

Supporting Information

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FRET-Based Trilateration of Probes Bound within Functional Ryanodine Receptors

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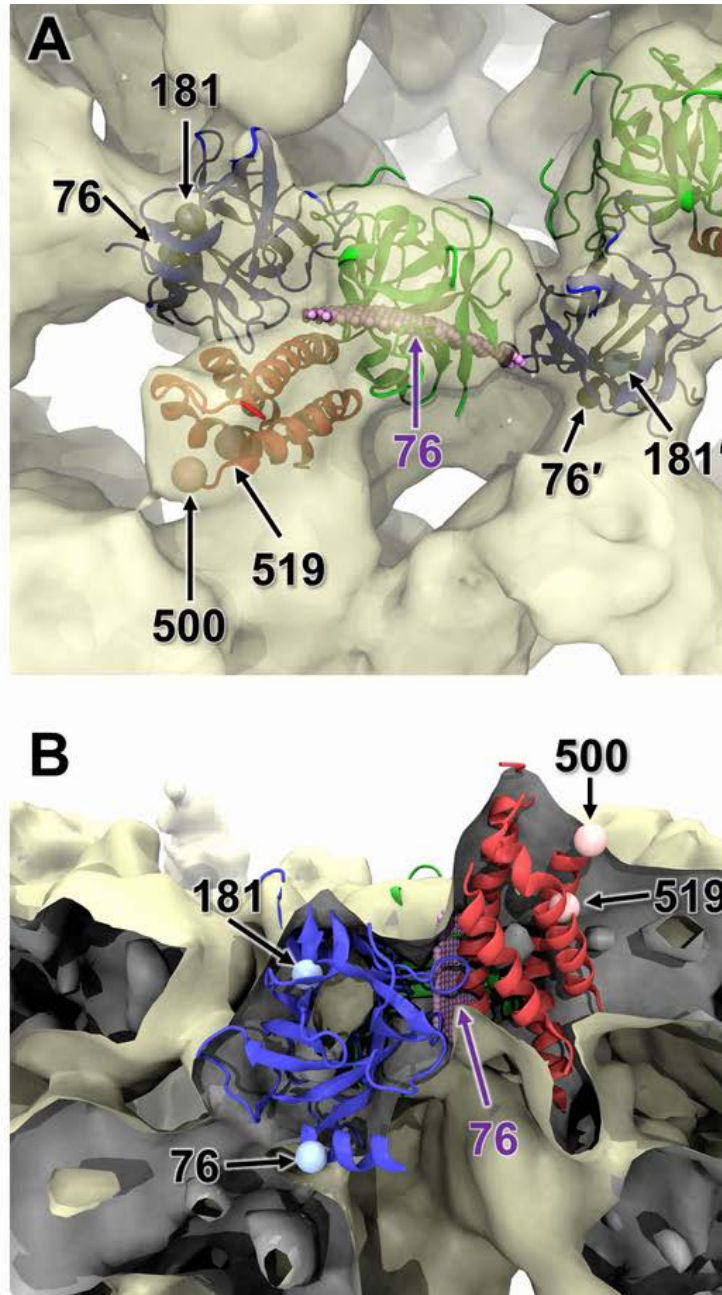


Figure S1: Trilaterated locus of the Cy3NTA acceptor probe attached to His₁₀ inserted at position 76. Panels A and B correspond to panels B and D in Fig. 5, from which the locus corresponding to position 181 has been removed. The ABC domains, “A” domain (blue), “B” domain (green), and “C” domain (red), are shown relative to the cryo-EM structure of the RyR1 (EMDB ID: 1606). (A) RyR1 is viewed from the “top” (cytosolic side), with two adjacent “ABC” domains represented in their respective color. (B) Magnified cut-out view of the region indicated in Fig. 5C (dashed box). In both panels, the locus of Cy3NTA attached to His₁₀ inserted at position 76 in the “A” domain is represented in purple.

