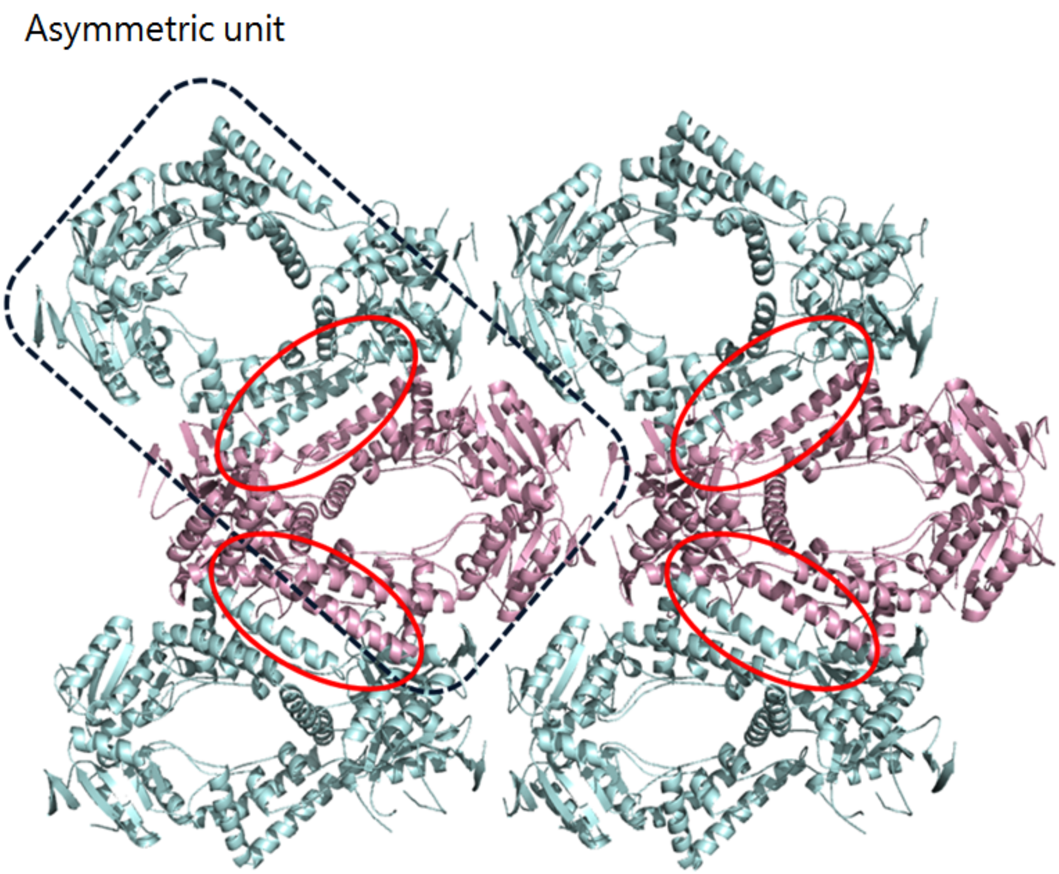
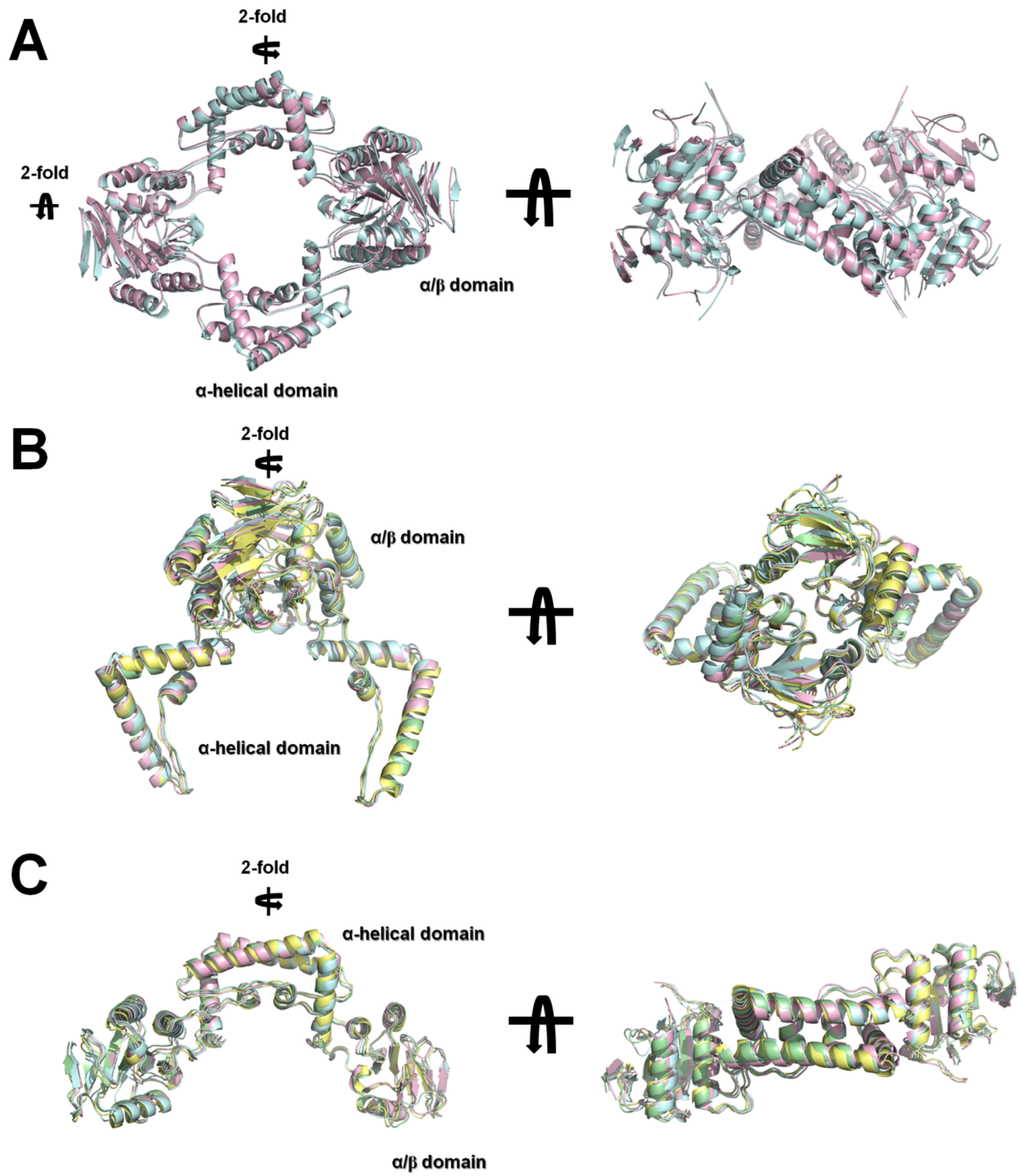
Supplemental Figures for

Crystal Structure of CRISPR-associated