

Investigating the structure and pharmacology of SK channels through molecular docking and *in vitro* patch clamp

Mouchet A.¹ (antoine.mouchet@uliege.be), Nadenoen T.¹, Vitello R.², Brans A.¹, Seutin V.³, Liégeois J.-F.², Kerff F.¹

¹ Centre for Protein Engineering (CIP), InBios, ULiège ; ² Laboratory of Medicinal Chemistry, C.I.R.M., ULiège ; ³ Laboratory of Neurophysiology, GIGA-Neurosciences, ULiège

Introduction

- Small conductance calcium-activated potassium channels (SK) channels are selective for K⁺ ions and are gated by Ca²⁺ via calmodulin molecules¹. Three isoforms exist and are expressed differentially within the central nervous system (CNS), SK1 & SK2 being mostly expressed in the cortex and hippocampus while SK3 expression is higher in the thalamus, the hypothalamus and the brainstem^{2,3}.
- SK channels underlie the medium duration component of the afterhyperpolarization (AHP, shown on *Figure 1*) and play an important role in modulating the firing rate/pattern of different types of neurons^{4,5}. They slow down the return to the resting potential of the membrane and lower the frequency of the excitation peaks.
- SK channels have been shown to be involved in the development of some mental illnesses such as schizophrenia⁶ and mood disorders.

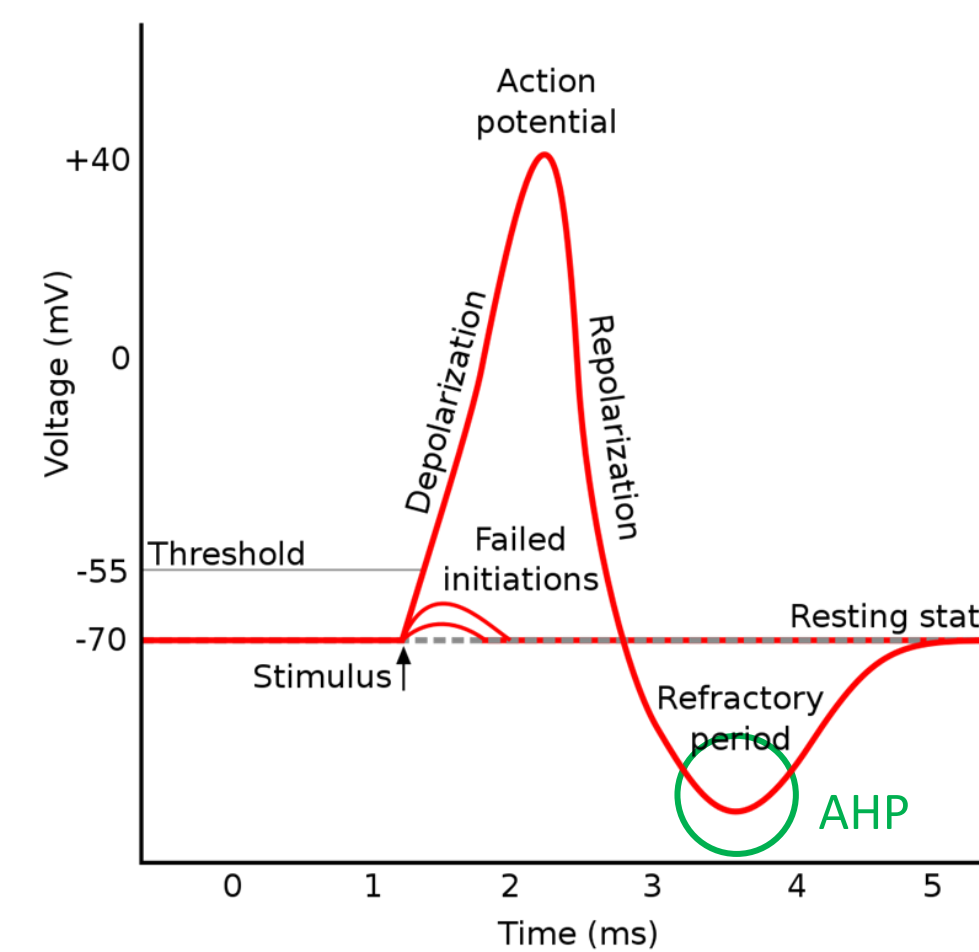


Figure 1. Excitation cycle of a neuron.

