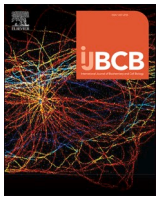
International Journal of Biochemistry and Cell Biology 161 (2023) 106442

Contents lists available at ScienceDirect

International Journal of Biochemistry and Cell Biology

journal homepage: www.elsevier.com/locate/biocel



Shining a light on RhoA: Optical control of cell contractility

Shreya Chandrasekar, Jordan R. Beach, Patrick W. Oakes *

Department of Cell & Molecular Physiology, Loyola University Chicago, Stritch School of Medicine, Maywood, IL 60153, USA

ARTICLE INFO

Keywords:
Cell mechanics
Optogenetics
RhoA
TFM

ABSTRACT

In addition to biochemical and electrochemical signaling, cells also rely extensively on mechanical signaling to regulate their behavior. While a number of tools have been adapted from physics and engineering to manipulate cell mechanics, they typically require specialized equipment or lack spatiotemporal precision. Alternatively, a recent, more elegant approach is to use light itself to modulate the mechanical equilibrium inside the cell. This approach leverages the power of optogenetics, which can be controlled in a fully reversible manner in both time and space, to tune RhoA signaling, the master regulator of cellular contractility. We review here the fundamentals of this approach, including illustrating the tunability and flexibility that optogenetics offers, and demonstrate how this tool can be used to modulate both internal cytoskeletal flows and contractile force generation. Together these features highlight the advantages that optogenetics offers for investigating mechanical interactions in cells.

1. Introduction

Mechanical interactions are vital to cell biology and impact a diverse array of physiological processes (Lecuit et al., 2011; Iskratsch et al., 2014). They originate both from internal cellular forces, such as those produced by myosin motors pulling on actin filaments in the cytoskeleton (Murrell et al., 2015), and from external forces, such as shear stress on the surface of endothelial cells (Humphrey and Schwartz, 2021). Their impact also spans multiple length scales. At the molecular scale they can alter the binding kinetics between proteins (Kong et al., 2009), open ion channels (Coste et al., 2010), or even unfold cryptic domains to reveal new binding sites (Yao et al., 2016). At the cellular scale they can regulate the organization of the cytoskeleton (Yoshigi et al., 2005) and change transcription activity in the nucleus (Dupont and Wickström, 2022). At the tissue scale they can change the stiffness of arteries (Tzima et al., 2005), and regulate morphogenesis (Goodwin and Nelson, 2021). Many of these mechanosensitive interactions also lead to changes in downstream signaling, further expanding their influence (Humphrey et al., 2014; Sun et al., 2016; De Belly et al., 2022). As these many works readily illustrate, we have only really just begun to scratch the surface of the many ways that mechanics impacts and regulates biological processes.

While some mechanical interactions can be studied statically, the majority require having knowledge of these interactions in both time and space. It is not surprising, therefore, that the study of mechanics and mechanotransduction has been inextricably tied to microscopy (Paluch et al., 2015; Wu et al., 2018). Indeed, the earliest approaches to alter mechanics in cells came from the work of Albert Harris who used transparent flexible silicone substrates to show that the inherent contractile nature of fibroblasts caused the substrates to wrinkle (Harris et al., 1980). Modulating the substrate stiffness has since become a staple of mechanotransduction and has been tied to cellular processes as diverse as spreading, motility and differentiation (Janmey et al., 2020; SenGupta et al., 2021). To gain more local and temporal control, additional techniques have been adopted from physics and engineering, including atomic force microscopy (Guolla et al., 2012), micropipette manipulation (Riveline et al., 2001), and optical (Schwingel and Bastmeyer, 2013) and magnetic tweezers (D'Angelo et al., 2019). While each of these approaches has their strengths, they all require specialized equipment and expertise, making their widespread adoption challenging.

More recently, optogenetics offers an alternative approach to induce mechanical changes in a cell without the need for special equipment. Relying instead on light sensitive molecules, these systems offer tight spatial and temporal control, with the additional feature of being reversible. The most popular optogenetic techniques to perturb cell mechanics hijack either Rho signaling (Wagner and Glotzer, 2016; Valon et al., 2017; Oakes et al., 2017; Guo et al., 2022; Herrera-Perez et al., 2021; Méry et al., 2023) or other molecules in the contractility pathway (Guglielmi et al., 2015; Yamamoto et al., 2021; Qiao et al., 2023) to

* Corresponding author.
E-mail address: poakes@luc.edu (P.W. Oakes).

https://doi.org/10.1016/j.biocel.2023.106442
Received 1 March 2023; Received in revised form 10 June 2023; Accepted 19 June 2023
Available online 20 June 2023

drive changes in actomyosin activity. In this review we highlight the features of this approach that make it a powerful tool to optically perturb cell mechanics.

2. Controlling RhoA signaling with light

2.1. RhoA Signaling

Rho GTPases are crucial signaling molecules that regulate cytoskeletal activity and architecture (Lawson and Ridley, 2018; Müller et al., 2020; Schwartz, 2004). Activity of these proteins are controlled via GTP hydrolysis, whereby guanine exchange factors (GEFs) and GTPase-activating proteins (GAPs) effectively turn on and off protein activity (Buchsbaum, 2007). In addition, guanine nucleotide dissociation inhibitors (GDIs) help maintain a cytoplasmic pool of Rho GTPases and to shuttle them back and forth to the membrane (Garcia-Mata et al., 2011). Different GEFs and GAPs have varying affinity and promiscuity for RhoA, which can impact the magnitude of activation (Müller et al., 2020). Once activated, there are numerous downstream targets of RhoA that affect the cytoskeleton, including the formin Diaphanous (Dia) which leads to increased polymerization of actin filaments, and Rho-associated coiled-coil kinase (ROCK) which leads to increased myosin phosphorylation (Schwartz, 2004). The combination of actin polymerization and myosin activity results in force production and increased tension in the cytoskeleton (Aratyn-Schaus et al., 2011; Murrell et al., 2015). It is thus unsurprising that RhoA plays a critical role in processes that regulate cell shape and mechanics, such as cytokinesis, migration and development (Ridley and Hall, 1992; Chrzanowska-Wodnicka and Burridge, 1996; Fededa and Gerlich, 2012; Duquette and Lamarche-Vane, 2014; Ridley, 2015). To avoid unintentionally impacting RhoA's myriad functions, most optogenetic approaches choose to modulate RhoA activity via localizing guanine exchange factors (GEFs) (Wagner and Glotzer, 2016; Valon et al., 2017; Oakes et al., 2017; Izquierdo et al., 2018; Herrera-Perez et al., 2021; Méry et al., 2023), though variants also exist which target the protein itself (Berlew et al., 2021). An alternative approach would be to target actin polymerization or myosin localization/activity directly. However, a key benefit of targeting RhoA is the combinatorial nature of its downstream actomyosin activation via enzymatic kinases (e.g. ROCK) (Schwartz, 2004), whereas direct formin or myosin recruitment, for example, may not be sufficient to invoke a response.