Csn2 Protein Revealed Ca^{2+}-dependent Double-stranded DNA-binding Activity

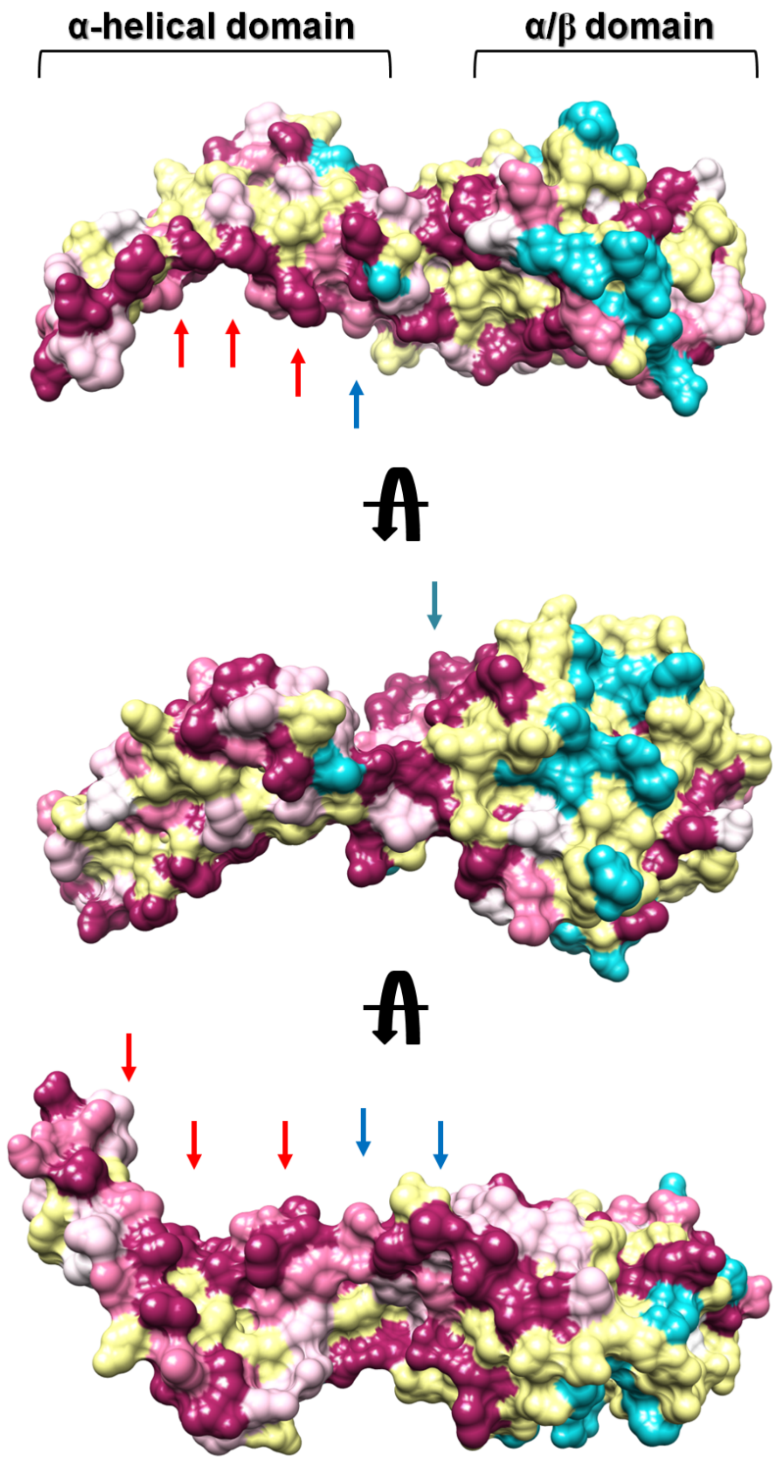
Ki Hyun Nam, Igor Kourinov, and Ailong Ke