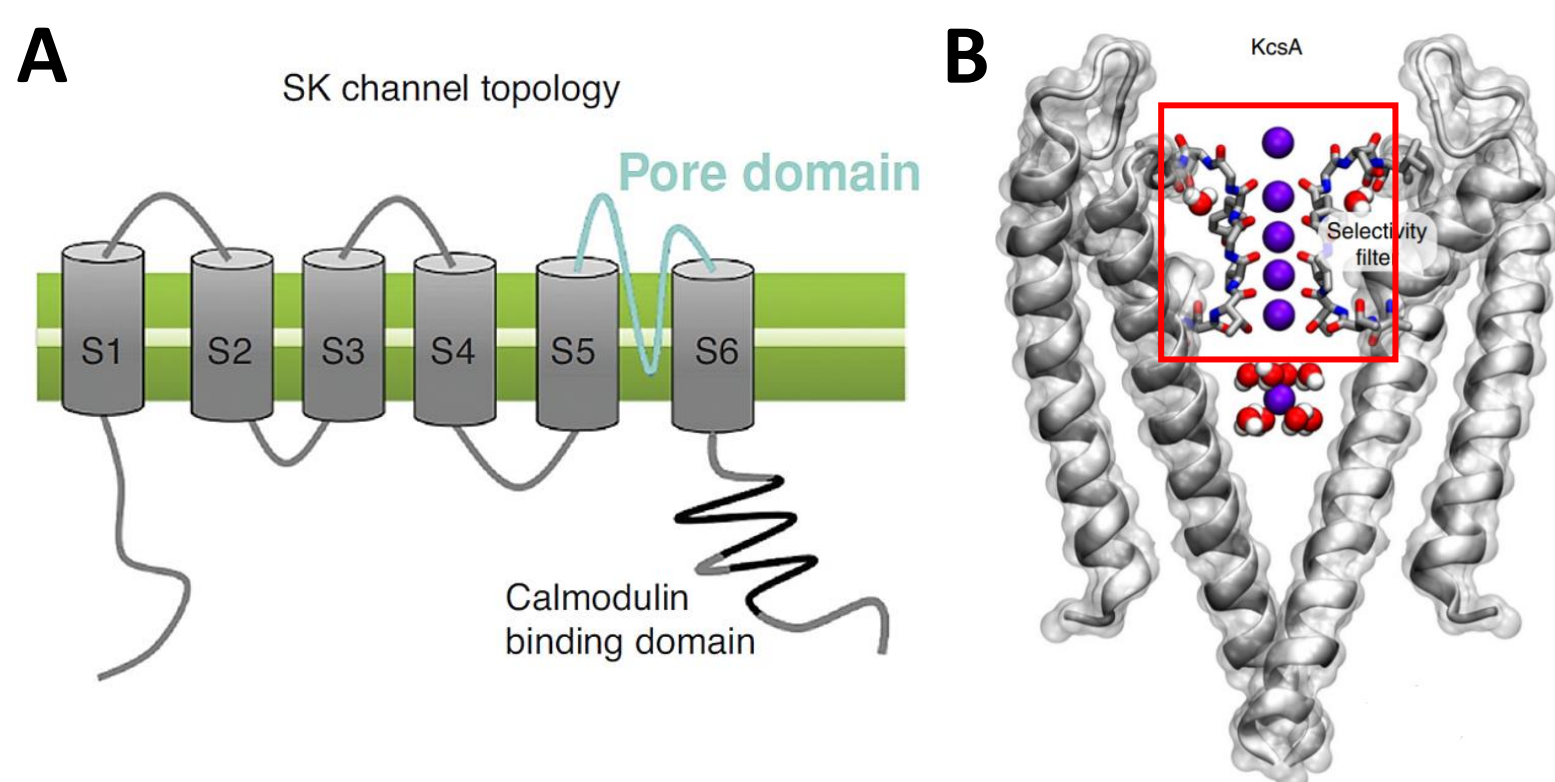
