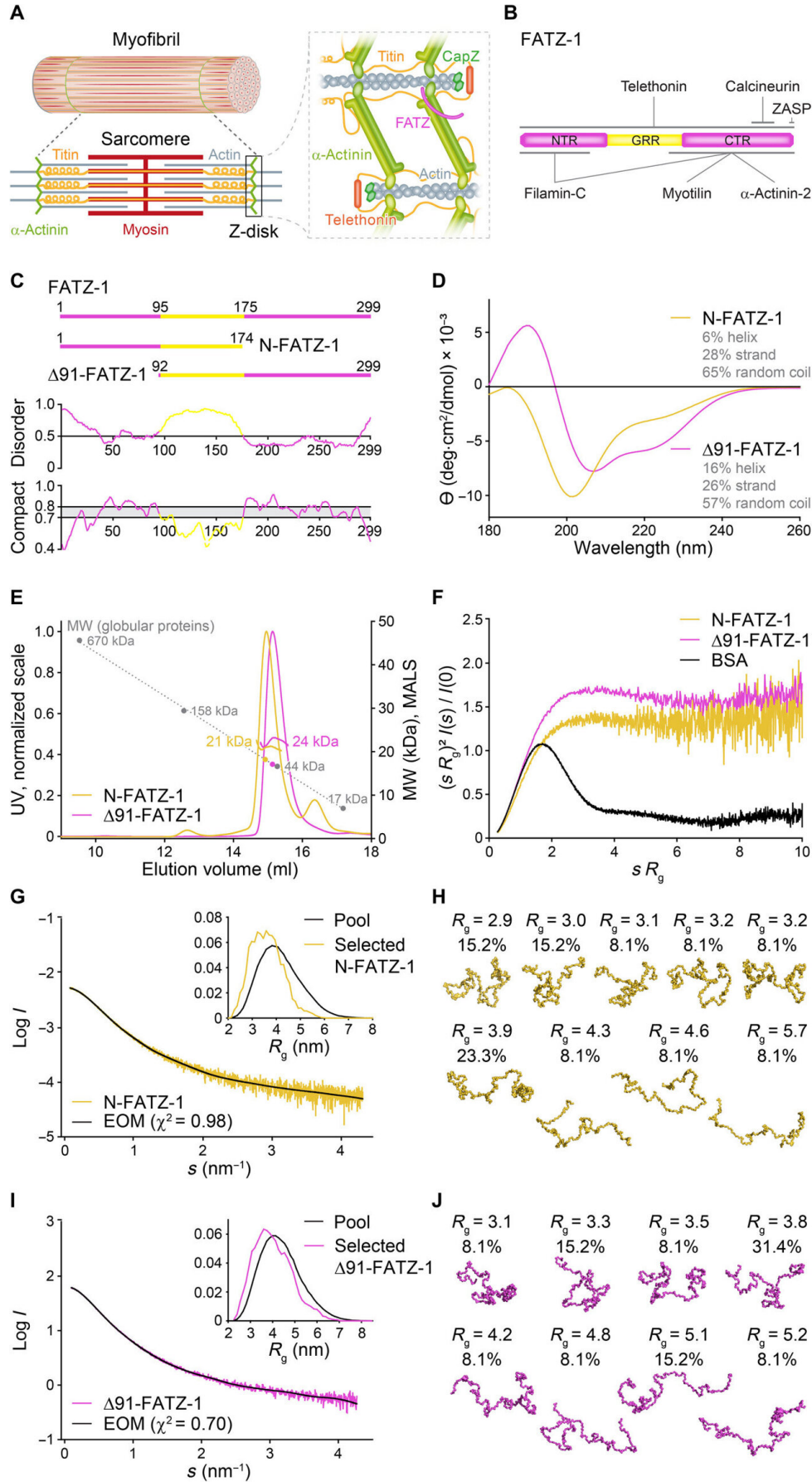


Order from disorder in the sarcomere

June 18 2021, by Thamarasee Jeewandara



Proteins of the FATZ family display intrinsic disorder. (A) Schematics of the striated muscle sarcomere and close-up view of F-actin/ α -actinin/FATZ interactions in Z-disk. (B) Schematics of the FATZ-1 interactome and binding sites reported to date. (C) Schematics of the main FATZ-1 constructs, along with their amino acid boundaries and domain composition. Predicted disordered regions (above 0.5) and compactness (above 0.8) are shown below. (D) Circular dichroism (CD) spectra of N-FATZ-1 and Δ 91-FATZ-1, along with calculated secondary structure content. (E) Size exclusion chromatography (SEC)–multiangle light scattering (MALS) analysis of N-FATZ-1 and Δ 91-FATZ-1, yielding molecular weights (MWs) of 21 and 24 kDa, respectively. Elution volumes were lower than anticipated relative to globular standards [thyroglobulin (670 kDa), γ -globulin (158 kDa), ovalbumin (44 kDa), and myoglobin (17 kDa)], corresponding to MWs of 50 and 46 kDa for N-FATZ-1 and Δ 91-FATZ-1, respectively. UV, ultraviolet. (F) Dimensionless Kratky plots of N-FATZ-1 and Δ 91-FATZ-1, as well as of globular bovine serum albumin (BSA) (SASBDB code SASDFQ8). Experimental SEC–small-angle x-ray scattering (SAXS) data of N-FATZ-1 (G) and Δ 91-FATZ-1 (I) and corresponding fit to the data of