Exercise 7: Mechanobiology

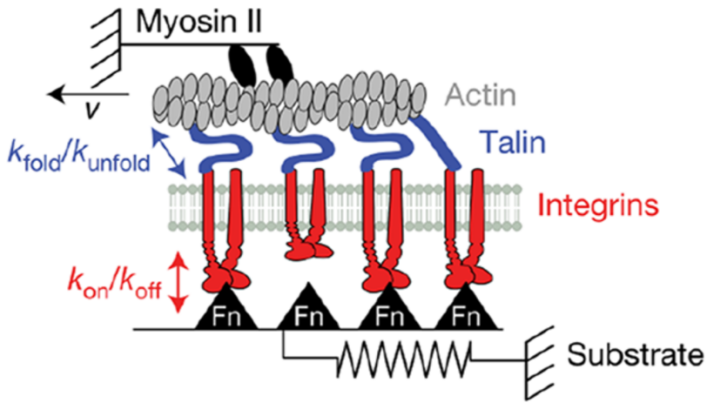


Figure 1*: Schematic figure that illustrates how forces created by cell contraction are balanced by resistance from the cell substrate (matrix or a biomaterial) – with physical and biological consequences for the structural proteins in between.*

Myosins are a superfamily of motor proteins best known for their roles in muscle contraction and in a wide range of other motility processes in eukaryotes. They are ATP-dependent and responsible for actin-based motility.

Although myosin was originally thought to be restricted to muscle, there is no single "myosin"; rather it is a very large superfamily of genes whose protein products share the basic properties of actin binding, ATP hydrolysis (ATPase enzyme activity), and force transduction.
Virtually all eukaryotic cells contain myosin isoforms. Some isoforms have specialized functions in certain cell types (such as muscle), while other isoforms are ubiquitous.

Myosin II is the myosin type responsible for producing cellular contraction in most animal cell types. It is found in non-muscle cells in contractile **actin** bundles called stress fibers.

Integrins are transmembrane receptors that facilitate cell-**extracellular matrix (ECM)** adhesion.

Upon ECM ligand binding (for instance, fibronectin or **FN**), integrins activate signal transduction pathways that mediate cellular signals such as regulation of the cell cycle, organization of the intracellular cytoskeleton, and movement of new receptors to the cell membrane. The presence of integrins allows rapid and flexible responses to events at the cell surface.

Talin is a high-molecular-weight cytoskeletal protein concentrated at regions of cell–substrate contact. Talin is a ubiquitous cytosolic protein that is found in high concentrations in **focal adhesions** (FA). It is capable of linking integrins to the actin cytoskeleton either directly or indirectly by interacting with other FA proteins such as vinculin and α-actinin.

Talin is a mechanosensitive protein. Its mechanical “vulnerability” and cellular position bridging integrin receptors and the actin cytoskeleton make it a fundamental protein in mechanotransduction. Mechanical stretching of talin promotes vinculin binding, and opens cryptic sites that interact with signaling molecules (such as **kinases that mediate phosphorylation**).

Questions on Figure 1

K_{on}/K_{off} represents here the relative likelihood that an integrin will bind **on**to (or release from: "**off**") an exposed fibronectin cell binding site.

K_{fold}/K_{unfold} represents here the relative likelihood that a talin molecule will be coiled (folded) vs. elongated under tension (unfolded).

Consider the following factors as variables:

- Force balance
    - Relative myosin activity ("molecular motor")
    - Relative substrate stiffness
- K_{on}/K_{off}
    - Relative integrin density
    - Relative matrix ligand density
- K_{fold} / K_{unfold}
    - Relative talin density (part of the so-called "molecular clutch")

Consider signaling kinase activity as "biological output" (e.g. MAPKs or mitogen activated protein kinases).

Assume that all variable factors are currently being regulated by the cell to remain steady aside from the following single factors: What is the effect on relative kinase activity if:

- Talin density *increases* (e.g. by additional expression)? Explain.
- Matrix density *increases* (e.g. by cellular secretion)? Explain.
- Integrin density *increases* (e.g. by additional expression)? Explain.
- Substrate stiffness *decreases* (e.g. through proteolytic activity)? Explain.

