Title: The emerging structural pharmacology of ATP-sensitive potassium channels

Running title: Cryo-EM reveals $K_{\text{ATP}}$ drug binding sites

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Abbreviations:

ABCC: sub-family C of the ATP-binding cassette transporter
ABLOS: ATP-binding loop of sulfonylurea receptor

IS: insulin secretagogues

ISBS: insulin secretagogue-binding site

K_{ATP}: ATP-sensitive potassium channels

KCO: K_{ATP} opener

KCOS: K_{ATP} opener binding site

Kir: inward-rectifier potassium channel

KNtp: Kir6.2 N-terminal peptide

NBD: nucleotide-binding domain

SUR: sulfonylurea receptor

TMD: transmembrane domain

KNtp: Kir6 N-terminal peptide

CTD: cytoplasmic domain

**Abstract:**

ATP-sensitive potassium channels (K_{ATP}) are energy sensors that participate in a range of physiological processes. These channels are also clinically validated drug targets. For decades, K_{ATP} inhibitors are prescribed for diabetes and K_{ATP} activators are used for the treatment of hypoglycemia, hypertension, and hair loss. In this Emerging Concepts article, we highlight our
current knowledge about the drug binding modes observed using cryo-EM techniques. The inhibitors and activators bind to two distinct sites in the transmembrane domain of sulfonylurea receptor (SUR) subunit. We also discuss the possible mechanism of how these drugs allosterically modulate the dimerization of SUR nucleotide-binding domains (NBD) and thus \( K_{ATP} \) channel activity.

**Significant statement:**

\( K_{ATP} \) are fundamental to energy homeostasis, and they participate in many vital physiological processes. \( K_{ATP} \) channels are important drug targets. Both \( K_{ATP} \) inhibitors (insulin secretagogues) and \( K_{ATP} \) activators are broadly used in clinic for the treatment of related diseases. Recent cryo-EM studies allow us to understand the emerging concept of \( K_{ATP} \) structural pharmacology.

**The emerging concept of \( K_{ATP} \) structural pharmacology**

The potassium currents passing through \( K_{ATP} \) channels are inhibited by intracellular ATP and activated by intracellular Mg-ADP (Nichols, 2006). As such, \( K_{ATP} \) channels tune their potassium conductance according to changes in the intracellular ATP/ADP ratio of the cell. \( K_{ATP} \) channels play essential roles in several important physiological processes, including insulin secretion and vasodilation (Ashcroft, 2006; Flagg et al., 2010). The genetic mutations of \( K_{ATP} \) channels lead to human diseases, such as neonatal diabetes and cardiovascular diseases (Ashcroft, 2006; Flagg et al., 2010).

\( K_{ATP} \) channels are assembled from four pore-forming inward-rectifier potassium channel 6 (Kir6) subunits and four regulatory SUR subunits (Figure 1A-C). The Kir6 subunit in the center has the inhibitory nucleotide-binding site, while the SUR subunit in the peripheral harbors the activating Mg-nucleotide-binding site (Figure 1A-C). The interplay of these nucleotide-binding sites during
the fluctuation of intracellular nucleotide concentrations shapes the overall $K_{\text{ATP}}$ channel activities. In the past several decades, fruitful medicinal research on $K_{\text{ATP}}$ channels has established that SUR proteins, either SUR1 or SUR2, are validated drug targets (Li et al., 2021). SUR proteins belong to the sub-family C of the ATP-binding cassette transporter (ABCC) subfamily of the ATP binding cassette (ABC) transporters and have a transmembrane domain 0 (TMD0), which is unique to certain ABCC family members. SUR proteins have a canonical structural core formed by TMD1-NBD1-TMD2-NBD2 (Figure 1D) (Aittoniemi et al., 2009; ter Beek et al., 2014). The helices from transmembrane domain 1 (TMD1) and TMD2 are intertwined to form two structural halves. The first half consists of TM6-8, TM11 of TMD1, and TM15-16 of TMD2; and the remaining helices in the transporter core form the second half. SUR proteins were found to have two major conformations: the NBD-separated inward-facing conformation represents an inactive state, while the NBD-dimerized occluded conformation is associated with $K_{\text{ATP}}$ activation (Wu et al., 2020). SUR subunits also dominate the pharmacological profile of $K_{\text{ATP}}$ channels. $K_{\text{ATP}}$ channels in pancreatic cells are mainly formed by the SUR1-Kir6.2 combination. SUR1 inhibitors can trigger depolarization of $\beta$ cells and thus insulin secretion. They are used for the treatment of diabetes (Li et al., 2021) and are therefore named “insulin secretagogues” (designated IS). Conversely, SUR1 activators can inhibit insulin secretion and thus can treat certain hypoglycemia (Li et al., 2021). SUR2 is expressed in blood vessels, heart, and skeleton muscles. SUR2 activators are used for the treatment of hypertension and hair loss (Li et al., 2021). SUR activators are called “$K_{\text{ATP}}$ openers” (designated KCO). Recent revolutionary advances in single particle cryo-EM allowed the visualization of membrane protein structures at near-atomic resolution (Kuhlbrandt, 2014). Using this technique, we and
others have experimentally determined the binding sites and poses of not only \( K_{ATP} \) inhibitors but also \( K_{ATP} \) activators inside the SUR proteins.