selected ensembles obtained from Ensemble Optimization Method (EOM). R_g distributions of selected ensembles relative to the distribution of a random pool are shown in the insets. Model representatives of the selected EOM ensembles for N-FATZ-1 (H) and Δ 91-FATZ-1 (J), along with their R_g (in nanometers) and volume fractions (in percentage). Credit: *Science Advances*, doi: 10.1126/sciadv.abg7653

Alpha-actinin can cross-link actin filaments and anchor them to the Z-disk in sarcomeres. Sarcomeres are a structural unit of myofibril in striated muscle. The FATZ (filamin, α -actinin- and telethonin-binding protein of the Z-disk) protein can interact with α -actinin and other core Z-disk proteins that contribute to myofibril assembly and maintenance. In a new report now on *Science Advances*, Antonio Sponga and an international research team in Austria, Germany, Russia, Poland and the

U.K. detailed the first structure and cellular validation of the α -actinin-2 complex with a Z-disk partner, FATZ-1, to form a conformational ensemble. The FATZ-1 formed a tight fuzzy complex with α -actinin-2 with a proposed interaction mechanism via molecular recognition elements and secondary binding sites. The obtained integrative model revealed a polar architecture of the complex in combination with the FATZ-1 multivalent scaffold function to organize interaction partners and stabilize.