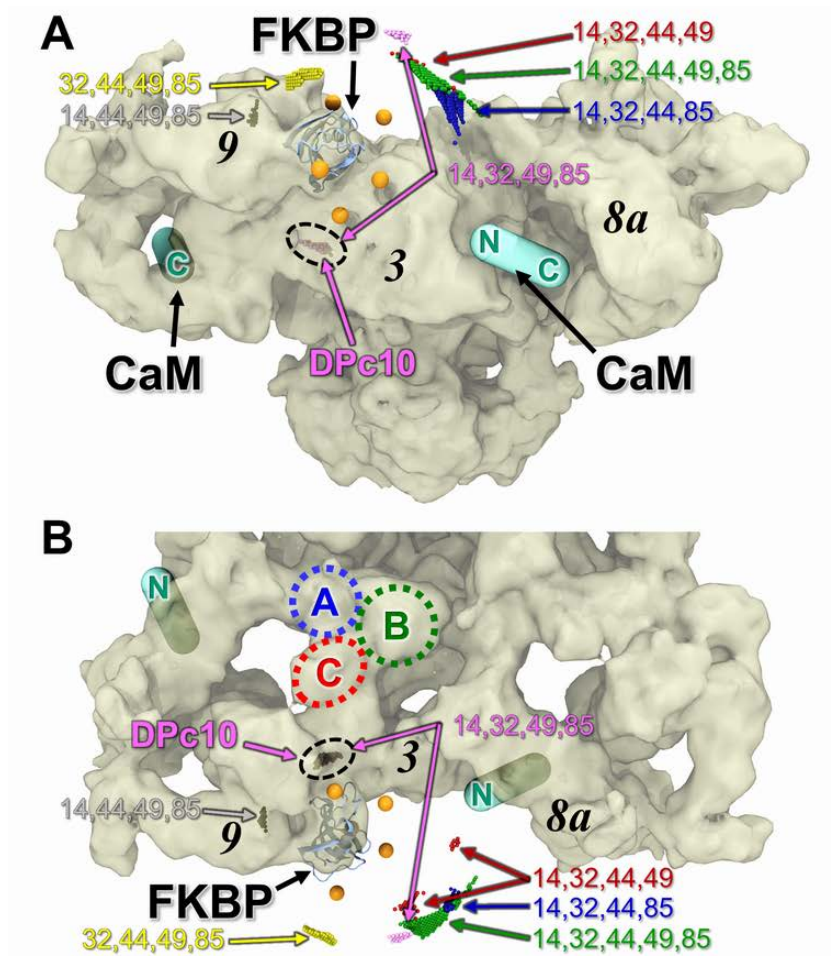


Figure S2: Trilateration results for FRET distances from D-FKBP to A-DPc10. Loci trilaterated using all five and all combinations of four FRET distances between D-FKBP and A-DPc10 are shown relative to the cryo-EM structure of the closed RyR (EMBD ID: 1606). (A) RyR shown in "side" view. (B) RyR viewed from the "top" (cytosolic side). The location of CaM is shown in cyan and labeled N and C to indicate the predicted N- and C-lobe positions (13). A trilaterated locus yielded using all five distances from D-FKBP sites 14, 32, 44, 49, and 85 is shown in *green*. This locus is found approximately 40 Å outside the cryo-EM map. The loci found using all combination of four donors are shown as follows; 14,32,44, and 49 (*red*), 14,32,44, and 85 (*blue*), 14,32,49, and 85 (*magenta*, and inside dashed circle), 14,44,49, and 85 (*grey*), and 32,44,49, and 85 (*yellow*). Only the 14,32,49, and 85 and 14,44,49, and 85 sets give loci that are in proximity of the RyR cryo-EM density. The 14,32,49, and 85 trilateration also yields a second solution that is on the opposite side of FKBP, well outside of the cryo-EM map. The only solution that is valid after considering proximity of the RyR map and FRET distances to CaM is indicated (*black dashed oval*).