calcium release playing a critical role in cardiac excitation-contraction coupling. Recently, we presented evidence that the RyR2 N-terminus self-associates into a tetrameric form, which stabilizes the closed conformation of the channel. In order to identify the interacting domains mediating RyR2 N-terminus tetramerisation, we used truncated constructs in yeast two-hybrid and chemical cross-linking assays. RyR2 residues 1-418 remained in monomeric form, whereas residues 1-530 retained self-association ability although it was much weaker compared to longer fragments. Tetramerisation was not abolished by removal of the N-terminal 160 amino acids but further truncations were detrimental. A targeted approach for putative inter-subunit contact sites was also employed testing the effect of small internal deletions within RyR2 residues 1-906. We found that two small deletions (167-178 or 335-358 amino acids) severely compromised tetramerisation ability. These findings suggest that residues 167-178 and 335-358 are the primary interacting sites, whereas additional downstream determinants further strengthen the RyR2 N-terminus self-association.

564-Pos Board B319
FRET-Based Trilateration of a Domain Peptide Bound within Functional Ryanodine Receptors in Cardiomyocytes
Bengt Svensson1, Tetsuro Oda2, Florentin R. Nit1, Justin Cornelia1, Donald M. Beers1, David D. Thomas1, Razvan L. Cornelia1.
1Biochemistry, Molecular Biology and Biophysics, Univ. of Minnesota, Minneapolis, MN, USA, 2Pharmacology, Univ. of California, Davis, CA, USA.
We have used trilateration, the method of using distances to determine a location in space, simulated annealing calculations, and confocal FRET measurements in permeabilized rat myocytes to determine the topology of a modifying surface fragment within the ryanodine receptor (RyR) Ca$^2+$ channel. A small peptide (~4kDa, termed DPC10), corresponding to a RyR central stretch, hypothetically destabilizes a key interaction between RyR N-terminal and central domains to promote the open channel. We used DPC10 labeled with a small fluorophore, HiLyte Fluo 647, as FRET acceptor. Five single-cysteine variants of the 12 kDa FK506-binding protein (FKBP) were labeled with FRET donor, Alexa Fluor 488 C5 maleimide, and targeted to RyR. Effective average positions of the donors were calculated from simulated annealing, constrained by the RyR cryo-EM map and by the FKBP atomic structure. FRET from the FKBP donors to the DPC10 acceptor was measured via confocal microscopy, and the calculated distances were used to trilaterate the acceptor location within the RyR 3D map. The trilateration method uses the distances determined by FRET from each donor’s calculated average position, where the ranges of the distances were based on measurement precision for each donor labeling site. The DPC10-bound acceptor locus is found at a region in space matching four distance ranges from FRET, and was further constrained by FRET between donor-labeled calmodulin and the DPC10. This locus is near the RyR N-terminal domain structure docked into the cryo-EM reconstructed map of RyR. Computational resources were provided by the Minnesota Supercomputing Institute. This work was supported by NIH grants R01HL092097 (to D.M.B. and R.L.C.), and was further constrained by FRET between donor-labeled calmodulin and is found at a region in space matching four distance ranges from FRET, and ment precision for each donor labeling site. The DPc10-bound acceptor locus method uses the distances determined by FRET from each donor's calculated to trilaterate the acceptor location within the RyR 3D map. The trilateration measured via confocal microscopy, and the calculated distances were used

567-Pos Board B322
A Regulatory Component of the Human Ryanodine Receptor 2 N-Terminus
Lubomir Borko1, Vladena Bauerova-Hlinkova1, Alexandra Zahradnikova1,2, Julius Kostam1, Konrad Beck1, Juraj Gasperik1, Eva Hostinova1, F. Anthony Lai5, Jozef Sevcik1.
1Department of Biochemistry and Structural Biology, Institute of Molecular Biology, Slovak Academy of Sciences, Bratislava, Slovakia, 2Department of Muscle Cell Research, Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovakia, 3Biochemistry & Biophysics, Structural & Computational Biology Department, Max F. Perutz Laboratories, University of Vienna, Vienna, Austria, 4Cardiff University School of Dentistry, Cardiff, United Kingdom, 5Department of Cardiology, Wales Heart Research Institute, Cardiff University School of Medicine, Cardiff, United Kingdom.
Human cardiac ryanodine receptor (hRyR2) is a channel mediating Ca$^{2+}$ release from the sarcoplasmic reticulum during excitation-contraction coupling. The N-terminal (1-655) and central (2100-2500) regions of hRyR2 are thought to be involved in regulating channel gating. Mutations linked to several heart diseases are clustered within these two, as well as in the channel pore-containing C-terminal regions. High resolution structures of key regions involved in the regulation of RyR2 activity could further the understanding of the gating mechanism of hRyR2 and of its malfunction in disease. Here we present the structure of the hRyR2 N-terminal region; it shares a significant structural similarity with other solved N-terminal RyR structures (pdb code: 2XOA, 4J4K) but lacks the chloride binding site proposed for mouse RyR2 (4J4K). Structural data suggested high flexibility of the region starting at around residue 544 predicted to consist of helices forming an armadillo motif - a protein-protein interaction domain. Our SAXS, NMR, and cryo-EM data showed high flexibility and partial unfolding of the C-terminal part of hRyR2-Nterminal beyond aa 544, suggestive of incompleteness of the C-terminus. Biochemical data showed a weak tendency of hRyR2-Nterminal to form dimers. Prolonging the fragment to the end of the predicted RHI domain increased the propensity to dimerize. A mutation introduced to a specific spot of hRyR2-Nterminal resulted in significant thermal destabilization and augmented dimerization. Docking of the hRyR2-Nterminal structure into cryo-EM maps of RyR ruled out interactions of its C-terminus with neighbouring hRyR2-Nterminal monomers. We interpret the formation of dimers as a surrogate for interaction of the N-terminal region with an armadillo motif of another part of RyR2, and propose the existence of a regulatory component at the secondary structure level controlling interaction power at inter-monomer interfaces within the RyR2 tetramer.