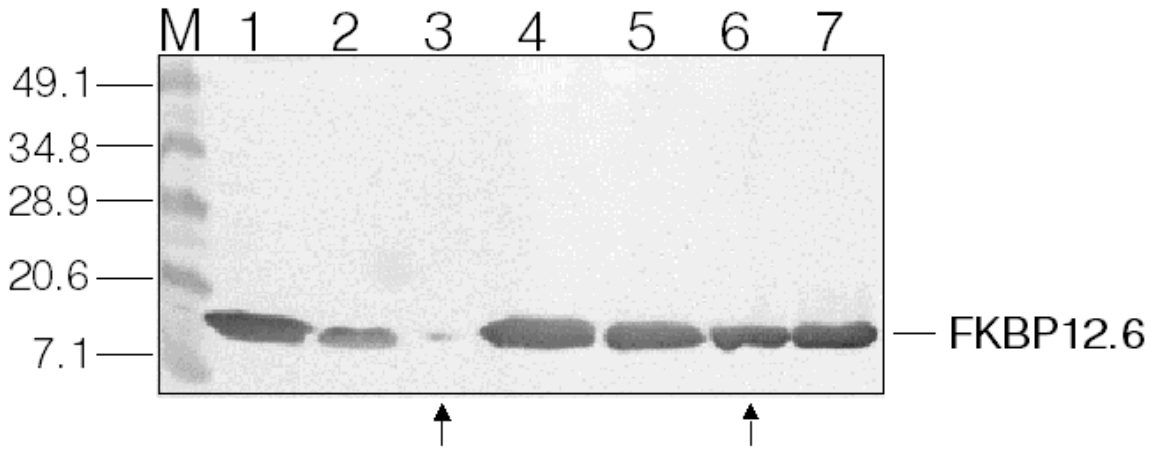
Supplemental Materials

THREE-DIMENSIONAL VISUALIZATION OF FKBP12.6 BINDING TO AN OPEN CONFORMATION OF CARDIAC RYANODINE RECEPTOR

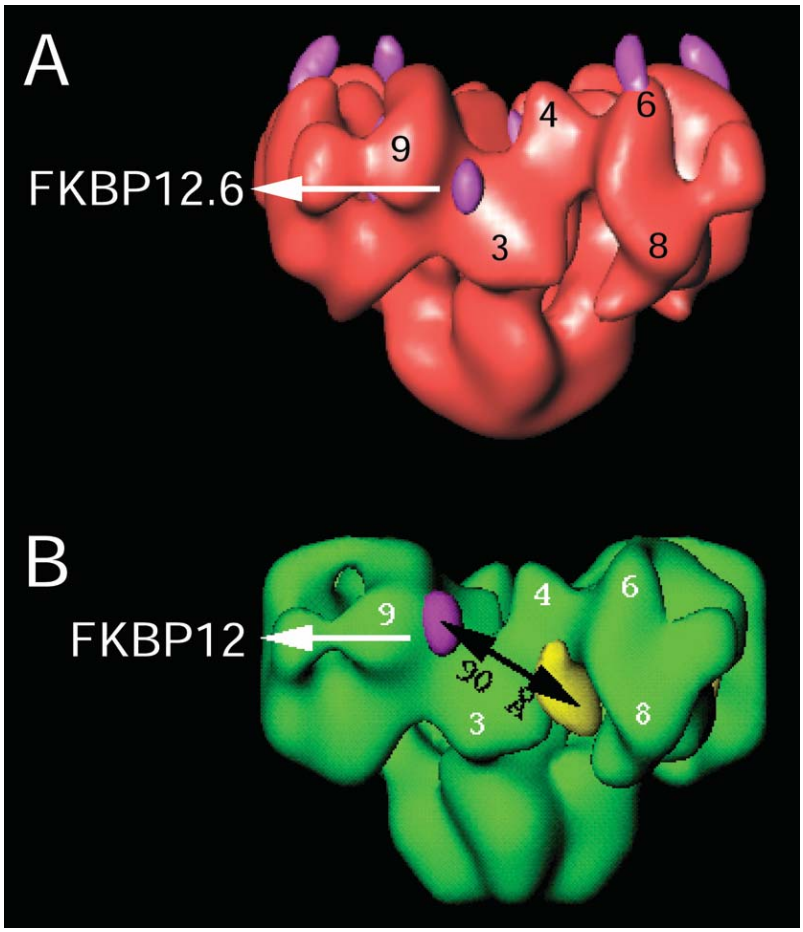
Manjuli Rani Sharma*, Loice H. Jeyakumar[#,§], Sidney Fleischer[#], and Terence Wagenknecht*,[¶].

*Wadsworth Center, New York State Department of Health, and [¶]Department of Biomedical Sciences, School of Public Health, State University of New York at Albany, Albany, NY-12201-0509

[#]Department of Biological Sciences, Vanderbilt University, TN, [§]Department of Medicine, Division of Gastroentrology, Vanderbilt University Medical Center, Nashville, TN 37232



Supplemental Fig. S1. Western blot analysis of RyR2-FKBP12.6 complex.
The proteins were transferred into Immobilon P-membrane (see Material and Methods) and probed with FKBP12 antiserum to detect FKBP12.6. Lane M: Prestained molecular weight marker. Lanes 1, 2, and 3 represent the supernatant, wash and final eluate from the RyR2 (-FKBP12.6) complex that was treated with the drug, FK506. Absence of FKBP12.6 from lane 3 (marked with arrow) confirms that FKBP12.6 was dissociated from RyR2 when the complex was used for making cryo-grids. Lanes 4, 5 and 6 correspond to the supernatant, wash and final eluate from the RyR2 (+FKBP12.6) complex. Presence of FKBP12.6 in lane 6 (also marked with arrow) clearly confirms that FKBP12.6 was bound to RyR2 when this complex was used for cryo-grid preparation. Last lane, human recombinant FKBP12.6 serves as standard.



Supplemental Fig. S2. FKBP locations on the 3D maps of (A) RyR2 and (B) RyR1. Note the difference in the binding position of FKBP12.6 (purple) on RyR2 (red, panel A) to that of FKBP12 (purple) on RyR1 (green, panel B). The complexes for RyR2 and RyR1 with FKBP12 were prepared under different buffer conditions: only the "close" conditions were used for RyR1-FKBP12 complex, while RyR2 complexes with FKBP12.6 were prepared under the buffer conditions favoring the "open" state of the receptor (for details, see Material and Methods). The density mass of FKBP12.6 and FKBP12 are displayed at same threshold and molecular mass (relative to molecular weight) though they appear to enclose slightly different sized masses when compared visually. The picture of later is adapted from Wagenknecht and coworkers (ref. 44).