Dimerization of neural-cadherins occurs via formation of calcium dependent strand-crossover structures, which leads to cell-cell adhesion in multicellular organisms. Strand-crossover dimer forms exclusively between the first N-ter-
minal extracellular modules (EC1) of adhesive partners via swapping of their β-sheet and docking of tryptophan-2 in the hydrophobic pocket. Prolines recurrently occur in proteins that form strand-crossover dimer and are be-
lieved to be the source of the strain in the monomer. N-cadherin has two pro-
line residues in the β-sheet. Our studies address two interesting questions; why is the dimerization in neural-cadherin calcium dependent, and do all three calcium-binding sites at the EC1-EC2 interface play a role in dimerization. To investigate these questions we mutated three important calcium-binding amino acids, D134, D136, and D103, and three amino acids in the β-sheet W2, P5 and P6, in NACD12, a construct containing EC1 and EC2. Spectro-
copic and chromatographic experiments showed that the calcium-binding sites are occupied sequentially in the order of site3, then site2 and site1, and cooperation between site2 and site1 is essential for dimerization. Studies on the P5A, P6A double mutant showed that the proline mutations increased the dimerizing calcium affinity - fold and relieved the requirement for calcium in dimerization. Studies on W2A showed that the binding of calcium creates strain in the hydrophobic interaction between the hydrophobic pocket and W2 in the closed monomer, which is relieved upon formation of the strand-
crossover dimer. In summary, our findings confirm that the hydrophobic in-
teraction involving W2 is the source of calcium-dependent dimerization and the proline residues at β-sheet act as a switch to control the dynamics of the equilibrium between monomer and dimer which is crucial for the plasticity of synapses.

Alternative Splicing at CBD2 Domain Modifies Ca2+ Sensing Properties at CBD1 in Regulatory Two-Domain Tandem of NCX Proteins
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The Na+/Ca2+ exchanger isoforms (NCX1-3) and their splice variants are ex-
pressed in a tissue-specific manner and extrude Ca2+ from the cell to regulate Ca2+-dependent events in many cell types. The cytosolic f-loop of NCX pro-
teins consists of two regulatory domains, CBD1 and CBD2, which form a two-domain tandem (CBD12) with a short linker. The CBD1 domain contains two high-affinity Ca2+ sites (Ca3-Ca4), which largely determine Ca2+-depen-
dent allosteric activation of NCX, whereas the alternative splicing sequence is exclu-
sively located on CBD2. Previous structural, biochemical and mutational studies have shown that in an isolated CBD1 tandem, CBD2 interacts with CBD1 through a novel interdomain salt-bridge to modify its on-rate, off-rate and off-rates at high affinity Ca2+ sensor of CBD1. We posit here that splice variations of A-F exons on CBD2 specifically modify dynamic properties of Ca2+ sensing at CBD1. In this respect, we analyzed three splice variants of an isolated two-domain construct, NCX1-CBD12: kidney (BD), brain (AD) and cardiac (ACDEF). In addition, we have analyzed CBDs from CALX-
CBD12, a Drosophila NCX protein which is inactivated, rather than activated 
by Ca2+ binding to the high affinity sensor on CBD1. By using equilibrium 
binding, stopped-flow and SAXS (small-angle X-ray scattering), we demon-
strate that different splice variants of CBD2 specifically modify the kinetic and equilibrium properties of Ca2+ sensing at CBD1. Moreover, Ca2+ binding decreases maximum distance (Dgeo) of the NCX-CBD12 kidney, brain and cardio-
diae splice variants, whereas Ca2+ has the opposite effect on CALX-CBD12. Therefore, experimental approaches described here may help in identifying 
specific structural motifs in CBD2, responsible for the dynamic properties of Ca2+ sensing and also the mode of allosteric signal decoding.