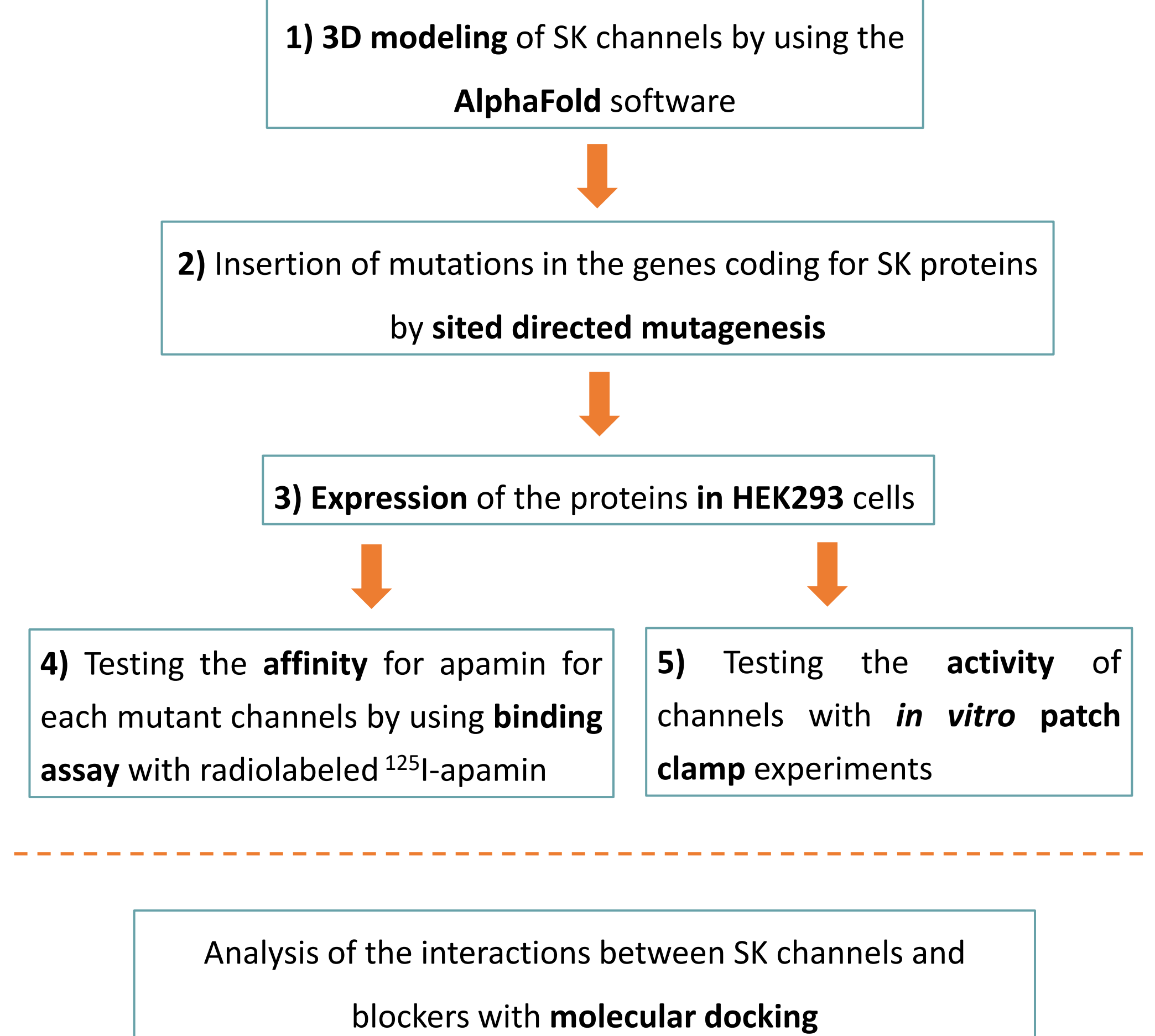