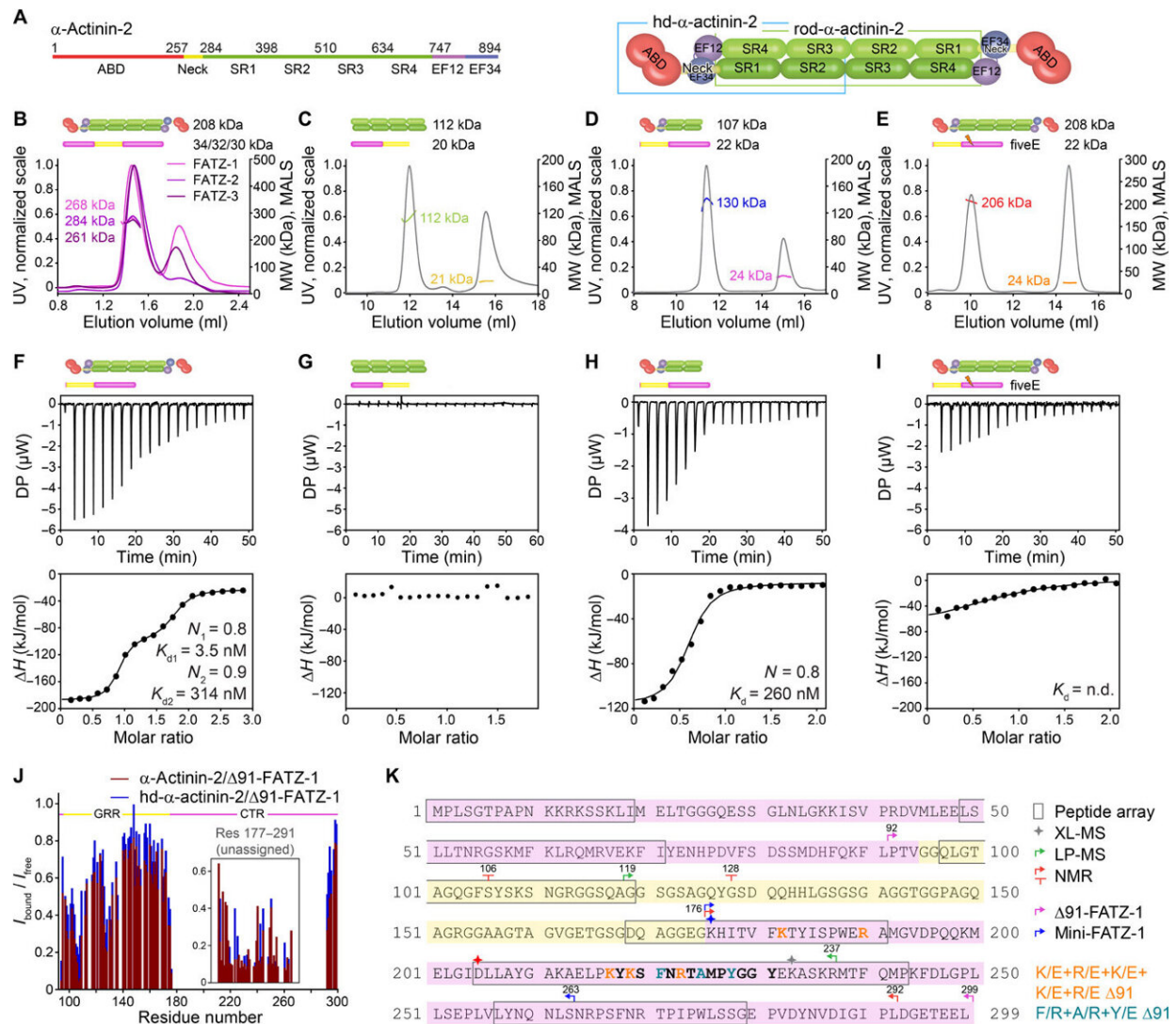
Sarcomere

The contracting muscles can regulate voluntary animal movement and involuntary heart beating, and sarcomeres are the basic contractile units of striated muscle cells. They are composed of arrays of thin (actin) and thick (myosin) filaments that slide past each other during contraction. The Z-disk can form the boundary between adjacent sarcomeres, where anti-parallel actin filaments are anchored. A suitably stable anchoring structure must be generated by the interaction between myosin and actin. The Z-disk can fulfill this role by acting as a mechanical hub and a signaling platform to allow the transmission of tension during contraction and the duration and transmission of information of biomechanical stress. As a result, any mutations that disrupt the Z-disk architecture and function could risk causing skeletal and cardiac dysfunction.

The protein complex

Alpha-actinin is an F-actin cross-linking protein in muscle Z-disks, which forms a major Z-disk component that cross-links antiparallel actin filaments from adjacent sarcomeres to serve as a binding platform for multiple Z-disk proteins, including FATZ-1. The FATZ proteins can bind to α -actinin through their c-terminal region and to domains of the Enigma family members via a specific c-terminal recognition motif. In

this work, Antonio Sponga et al. demonstrated how FATZ proteins contained intrinsically disordered regions (IDRs) best described as a conformational ensemble, which are less stable and lack a stable tertiary structure. In addition to biophysical characterization methods, the team used X-ray crystallography and small-angle X-ray scattering to describe a "fuzzy" α -actinin-2/FATZ-1 complex. The FATZ-1 protein can play an organizational role in the Z-disk due to its multivalent scaffolding properties and form a tight complex of polar architecture with α -actinin-2.

