Structural Insights into SK Channels: Unveiling Key Determinants for Selective Blocker Development

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Introduction

- Small conductance calcium-activated potassium (**SK**) **channels** are **selective for K^+** ions and are **gated by Ca^{2+}** via calmodulin molecules[1]. **Three isoforms (SK1-3)** exist and are **expressed differentially within the central nervous system**[2,3].
- **SK channels** regulate the afterhyperpolarization (AHP) and **modulate the firing rate/pattern of different types of neurons**[4,5] (*Figure 1*).
- SK channels are involved in the development of mental illnesses such as **schizophrenia**[6] and **mood disorders**[7].
- Their activity can be regulated by using **blockers** like **apamin**[8], a neurotoxin found in bee venom, or **UCL1684**[9].

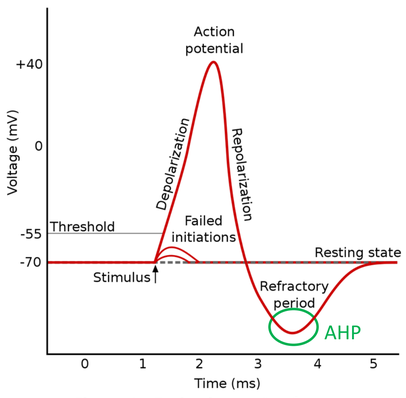

Figure 1 - Excitation cycle of a neuron

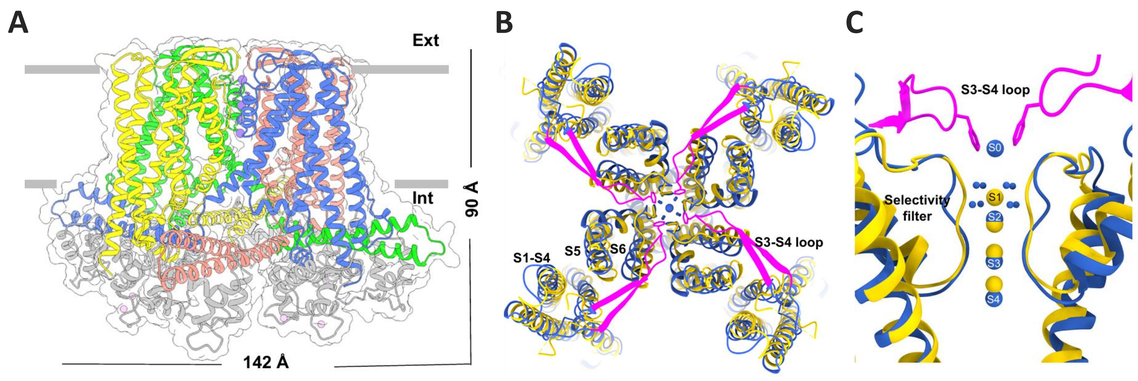

Figure 2 - SK2 structure *(Adapted from Nam YW, et al., 2025).* ***(A)*** *Cryo-EM density map with fitted model of SK2 viewed from the plane of the membrane. Four SK2 subunits are shown in blue, yellow, green and salmon, and CaM is shown in gray.* ***(B-C)*** *Superimposition of SK2 (blue) on SK4 (yellow).* ***(B)*** *Extracellular view - The four S3-S4 ß-hairpins (magenta) form a canopy over the outside of the pore. Phe243 residues at the tips of four S3-S4 ß-hairpins form an aromatic box with a central opening at the outer end of the pore.* ***(C)*** *Focus on the selectivity filter viewed from the plane of the membrane with two subunits shown.*

- Recently, a **cryo-EM structure of SK2** has been obtained (*Figure 2A – PDB accession code : 8v2g*) [10]
- S3-S4 loops (β-hairpins) form a canopy over the outside of the pore (*Figure 2B*).
- At the tip of the β -hairpin a Phe residue was shown to be essential for the proper folding of the loop and induces conformational changes that contribute to the low unitary conductance characteristic of SK channels.