Figure 2. (A) General topology of SK proteins⁷, (B) 3D representation of the S5-S6 domains with the selectivity filter in KcsA channel.

- One monomer consists of 6 transmembrane domains, S1 to S6, with the N and C-terminal ends in the cytoplasm and the pore domain between the S5 and S6 helices (*Figure 2A*)⁷.
- The pore of the channel contains a region called the selectivity filter (circled in red on *Figure 2B*), essential for the regulation of the flux of K⁺ ions. Functional SK channels are tetramers.
- SK channels can be blocked by a whole series of inhibitors, including apamin, a neurotoxin found in bee venom⁸. By studying the structure and the pharmacology of SK channels, we aim to develop new non-peptidic blockers capable of acting specifically on the different subtypes of SK channels.

Methods



AlphaFold Models

- The models obtained with AlphaFold highlight a particular conformation of the extracellular S3S4 loop (shown in red on *Figure 3A*) that is conserved in the three subtypes SK1-3 but different from the loop in SK4 (*Figure 3C*). This loop contains a phenylalanine residue located just at the exit of the pore of the channel as shown in *Figure 3B-C*.
- As previous papers demonstrated the importance of amino acids next to this phenylalanine for the interaction with apamin⁹, our hypothesis is that the phenylalanine residue could also play a key role in the interaction and in the blocking mechanism by apamin and potentially by some other blockers.