**The IS binding site of \( K_{ATP} \) channel**

Structurally, IS are classified into sulfonylureas (SUs) (such as glibenclamide, GBM) and glinides (such as repaglinide, RPG, and mitiglinide, MTG) (Gribble and Reimann, 2003; Winkler et al., 2007; Wu et al., 2020). Based on the structure-activity relationship of IS, researchers guessed that there were the "A" site and "B" site on SUR long before the structure determination of \( K_{ATP} \) (Brown and Foubister, 1984; Rufer et al., 1974; Winkler et al., 2007). Up to today, high-resolution structures of \( K_{ATP} \) have revealed the binding sites of three IS inside the central vestibule of SUR, including GBM, RPG, and MTG (Figure 1) (Ding et al., 2019; Li et al., 2017; Martin et al., 2017a; Martin et al., 2019; Martin et al., 2017b; Sung et al., 2021; Wang et al., 2022a; Wu et al., 2018). The binding of IS stabilizes SUR in the inward-facing state with two NBDs separated (Figure 1D). These structures have depicted the IS Binding Site (ISBS) at near-atomic resolution and allowed us to analyze IS-SUR interactions in detail (Figure 1F-K). We conceptually divide the ISBS into three connecting regions according to the structures: the Hydrophobic A site (H-A), the Polar site (P), and the Hydrophobic B site (H-B) (Figure 1E). Based on the common pharmacophores, we also tentatively list and align several representative IS, the structures of which in complex with \( K_{ATP} \) are not available yet (Basit et al., 2012; Grell et al., 1998; Hu et al., 1999; Reimann et al., 2001b; Vila-Carriles et al., 2007; Winkler et al., 2007) (Figure 1B).

Intriguingly, all of these IS interact with the P site and the H-B site (Figure 1E). The P site is formed by positively charged R1246, R1300, and polar N1245 of SUR1, and interacts with the
electrostatically negative group of IS, such as the sulfonyl group of GBM, and the carboxyl
group of RPG and MTG (Figure 1E-K). Mutations at the P site abolished the inhibition of GBM,
RPG, and MTG (Ding et al., 2019; Wang et al., 2022a).

IS have diverse chemical groups at the H-A site, which is one of the key determinants for the
subtype selectivity of IS. S1238 of SUR1 at the H-A site is replaced by a much larger tyrosine in
SUR2. Therefore, IS with a bulkier group at the H-A site could be accommodated by SUR1 but
might not bind SUR2 favorably, in agreement with the order of SUR1/SUR2 selectivity: MTG >
GBM > RPG (Quast et al., 2004; Wang et al., 2022a).

The common hydrophobic phenyl group of IS is bound in the hydrophobic pocket formed by
residues on the H-B site, including F433 and I381 (Figure 1E-K). A large hydrophobic cavity
formed by residues from helices TM6-8 and TM11 (Y377, F433, L434, M441, L592, V596, and
W1297) accommodates the chloro benzamide group of GBM or the large hydrophobic group
including the piperidinylphenyl moiety and the butyl carbamoyl-methyl moiety of RPG (Figure
1). GBM and RPG make differential interactions with the H-B site. R306 on TM8 of the H-B site
of SUR1 only forms polar interactions with the chloride atom of GBM but not with RPG (Figure
1E-K), which is consistent with the decreased potency of GBM but not RPG to the R306A
mutant of SUR1 (Ding et al., 2019). It was initially found that Kir6 N-terminal peptide (KNtp) is
close to the H-B site according to the map of SUR1/Kir6.2 K_{ATP} in the presence of GBM (Wu et
al., 2018). This was later confirmed in SUR1/Kir6.2 K_{ATP} in the presence of RPG and even
SUR2B/Kir6.1 K_{ATP} in the presence of GBM (Ding et al., 2019; Martin et al., 2019; Sung et al.,
2021). Therefore, the H-B site is formed not only by the SUR subunits but also by the KNtp,
providing a plausible mechanism for the IS-enhanced inhibitory coupling between SUR and Kir6.
However, due to the flexibility of KNtp, the exact residues that interact with IS remain poorly defined.

