# Identification of "on-off residues" in rat Cav1.2 α1C subunit channel using in silico analysis and docking simulation

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## Introduction

Voltage-dependent calcium channels (VSCC) is involved in important biological function as calcium ion transmembrane transport and cardiac contraction. VSCC is a multi-pass membrane protein, made up from α-1, α-2, β and δ subunits. α-1 subunit regulates the entry of ion calcium. Voltage-dependent L-type calcium channel subunit alpha-1C (Cav1.2 α1C subunit channel) is an isoform of VSCC, and is characterized from an high-voltage activation. Previous study have shown that class of molecules as benzothiazepines (Tikhonov D. et al, 2008), are able to block the alpha-1C subunit. Recent works have demonstrated that molecules belonged at the flavonoid class are able to inhibit or to raise channel activity (Saponara S. et al, 2011). In this work, we reported the sensing- residues that could play a key role in Cav1.2 α1C activity. Furthermore, we proposed a potential mechanism of action inside Cav1.2 α1C binding-site with differences between inhibitors and stimulants. Our work has clarifiedas the ligands operate on Cav1.2 α1C, this information could be useful in order to improve their usefulness.

## Methods

The 3D structure of Cav1.2 α1C subunit channel was obtained on basis of previous work (Saponara S. et al, 2015). The structure of flavons were downloaded from Pubchem(Kim S. et al, 2015). Docking simulation was carried out through Autodock/Vina v.1.1.2 (O.Trott et al, 2010). PDBePISAwas used in order to evaluate buried surface area values (B.S.A). Protein-ligand interactions were obtained using protein–ligand interaction profiler (P.L.I.P)(Salentin S. et al, 2015). Pymol was used as molecular graphics system (The PyMOL Molecular Graphics System, Version 1.8 Schrödinger, LLC.).

## Results and discussion

In vitro analysis on rat Cav1.2 L-type channel of 20 flavonoids have shown stimulatory and inhibitory activities (Saponara S. et al, 2015).The 11 inhibitors and 8 stimulators derivatives are positioned in their corresponding binding-sites with peculiar sensing-residues interactions, furthermore, as reported in figure 1, the inhibitors-stimulators binding-site, results to be very close between them. Analyzing the network of interactions among the two classes of flavonoids we have observed hydrogen bonds, hydrophobic interactions and π–π stacking bonds characterizing their activities that could differently promote pore open/closed conformation and decreasing voltage-sensitive calcium channel activity (Tikhonov D. et al, 2009).

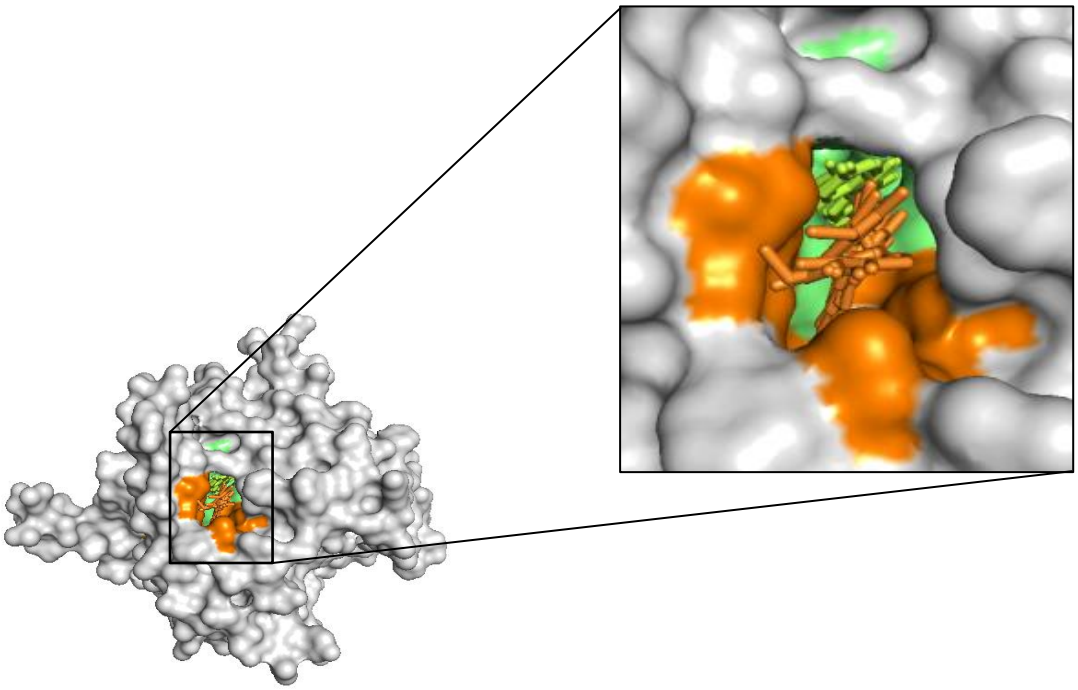

*Figure 1. Overview inhibitor-stimulant binding-site in Cav1.2 α1C subunit channel structure. In gray surface is reported Cav1.2 α1C subunit channel structure, in light green and orange surface are shown inhibitor-stimulator binding-site respectively. Inhibitors and agonists are represented in light green and orange sticks respectively. Is evident that inhibitors and stimulators are presents in two different pockets in continuity between them.*

Furthermore, on the basis of the selectivity filters model, we have evaluated B.S.A residues values present in the binding-sites, we have observed that B.S.A of some residues dramatically decrease, showing that these residues play a key role in the pore stabilization. The different mechanism of action of these molecules can be attributed to their chemical-physical proprieties as steric hindrance and different positions of hydroxyl groups. To know in depth how inhibitors and stimulants bind in Cav channel active site is very important, in this way we can improve drug design of these molecules in order to increase their efficacy.