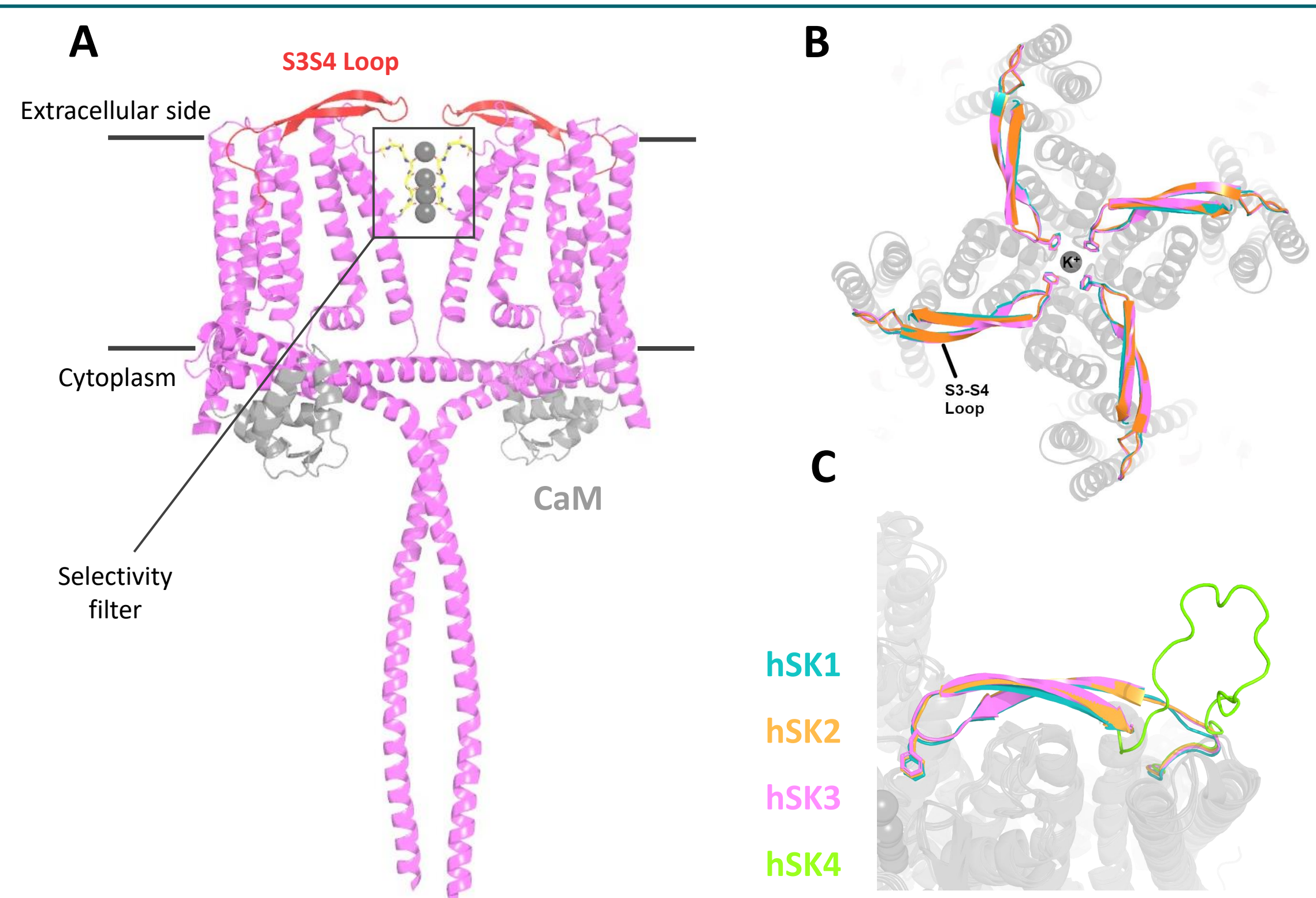


Figure 3. (A) Cartoon representation of the model obtained for human SK3 channel with AlphaFold. (B) Top view of SK channels highlighting the S3S4 loop. (C) Zoom in on the S3S4 loop in SK1 (blue), SK2 (orange), SK3 (pink) and SK4 (green).

Molecular Docking

- In order to complement the patch clamp experiments shown below, molecular docking experiments were carried out with UCL1684, a chemical compound known to block SK channels currents. Simulation results show a similar position of UCL1684 when docking with the three SK channel subtypes and confirm the importance of the phenylalanine residue for the interaction with the compound.