The KCO binding site of $K_{\text{ATP}}$ channel

KCO are chemically diverse and comprise distinct classes including cyanoguanidines (such as pinacidil and P1075), benzopyrans (such as levromakalim, abbreviated as Lev), thiadiazines (such as diazoxide and NN414), and others (Gilbert et al., 2000; Mannhold, 2004, 2006; Tusnady et al., 1997). Their exact binding sites on the SUR subunit were elusive despite the enlightening work on chimeric SUR and mutagenesis (Ashcroft and Gribble, 2000; Babenko et al., 2000; D'Hahan et al., 1999a; D'Hahan et al., 1999b; Moreau et al., 2000; Reimann et al., 2001a; Uhde et al., 1999). To experimentally determine the KCO binding site, our lab has solved the structure of SUR2 in complex with SUR2-selective P1075 and Lev (Ding et al., 2022), and SUR1/Kir6.2 $K_{\text{ATP}}$ with SUR1-selective NN414 (Wang et al., 2022b), providing comprehensive views of KCO binding sites (KCOS) at near-atomic resolutions (Figure 2). The binding of KCO synergizes with Mg-nucleotides to stabilize the SUR in the NBD-dimerized occluded conformation (Figure 2A), in agreement with the fact that KCO bind to SUR with slower off-rates in the presence of Mg-nucleotides (Gribble et al., 2000). Despite the distinct chemical structures and selectivities of these three KCO, they all bind at a common KCOS in the middle of the transmembrane domain of SUR (Figure 2A), in agreement with the competitive binding behavior of KCO (Bray and Quast, 1992). KCOS is embraced by TM10, TM11, TM12, TM13, TM14, and TM17 helices (Figure 2C, 2E, and 2G), and is about 20 Å away from the aforementioned ISBS (Ding et al., 2022). Based on the structures, we define the KCOS as three connecting regions on SUR: the A site, the Polar site (P site), and the B site (Figure 2B).
Two residues in the P site, a His (H584 in SUR1 and H576 in SUR2) and an Asp (D1031 in SUR1 and D1008 in SUR2), play critical roles in interacting with the central polar groups of KCO. H584 of SUR1 interacts with the sulfonyleurea group on the thiadiazine ring of NN414 and D1031 forms hydrogen bonds between its carboxyl group and the thiadiazine ring of NN414 (Figure 2C and 2D). H576 on TM11 interacts with the guanidine group of P1075. D1008 on TM12 forms hydrogen bonds between its carboxyl group and guanidine group of P1075 (Figure 2E and 2F). D1008 interacts with the hydroxyl group on the chiral carbon of Lev and H576 interacts with the hydroxyl group and pyrrolidinone ring on the other chiral carbon of Lev (Figure 2G and 2H). Structural information is consistent with previous studies showing that NH groups of pyridylamino group of P1075 can act as a hydrogen-bond donor and make a crucial contribution toward \( K_{\text{ATP}} \) activation (Manley and Quast, 1992); the substitution of pyrrolidinone of Lev changed drug potency significantly (Mannhold, 2006); the substitution of the methyl group on the thiadiazine rings by alkylamino group enhanced the potency of dioxide derivatives (Hansen, 2006). Mutation of these two residues greatly decreased the activation effect of both SUR1-selective opener NN414 (Wang et al., 2022b) and SUR2-selective KCO, including P1075 and Lev (Ding et al., 2022), emphasizing that the polar interactions between the P site of SUR and KCO are essential for channel activation.

The A site is formed by residues in TMD2 and these residues are not identical between SUR1 and SUR2 (Ding et al., 2022). I1004 and T1253 in SUR2 are L1027 and M1290 in SUR1, respectively. I1004 of TM12 and T1253 of TM17 of SUR2 are close to the cyano group and pyridine ring of P1075. Mutations of I1004L and T1253M introduce steric hindrance to the binding of P1075 (Ding et al., 2022). Rb\(^+\) efflux assay showed that I1004L in SUR2B reduced the activating effect of P1075 significantly and T1253M in SUR2B had a more profound effect
In the structure of SUR2B in complex with Lev, I1004 and T1253 are located close to the benzonitrile group and pyrrolidinone ring of Lev, respectively (Figure 2G and 2H). I1004L and T1253M mutations in SUR2B reduced the activating effect of Lev to a similar extent (Ding et al., 2022). M1290 at the A site of SUR1 interacts with the hydrophobic methyl cyclopropane group of NN414 (Figure 2C and 2D). This group is replaced by a smaller methyl group in diazoxide (Fig. 2B), which could not efficiently make hydrophobic interactions with M1290, partially explaining the higher potency of NN414 than diazoxide in the activation of the SUR1-containing $K_{\text{ATP}}$ channel (Dabrowski et al., 2003).

