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-sheets 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 
aming 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- 
scopic and chromatographic experiments showed that the calcium-binding 
sites are occupied sequentially in the order of site3, then site2 and site1, and cooperativity between site2 and site1 is essential for dimerization. Studies on 
the P5, P6A double mutant showed that the proline mutations increased the 
dimerization affinity 20-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 inter- 
action 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.

**2341-Pos Board B111**

**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+ exchange 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- 
etins 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 
exclusively located on CBD2. Previous structural, biochemical and mutational 
studies have shown that in an isolated CBD2 tandem, CBD2 interacts with 
CBD1 through a novel interdomain salt-bridge and with the linker to modify 
its overall affinity and off-rates at high affinity Ca2+ sensor of CBD1. We pos- 
ition 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, brain (AD) and 
cardiac (ACDEF). In addition, we have analyzed CBDs from CALX-
CBD2, 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 (D_{\text{max}}) of the NCX-CBD12 kidney, brain and 
cardiac 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.

**2342-Pos Board B112**

**Dissecting the Roles of the Extracellular Regions in Apelin Peptide-Receptor Binding**

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The apelin receptor is a class A G-protein coupled receptor with homology to the angiotensin II receptor and the CXCR4 and CCR5 chemokine receptors. It is 
expressed at high levels in lung, heart, adipose tissue, kidney, spleen and in 
some areas of the brain. The apelin receptor gene is also upregulated and over- 
expressed in one-third of human tumours. The receptor is assumed to consist of 
a transmembrane region with 7 helical segments, an intracellular and an extra- 
cellular domain. The extracellular domain, made up of the N-terminal tail and 
three extracellular loops (EL1, 2 and 3), exhibits low sequence homology to 
other GPCRs. Several somatic mutations in loops have been linked to diseases, 
pointing out the importance of loops in the activation and/or binding of GPCRs 
to their cognate ligands. We carried out a physicochemical characterization of 
the N-terminus and extracellular loops of the apelin receptor in lipid mimetic 
environments. The N-terminus (attached to the first transmembrane domain) 
and EL2 were produced as recombinant proteins while the EL peptides and a 
fluorescently tagged apelin were synthesized by solid-phase peptide synthe- 
sis. All species were purified by high-performance liquid chromatography.

Characterization both of the peptides in isolation and of the binding between 
apelin and extracellular loop peptides was carried out using circular dichroism 
spectroscopy, fluorescence resonance energy transfer and nuclear magnetic res- 
onance spectroscopy. These results provide insight into understanding the role 
of the each of the extracellular regions of this receptor at the molecular level 
and constitute important information the apelin receptor for the development 
of therapeutics targeting this system.