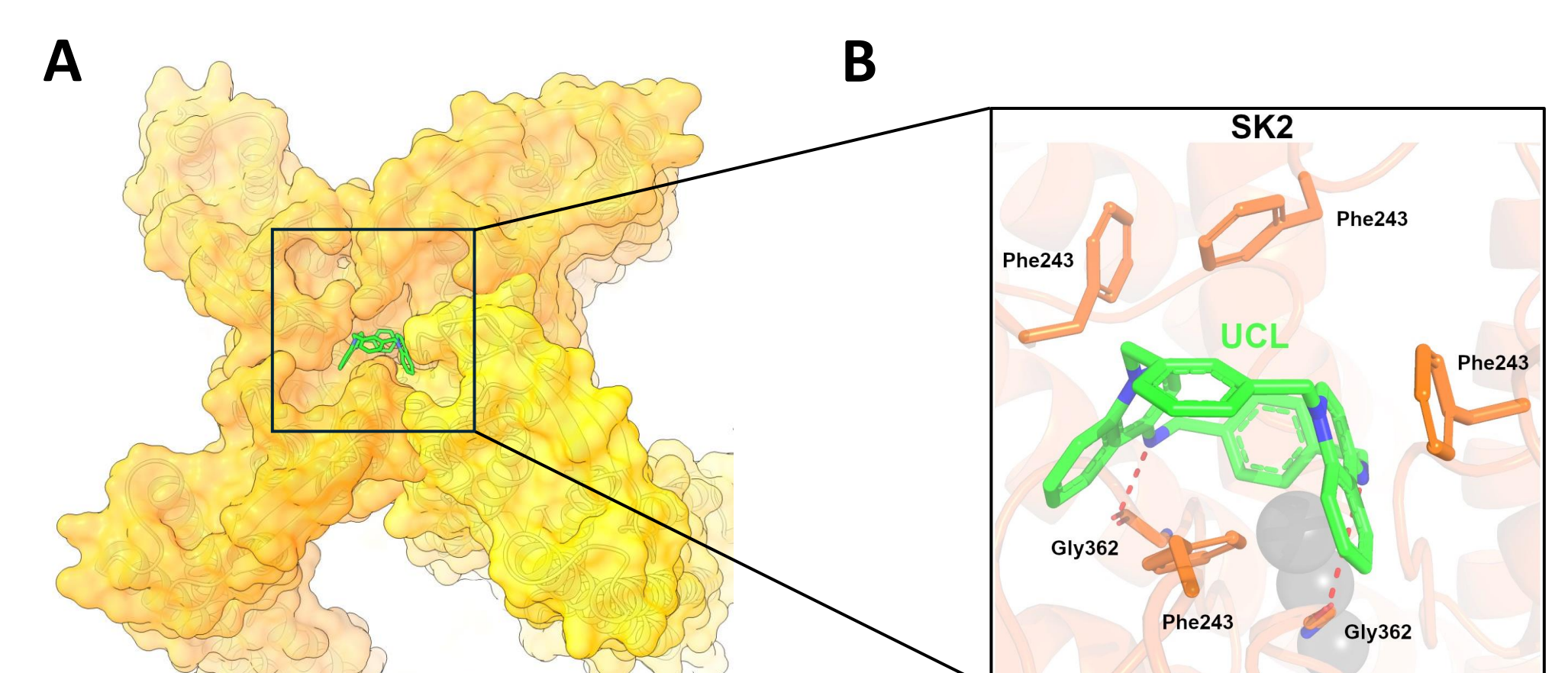


Figure 4. (A) Surface representation of hSK2 channel with UCL1684 in the binding pocket. (B) Zoom in on the binding pocket highlighting interactions between UCL1684 and hSK2 proteins.

Affinity and activity tests

Mutants of hSK2 and hSK3 were generated by replacing the phenylalanine (F) 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 Ca²⁺ in the pipette).

Binding assay

Mutants	Radioactive activity (dpm)		
	Total binding	Non specific	Specific Binding
hSK2 WT	4456	181,5	4274,5
	4154	217	3937
hSK3 WT	4726,5	293,5	4433
	4594,5	386	4208,5
hSK2 F243A	275,5	305	-29,5
	321	230	91
hSK2 F243Y	5673	234,5	5438,5
	6035,5	270	5765,5
hSK3 F392A	499,5	288,5	211
	270,5	187	83,5
hSK3 F392Y	212,5	251,5	-39
	296	214,5	81,5