Objective

- **Study of the conserved Phe residue at the tip of the S3-S4 loop and its role in the blocking by apamin and other compounds for the development of non-peptidic molecules with specificity for SK3.**

Methods

1) Insertion of mutations in the genes coding for SK proteins by **sited directed mutagenesis**

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2) Expression of the proteins **in HEK293** cells

↓

3) Testing the **affinity** for apamin for each mutant channels by using **binding assay** with radiolabeled ^{125}I-apamin

4) Testing the **activity** of channels with ***in vitro*** **patch clamp** experiments

Analysis of the interactions between SK channels and blockers with **molecular docking** and **modeling of the mutants with AlphaFold 3**

Affinity and activity tests

Mutants of hSK2 and hSK3 were generated by replacing the phenylalanine (F243) of interest by either an alanine (A) or a tyrosine (Y). The affinity of mutant channels for apamin was screened through binding assays and their activity was tested with *in vitro* patch clamp experiments (whole-cell configuration, symmetrical K^+ and 10 µM free Ca^{2+} in the pipette).

in vitro Binding Assays

A

Channels	Radioactive activity (dpm)		
	Total Binding	Non Specific	Specific Binding
SK2 WT	4305 ± 239	199 ± 35	4106 ± 270
SK2 F243Y	5854 ± 245	252 ± 29	5602 ± 224
SK2 F243A	298 ± 68	267 ± 45	31 ± 93
SK3 WT	4660 ± 199	340 ± 125	4321 ± 304
SK3 F392Y	254 ± 69	233 ± 40	21 ± 105
SK3 F392A	385 ± 246	238 ± 93	147 ± 155

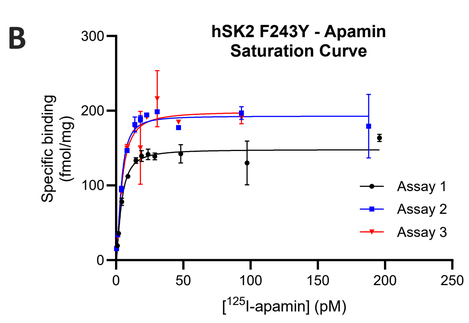
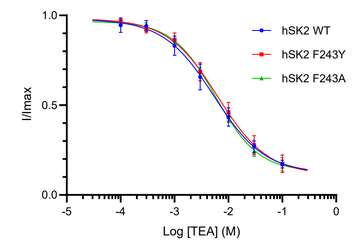


Figure 3 - Binding assay with radiolabeled apamin
*(**A**) Mutants screening. **(B)** Saturation assay with hSK2 F243Y.*

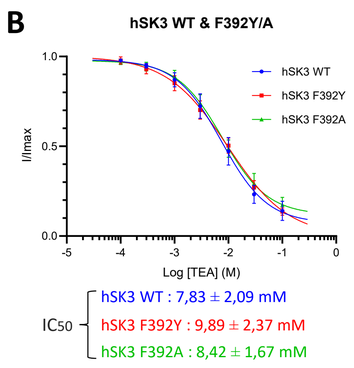
- Screening of the mutants showed that only hSK2 F243Y bound to apamin (*Figure 3A*). Saturation assays with this tyrosine mutant (*Figure 3B*) showed that it has a K_D value similar to that of native SK channels (from 3.7 to 4.7 pM for the mutant, ~5pM for native channels).

in vitro Patch-clamp Assays

Tetraethylammonium (TEA)

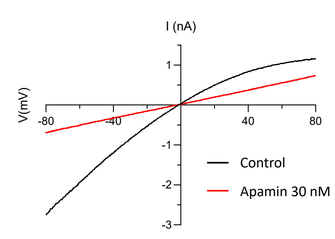


IC50 — hSK2 WT : 5,91 ± 1,72 mM
hSK2 F243Y : 6,94 ± 1,46 mM
hSK2 F243A : 5,86 ± 0,48 mM

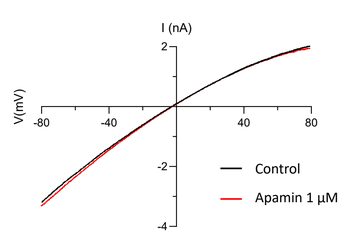


IC50 — hSK3 WT : 7,83 ± 2,09 mM
hSK3 F392Y : 9,89 ± 2,37 mM
hSK3 F392A : 8,42 ± 1,67 mM

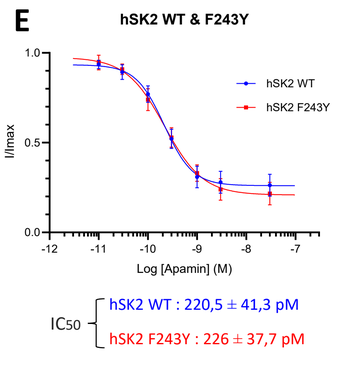
Apamin



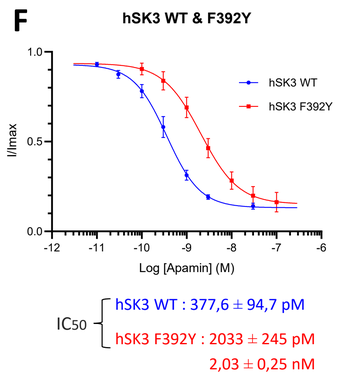
→ Apamin 30nM blocks > 75% of current intensity at -80mV
→ Similar observation for hSK3 WT