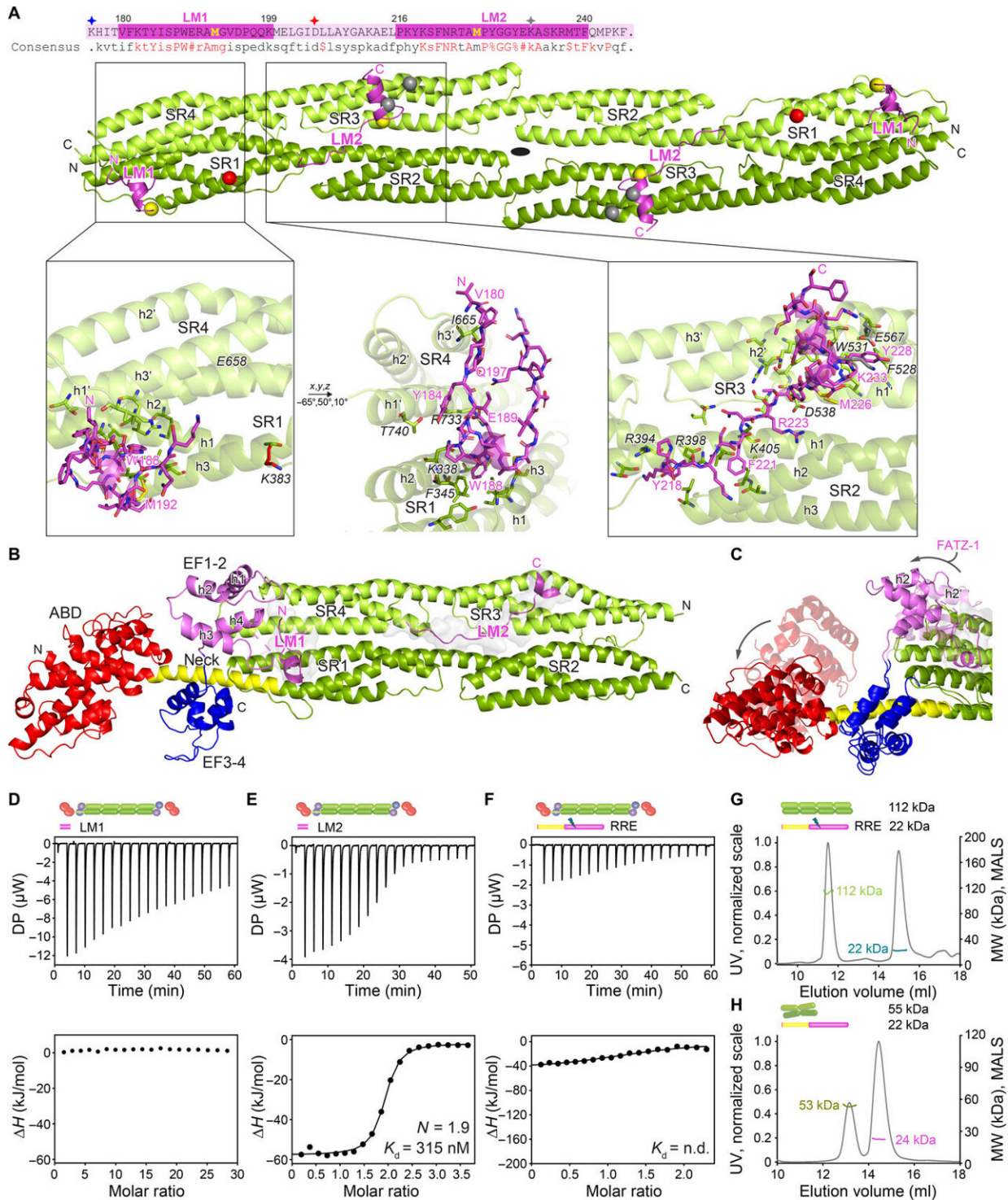


FATZ-1 forms a tight 2:1 complex with α -actinin-2 dimer via multiple binding sites. (A) Schematics of the α -actinin-2 constructs, along with their amino acid boundaries and domain composition. SEC-MALS analysis for the interaction of FATZ-1, FATZ-2, and FATZ-3 with α -actinin-2 (B), N-FATZ-1 with rod- α -actinin-2 (C), Δ 91-FATZ-1 with hd- α -actinin-2 (D), and fiveE Δ 91-FATZ-1 mutant with α -actinin-2 (E). ITC analysis for the interaction of Δ 91-FATZ-1 with α -actinin-2 (F), N-FATZ-1 with rod- α -actinin-2 (G), Δ 91-FATZ-1 with hd- α -actinin-2 (H), and fiveE Δ 91-FATZ-1 mutant with α -actinin-2 (I). n.d., not determined. (J) ^1H - ^{15}N HSQC signal intensity ratio of ^{15}N Δ 91-FATZ-1 bound/free, mapping FATZ-1 primary binding site for α -actinin-2. Unassigned part in FATZ-1 is boxed, and residues are plotted at a random position. (K) Sequence of FATZ-1 showing multiple interaction sites for α -actinin-2 as determined from the peptide array (squared residues), XL-MS (star), LP-MS (residues delimited by arrows), and NMR (arrows). Residues matching the strongest signal peptide in the peptide array are shown in bold. Boundaries for Δ 91-FATZ-1 and mini-FATZ-1 are delimited by arrows. Mutations within fiveE Δ 91-FATZ-1 and RRE Δ 91-FATZ-1 are indicated in orange and dark cyan, respectively. Credit: Science Advances, doi: 10.1126/sciadv.abg7653

The FATZ protein family

The FATZ protein family are found across all vertebrates where human FATZ-1, FATZ-2, and FATZ-3 share 34 to 40 percent sequence identity. The scientists recognized proteolysis-resistant fragments, after conducting proteolysis experiments. When they combined size exclusion chromatography (SEC) with multiangle light scattering, they noted the predominant monomers under experimental conditions. They then further characterized the monomers using SEC combined with small angle X-ray scattering and also highlighted the intrinsically disordered/ensemble-state nature of the monomers using single-quantum coherence (HSQC) spectra, for both constructs. To understand the binding stoichiometry of the FATZ-1-to-3 proteins to α -actinin-2, Sponga et al. used size exclusion chromatography-multiangle light

scattering (SEC-MALS). To characterize the binding stoichiometry of the FATZ-1-to-3 proteins to α -actinin-2, Sponga et al. used SEC-MALS. The outcome showed how each of the three FATZ proteins formed a tight complex with α -actinin-2, with a binding stoichiometry of two FATZ molecules per α -actinin-2 dimer. That is one FATZ molecule per α -actinin-2 subunit. The team next used isothermal titration calorimetry (ITC) to quantify the interaction affinity.



