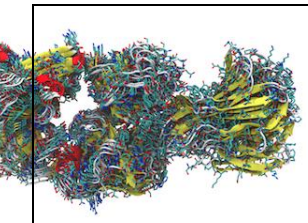

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Fast time scale backbone dynamics the sodium-calcium exchanger (CALX) CBD2 domain

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Abstract

Sodium-calcium exchangers (NCXs) are an important family of membrane proteins that regulate the concentration of intracellular Ca^{2+} through the counter transport of Na^{+} and Ca^{+2} (3:1) across the cell membrane. The exchanger consists of a transmembrane domain and a very large intracellular loop, which is involved in regulation of the exchanger activity. Binding of Ca^{2+} to a cytosolic Ca^{2+}-sensor, CBD12, located in the loop, activates the NCX. In contrast, Ca^{2+}-binding to CBD12 inhibits the exchanger of *Drosophila*, CALX. CALX has two splice variants called CALX-1.1 and CALX-1.2, which present different regulatory responses to intracellular Ca^{2+}. While intracellular Ca^{2+} inhibits the CALX isoform 1.1, CALX 1.2 is insensitive. These two splice variants differ in a small segment of 5 amino acids located in the CBD2 domain **(Figure 1)**. The three-dimensional structures of the two Ca^{2+}-sensors, CBD12-1.1 and CBD12-1.2, are strikingly similar, which do not explain the different regulatory behaviors of CALX 1.1 and 1.2. Therefore, we sought to investigate whether there are differences in backbone dynamics between the two Ca^{2+}-sensors, CBD12-1.1 and CBD12-1.2. These differences could be related to the different regulatory behaviors of CALX 1.1 and CALX 1.2. Since CBD12 is a very large protein for high-resolution NMR spectroscopy, we focused on the CALX-CBD2 domain. We performed an almost complete backbone resonance assignment of the two isoforms of CBD2. We measured ^{15}N spin relaxation rates, R_1 and R_2, and the heteronuclear NOE, of CBD2 1.1 and 1.2 in order to gain insights on dynamics at fast time scales. In order to complement our measurements of ^{15}N relaxation, we have been running long (500 ns) MD simulations of CBD2 1.1 and 1.2. Analysis of the ^{15}N relaxation rates, NOE and R-factor (molecular simulation) showed that the isoform 1.2 displays more dynamics at the FG-loop relative to the isoform 1.1.

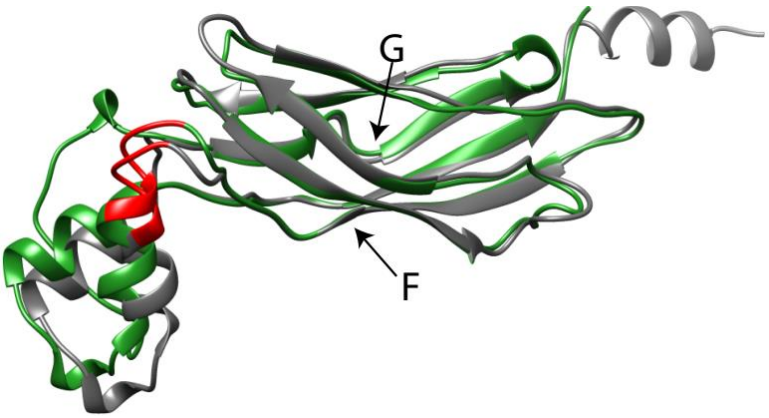

Figure 1. Superposition of the crystal structures of CBD2-1.1 (Grey) (PDB 3E9U) and CBD2-1.2 (Green) (Modelling with Robetta). The segment that is different in each isoform is shown in red.