1	SUR2A	IC_{50}	SE	Hill	SE	n (min cells/point)
WT	WT	6.14	1.13	0.80	0.08	6.00
E332K	WT	318.40	1.20	0.63	0.06	6.00
R347E	WT	241.60	1.09	0.91	0.06	7.00
E332K	Q1336E	Insensitive				6.00
R347E	E1318R	13.75	1.11	0.89	0.07	6.00
E332K	K1322D + Q1336E	Insensitive				6.00
E332K	K1322D	9.12	1.12	0.71	0.06	7.00

Conclusion

Evidence for the transmission of

allosteric information via

salt bridges (electrostatic interactions)

between the cytoplasmic NBD-2 of SUR2A and

the distal C-terminus of Kir6

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