2.2. Components

Numerous optogenetic systems have been developed in recent years to modulate cell signaling (Bugaj et al., 2013; Wagner and Glotzer, 2016; Valon et al., 2017; Oakes et al., 2017; Izquierdo et al., 2018; Kamps et al., 2020; Cavanaugh et al., 2020a, 2020b; Rich et al., 2020; Castillo-Badillo and Gautam, 2021; Herrera-Perez et al., 2021; Inaba et al., 2021; Berlew et al., 2021; Ju et al., 2022). A unifying feature is that they all incorporate at least one component that is sensitive to specific wavelengths of light. To perturb cytoskeletal signaling, the most common probes have typically relied upon either the light-oxygen-voltage 2 (LOV2) domain from Avena sativa (Zayner et al., 2012; Guntas et al., 2015) or the protein cryptochrome 2 (CRY2) from Arabidopsis (Kennedy et al., 2010; Duan et al., 2017). Both of these molecules undergo conformational changes upon exposure to blue light (300–500 nm) (Liu et al., 2008; Salomon et al., 2000), unmasking interaction motifs that are hidden in the dark state. In the case of CRY2 this involves interaction with another protein from Arabidopsis, cryptochrome-interacting basic-helix-loop-helix (CIB1). For LOV2, the conformational change results in the unwinding of a Jα helix on the side of the protein, which can be modified to interact with different binding partners. The most adopted of these modifications has been the improved Light Inducible Dimer (iLID) system which appends the end of the Jα helix with a short bacterial peptide, SsrA, which binds tightly to a companion bacterial protein SspB (Guntas et al., 2015). A number of mutations have been identified within the SsrA and SspB proteins that alter their binding affinity, ranging from 10 nM to 1 mM, thereby producing a large dynamic range of affinities that researchers can take advantage of (Zimmerman et al., 2016; Guntas et al., 2015). In practice, the higher affinity partners should enable longer interactions and require less frequent stimulation, but are also potentially subject to enhanced binding in the absence of stimulation and reduced control of the system.

To control localization, these probes are generally split into two components (Fig. 1B). The first consists of an anchor protein that targets the interaction to the region of interest. Since RhoA is most active at the cortex, plasma membrane anchors ranging in complexity from a simple CAAX domain (Valon et al., 2017) to larger transmembrane proteins like Stargazin (Wagner and Glotzer, 2016) can be used to enhance Rho signaling at the cortex. The choice of anchor in part is dictated by the level of spatial control required, as larger anchor proteins exhibit reduced diffusion in the membrane and therefore lead to tighter spatial localization (Natwick and Collins, 2020). Notably, in contrast to a Rho activation system, one can engineer a sequestration system to inhibit contractility. For example, a mitochondrial anchored CIBN enabled light-induced sequestration of RhoA from the cortex and resulted in downregulation of cell contractility (Valon et al., 2017). Attached to the anchor protein is a fluorophore which serves to mark cells expressing the construct and also acts as a linker allowing greater mobility of the LOV2 (or CRY2) molecule attached at its end (Fig. 1B).

The second construct consists of the binding partner SspB (or CIB1) tethered to the protein being recruited, followed by another fluorophore. Activation by blue light drives dimerization of the two constructs, recruiting the protein to the anchor site. In the absence of blue light, the system thermodynamically relaxes back to the dark state without input from the user, allowing the two components to separate (Fig. 1B). The kinetics of this relaxation are driven by the molecular interactions of the two components (Benedetti et al., 2018). Mutations have been identified in each system that allow for some variation in kinetics, with the iLID variants relaxing with half-lives ranging from 10s of seconds to minutes (Guntas et al., 2015; Salomon et al., 2000; Swartz et al., 2001; Zimmerman et al., 2016), and the CRY2-CIB1 variants relaxing with slightly longer half-lives ranging from 2 to 25 min (Kennedy et al., 2010; Taslimi et al., 2016). The choice of whether to use iLID or CRY/CIB ultimately comes down to experimental needs. When localization and fast temporal control are prioritized, iLID constructs will often be better suited due to their more rapid on/off kinetics and limited ability to diffuse far once activated. If longer periods of activation are required with less frequent imaging, CRY/CIB constructs are better suited on account of their generally slower kinetics. Alternative systems such as the Magnet optogenetic system (Benedetti et al., 2018) offer yet another potential avenue, but have yet to be used in this type of approach.

A variety of GEF or GEF truncations have been used as the recruitable protein fused to the SspB/CIB1 to control RhoA signaling. These include the DH domain of LARG (Wagner and Glotzer, 2016; Oakes et al., 2017; Cavanaugh et al., 2020a, 2020b; Rich et al., 2020; O'Neill et al., 2018; Inaba et al., 2021), the DHPH domain of ARHGEF11 (Valon et al., 2017; Berlew et al., 2021; Méry et al., 2023), the DHPH domain of RhoGEF2 (Izquierdo et al., 2018), and full length RhoGEF2 and RhoGAP71E (Herrera-Perez et al., 2021). The DH domain is often used alone to reduce basal activity and to ensure that any resulting signaling comes from the optogenetic recruitment and not additional biochemical interactions (Wagner and Glotzer, 2016).

Alternatively, full length RhoA (Bugaj et al., 2013; Berlew et al., 2021) and a dominant negative RhoA have also been used (Guo et al., 2022). Unfortunately, no systematic comparison of the different GEFs has been made within the same systems, but previous research suggests that individual GEFs will activate RhoA at different rates and magnitudes (Müller et al., 2020). Ultimately, consideration of the

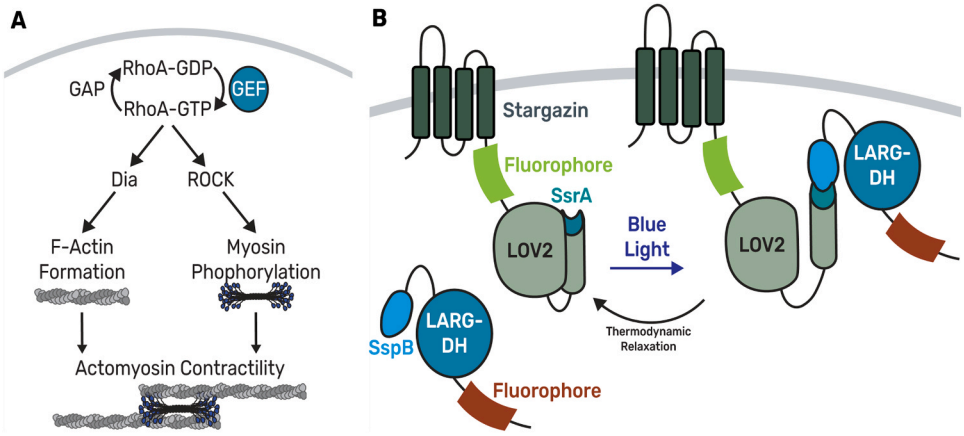


Fig. 1. Using light to achieve spatiotemporal control of RhoA activity. (A) The RhoA Signaling Pathway. A GEF catalyzes the exchange of RhoA-GDP with its active form, RhoA-GTP. Active RhoA in turn promotes actin polymerization by activating Diaphanous-related formins and increased myosin phosphorylation via ROCK. Together these result in increased actomyosin contracitility within the cell. (B) A schematic of the components of a typical optogenetic approach to activate RhoA. A LOV2 molecule with a SsrA peptide at the Jα helix is anchored to the plasma membrane via a transmembrane protein, Stargazin. The binding partner of SsrA, SspB, is fused with the DH domain of LARG and freely diffusing in the cytosol. Upon stimulation with blue light, the LOV2 molecule undergoes a conformational change, exposing the SsrA peptide and allowing it to interact with SspB. This results in LARG moving to the plasma membrane where it can activate endogenous RhoA. When the activating blue light is removed, LOV2 thermodynamically relaxes back to its dark state without input from the user, thus preventing further recruitment of LARG.

experimental goals must be the main driving factor in determining the optimal components for a given experiment.

2.3. Microscopy and equipment

Unlike many of the other approaches to mechanically modulate the cell, the optogenetic approach does not require additional specialized equipment. Both CRY2 and LOV2 are sensitive to UV and blue wavelengths (~380–500 nm) (Crosson and Moffat, 2002; Kennedy et al., 2010; Pathak et al., 2013), though we have also been able to excite LOV2 at 514 nm which is reaching into the green spectrum. Relatively minimal amounts of light are needed for activation, with reports typically ranging around a few mW/mm^2 (Kennedy et al., 2010; Duan et al., 2017; Cavanaugh et al., 2020a, 2020b; Castillo-Badillo and Gautam, 2021; Méry et al., 2023). This ensures that any standard blue light source should be sufficient to induce activation, and that the excess exposure to blue/UV light should have negligible phototoxic impact on cell behavior, as the requisite intensity of light is minimal. With these features, any standard laser scanning confocal microscope should be capable of performing local activation. Other modalities, such as spinning disk confocal or widefield microscopes can take advantage of spatial light modulators, such as those used in fluorescent recovery after photobleaching (FRAP) to induce local activation. While the blue light sensitivity makes these probes easy to implement, it limits the choice of additional fluorophores to use as markers. GFP and its variants all have excitation spectra that overlap with both LOV2 and CRY2, removing their availability as useful markers for other downstream signaling partners. For this reason GFP is often used to label the LOV2 or CRY2 protein itself. If the GEF is also labeled, typically with a red fluorophore, additional downstream markers are restricted to the far-red channel. Available options then include proteins labeled with far-red fluorophores (e.g. iRFP670 or HaloTag coupled to JF646), cell permeable dyes (e.g. SiR-Actin), or fluorescent beads like those used in traction force microscopy (TFM) (Oakes et al., 2017; Cavanaugh et al., 2020a, 2020b). To open up additional channels, the fluorophore on either the CRY2/LOV2 or GEF can be mutated to be silent (i.e. non-fluorescent), preserving its role as a linker while allowing another marker of interest to visualized in the red channel (Oakes et al., 2017; Rich et al., 2020). The downside of this approach is that it does not allow for direct monitoring of GEF recruitment.

3. Tunability and repeatability of optogenetics

The power of optogenetics comes from its ability to modulate interactions both globally and locally. Before attempting to activate RhoA or another signaling pathway, it is worthwhile to optimize a given microscope system with a recruitable fluorophore lacking any signaling components. Here, we provide an example of this optimization, and demonstrate features of the iLID system by recruiting a tagRFP-SspB to the plasma membrane with varying laser settings (Fig. 2). Global activation can be done on any imaging system and simply requires taking an image with standard blue (e.g. <500 nm or GFP) excitation source (Fig. 2A-B). The membrane anchored SsrA shows no discernible change in localization upon activation (Fig. 2A), while the SspB moves from the cytoplasm to the membrane (Fig. 2B). In contrast, local activation can be done by illuminating a defined region, resulting in local accumulation of SspB (Fig. 2C). Recruitment is a function of the power incident on the region, and thus can be tuned by changing either the intensity of the incident light, or the length of time the region is illuminated. In Fig. 2C we show a cell subjected to periodic (100 s) intervals of blue light pulsed before each acquisition. During each interval the duration of the pulses is increased (e.g. more photons are incident on the region of interest) and we see a corresponding increase in the magnitude of recruitment. After each activation interval we stop the blue light pulses with each acquisition and the system relaxes back to equilibrium, which in this case is a uniform distribution of fluorescence. If we measure the change in intensity in the activation region, we observe that the average intensity of the SspB fluorophore in this region increases with increasing exposure time of blue light (blue; Fig. 2D). If we similarly plot the intensity of light in a control region that is not being illuminated with blue light, we see a corresponding drop in average intensity, indicating that protein is leaving this area and translocating to the activation region (black; Fig. 2D), consistent with the use of this system for protein sequestration (Valon et al., 2017). Since the magnitude of recruitment is dependent on the number of incident photons, repeated intervals of activation using the same frequency and duration of blue light pulses at each acquisition will result in similar levels of recruitment for each activation interval. This is shown in Fig. 2E where a cell is exposed to five repeated intervals of 100 s activations (i.e. pulsed prior to each acquisition with blue light) and 200 s relaxations with similar magnitudes of recruitment for each activation interval. These examples demonstrate the flexibility, reproducibility and reversibility that an optogenetic approach offers to modulating signaling inside of cells.

4. RhoA activation alters cellular tension

To directly alter mechanical activity in cells we target a RhoA GEF (e.g. LARG, Ect2, ARHGEF11) to a membrane bound anchor (Fig. 1B).

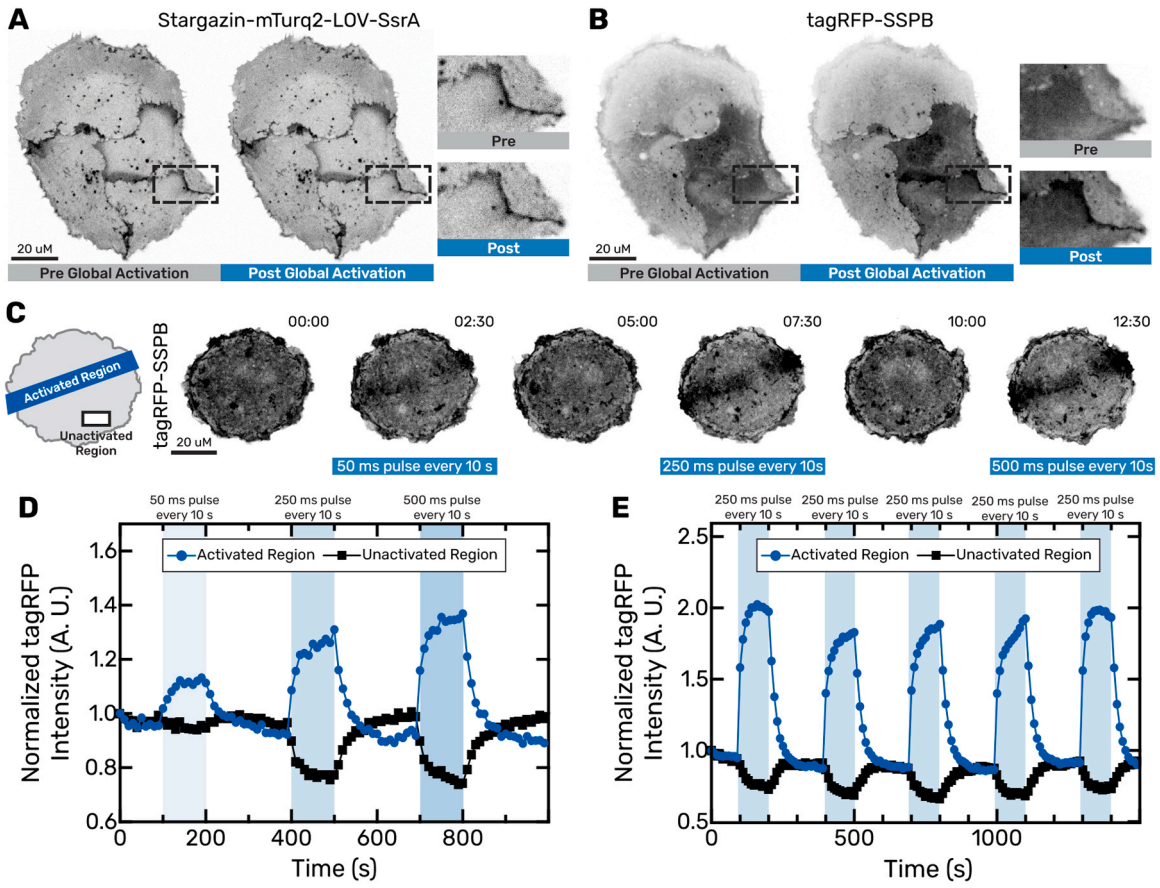


Fig. 2. Optogenetic approaches exhibit excellent spatial and temporal flexibility. They can be used to achieve global and spatiotemporally tunable recruitment of proteins. (A and B) A U2OS cell expressing Stargazin-mTurq2-LOV-SsrA and SspB-tagRFP is globally illuminated with blue light (405 nm) for 200 ms. While the Stargazin distribution does not change (A, inset), the SspB-tagRFP relocalizes from the cytoplasm to the membrane (B, inset). (C) A series of images of a representative U2OS cell illuminated locally by periodic activation intervals demonstrating that recruitment is tunable. The cell was imaged with a 561 nm laser every 10 s for the duration of the experiment. The activated region (indicated in blue in the cell schematic) was exposed to blue light of equal intensity (~ 6 mW/mm^2) for durations of 50, 250 or 500 ms prior to every acquisition during the three activation cycles respectively. Each activation cycle was 100 s long and was followed by a 200 s period of relaxation. (D) Quantification of fluorescent intensity changes in the activated and unactivated regions indicated in the cell schematic in (C). Fluorescence intensity in the activated region increases with increasing duration of exposure (indicated above the graphs) to the activating blue light (blue line). Simultaneously, intensity decreases in the cytoplasm outside of the activation region (black line). (E) Quantification of fluorescent intensity changes in tagRFP in another representative cell exposed to repeated activations of equal intensity and duration, illustrating that recruitment is reproducible within a cell.

While no systematic comparison of photorecruiting GEFs has been performed, different affinities in GEF-RhoA interactions will likely modulate the magnitude of downstream signaling (Müller et al., 2020), similar to titrations of light as shown in Fig. 2C-E. Regardless of choice of GEF, activation of RhoA will result in increased actomyosin contractility (Fig. 1A), altering both internal tension and the stresses applied to the substrate.

4.1. Intracellular contraction

Constant cytoskeletal activity produces a basal steady state flow from the periphery towards the cell body which is driven by actin retrograde flow and myosin activity (Mitchison and Cramer, 1996; Mogilner and Oster, 1996). Local activation of RhoA results in a local increase in actin and myosin in the activation region, and the resulting contraction pulls the cytoskeleton towards that location (Fig. 3A-C). As the RhoA activation is removed, the system relaxes elastically, flowing away from the activated region (Fig. 3 B, right). This is more easily seen in a kymograph of a line drawn perpendicular through the activated region (Fig. 3C). As RhoA is activated, myosin puncta along the stress fiber flow towards the center of the activation region from both sides, and away from the activation region when the activation stops (Fig. 3C).

This flow and local contraction leads to the induction of strain (e.g. stretching/compression) in different regions within the cell. As the cartoons in the bottom of Fig. 3B illustrate, pre-activation cytoskeletal flow is generally pointed inward. As RhoA is activated in the center of the cell, the cytoskeleton flow increases towards the activated region (Fig. 3B, middle). As the RhoA stimulation is removed, the cytoskeleton reverts back to its initial tension state, flowing away from the activation region and changing the local strain again (Fig. 3B, right). Interestingly, by examining the spacing of the puncta along the stress fiber in the kymograph, we see that it largely does not change (Fig. 3C). This indicates that the stress fibers are largely undergoing translation and that strain is concentrated at the focal adhesions, where the stress fibers are being stretched, and in the activation region where they appear to be compressed (Fig. 3B, bottom; Oakes et al., 2017). Local activation of RhoA thus produces both positive (i.e. stretching) and negative (i.e. compressing) strains in the cytoskeleton, offering an ideal tool to probe protein response to these changes in local actin tension and architecture (Oakes et al., 2017; Seetharaman et al., 2023).

4.2. Extracellular tension

In addition to changing the intracellular flow and tension within the cytoskeleton, activation of RhoA increases the external traction stresses the cell exerts, as the internal stresses are propagated through adhesions

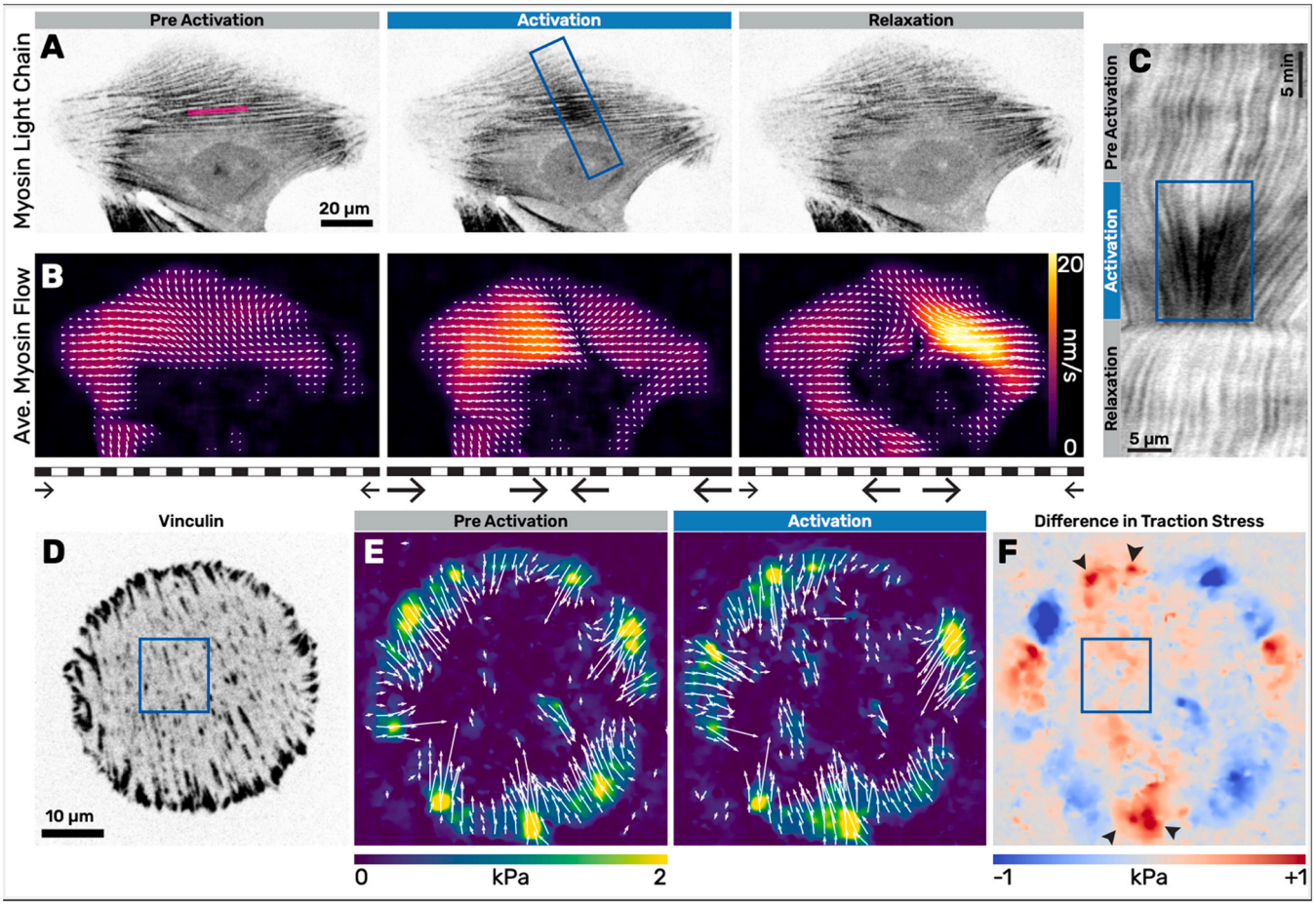


Fig. 3. Local activation of RhoA alters cell mechanics. (A) A U2OS cell expressing Stargazin-mTurq2-LOV-SsrA, SspB-LARG-mScarlet and myosin RLC-3xiRFP670 is imaged with a 561 nm laser every 10 s for 45 min. After 15 min, each acquisition was preceded by a local illumination (blue box) of 405 nm light for 250 ms at a power of ~ 6 mW/mm^2 for an additional 15 min. During activation myosin flowed into the region of activation. Finally, the local activation was stopped and the cell was imaged only with the 561 nm light for an additional 15 min to capture the relaxation of the cell. During relaxation, myosin intensity dissipated and flowed away from the region of activation. (B) Average flow maps of myosin showing baseline flow and induced flow in response to local RhoA activation. The flow reverses when the stimulating light is removed. (C) A kymograph along the pink line shown in (A) illustrates that local illumination stimulates a contractile flow which reverses when the illumination is stopped. (D) An image of vinculin in a cell expressing mApple-vinculin and the optogenetic RhoA constructs. The blue box indicates the region of local RhoA activation. (E) A map showing the traction stresses before and during local activation of RhoA. The imaging protocol was similar to that described in (A) and traction force microscopy was performed as described in (Oakes et al., 2017). (F) An image showing the difference in traction stresses between the frames depicted in (E). Red areas (black arrow heads) indicate regions where traction stresses have increased, while blue areas indicate regions where forces have decreased. Traction stresses change outside the local activation region on account of the stresses being propagated along the actin stress fibers to the focal adhesions at the cell periphery.

to the extracellular matrix (or neighboring cells in multicellular environments) (Valon et al., 2017; Oakes et al., 2017). We can measure these stresses experimentally using TFM (Huang et al., 2019; Sala and Oakes, 2021) As tension takes time to build, the kinetics of force build-up are significantly slower than the recruitment of the GEF to the membrane. Unlike the local accumulation of myosin, the increase in traction stress is dependent on the architecture of the cytoskeleton to propagate the increased stress to the connected focal adhesions that are outside the region of activation Fig. 3D; (Oakes et al., 2017). Traction stresses increase primarily in locations above and below the activation region, as intracellular forces from the activation region are propagated along the actin stress fibers (Fig. 3E-F). While the total change in exerted forces is relatively minor in this cell, on the order of ≈ 10%, the redistribution of stress can be much larger. This is most readily seen by comparing the traction maps during activation and before activation (Fig. 3F), where forces at individual regions of focal adhesions can increase/decrease by up to 50% from their previous magnitudes. By judiciously choosing activation regions we can therefore control the distribution of traction stresses exerted by the cell (Oakes et al., 2017).

5. Conclusions

In summary, optogenetic manipulation of RhoA offers unparalleled spatial and temporal control of mechanical perturbations in a cell, with the added benefit of being fully reversible. Its general accessibility and lack of specialized equipment also makes it an attractive approach to alter cellular mechanics. Any lab with access to a laser scanning confocal microscope can theoretically implement these probes. As an added advantage, by comparing pre-activation, activation, and post-activation responses, experiments can also be fully self-controlled to account for the broad heterogeneity in cell morphology and expression levels of constructs.

Optogenetics is, of course, not without its challenges. Since blue light illumination activates LOV2 and CRY2, it is not possible to visualize proteins in the green channel, which is often the most used marker. This limits the number of channels, and therefore associated markers, that can be imaged during these experiments. This approach also requires expression of multiple constructs. While some of this can be avoided by making stable cell lines, in our experience we have found that cells

typically do not tolerate over-expression of the RhoA GEF LARG, for long periods of time (Cavanaugh et al., 2020a, 2020b), although the membrane-anchored SsrA constructs are often well-tolerated. Other groups have had success stably expressing RhoGEF2 (Izquierdo et al., 2018), so this might depend on cell type/organism and the chosen GEF. Careful planning must therefore be exercised to maximize the utility of this approach. Despite these caveats, using light to control and alter cell mechanics inside cells opens many previously inaccessible research avenues and offers an exciting path forward to continue exploring mechanotransduction and mechanosensitivity in cells.

Declaration of Competing Interest

None.

Data availability

Data will be made available on request.

Acknowledgements

PWO is funded in part by a National Science Foundation (NSF) CAREER award #2000554. JRB is supported by a Maximizing Investigators' Research Award (MIRA - R35) from the National Institute of General Medical Sciences (NIGMS) of the National Institutes of Health (NIH) under grant number R35GM138183.

References

Aratyn-Schaus, Y., Oakes, P.W., Gardel, M.L., 2011. Dynamic and structural signatures of lamellar actomyosin force generation. Mol. Biol. Cell 22, 1330–1339.
Benedetti, L., Barentine, A.E.S., Messa, M., Wheeler, H., Bewersdorf, J., De Camilli, P., 2018. Light-activated protein interaction with high spatial subcellular confinement. Proc. Natl. Acad. Sci. U. S. A. 115, E2238–E2245.
Berlew, E.E., Kuznetsov, I.A., Yamada, K., Bugaj, L.J., Boerckel, J.D., Chow, B.Y., 2021. Single-Component optogenetic tools for inducible RhoA GTPase signaling. Adv. Biol. 5, e2100810.
Buchsbaum, R.J., 2007. Rho activation at a glance. J. Cell Sci. 120, 1149–1152.
Bugaj, L.J., Choksi, A.T., Mesuda, C.K., Kane, R.S., Schaffer, D.V., 2013. Optogenetic protein clustering and signaling activation in mammalian cells. Nat. Methods 10, 249–252.
Castillo-Badillo, J.A., Gautam, N., 2021. An optogenetic model reveals cell shape regulation through FAK and fascin. J. Cell Sci. 134.
Cavanaugh, K.E., Staddon, M.F., Munro, E., Banerjee, S., Gardel, M.L., 2020a. RhoA mediates epithelial cell shape changes via mechanosensitive endocytosis. Dev. Cell 52, 152–166.e5.
Cavanaugh, K.E., Oakes, P.W., Gardel, M.L., 2020b. Optogenetic control of RhoA to probe subcellular mechanochemical circuitry. Curr. Protoc. Cell Biol. 86, e102.
Chrzanowska-Wodnicka, M., Burridge, K., 1996. Rho-stimulated contractility drives the formation of stress fibers and focal adhesions. J. Cell Biol. 133, 1403–1415.
Coste, B., Mathur, J., Schmidt, M., Earley, T.J., Ranade, S., Petrus, M.J., Dubin, A.E., Patapoutian, A., 2010. Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels. Science 330, 55–60.
Crosson, S., Moffat, K., 2002. Photoexcited structure of a plant photoreceptor domain reveals a light-driven molecular switch. Plant Cell 14, 1067–1075.
D'Angelo, A., Dierkes, K., Carolis, C., Salbreux, G., Solon, J., 2019. In vivo force application reveals a fast tissue softening and external friction increase during early embryogenesis. Curr. Biol. 29, 1564–1571.e6.
De Belly, H., Paluch, E.K., Chalut, K.J., 2022. Interplay between mechanics and signalling in regulating cell fate. Nat. Rev. Mol. Cell Biol. 23, 465–480.
Duan, J.Hope, Ong, Q., Lou, H.-Y., Kim, N., McCarthy, C., Acero, V., Lin, M.Z., Cui, B., 2017. Understanding CRY2 interactions for optical control of intracellular signaling. Nat. Commun. 8, 547.
Dupont, S., Wickström, S.A., 2022. Mechanical regulation of chromatin and transcription. Nat. Rev. Genet. 1–20.
Duquette, P.M., Lamarche-Vane, N., 2014. Rho GTPases in embryonic development. Small GTPases 5, e972857.
Fededa, J.P., Gerlich, D.W., 2012. Molecular control of animal cell cytokinesis. Nat. Cell Biol. 14, 440–447.
Garcia-Mata, R., Boulter, E., Burridge, K., 2011. The 'invisible hand': regulation of RHO GTPases by RHOGDIs. Nat. Rev. Mol. Cell Biol. 493–504.
Goodwin, K., Nelson, C.M., 2021. Mechanics of development. Dev. Cell 56, 240–250.
Guglielmi, G., Barry, J., Huber, W., De Renzis, S., 2015. An optogenetic method to modulate cell contractility during tissue morphogenesis. Dev. Cell 35, 646–660.
Guntas, G., Hallett, R.A., Zimmerman, S.P., Williams, T., Yumerefendi, H., Bear, J.E., Kuhlman, B., 2015. Engineering an improved light-induced dimer (iLID) for controlling the localization and activity of signaling proteins. Proc. Natl. Acad. Sci. U. S. A. 112, 112–117.
Guo, H., Swan, M., He, B., 2022. Optogenetic inhibition of actomyosin reveals mechanical bistability of the mesoderm epithelium during Drosophila mesoderm invagination. eLife 11, e69082.
Guolla, L., Bertrand, M., Haase, K., Pelling, A.E., 2012. Force transduction and strain dynamics in actin stress fibres in response to nanonewton forces. J. Cell Sci. 125, 603–613.
Harris, A.K., Wild, P., Stopak, D., 1980. Silicone rubber substrata: a new wrinkle in the study of cell locomotion. Science 208, 177–179.
Herrera-Perez, R.M., Cupo, C., Allan, C., Lin, A., Kasza, K.E., 2021. Using optogenetics to link myosin patterns to contractile cell behaviors during convergent extension. Biophys. J. 120, 4214–4229.
Huang, Y., Schell, C., Huber, T.B., Şimşek, A.N., Hersch, N., Merkel, R., Gompper, G., Sabass, B., 2019. Traction force microscopy with optimized regularization and automated bayesian parameter selection for comparing cells. Sci. Rep. 9, 1–16.
Humphrey, J.D., Schwartz, M.A., 2021. Vascular mechanobiology: Homeostasis, adaptation, and disease. Annu. Rev. Biomed. Eng. 23, 1–27.
Humphrey, J.D., Dufresne, E.R., Schwartz, M.A., 2014. Mechanotransduction and extracellular matrix homeostasis. Nat. Rev. Mol. Cell Biol. 15, 802–812.
Inaba, H., Miao, Q., Nakata, T., 2021. Optogenetic control of small GTPases reveals RhoA mediates intracellular calcium signaling. J. Biol. Chem. 296, 100290.
Iskratsch, T., Wolfenson, H., Sheetz, M.P., 2014. Appreciating force and shape—the rise of mechanotransduction in cell biology. Nat. Rev. Mol. Cell Biol. 15, 825–833.
Izquierdo, E., Quinkler, T., De Renzis, S., 2018. Guided morphogenesis through optogenetic activation of Rho signalling during early Drosophila embryogenesis. Nat. Commun. 9, 2366.
Janmey, P.A., Fletcher, D.A., Reinhart-King, C.A., 2020. Stiffness sensing by cells. Physiol. Rev. 100, 695–724.
Ju, J., Lee, H.N., Ning, L., Ryu, H., Zhou, X.X., Chun, H., Lee, Y.W., Lee-Richerson, A.I., Jeong, C., Lin, M.Z., Seong, J., 2022. Optical regulation of endogenous RhoA reveals selection of cellular responses by signal amplitude. Cell Rep. 40, 111080.
Kamps, D., Koch, J., Juma, V.O., Campillo-Funollet, E., Graessl, M., Banerjee, S., Mazel, T., Chen, X., Wu, Y.-W., Portet, S., Madzvamuse, A., Nalbant, P., Dehmelt, L., 2020. Optogenetic tuning reveals rho Amplification-Dependent dynamics of a cell contraction signal network. Cell Rep. 33, 108467.
Kennedy, M.J., Hughes, R.M., Peteya, L.A., Schwartz, J.W., Ehlers, M.D., Tucker, C.L., 2010. Rapid blue-light-mediated induction of protein interactions in living cells. Nat. Methods 7, 973–975.
Kong, F., García, A.J., Mould, A.P., Humphries, M.J., Zhu, C., 2009. Demonstration of catch bonds between an integrin and its ligand. J. Cell Biol. 185, 1275–1284.
Lawson, C.D., Ridley, A.J., 2018. Rho GTPase signaling complexes in cell migration and invasion. J. Cell Biol. 217, 447–457.
Lecuit, T., Lenne, P.-F., Munro, E., 2011. Force generation, transmission, and integration during cell and tissue morphogenesis. Annu. Rev. Cell Dev. Biol. 27, 157–184.
Liu, H., Yu, X., Li, K., Klejnot, J., Yang, H., Lisiero, D., Lin, C., 2008. Photoexcited CRY2 interacts with CIB1 to regulate transcription and floral initiation in arabidopsis. Science 322, 1535–1539.
Méry, A., Ruppel, A., Revilloud, J., Balland, M., Cappello, G., Boudou, T., 2023. Light-driven biological actuators to probe the rheology of 3D microtissues. Nat. Commun. 717.
Mitchison, T.J., Cramer, L.P., 1996. Actin-based cell motility and cell locomotion. Cell 84, 371–379.
Mogilner, A., Oster, G., 1996. Cell motility driven by actin polymerization. Biophys. J. 71, 3030–3045.
Müller, P.M., Rademacher, J., Bagshaw, R.D., Wortmann, C., Barth, C., Unen, J., Alp, K. M., Giudice, G., Eccles, R.L., Heinrich, L.E., Pascual-Vargas, P., Sanchez-Castro, M., Brandenburg, L., Mbamalu, G., Tucholska, M., Spatt, L., Czajkowski, M.T., Welke, R. W., Zhang, S., Nguyen, V., Rrustemi, T., Trnka, P., Freitag, K., Larsen, B., Popp, O., Mertins, P., Gingras, A.-C., Roth, F.P., Colwill, K., Bakal, C., Pertz, O., Pawson, T., Petsalaki, E., Rocks, O., 2020. Systems analysis of RhoGEF and RhoGAP regulatory proteins reveals spatially organized RAC1 signalling from integrin adhesions. Nat. Cell Biol. 22, 498–511.
Murrell, M., Oakes, P.W., Lenz, M., Gardel, M.L., 2015. Forcing cells into shape: the mechanics of actomyosin contractility. Nat. Rev. Mol. Cell Biol. 16, 486–498.
Natwick, D.E., Collins, S.R., 2020. Optimized iLID membrane anchors for local optogenetic protein recruitment. ACS Synth. Biol. 10, 1009–1023.
O'Neill, P.R., Castillo-Badillo, J.A., Meshik, X., Kalyanaraman, V., Melgarejo, K., Gautam, N., 2018. Membrane flow drives an adhesion-independent amoeboid cell migration mode. Dev. Cell 46, 9–22.e4.
Oakes, P.W., Wagner, E., Brand, C.A., Probst, D., Linke, M., Schwarz, U.S., Glotzer, M., Gardel, M.L., 2017. Optogenetic control of RhoA reveals zyxin-mediated elasticity of stress fibres. Nat. Commun. 8, 15817.
Paluch, E.K., Nelson, C.M., Biais, N., Fabry, B., Moeller, J., Pruitt, B.L., Wollnik, C., Kudryasheva, G., Rehfeldt, F., Federle, W., 2015. Mechanotransduction: use the force (s). BMC Biol. 13, 47.
Pathak, G.P., Vrana, J.D., Tucker, C.L., 2013. Optogenetic control of cell function using engineered photoreceptors. Biol. Cell 105, 59–72.
Qiao, J., Peng, H., Dong, B., 2023. Development and application of an optogenetic manipulation system to suppress actomyosin activity in ciona epidermis. Int. J. Mol. Sci. 24, 5707.
Rich, A., Fehon, R.G., Glotzer, M., 2020. Rho1 activation recapitulates early gastrulation events in the ventral, but not dorsal, epithelium of drosophila embryos. Elife 9, e56893.
Ridley, A.J., 2015. Rho GTPase signalling in cell migration. Curr. Opin. Cell Biol. 36, 103–112.

Ridley, A.J., Hall, A., 1992. The small GTP-binding protein rho regulates the assembly of focal adhesions and actin stress fibers in response to growth factors. Cell 70, 389–399.
Riveline, D., Zamir, E., Balaban, N.Q., Schwarz, U.S., Ishizaki, T., Narumiya, S., Kam, Z., Geiger, B., Bershadsky, A.D., 2001. Focal contacts as mechanosensors: externally applied local mechanical force induces growth of focal contacts by an mdia1-dependent and ROCK-independent mechanism. J. Cell Biol. 153, 1175–1186.
Sala, S., Oakes, P.W., 2021. Stress fiber strain recognition by the LIM protein testin is cryptic and mediated by RhoA. Mol. Biol. Cell 32, 1758–1771.
Salomon, M., Christie, J.M., Knieb, E., Lempert, U., Briggs, W.R., 2000. Photochemical and mutational analysis of the FMN-binding domains of the plant blue light receptor, phototropin. Biochemistry 39, 9401–9410.
Schwartz, M., 2004. Rho signalling at a glance. J. Cell Sci. 117, 5457–5458.
Schwingel, M., Bastmeyer, M., 2013. Force mapping during the formation and maturation of cell adhesion sites with multiple optical tweezers. PLoS One 8, e54850.
Seetharaman, S., Sala, S., Gardel, M.L., Oakes, P.W., 2023. Quantifying strain-sensing protein recruitment during stress fiber repair. Methods Mol. Biol. 2600, 169–182.
SenGupta, S., Parent, C.A., Bear, J.E., 2021. The principles of directed cell migration. Nat. Rev. Mol. Cell Biol. 22, 529–547.
Sun, Z., Guo, S.S., Fässler, R., 2016. Integrin-mediated mechanotransduction. J. Cell Biol. 215 (4), 445–456.
Swartz, T.E., Corchnoy, S.B., Christie, J.M., Lewis, J.W., Szundi, I., Briggs, W.R., Bogomolni, R.A., 2001. The photocycle of a flavin-binding domain of the blue light photoreceptor phototropin. J. Biol. Chem. 276, 36493–36500.
Taslimi, A., Zoltowski, B., Miranda, J.G., Pathak, G.P., Hughes, R.M., Tucker, C.L., 2016. Optimized second-generation CRY2-CIB dimerizers and photoactivatable cre recombinase. Nat. Chem. Biol. 12, 425–430.
Tzima, E., Irani-Tehrani, M., Kiosses, W.B., Dejana, E., Schultz, D.A., Engelhardt, B., Cao, G., DeLisser, H., Schwartz, M.A., 2005. A mechanosensory complex that mediates the endothelial cell response to fluid shear stress. Nature 437, 426–431.
Valon, L., Marín-Llauradó, A., Wyatt, T., Charras, G., Trepat, X., 2017. Optogenetic control of cellular forces and mechanotransduction. Nat. Commun. 8, 14396.
Wagner, E., Glotzer, M., 2016. Local RhoA activation induces cytokinetic furrows independent of spindle position and cell cycle stage. J. Cell Biol. 213, 641–649.
Wu, P.-H., Aroush, D.R.-B., Asnacios, A., Chen, W.-C., Dokukin, M.E., Doss, B.L., Durand-Smet, P., Ekpenyong, A., Guck, J., Guz, N.V., Janmey, P.A., Lee, J.S.H., Moore, N.M., Ott, A., Poh, Y.-C., Ros, R., Sander, M., Sokolov, I., Staunton, J.R., Wang, N., Whyte, G., Wirtz, D., 2018. A comparison of methods to assess cell mechanical properties. Nat. Methods 1–13.
Yamamoto, K., Miura, H., Ishida, M., Mii, Y., Kinoshita, N., Takada, S., Ueno, N., Sawai, S., Kondo, Y., Aoki, K., 2021. Optogenetic relaxation of actomyosin contractility uncovers mechanistic roles of cortical tension during cytokinesis. Na. Commun. 7145.
Yao, M., Goult, B.T., Klapholz, B., Hu, X., Toseland, C.P., Guo, Y., Cong, P., Sheetz, M.P., Yan, J., 2016. The mechanical response of talin. Nat. Commun. 7, 11966.
Yoshigi, M., Hoffman, L.M., Jensen, C.C., Yost, H.J., Beckerle, M.C., 2005. Mechanical force mobilizes zyxin from focal adhesions to actin filaments and regulates cytoskeletal reinforcement. J. Cell Biol. 171, 209–215.
Zayner, J.P., Antoniou, C., Sosnick, T.R., 2012. The amino-terminal helix modulates light-activated conformational changes in AsLOV2. J. Mol. Biol. 419, 61–74.
Zimmerman, S.P., Hallett, R.A., Bourke, A.M., Bear, J.E., Kennedy, M.J., Kuhlman, B., 2016. Tuning the binding affinities and reversion kinetics of a light inducible dimer allows control of transmembrane protein localization. Biochemistry 55 (37), 5264–5271.