→ No current inhibition by apamin 1µM
→ Same observation for hSK3 F392A

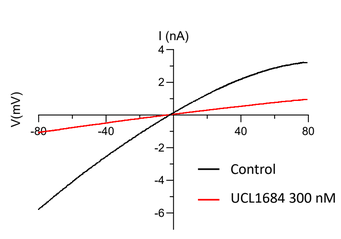


IC50 — hSK2 WT : 220,5 ± 41,3 pM
hSK2 F243Y : 226 ± 37,7 pM

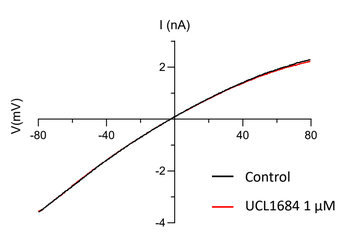


IC50 — hSK3 WT : 377,6 ± 94,7 pM
hSK3 F392Y : 2033 ± 245 pM
2,03 ± 0,25 nM

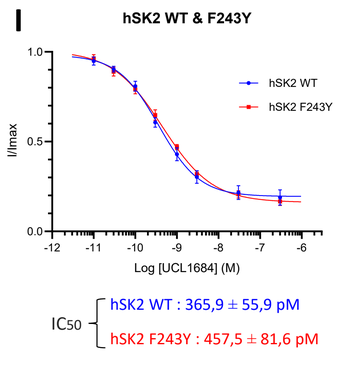
UCL1684



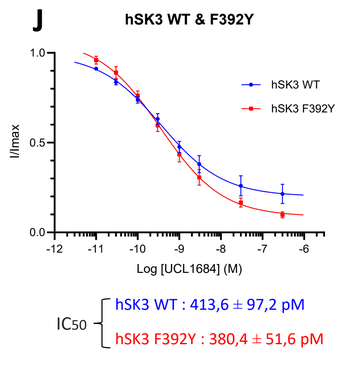
→ UCL1684 300nM blocks > 80% of current intensity at -80mV



→ No current inhibition by UCL1684 1µM



IC50 — hSK2 WT : 365,9 ± 55,9 pM
hSK2 F243Y : 457,5 ± 81,6 pM



IC50 — hSK3 WT : 413,6 ± 97,2 pM
hSK3 F392Y : 380,4 ± 51,6 pM

Figure 4*. (**A-B**) Concentration-inhibition curves of TEA on SK2 and SK3 show similar sensitivity for wild-type channels and their Phe-Tyr and Phe-Ala mutants (n = 5 ; p > 0.05 ; Kruskal-Wallis test). (**C-D**) I–V relationships before (black) and after (red) adding high concentration of apamin on hSK2 WT and hSK2 F243A channels. Curves are obtained by averaging 5 experiments. (**E-F**) Concentration-inhibition curves show similar sensitivity to apamin for wild-type and tyrosine mutant in SK2 (n = 5 ; p > 0.05 ; Mann-Whitney test) but a differential sensitivity in SK3 (n = 5 ; p < 0.01). (**G-H**) I–V relationships before (black) and after (red) adding high concentration of UCL1684 on hSK3 WT and hSK3 F392A channels. Curves are obtained by averaging 5 experiments. (**I-J**) Concentration-inhibition curves show similar sensitivity to UCL1684 for wild-type and tyrosine mutant in both SK2 and SK3 (n = 5 ; p > 0.05 ; Mann-Whitney test). All error bars correspond to SEM.*

Models of the SK2/3 tyrosine mutants

- Structural models of tyrosine mutants in SK2 and SK3 were generated using AlphaFold3, with the SK2 wild-type structure (PDB ID – 8v2g) serving as a template to preserve the conformation of the selectivity filter *(Figure 5*).
- The mutation in tyrosine maintains the hydrophobic interactions of the phenylalanine it replaces and previously shown to induce conformational changes in the SK2 selectivity filter. In addition, tyrosine forms a supplementary hydrogen bond with the carbonyl group of a glycine (G362 in SK2, G511 in SK3) within the same monomer (*Figure 5 C and D*). **This additional interaction likely enhances the stability of the S3–S4 loop conformation.**
- A tyrosine residue substituting phenylalanine has been identified in native SK channels from various bacterial species (e.g., *Alteromonas, Amphritea*) and animal species, predominantly fish (e.g., *Cyprinodon variegatus, Oryzias latipes, Poecilia latipinna*), where the rest of the S3–S4 loop remains highly conserved.