The B site is mainly formed by TMD1. P551, I552, and V555 on TM10, L580 on TM11, I1030 on TM12, and C1072 on TM13 of SUR1 form a hydrophobic pocket and embrace the chlorothiophene ring of NN414 (Figure 2C and 2D). The hydrophobic pocket of SUR2 is formed by I545 and V548 of TM10, V579 of TM11, and I1004 and I1007 of TM12. Dimethylpropyl group of P1075 and benzopyran group of Lev are inserted into the hydrophobic pocket of SUR2 (Figure 2E-H). Although residues that form the B and P sites are conserved between SUR1 and SUR2 subunits, the B and P sites of KCOS in SUR1 is enlarged compared with SUR2. The $\text{C}\alpha$ distance between H576 and D1008 is increased from 10.2 Å in SUR2 (Ding et al., 2022) (PDB ID: 7VLS) to 12.4 Å in SUR1 (Wang et al., 2022b) (PDB ID: 7W4O), due to the conformational difference of their transmembrane helices.

**Future Efforts**

As described above, the currently available structures have uncovered the ISBS and KCOS on the SUR subunits, but the exact mechanisms of how IS and KCO work remain incompletely understood. Based on previous functional studies and available structures, IS likely inhibit $K_{\text{ATP}}$...
through two interdependent mechanisms. First, IS wedge in the TMD to allosterically inhibit the
dimerization of two NBDs. Second, IS facilitate the recruitment of Kir6 N-terminal peptide
(KNtp) to the central vestibule of SUR1, and KNtp further restrains the rotation of Kir6.2
cytoplasmic domain (CTD) to inhibit channel opening (Wu et al., 2020; Wu et al., 2018). Despite
recent progress in the cryo-EM studies of KATP with intact KNtp (Li et al., 2017; Martin et al.,
2017a; Martin et al., 2019; Sung et al., 2021) or KNtp-SUR1 chimera (Ding et al., 2019), the
exact residues on KNtp that make interactions with IS and SUR1 could not be explicitly
identified in currently available maps, because of their poor local map qualities due to the
flexibility of KNtp. Similarly, the pharmacophores on IS that are responsible for KNtp binding
are also poorly defined, although it is obvious that different IS use distinct chemical groups at H-
B site for KNtp recruitment (Fig. 1E). These fundamental questions about the structural
pharmacology of IS await further structural and functional investigation. This allosteric model
for IS inhibition is also in agreement with the shortcoming of IS: some neonatal diabetes patients
with Kir6.2 mutations are resistant to the sulfonylurea therapy (SU-insensitive mutants)
(Pipatpolkai et al., 2020). This is likely because the Kir6.2 CTDs of these SU-insensitive mutants
seldom sample the closed conformation which allows the insertion of KNtp into the SUR1
central vestibule. Therefore, drugs that directly inhibit the Kir6.2 channel would be a better
choice to suppress the activity of these mutants. However, available small molecule inhibitors of
Kir6.2, such as imidazoline-type compound phentolamine, are low-affinity and their binding
sites are unknown yet (Cui et al., 2021). Recently, a centipede toxin SpTx-1 is reported to inhibit
the Kir6.2 channel with high potency (Ramu et al., 2018) and SpTx-1 could trigger insulin
secretion and lower blood glucose levels in the diabetic mice model (Ramu et al., 2022). We
believe further elucidation of the structural mechanism of Kir6.2-specific small molecules or
SpTx-1 toxins might shed light on the development of novel anti-diabetic drugs targeting SU-insensitive $\text{K}_{\text{ATP}}$ mutants.