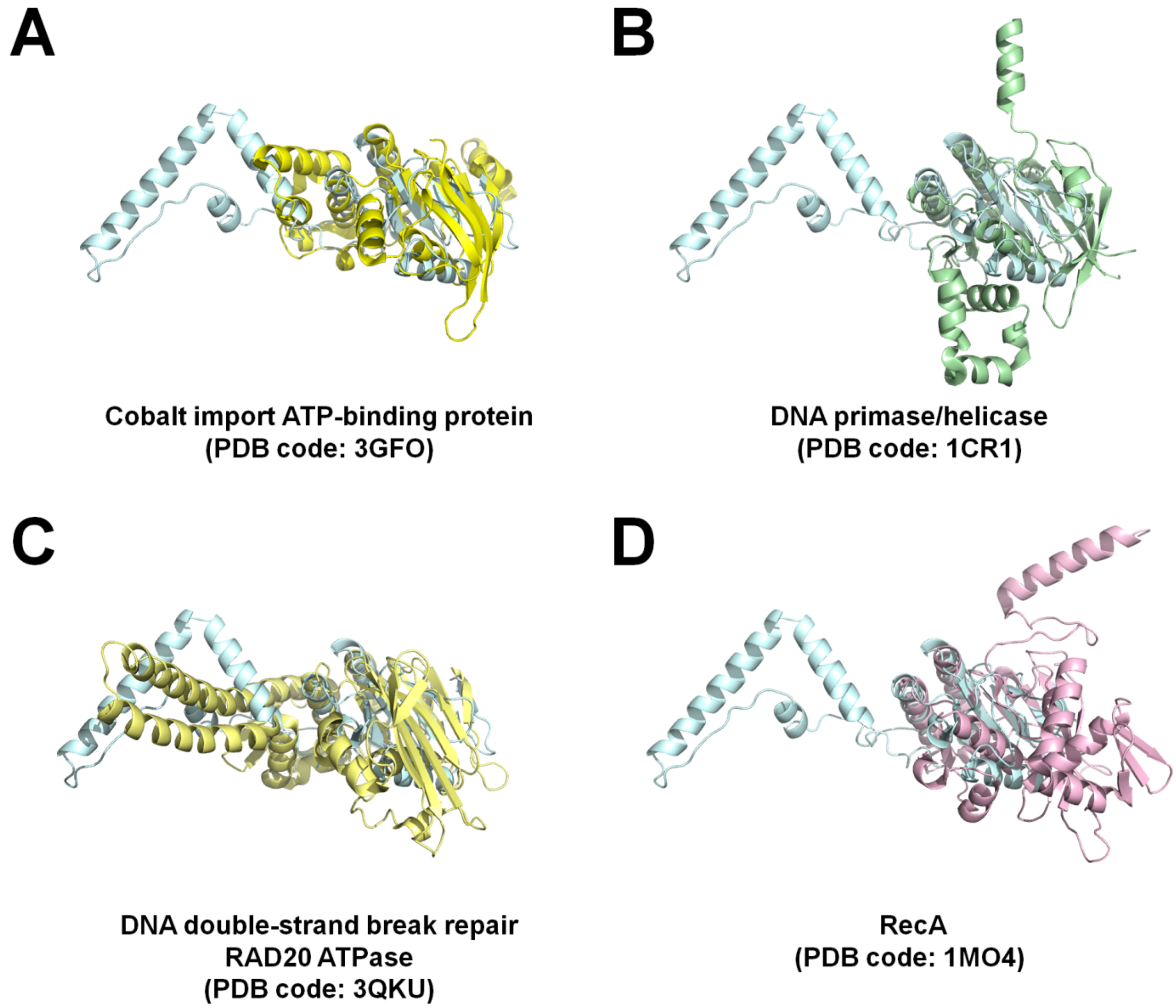
Supplemental Fig. S1. Packing interactions in the Csn2 crystal lattice. The two Csn2 tetrameric rings in the asymmetric unit are circled by the blue dotted line. Crystal packing interfaces between the Csn2 tetrameric rings are circled in red.