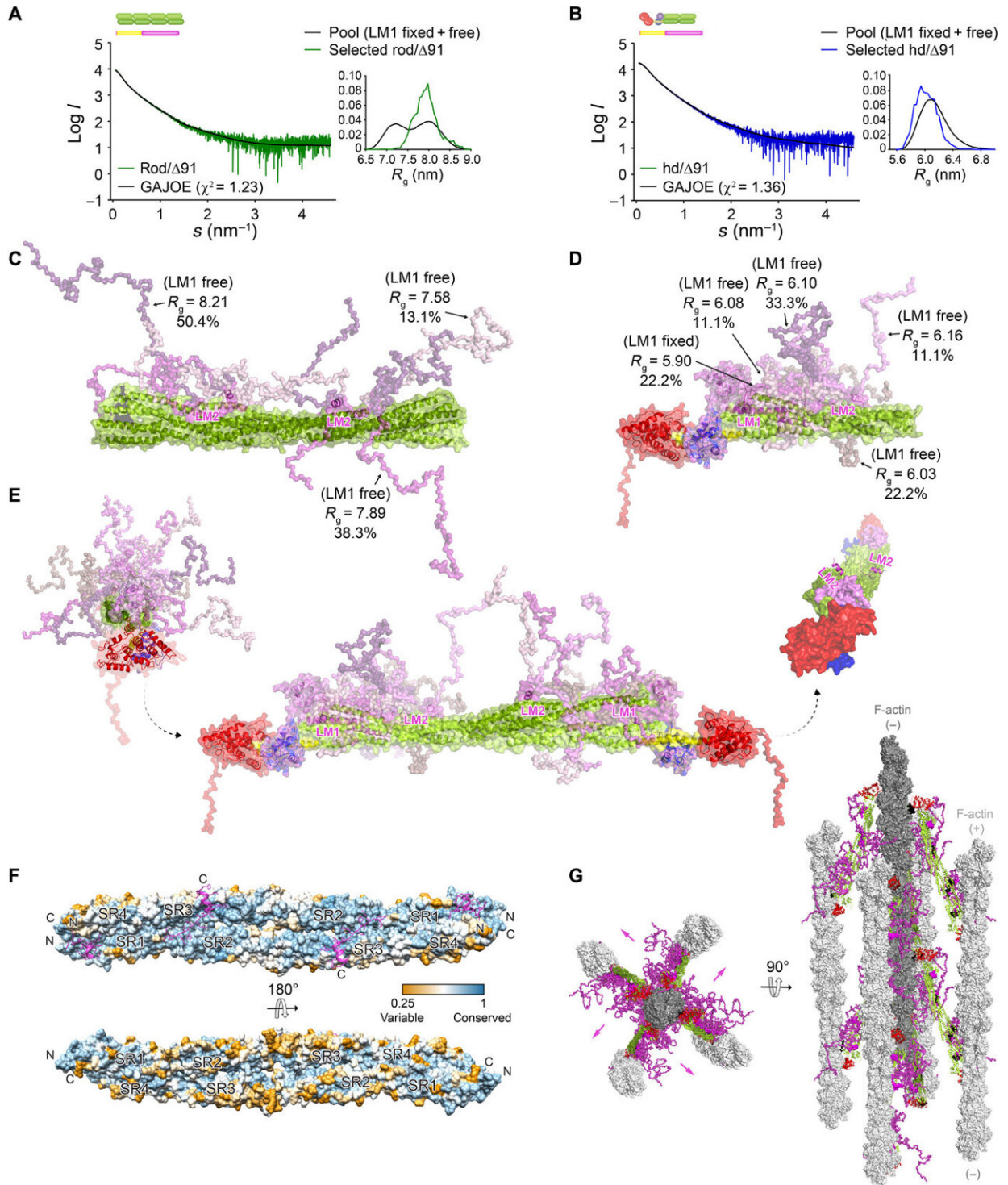
Crystal structures of α -actinin-2/FATZ-1 reveal two linear binding motifs in FATZ-1. (A) Crystal structure of rod- α -actinin-2/mini-FATZ-1 (in green/magenta), along with the determined FATZ-1 consensus sequence (35 to 80% and 60 to 84% pairwise sequence identity for LM1 and LM2, respectively).

Cross-linked residues are indicated by blue, red, and gray stars/balls/sticks on the sequence/structure. Identified Se-Mets are shown in yellow. The rod- α -actinin-2 dimer is assembled through a crystallographic twofold axis between symmetry mates (black circle). Interacting residues (rod- α -actinin-2 in italics), along with helices from SR1/SR2 (h1, h2, and h3) and SR3/SR4 (h1', h2', and h3'), are shown in close-up views. (B) Crystal structure of hd- α -actinin-2/ Δ 91-FATZ-1 (LM1 and LM2 as magenta cartoon and transparent gray surface; hd- α -actinin-2 color-coded as in Fig. 2A). (C) Comparison of unbound [Protein Data Bank (PDB) code 4D1E] and bound (this work) hd- α -actinin-2. ABD and EF1-2 of unbound hd- α -actinin-2 are shown with transparency. ITC analysis for the interaction of LM1 peptide with α -actinin-2 (D), LM2 peptide with α -actinin-2 (E), and RRE Δ 91-FATZ-1 mutant with α -actinin-2 (F). SEC-MALS analysis for the interaction of RRE Δ 91-FATZ-1 mutant with rod- α -actinin-2 (G) and Δ 91-FATZ-1 with *E. histolytica* rod- α -actinin-2 (H). Credit: Science Advances, doi: 10.1126/sciadv.abg7653