The structures of SUR in complex with KCO reveal that KCO activate the channel by promoting SUR to adopt the occluded state, which is in synergy with Mg-ADP (Ding et al., 2022; Wang et al., 2022b). Recent structures of the pancreatic $\text{K}_{\text{ATP}}$ channel in the activated non-conductive state (radii of the ion permeation pathway at the constriction are smaller than the hydrated potassium ion 3.3 Å) provide hints of how the occluded SUR activates $\text{K}_{\text{ATP}}$ (Wang et al., 2022b; Zhao and MacKinnon, 2021): first, the KNtp is released from the central vestibule of SUR, alleviating its allosteric effects on the inhibition of Kir6; second, the ATP binding loop of SUR (ABLOS) is away from Kir6, which decreases the affinity of $\text{K}_{\text{ATP}}$ for the inhibitory nucleotides. However, the exact activation mechanism of $\text{K}_{\text{ATP}}$ by the occluded SUR awaits the determination of $\text{K}_{\text{ATP}}$ structure in the fully open conformation.

Taken together, recent progress on the structural determination of $\text{K}_{\text{ATP}}$-drug complexes shed lights on the concept of $\text{K}_{\text{ATP}}$ pharmacology at near-atomic resolution and sets the foundation for future structure-based drug design and optimization. However, a complete mechanistic understanding of the $\text{K}_{\text{ATP}}$ pharmacology awaits further investigation.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

Wrote or contributed to the writing of the manuscript: Wu, Ding, and Chen.
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References


Legends

Figure 1. The insulin secretagogue binding site of SUR
A-B. Bottom view (from the intracellular side) and side view of the $K_{ATP}$ hetero-octamer in complex with RPG (PDB code: 6JB1, EMDB code: 9787). The Kir6, SUR TMD0, TMD1-NBD1 (nucleotide binding domain 1), TMD2-NBD2, RPG, and ATP are colored in cyan, orange, pink, blue, yellow, and brown, respectively. TMD (transmembrane domain), NBD (nucleotide binding domain).

C. The cartoon topology of Kir6 and SUR. PH (pore helix), IH (interfacial helix), CTD (cytoplasmic domain), ABLOS (ATP binding loop of SUR), ICL (intracellular loop), ECL (extracellular loop).

D. Side view of the SUR1 ABC transporter module in the presence of RPG and ATPγS (PDB code: 6JB1).

E. The chemical structures of IS. The hydrophobic A (H-A) and hydrophobic B (H-B) sites are in the gray background, the Polar site (P) is in pink background, respectively. The IS without available $K_{ATP}$ complex structure are colored in gray.

F-K. Close-up view of the binding site of (F) GBM (PDB code: 5YW7), (H) RPG (PDB code: 6JB3), and (J) MTG (PDB code: 7WIT). SUR1 and ISs are colored in grey and yellow, respectively. Residues of SUR1 that interact with ISs are shown as sticks and colored as Figure 1E according to the binding sites. The SUR1-IS interactions analyzed by Ligplot. (G) GBM, (I) RPG, (K) MTG.

Figure 2. The KCO binding sites of SUR.
A. Structure of SUR2B in complex with levcromakalim (Lev) and Mg-nucleotides. TMD1-NBD1, TMD2-NBD2, Lev, Mg-ATP and Mg-ADP are colored in pink, blue, yellow, cyan and orange, respectively.

B. Chemical structures of KCO. A site, P site, and B site are in gray, pink, and green background, respectively. KCO without available K<sub>ATP</sub> complex structure are colored in gray.

C-D. Close-up view of NN414 binding site. SUR1 and NN414 are colored in grey and green, respectively. Residues of SUR1 interacting with NN414 are shown as sticks and colored as Figure 1E according to the binding sites.

E-F. Close-up view of P1075 binding site. SUR2B and P1075 are colored in grey and green, respectively. Residues of SUR2B interacting with P1075 are shown as sticks.

G-H. Close-up view of Lev binding site. SUR2B and Lev are colored in grey and green, respectively. Residues of SUR2B interacting with Lev are shown as sticks.
Extracellular

RPG

TMD1

NBD1

TMD2

NBD2

A

73Ȗ6

FD

Tyr377

Leu434

Leu592

Trp430

Met441

Glu1249

Phe433

Val596

O

RPG

O

O

O

N

N

N

N

N

O

Asn437

N

O

N

O

Asn1245

N

O

N

O

Asn1245

N

O

N

O

Arg1246

N

N

N

N

N

O

Asn1245

N

O

N

O

Met441

Thr588

Trp1297

Repaglinide

N

H

O

OO -

Nateglinide

Mitiglinide

Glibenclamide

N

H

O

N

H

S

OO

Glimepiride

Tolbutamide

Meglitinide

O

O-

O

O

N

H

H

H

H

N

H

H

N

H

H

S

OO

Fig. 1
Fig. 2