Ca\textsuperscript{2+}. Because major components of the spasmoneme, the contractile organelle inside the stalk, are EF-hand Ca\textsuperscript{2+}-binding proteins including spasmin and cofilin, their interaction is thought to be related to other centrosome-motility mechanisms. This study describes how stalk force affects contractions of live *Vorticella*. To impede contractions, we applied hydrodynamic drag force to *Vorticella* in a microfluidic channel with Poiseuille flow of viscous PVP solution. This method enables controlling the stalk force by changing flow rate and the viscosity of the solution. Cell dimension measurements show that the zooid is elongated by the flow in relaxed and contracted states keeping roughly constant volume. As the stalk force increases, the end-to-end length of the contracted stalk increases while that of the relaxed stalk is almost constant, and maximum contraction speed decreases while contractions take longer time. Furthermore, the time lag in contraction commencement between the zooid and the stalk also increases. We measured time differences in movement start among polystyrene beads attached to the stalk, and they increase with increase in stalk force. These observations suggest that the stalk cannot contract until it develops force great enough to overcome the stalk force. The stalk force affects the relaxation of *Vorticella* because relaxations take longer time as the stalk force increase and the extending stalk resums its contraction after the stalk force is removed. It seems that although the spasmoneme retains contractile force, the stalk force extends the stalk.

2677-Pos Board B647
Tuning Cellular Mechano-Response Using Biomembrane-Mimicking Substrates of Adjustable Fluidity
Daniel E. Minner\textsuperscript{1}, Philipp Rauch\textsuperscript{2}, Amanda P. Siege\textsuperscript{1}, Johannes Stelzer\textsuperscript{1}, Joseph Kas\textsuperscript{2}, Guilhem Protopopov\textsuperscript{2}, Kevin Harvey\textsuperscript{1}, Rafat Siddiqui\textsuperscript{1}, Simon Atkinson\textsuperscript{1}, Christoph A. Naumann\textsuperscript{1}.
\textsuperscript{1}Indiana University Purdue University Indianapolis, Indianapolis, IN, USA, \textsuperscript{2}University of Leipzig, Leipzig, Germany.

An important aspect of mechanobiology is that tissue cells are anchorage-dependent and respond to viscoelastic changes in their environments. The mechanosensitivity of cells is believed to play an important role in processes such as cancer cell migration and stem cell differentiation. Previously, cellular mechano-response has been mainly studied using \(\mu\)-thick polymeric films of adjustable viscoelasticity. Here we report on the design and characterization of alternative cell substrates based on 30–40nm thick polymer-tethered phospholipid membranes where cellular mechano-response can be regulated by tuning bilayer fluidity. Two complementary membrane systems are employed to span a wide range from low to high bilayer fluidity. Low to medium bilayer fluidity is achieved by using a single polymer-tethered lipid bilayer of adjustable tethering concentration. Medium to high bilayer fluidity is obtained through the regulation of the number of bilayers in a stack of polymer-tethered lipid bilayers. Changes in bilayer fluidity in these substrates have been confirmed through wide-field single molecule tracking of fluorescently labeled lipids. To facilitate the adsorption and migration of cells, these biomembrane-mimicking substrates contain bilayer-cell linkages of well-defined concentrations. Phase contrast microscopy experiments on PC12 neurons show that dendritic growth can be tuned by modifying the tethering concentration in a single polymer-tethered lipid bilayer. Comparing phase contrast and epifluorescence microscopy images of these systems, we hypothesize that actin may be organized into stratified layers within FA that serve as dissipative elements in a “molecular clutch” to form a regulatable, force-transducing link between the actin cytoskeleton and the ECM. Despite the central role of FA in cell migration and the wealth of biochemical and cell biological data on FA proteins, it remains virtually unknown how these proteins are organized within FA. Based on the differential dynamics of distinct FA proteins we previously observed using iPALM, we hypothesize that FA proteins may be organized into stratified layers within FA that serve as dissipative elements in a “molecular clutch” to form a regulatable, force-transducing link between the actin cytoskeleton and the ECM. To test this hypothesis, we employed a 3-dimensional superresolution fluorescence microscopy technique, interferometric photoactivated localization microscopy (iPALM), to determine sub-20 nm z-axis localizations of several key structural components of FA labeled with photoactivatable fluorescent proteins and expressed in U2OS cells plated on a fibrinogen-coated substrate. Within FA, we found that the cytoplasmic face of the plasma membrane, marked by Farnesylated tdEosFP, was localized at ~20–30 nm from the substrate, in agreement with previous electron microscopic analyses. Talin and vinculin, putative force transducing elements of FA, were observed within distinct planes parallel to the substrate, with the highest densities at ~35, and ~50 nm above the substrate plane, and was largely excluded from area adjacent to plasma membrane up to a height of ~50 nm. iPALM reveals for the first time the organization of specific proteins within the nanoscale core of the FA. The protein stratifications in FA provide a structural context for the mechanosensing and mechanotransducing functions of FA.