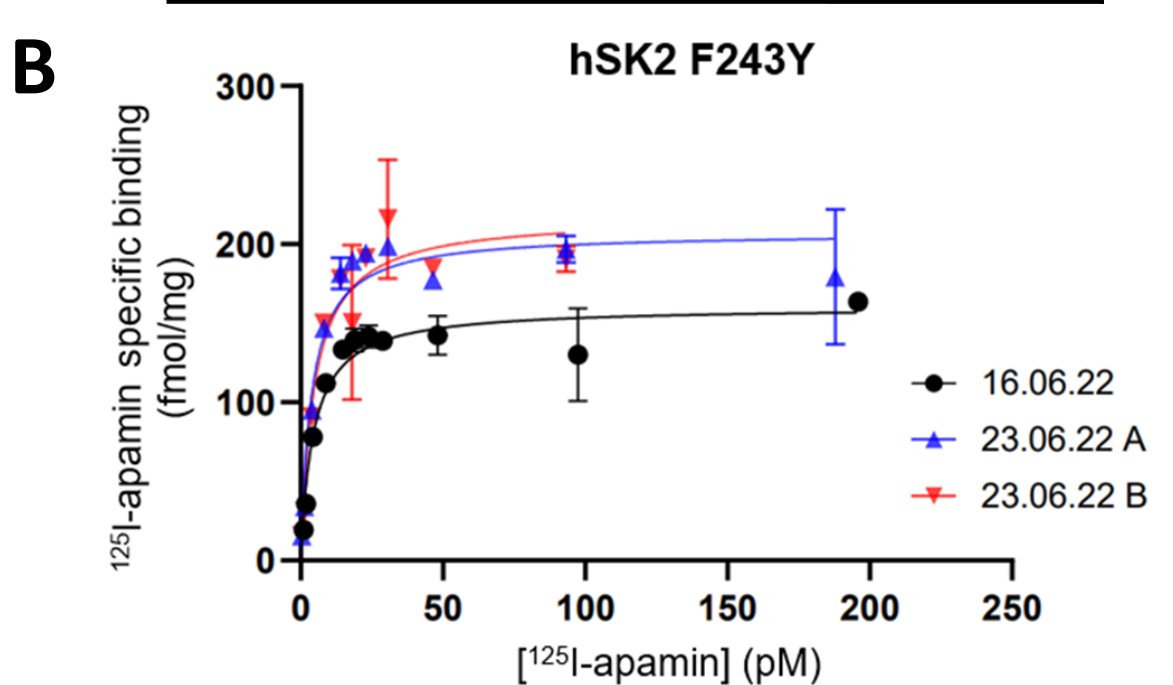
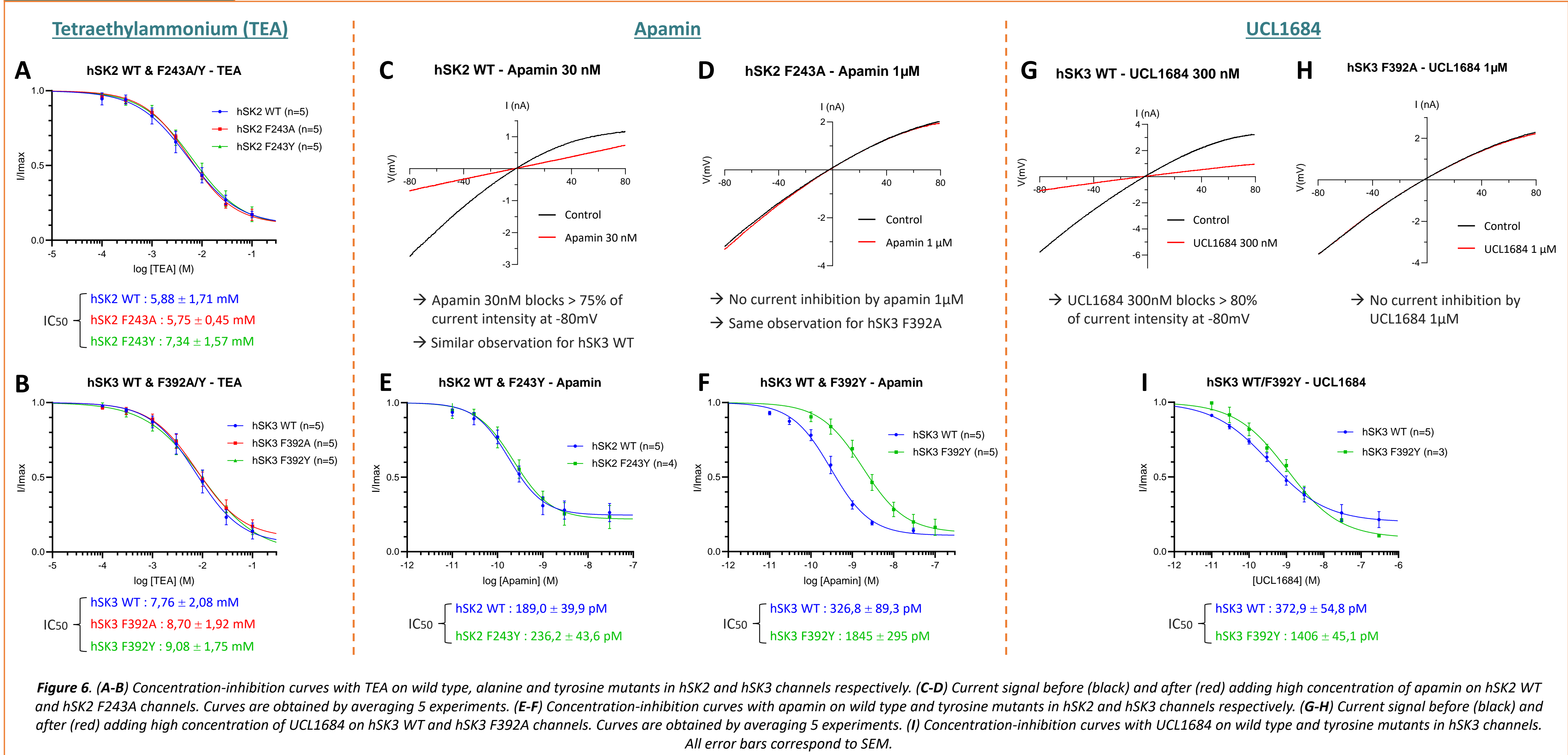