Multiple binding sites for the protein complex

The team noted how FATZ-1 interacted with α -actinin-2 via multiple binding sites. To narrow down the FATZ-1 binding sites, Sponga et al. used limited proteolysis and chemical cross-linking coupled with mass spectrometry on the protein complex. To then aid the crystallization of this [protein complex](#), the team then also combined the information from the peptide array and generated a shorter construct known as mini-FATZ-1 for further studies on their structural biology. The scientists then validated the fuzzy models developed in the work using calculated and experimentally derived intrinsic viscosity—a hydrodynamic parameter of protein conformation. To then understand the contribution of α -actinin-2 to localize FATZ proteins onto the Z-disk of the sarcomere, Sponga et al. transfected GFP-tagged FATZ-1 or FATZ-2 proteins into immortalized mouse myoblasts or neonatal rat cardiomyocytes. Both FATZ-1 and -2 proteins correctly targeted the Z-

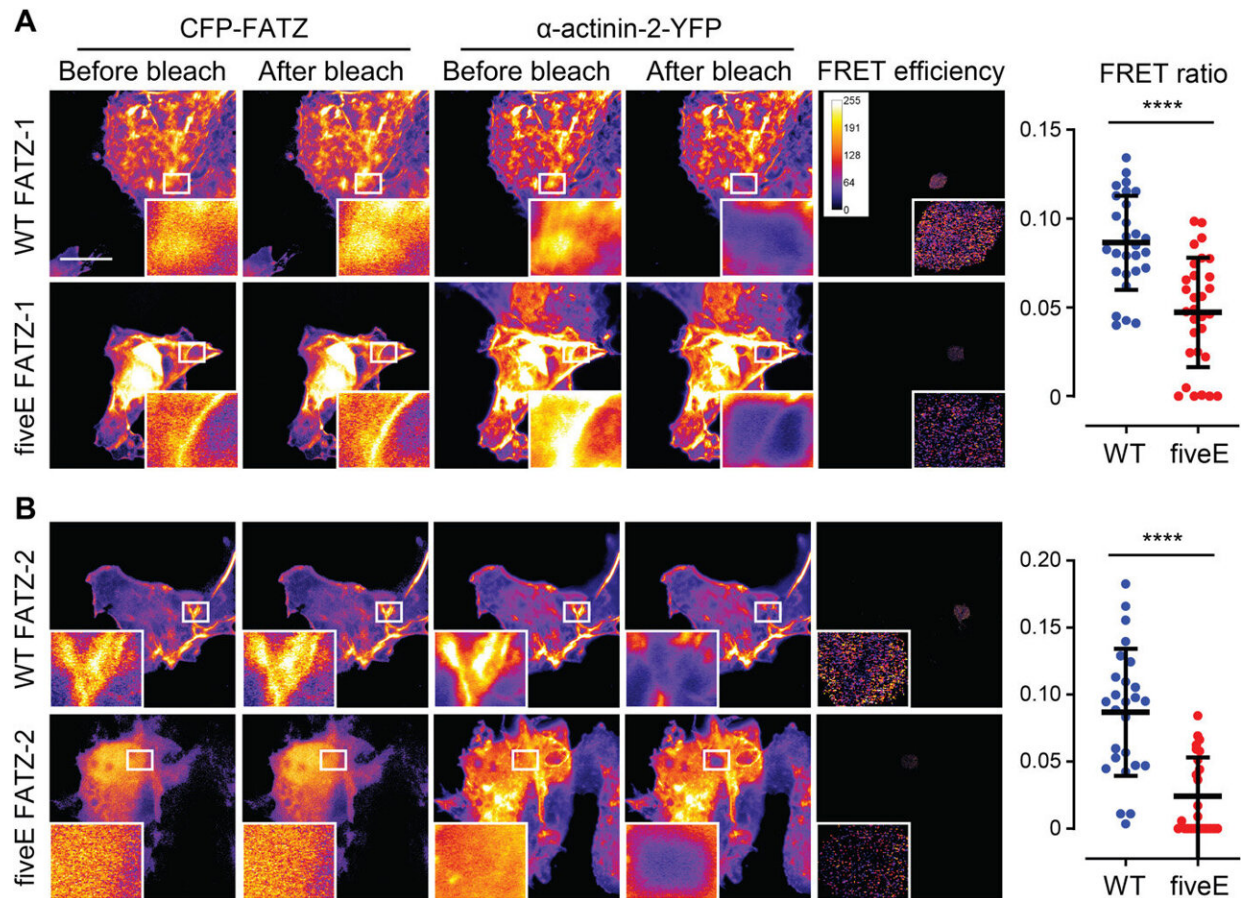
disk and co-localized with α -actinin-2.