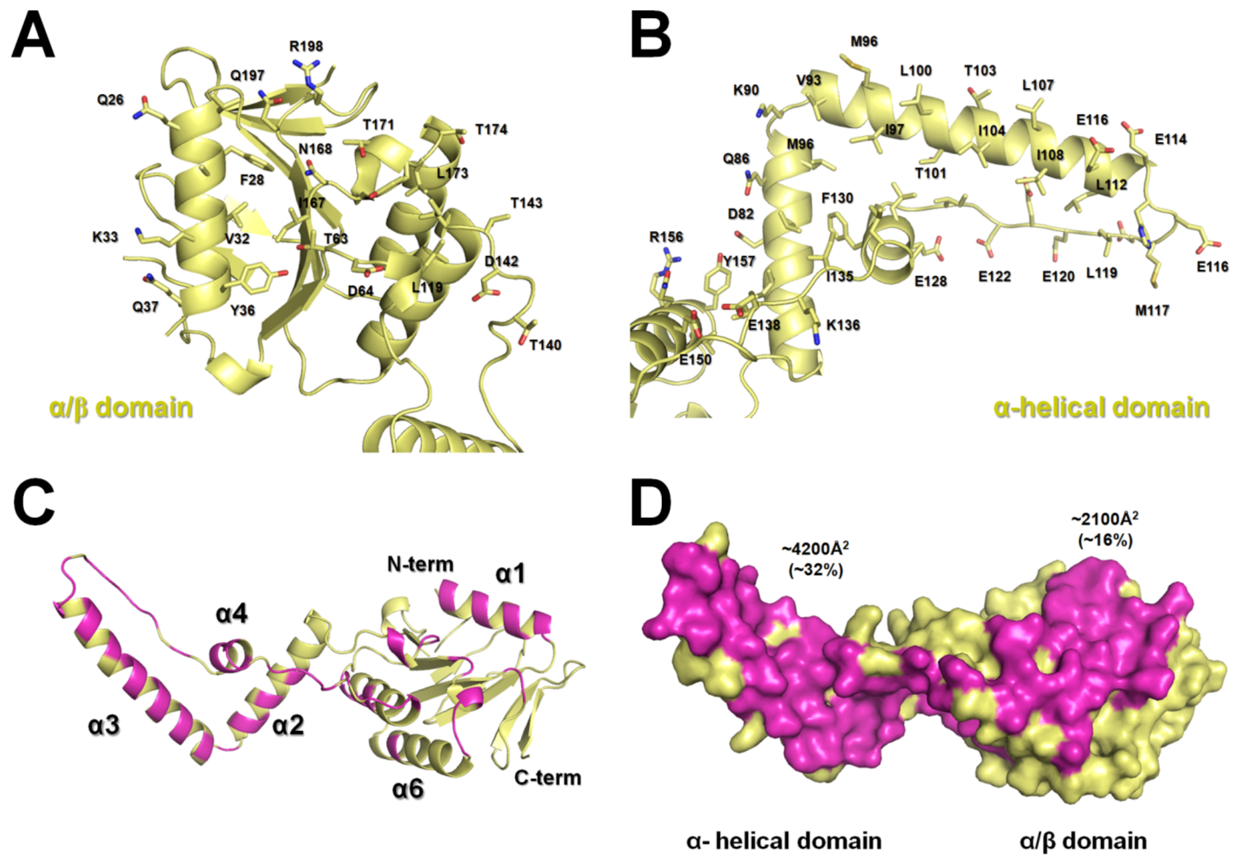
Supplemental Fig.S2. Comparison of the protomer conformations in the Csn2 tetrameric rings. (A) Superimposition of the two Csn2 tetrameric ring in the asymmetric unit showing an r.m.s.d of 1.3 Å for Cα atoms. More detailed comparison was done by classifying the Csn2 oligomerization into either molecules A-B type dimers, or A-C type dimers. (B, C) Superimposition of the four copies of the A-B type dimers or A-C type dimers revealed an r.m.s.d of 0.6-1.1 Å and 0.5-1.1 Å for Cα atoms, respectively.