Figure 2A. *The mechanical properties of the ECM are sometimes modeled using a "viscoelastic" material model. One such model is an elastic "spring" (in blue) in series with a "dashpot" (in red) – the so called "Maxwell material model".*

The spring can absorb a displacement (it will instantaneously elongate under an applied force, according to Hooks law:

$$\Delta F = k\Delta d\mathrm{F}$$

and storing the work done on it

$$\Delta W = F\Delta d$$

for eventual release. However, the dashpot represents an energy dissipation element, whereby fluid in the matrix may be squeezed through the matrix, with drag (friction) forces that lead to a loss of stored energy that is releasing as heat. For instance, as you run up the stairs, the cartilage in your knees will begin to warm by as much as 3 °C. This heat is eventually taken up by the synovial fluid in your knees and transferred to other tissues. The rate at which stored energy is lost depends on how quickly the fluid in the matrix is squeezed through pores in the matrix.

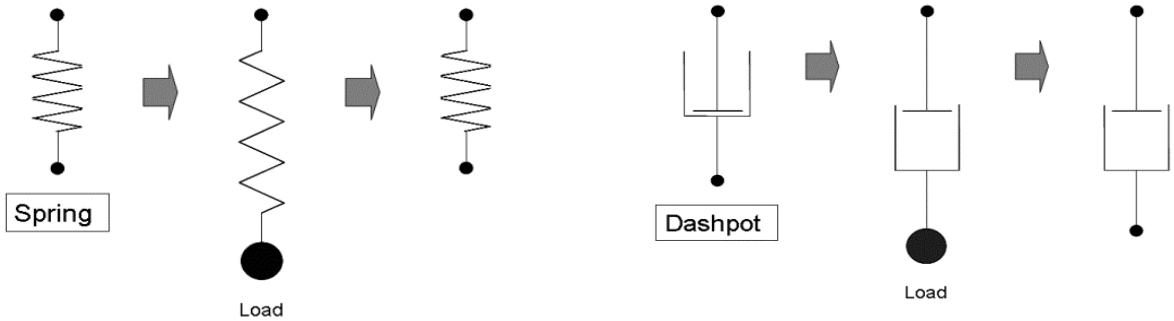
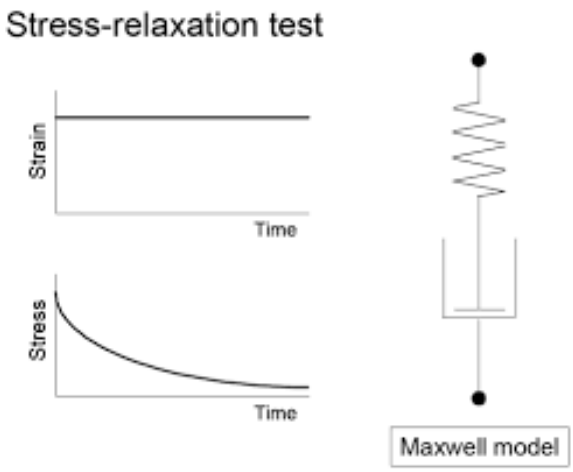


Figure 2B: *(*left*) "elastic" response: a loaded spring instantaneously deforms under load, and instantaneously returns to its original shape as soon as load is removed.*
*(*right*) a loaded dashpot continually deforms under load (the rate depends on the viscosity of the fluid inside the dashpot), and then retains its deformed shape after load is removed.*

Questions on Figure 2:



- Explain the observed behavior of the "stress relaxation test" shown in the strain-time and stress-time curves above. (strain is displacement normalized to length of the system; stress is force normalized to cross-section of the system);
- Make a sketch of these two curves that illustrates the difference between a highly viscous fluid vs a low viscosity fluid (assume an equivalent spring)
- Make a sketch of these two curves that illustrates the difference between a high stiffness vs low stiffness spring.

Questions combining concepts of figure 1 (biology) & 2 (mechanical models):

- Assuming a sudden onset of cellular traction with then constant forces provided by the actin-myosin machinery, draw the **force vs. time** curves for forces at a single focal adhesion for the following cases:
    - matrix is very dense vs. very porous; in both cases assume a normal interstitial fluid (mostly water, ions, and soluble factors)
    - fluid in the matrix is of normal viscosity (healthy interstitial fluid), versus inflammatory (containing proteins that confer low viscosity properties).

- Assuming a matrix with viscoelastic mechanical properties, and assuming a sudden force onset driven by constant myosin activity:
    - Describe the relative changes (as a function of time) of:
        - the integrin-ECM binding site locations with respect to the cell center
        - the structural "conformation" (shape) of talin
        - kinase activity

- Assuming that the cell will attempt to maintain constant cell contractility, and that kinase activity is required to maintain myosin activity, how do you suspect a cell reacts to a time-dependent displacement of the viscoelastic substrate?