FATZ-1 forms a fuzzy complex with α -actinin-2 resulting in a polar architecture of the complex. Experimental SAXS data of rod- α -actinin-2/ Δ 91-FATZ-1 (A) and hd- α -actinin-2/ Δ 91-FATZ-1 (B), with the corresponding model fits to the data of the selected ensembles. GAJOE, Genetic Algorithm Judging Optimisation of Ensembles. Flexible regions of Δ 91-FATZ-1, nonvisible in our determined crystal structures, were generated with EOM keeping LM1 either fixed or free (10,000 models for each). Selected ensemble model representatives for rod- α -actinin-2/ Δ 91-FATZ-1 (C) and hd- α -actinin-2/ Δ 91-FATZ-1 (D), along with their Rg and volume fractions within the ensemble. (E) Integrative model of fuzzy α -actinin-2/ Δ 91-FATZ-1 built using x-ray crystallography and SAXS models of hd- α -actinin-2/ Δ 91-FATZ-1. Rotation for LM2 helices of bound FATZ-1 molecules with respect to each other, as well as torsional twist in the rod along the longitudinal α -actinin-2 axis, is shown in the right inset (FATZ-1 flexible parts are omitted for clarity). (F) Surface of the rod- α -actinin-2/FATZ-1 structure showing the sequence conservation of α -actinin interacting residues for FATZ-1 (alignment done using 1505 α -actinins from vertebrates). (G) Model of F-actin/ α -actinin-2/FATZ-1 (F-actin in light and dark gray) based on a cryo-electron tomography structure of the Z-disk and the integrative model. Credit: Science Advances, doi: 10.1126/sciadv.abg7653

Outlook

In this way, Antonio Sponga and colleagues described how the sarcomere assembly started from Z-bodies of α -actinin-2, to include proteins such as FATZ, myotilin, and actin, to name a few. The outcome indicates that proteins of the FATZ family are available in Z-bodies and mature Z-disks with a role in protein signaling pathways to bind calcineurin. The team highlighted the role of FATZ-1, the most studied family member and its interaction with the major Z-disk protein α -actinin-2. The structure and binding mechanism of the fuzzy α -actinin-2/FATZ-1 complex supported FATZ-1 function as a classical scaffolding [protein](#) in the Z-disk assembly. Further studies will reveal if the same principles apply under physiological conditions in living cells.



α -Actinin-2 stabilizes FATZ proteins at the Z-disk. (A) COS-1 cells coexpressing enhanced CFP (ECFP)-tagged FATZ-1 (WT FATZ-1) or corresponding fiveE mutant, in combination with actinin-2-EYFP, as determined in fixed cells by acceptor photobleaching. Representative images are shown before and after bleaching for both proteins. Bleached regions of interest are shown (insets), along with FRET efficiencies used to calculate FRET ratios presented in the adjacent graph [n = 28 (WT) and 30 (fiveE), ****P

More information: Sponga A. et al. Order from disorder in the sarcomere: FATZ forms a fuzzy but tight complex and phase-separated condensates with α -actinin, *Science Advances*, DOI: [10.1126/sciadv.abg7653](https://doi.org/10.1126/sciadv.abg7653)

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