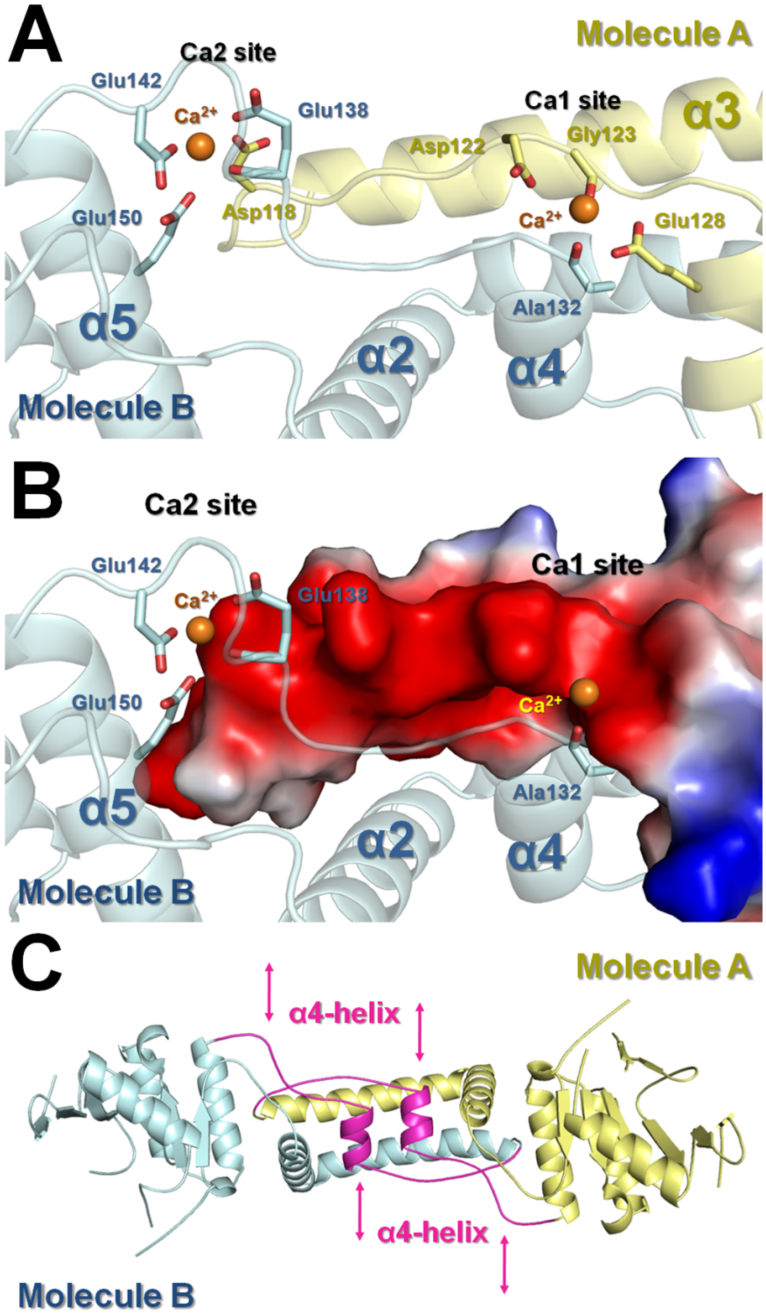
Supplemental Fig. S3. Surface conservation on the *E. faecalis* Csn2 protein shown in different orientations. Residues are colored from magenta to cyan with descending order of conservation.



Supplemental Fig. S4. Superimposition of the α/β domain of the *E. faecalis* Csn2 protein with its structural homologs including the Cobalt import ATP-binding protein (A), the DNA primase/helicase (B), the DNA double-strand break repair RAD20 ATPase (C), and the RecA protein (D). Structural homology search was performed using the Dali server. The Z-score and and the r.m.s.d. of the alignment are 7.7-8.8 and 3.1-3.4 Å, respectively.



Supplemental Fig. S5. Interfaces A-C and A-B in Csn2 tetramerization. (A) Residues involved in the dimerization of two α/β domains at the interface A-C are labeled. (B) Residues involved in the dimerization of two α-helical domains at the interface A-B are labeled. (C) Residues involved in tetramerization are highlighted in magenta onto the yellow cartoon representation of the Csn2 protomer. (D) Magenta coloring of the intermolecular interaction residues onto the surface representation of the Csn2 protein.



Supplemental Fig. S6. Ca^{2+} binding is crucial for Csn2 tetramerization. (A) Zoom view of the Ca^{2+} binding sites Ca1 and Ca2. (B) Same view but with one Csn2 molecular in the surface representation showing the concentration of negative charges in this region. The four Ca^{2+} ions can stabilize this dimerization interface by are shielding these negative charges. (C) A speculated scenario in which dissociation of the Ca^{2+} ions weakens the A-B interface and increases the conformational flexibility in the hinge region, which in turn affects the oligomerization state and DNA-binding properties of the Csn2 protein.