Figure 5. Binding assay with radiolabeled apamin. (A) Screening of the mutants. (B) Saturation assay with hSK2 F243Y.

Screening of the mutants showed that only hSK2 F243Y bound to apamin (*Figure 5A*). Saturation assays with this tyrosine mutant (*Figure 5B*) showed that it has a K₀ 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



Conclusion

- AlphaFold models of SK channels highlighted the particular conformation of the S3S4 loop and the presence of a conserved phenylalanine residue in hSK1, 2 and 3. Molecular docking experiments with UCL1684 supported the idea that this phenylalanine plays an important role in the interaction with the blocker. Binding assay and whole-cell patch clamp experiments on the alanine mutants in hSK2 and hSK3 proved that the phenylalanine residue is essential for the interaction and the subsequent blocking by apamin and UCL1684.

References

- Schumacher MA, et al. (2001), Nature 410:1120–1124.
- Kohler M, et al. (1996), Science (80-) 273:1709–1714.
- Faber ESL, Sah P (2007), Clin Exp Pharmacol Physiol. 34:1077–1083.
- Rouchet N, et al. (2008), Eur J Neurosci 28:1108–1115.
- Waroux O, et al. (2005), Eur J Neurosci 22:3111–3121.
- Grube S, et al. (2011), EMBO Mol Med 3:309–319.
- González C, et al. (2012), Compr Physiol. 2:2087–149.
- Lamy C, et al. (2010), Biol Chem 285:27067–27077.
- Weatherall KL, et al. (2011), PNAS 108(45):18494–9.

Acknowledgments

This work was supported by a FNRS grant "Projet de recherche" (grant PDR/T.0121.22) and an "Action de Recherche Concertée" from Fédération Wallonie-Bruxelles (grant ARC 21/25-08), a FRIA grant from the FNRS for R.V. F.K. and J.-F.L. are research associates and research director of the FRS-FNRS (Brussels, Belgium), respectively.