α-Actinin cross-links actin and titin in the Z-disk [1]. During contraction of the sarcomere, the disk experiences displacing forces orthogonal to the direction of compression due to the relative incompressibility of myosin and actin fibers condensed. As a consequence, the α-Actinin is stretched in that direction [2].

We labeled both termini of α-Actinin with GFP and mCherry tags respectively and speckle promoted the expression in order to obtain some isolated fluorescent α-Actinin molecules in the sarcomere nodes. The large variation of α-Actinin expression density allows for both a clear identification of the sarcomere chains as well as occasionally observing a single molecule node in which the orthogonal extension of α-Actinin can be optically monitored with the existing two tag tracking microscopy [3].

The sparse expression makes it more difficult to find nodes which host exactly one fluorescent molecule and that molecule will bleach before the experiment finishes. At 1Hz observation interval we achieve up to 15 measurements which is sufficient to correlate the sarcomere stretch cycle with the dynamics of a single molecule in a particular node. An even bigger challenge is that α-Actinin always forms as an antiparallel dimer which cannot be observed by means of localization microscopy. We used a data mining algorithm to find nodes that formed as optical monomers which then permitted the length measurements.

references: