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MECHANOTRANSDAUCTION: HOW CELLS SENSE AND REACT TO MECHANICAL STIMULATION

Abstract*: the ability of cells to sense and respond to mechanical signals is vital in development functioning of healthy tissue. Many diseases are correlated to either changing mechanical properties of the tissue, or changes in the ability of cells to sense mechanical signals. This ssensing happens in part, at integrin-associated complexes (IACs) that form sites of attachment between the cell and the extracellular matrix (ECM). In this review, we will discuss the complex mechanical signals of the ECM and it's components: how cells sense mechanical stimuli, how mechanical signals are transmitted intracellularly, and what effects those signals have on cell function.*

Since this is such a voluminous and complex topic, we have focused here on the generalities, rather than details of how a specific cell type responds to mechanical stimulation.

Keywords*: mechanotrasduction, Extracellular Matrix, ECM, Mechanical Stimuli.*

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DOI 10.31483/r-106759

МЕХАНОТРАНСДУКЦИЯ: КАК КЛЕТКИ ВОСПРИНИМАЮТ МЕХАНИЧЕСКУЮ СТИМУЛЯЦИЮ И РЕАГИРУЮТ НА НЕЕ

***Аннотация**: способность клеток воспринимать механические сигналы и реагировать на них жизненно важна для развития и функционирования здоровой ткани. Многие заболевания связаны либо с изменением механических свойств ткани, либо с изменениями в способности клеток воспринимать механические сигналы. Частично это происходит в интегрин-ассоциированных комплексах (IAC), которые образуют участки прикрепления между клеткой и внеклеточным матриксом (ECM). В этом обзоре обсуждаются сложные механические сигналы ECM и его компонентов: как клетки воспринимают механические стимулы, как механические сигналы передаются внутриклеточно и какое влияние эти сигналы оказывают на функции клеток.*

Поскольку это такая объемная и сложная тема, автор сосредоточился на общих чертах, а не на деталях того, как конкретный тип клеток реагирует на механическую стимуляцию.

***Ключевые слова**: механотрансдукция, внеклеточный матрикс, ECM, механические стимулы.*

1. *Introduction.*

In human tissues, cells undergo numerous mechanical stimuli, for instance shear stresses from the blood flow or stretching and compression forces from diverse tissues associated with muscle activity [1]. Cells are encircled by an extracellular matrix (ECM) that is composed of different proteins including laminins, collagens and fibronecti to mention a few. Cells sense the intrinsic mechanical properties of the ECM by applying traction forces. The ability of cells to respond to external forces, to probe and interpret the mechanical characteristics of the ECM, and to synthesise and remodel it, play crucial roles in many facets of cell behaviour [2]. ECM stiffening in disease states (*e.g.* cancer and fibrosis) [1; 3] or during aging [4] can adversely affect cell migration, differentiation and proliferation. Alternatively, aberration in intracellular signals that influence the cells ability to sense and react to extracellular mechanical stimuli can also contribute to disease states including cancer [1].

The ability of cells to sense and respond to mechanical stimuli is termed mechanotransduction (*see Box 1*). Mechanotransduction requires the sensing of external

forces or biomechanical properties and the transduction of this information which triggers a specific intracellular signalling response [1] The cytoskeleton plays a critical role in mechanotransduction by linking cellular compartments (e.g other cytoskeletal systems and the nucleus) [5] to the force-sensing apparatus.

Mechanotrasduction – the overall process of how cells sense a mechanical stimulus and converts it into a biochemical, intracellular response
Mechaniresponse – the specific response of cells to a mechanical stumuli (for example, increased protein phosphorylation; changes in gene transcription; changes in gene transcription, changes in cell behavior)
Mechanosensing – the act of sensing a mechanical stimulus by a cell
Mechanosignalling – an intracellular signaling event occurring in response to a mechanical stimulus
Mechanosensitive – a protein, or specific protein domaim, that is sensitive to force by undergoing force-dependent conformational changes
Mechanotransmission – the act of transmitting a force, such as transmitting intracellular forces from inside the cell to the outside to probe the ECM, or vice-versa

Box 1. Terms defined. Key terminologies in

In this paper we will discuss how cells sense mechanical stimuli, how mechanical signals are transmitted intracellularly, and what effects those signals have on cell function. We will focus our discussion on the benefits of 3D modelling in understanding cellular sensing mechanisms as well as highlighting the applications of mechanotransduction in Mechanomedicine.

2. Components a cell needs to respond to a mechanical stimulus.

Mechanoreception.

A cell must detect the stimulus and relay the message from outside the cell (where the stimulus acts) to inside the cell [6] (where a response will ultimately be generated). To do this, cells use mechanoreceptors.

Signal transmission.

Once sensed, the mechanical signal tneeds to be relayed in the cell to various targets throughout the cell; cells appear to use both biochemical pathways and the cytoskeleton to transmit this signal.

Target activation.

When the signal reaches its target (usually a protein), the target is activated. This causes alterations in cell behavior through a variety of molecular mechanisms as shown in *Figure 1*

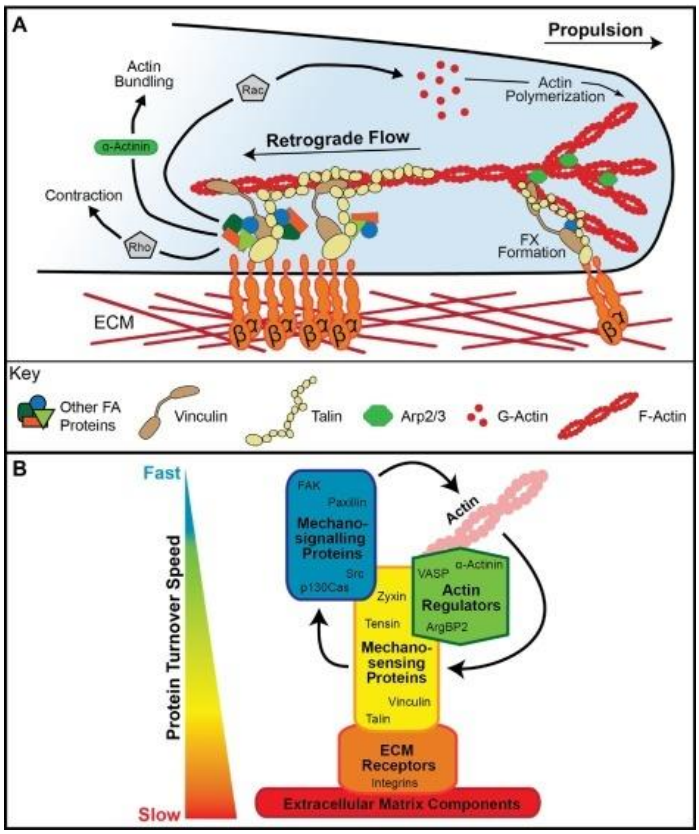

Fig. 1. The molecular clutch, protein turnover, and mechanotransduction [22]

2.1. Mechanoreceptors.

Several mechanoreceptors have been identified in this location, including integrins, stretch activated ion channels, and other cell-surface receptor proteins (*Fig.1*). are

physically located in the plasma membrane, at the junction of extracellular and intracellular spaces [6].

2.1.1 Integrins.

Integrins are transmembrane proteins which link the ECM to the cytoskeleton via focal adhesion proteins in the cytoplasm. Because of the physical connection between the ECM and cytosolic components, a mechanical stimulus applied to integrins can change the structure of the cytoskeleton directly. Deformation of the cytoskeleton can have numerous consequences since the physical properties of the cell will change [6].

2.1.2 Stretch-activated ion channels.

Ion channels are proteins that span the plasma membrane, connecting the cytosol to the cell exterior. Unlike other membrane pores, which are relatively large and permissive, ion channels are really selective, allowing diffusion of specific inorganic ions across the lipid bilayer [7]. These ions, which include Na+, K+, Ca2+, and Cl-, are involved in a multitude of cellular activities, including intracellular signaling, gene expression, transcription, translation, and protein synthesis. Ion channels are further specialized in that they are not always open – instead they are gated, meaning a specific stimulus can cause them to open briefly, thereby allowing the flow of ions either into or out of the cell depending on the electrochemical gradients. Opening of ion channels typically involves an alteration in the channel's physical configuration. In the case of mechanically gated channels, it is not entirely clear how this occurs. One possibility is that physical deformation of the plasma membrane causes conformational changes in the embedded channel protein, leading to its activation. Interestingly, while certain channels are activated by stretch, others are actually inactivated by stretch [6].

2.1.3. Cell-surface receptor proteins.

In order to respond to cues from their environment, cells rely on cell-surface receptors that connect signaling molecules to initiate an intracellular response. These cell-surface receptors are broadly classified as either G protein-linked or enzyme linked (Alberts et al [8]). Typically, receptors respond to soluble extracellular signal molecules, such as proteins, small peptides, steroids, or dissolved gases. However, there is evidence that certain cell-surface receptors are responsive to, or are at least involved

in, the sensing of mechanical signals. Again, the mechanisms are not fully clear, but like stretch-activated ion channels, the conformation of cell-surface receptors may be altered by membrane deformation, switching them from an inactive to an active state. Additionally, the cytoskeleton and focal adhesions may play roles in activation of these receptors. For example, subunits of G proteins have been shown to be localized to sites of focal adhesions, in close proximity to integrins and the cytoskeleton [9].

2.2. Intracellular signal transduction.

Once a mechanical stimulus is sensed and transferred from outside the cell, the signal require transmitting to other points within the cell where a molecular response can be generated. It appears that cells rely on both physical and biochemical mechanisms to transmit mechanical signals.

2.2.1. Cytoskeleton-mediated signal transduction.

Transmission of mechanical signals via integrins can lead to deformation of the cytoskeleton, which, in turn, can impact the biochemical state of the cell. For instance, because the cytoskeleton is a continuous, dynamic network that provides mechanical connections between intracellular structures, deformation of the cytoskeleton at one location may lead to deformations of connected structures at remote locations. This «hard-wiring» within the cell means a perturbation applied locally to an integrin can cause movement of organelles [10] and distortion of the nucleus [11], possibly influencing gene expression. As mentioned above, cytoskeletal deformation can also activate other receptors, such as ion channels and G protein-linked receptors.

This «decentralization» mechanism, by which a locally applied stimulus results in mechanotransduction at multiple, mechanically coupled sites, allows for greater diversity in the cellular response than is possible with a single uncoupled receptor, since different receptors will have different sensitivities and response times and will thus respond to different local environmental cues [12].

Another possible role for the cytoskeleton in mechanotransduction is based on the observation that many proteins and enzymes involved in protein synthesis and biochemical signal transduction seem to be immobilized on the cytoskeleton [13]. It has been suggested that these regulatory molecules will experience the mechanical load

imposed on the cytoskeleton as a consequence of their binding to it. The imposed load could modify the conformation of the regulatory molecules, which, in turn, would change their kinetic behavior and biochemical activity. Thus, the cytoskeleton and its associated regulatory molecules may serve as a scaffold for the transduction of mechanical signals to biochemical signals within the cell.

2.2.2. Biochemically mediated signal ttransduction:

By initiating biochemical signal transduction the biochemical signaling pathways interact with target proteins, which are responsible for altering the behavior of the cell [6].

2.3 Cellular response to mechanical signals.

Mechanical signals, like other extracellular signals, are able to influence cellular function at multiple levels, depending on the targets of the signaling pathways initiated by the stimulus. For example, a signaling pathway activated by a mechanical stimulus might target proteins that regulate gene expression and the transcription of mRNA from DNA (e.g., transcription factors). Additionally, the signaling targets might be molecules involved in protein production, so that alteration of those molecules will affect translation of mRNA to proteins or posttranscriptional

assembly or secretion of proteins. Since cell shape and motility are dependent on the cytoskeleton, its deformation by a mechanical stimulus can alter these cytoskeleton-dependent processes. Lastly, the production of proteins and their secretion from a cell can affect the function of neighboring cells (or even the secreting cell itself), thereby propagating the effect of the mechanical signal from one cell to several.

It is important to realize that the cellular response to a single type of stimulus can be rather complex, since activation of a single type of receptor usually activates multiple parallel signaling pathways and therefore can influence multiple aspects of cell behavior. Furthermore, at any one time, cells are receiving hundreds of different signals from their environment and their response is determined by integration of all the information they receive. Clearly, this makes things rather sophisticated, particularly if one wants to understand the response of a cell to a particular mechanical stimulus. As a result, efforts to understand the response of cells to mechanical stimuli often rely on experiments performed under controlled conditions in the laboratory [6].

3. The Extracellular Matrix ECM.

The ECM is more than just a passive network of ligands to support cell attachment: it contains different types of mechanical signals and it provides dimensionality. Our focus will be on the contribution of the physical properties of the ECM environment on cellular mechanosensing.

3.1. Mechanical properties of ECM networks.

3.1.1. Elasticity.

Elasticity (*see Fig. 1A*) can control many cellular processes, including the motility of a variety of cells, cell proliferation and differentiation [14], and even axon guidance of neuronal cells [15]. However, it is not only the elastic properties that play a role in modulating cell behaviour and unlike purely elastic polyacrylamide gels, tissues show stress relaxation behaviour [16].

3.1.2. Viscosity.

Indeed, fibroblast spreading on soft surfaces is enhanced if the ability to remodel the ECM is increased by introducing stress relaxation in the underlying surface (or increasing its *viscous* properties, see *Fig. 1B*) [*17*].

Also, hydrogels with more or less stress relaxation can guide stem cell fate independently of other known parameters, such as elasticity or ligand density [16]. The combination of elasticity and viscous properties, i.e. viscoelasticity (Fig. 1C), could be particularly relevant for the «more in-vivo like’

3.1.3 Viscoelasticity.

The cell generally behaves as a viscoelastic system [18]. However differently from inorganic materials, biological soft matter is inhomogeneous and generally hierarchically well organized [19]and thus reacts to mechanical stimuli by simultaneously involving several cell districts and processes, as well as protein filaments and supramolecular and molecular structures present at different scale levels. The hierarchical organization of the cell works as a complex transducer device that converts macromechanical signals (pressure gradients, oscillation of organelles, etc.) to activate a biomechanical orchestra that steers a cascade of biochemical and physical coordinated events which regulate the mechanobiology and the mechanosensing of the whole cell,

regulating differentiation, growth, morphogenesis and-through polymerization/depolymerization based cytoskeleton structural rearrangements-migration and adhesion phenomena affecting both single-cell dynamics and macroscopic behaviours of tissue and tumour masses [20].

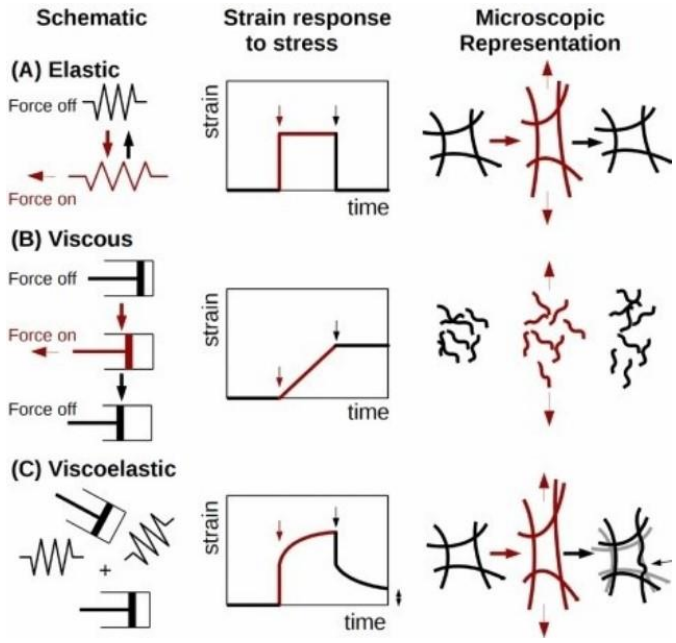

Fig. 2

[*22*] Biopolymer networks typically show signatures of both elastic (A) and viscous (B) properties (C). (A) For a completely elastic system, such as a mechanical spring, applied force or stress increases the strain or extension sharply in time and does not change thereafter. When the force is released, the spring returns to its original position.

Microscopically, such a network recovers completely when forces are released. (B) For a viscous system, such as a dashpot or a French press, the strain increases linearly and irreversibly in time under influence of force. In other words, the system flows, where any potential bonds between fibre segments are broken and reformed continuously. Note that there is not a connected «network' in this case, but a solution of monomers or small fibre segments. The original picture is not recovered when the forces are released. (C) Biopolymer networks could roughly be considered as a combination of springs and dashpots. This can give rise to a strain dependence that shows both elastic (i.e. strain responses that are recoverable) and viscous properties (time dependent changes and non-recoverable strain). Microscopically, the original network

structure can be partly recovered (compare grey with black in the microscopic representation), but bonds between fibres could be broken and reformed, or individual fibres could show flow behaviour (resulting in lengthening of the lengthened [21], see arrow).

4. Discussion:

In living cells, application of a mechanical stimulus causes not only a mechanical response but also a biological response. Using complex networks of sensors, transducers and actuating mechanisms, cells are capable of responding and adapting to their mechanical environment [6].

The precise mechanisms by which cells interpret mechanical information is a complex process and involves many different proteins at various compartments of the cell. In cell-ECM attachment sites mechanotransduction appears to be subdivided into modules. The mechanosensing module contains talin, vinculin and maybe other proteins that directly link integrins with actin. Other modules comprise mechanosignalling proteins (FAK and paxillin), actin polymerising factors (*e.g.* Arp2/3, VASP *etc*.) or actin-crosslinking proteins, all of which bind actin to mediate the mechanotransduction events so as to control different aspects of cellular roles in physiology [22].

An elegant intracellular machinery beneath transduction of the complex environmental signals to biochemical cascades inside the cell. Cellular mechanotransduction is a multiscale/ multiphysics process critical for many biological functions including cell migration and differentiation. Cytoskeletal structures transmit intracellular forces to both the cell membrane and the nuclear envelope through macromolecular bridges [23].

Mechanomedicine

There are a number of medical therapies based on mechanotransduction, including manipulation of ion channels sensitive to mechanical stimuli, manipulation of tissue constructs for the treatment of heart failure, and various uses in regenerative medicine [24].

«Mechanomedicine» is a new field of therapy that uses mechanotransduction to improve hhealth [24] Various studies have described the benefits of mechanomedicine to health, such as promoting bone formation in an osteoporotic mouse model [25] stimulating wound healing in mice with diabetes [26], controlling pressure in vivo to inhibit

cancer metastasis [27] and enhancing gene expression[28] In each of these studies, mechanical vibration was used as the stimulus in vivo.

Metabolism:

Regulation of cell metabolism has been of great interest in many therapeutic applications such as cancer,[29] injury healing,[30] and drug metabolism[31] Mechanotherapy, in particular, has been applied as a noninvasive approach in tissue engineering to alter, or improve, the metabolic function of a target cell[32] In mechanotherapy, an applied physical stimulus is employed to promote tissue regeneration by exploiting a series of biochemical reactions within the cell[33] Mechanotherapy methods such as microdeformational wound therapy,[33] shock wave therapywa [34] and nanovibration enhanced osteogenesingl35] have been successfully applied to relieve symptoms and promote rehabilitations toward predamaged or presurgical levels. However, despite its potential to alter the metabolic function of a target cell at the single cell level, [36] mechanotherapy is yet to be applied as a direct treatment method for a specific disease.

Accoustic-based Mechanotherapy:

A common approach that has gained popularity recently is acoustic-based mechanotherapy, in which bulk acoustic waves (BAW) with the frequency ranging from 0.1–5 MHz cause an intentional pressure disturbance within a tissue[37] to improve blood circulation,[38] and reduce cell death by either necrosis[39] or apoptosis[40] In contrast to BAW, surface acoustic waves (SAW) are typically used for operation at a higher frequency (typically 20–350 MHz) to achieve a higher precision in single cell level manipulation [41] for orting [42; 43], patterning [44], capturing [45], and sonoporation [46] of live cells, since SAW wavelength is in the same range as typical cells' sizes. This capability points to a potential for higher accuracy and efficiency in mechanotherapy-based applications. Indeed, Devendran et al [47] recently reported a consistent increase of metabolic activity for adherent, nonmotile cells post-SAW exposure, suggesting the potential utilization of this method for single cell level mechanotherapy.

3D Modelling

Signal modulation has been developed in living cells throughout to promote utilizing the same machinery for multiple cellular functions. Chemical and mechanical

modules of signal transmission and transduction are interconnected and necessary for organ development and growth. However, due to the high complexity of the intercommunication of physical intracellular connections with biochemical pathways, there are many missing details in our overall understanding of mechanotransduction processes, i.e., the process by which mechanical signals are converted to biochemical cascades. Cell-matrix adhesions are mechanically coupled to the nucleus through the cytoskeleton [48[. This modulated and tightly integrated network mediates the transmission of mechanochemical signals from the extracellular matrix to the nucleus. Various experimental and computational techniques have been utilized to understand the basic mechanisms of mechanotransduction (*figure 3*), yet many facets have remained elusive. Recently, in silico experiments have made important contributions to the field of mechanobiology. Herein, computational modeling efforts devoted to understanding integrin-mediated.

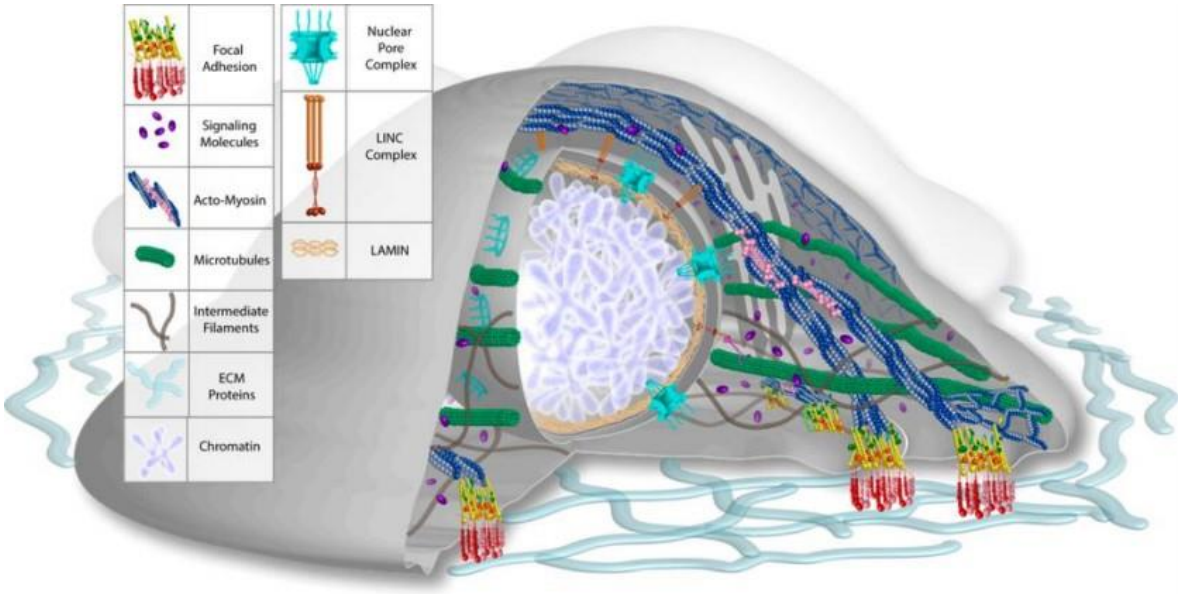

Fig. 3. Important modules involved in cellular mechanotransduction [23]

Cells sense and respond to mechanical forces through triggering intracellular biochemical cascades. Mechanical signals are transmitted through FAs from the ECM to the cytoplasm. Cytoskeletal networks consisting of actin, microtubules, and intermediate filaments are involved in generating and transmitting forces throughout the cell. These forces are ultimately transmitted to the nucleus, most likely via LINC complexes that directly couple the cytoskeletal components to the nucleoskeletal elements such as

lamins. Nuclear pore complexes mediate biochemical signaling between the nucleoplasm and cytoplasm and might as well play a role in physically linking the nucleus to the cytoskeleton [23].

5. Conclusion.

Although the force-bearing properties of individual mechanotransduction modules have been widely studiedand investigated there are still many unknowns regarding the structure and function of each module and the cross-talk between different modules is poorly understood [22].

One of the important gaps in the field of mechanotransduction is the full protein-protein interaction patterns and conformational states of individual proteins. For instance, each integrin receptor within the FA complex associates with a certain composition of proteins, which may result in a specific functionality of that integrin-complex for signaling with the ECM. Such an approach will provide great insights into specific roles of individual proteins in integrin-mediated signaling and potential redundancies in their function, which is not well understood [22].

Furthermore, the amino acid composition and interactions between different domains of FA proteins can serve as means of modularity that controls how stress is compiled within the protein structure. For instance, actin binding proteins may experience tension, bending, or other types of motion depending on the local stress environment [22].

Computational modeling *figure 3 above* is a powerful tool for understanding mechanobiology of mechanotransduction modules. Specifically, molecular dynamics simulations provide detailed structural insights into the molecular mechanisms of signal transmission.

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