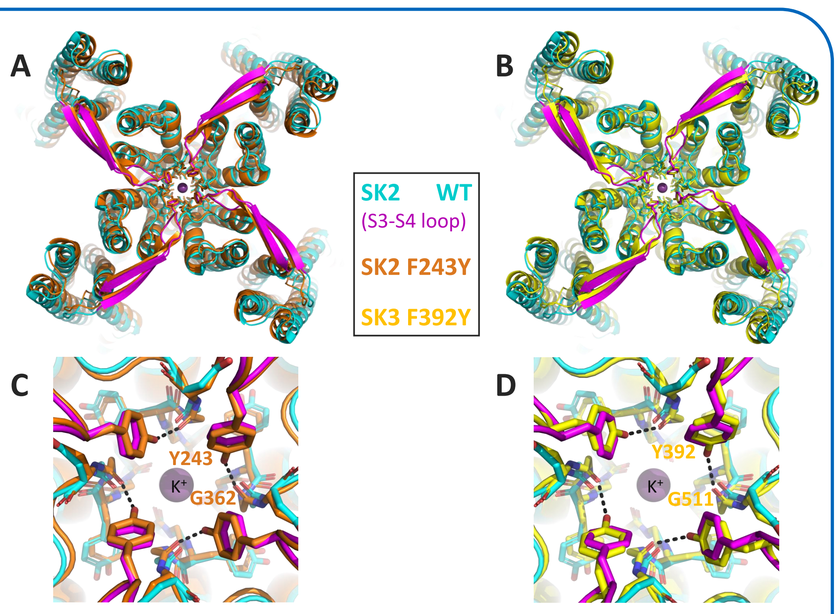


Figure 5*. **SK2/3 tyrosine mutant models.** (**A-B**) Extracellular view of the SK2 and 3 tyrosine mutants (respectively in orange and yellow) superimposed on SK2 WT structure - 8v2g (cyan). S3-S4 loop of SK2 WT is shown in magenta. (**C-D**) Zoom on the on the tyrosines and the pore of the channel. The hydrogen bonds between Y243 and G362 in SK2, and Y392 and G511 in SK3 are shown in black dotted lines.*

Molecular Docking of TEA

- **TEA** was docked onto **SK2** structure (PDB code – 8v2g)[10] using the VINA algorithm with a 20 Å cubic simulation cell centered on the exit of the selectivity filter and the S3-S4 phenylalanine residue (F243).
- The two best poses show that TEA localizes at the exit of the pore, either between the F243 residues (1 - green) or in the negatively polarized region generated by the carbonyl groups of the selectivity filter (2 - orange) (*Figure 6*).
- The F243 residues cause the exit of the channel pore to be very narrow and the passage of the TEA to be difficult. Pose 1 is therefore more likely unless significant dynamic of the loop is present.

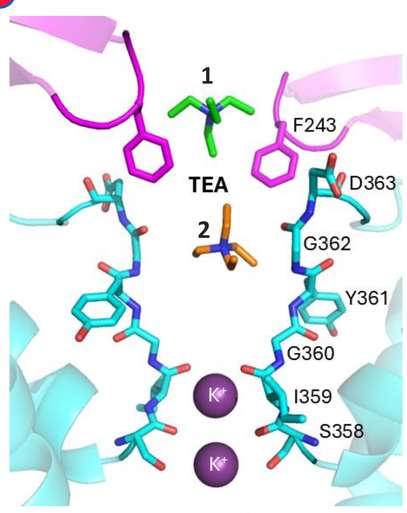


Figure 6 - Docking of TEA on SK2
Side view of the two poses obtained for TEA (in green and orange) at the exit of the pore of SK2. Residues composing the selectivity filter (cyan) and the Phe of the S3-S4 loops (magenta) are shown as sticks.

Conclusion

- **In this study, we confirm the critical role of the Phe residue in the S3–S4 loop of SK2 and SK3 channels, supporting findings reported in a very recent study[10]. *In vitro* binding and patch-clamp experiments demonstrated that substitution of phenylalanine with alanine abolishes the sensitivity of SK2 and SK3 channels to apamin and UCL1684, whereas substitution with tyrosine preserves sensitivity to these compounds. Interestingly, all channels showed a similar sensitivity to TEA while the alanine mutation is expected to modify the conformation of the pore region. AlphaFold models of tyrosine mutants show that the tyrosine would maintain the interactions observed for the phenylalanine and allow an additional H-bond with a glycine from the selectivity filter, potentially enhancing the stability of the S3-S4 loop.**

References

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