

Using force to visualize conformational activation of integrins

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The development of biophysical approaches to analyze integrin–ligand binding allows us to visualize in real time the conformational changes that shift the bond affinity between low- and high-affinity states. In this issue, Chen et al. (2012. *J. Cell Biol.* <http://dx.doi.org/jcb.201201091>) use these approaches to validate some aspects of the classical integrin regulation model; however, their data suggest that much of the regulation occurs after ligand binding rather than in preparation for ligand binding to occur.

Cell adhesion is a critical development that spurred the evolution of metazoans and is integrated into virtually all physiological functions, from energy and metabolism to movement and defense against invasive organisms. Adhesion receptors share with other cell surface receptors, such as the tyrosine kinase growth factor or G-protein–coupled receptors, the ability to transmit extracellular signals into cells (Menko and Boettiger, 1987). However, their primary function is mechanical and their signaling function appears to devolve from their adhesive function (Friedland et al., 2009). The mechanical function of adhesion receptors involves both the number of bound receptors and their spatial distribution on the cells. The strength of adhesion is determined primarily by the number of adhesive bonds (bonds between cell surface adhesion receptors and cell or extracellular matrix–bound ligands). Because cells need to move and change shape, they need to vary the number and positions of their adhesive bonds. This requires the cells to control the binding and unbinding of adhesion receptors. To accomplish this regulation, it is necessary to modulate the affinity of the binding reaction. The classical way to modulate binding affinity is through allosteric regulation in which the binding of a ligand to one domain on the receptor changes its conformation and modulates the binding of another ligand to another domain. This is the basis of the classical model for the regulation of the best understood of the adhesion receptor families, the integrins (Ye et al., 2010). More recently, another way to change the affinity of integrin–ligand bonds has been discovered. Because integrins that are physically bound to the substrate are also bound, through focal complexes inside the cell, to the actin cytoskeleton (Pavalko et al., 1991), intracellular actin-myosin contraction can exert tension on the integrin–ligand bond (Friedland et al., 2009). Tension will change the integrin conformation (by force) and change the integrin–ligand binding affinity (Kong et al., 2009).

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For most chemical bonds, tension reduces bond lifetime and increases the dissociation rate (these bonds are called “slip bonds”); but for integrin–ligand bonds, tension stabilizes the bond and increases the bond lifetime (these bonds are called “catch bonds”). In this issue of JCB, Chen et al. present a novel approach that allows us to visualize both the conformational switching of integrins and switching between short and long bond lifetimes. Their analysis brings together the classical and the catch bond models of regulation and may change our perception of how adhesive bonds are regulated.

The classical model for integrin regulation is a three-state model: inactive, active, and active/bound to ligand. Integrin activation is based on the interconversion between the inactive and the active state (Frelinger et al., 1991; Ye et al., 2010). The regulation is fundamentally allosteric, in which the final common step involves the binding of talin and/or kindlin to the cytoplasmic domain of the β subunit of integrin, causing a separation of the α and β subunit cytoplasmic domains. This generates an allosteric change that is propagated to the extracellular domain, resulting in a conversion from the low- to the high-affinity state that is primed to bind to ligand. In the x-ray diffraction structure of integrin extracellular domains, the overall structure is bent but can be converted by reasonable calculations to an extended form (Xiong et al., 2001). It was proposed that the bent form represented the inactive and the extended form represented the active form of integrin (Takagi et al., 2002). Thus, integrin activation would generate a 15–20-nm shift in the ligand-binding domain (α A domain) away from the plasma membrane (Fig. 1). Over the past 20 or more years, the classical model has been developed in significant molecular detail. However, these analyses have generally followed a biochemical bias and have been relatively blind both to the analysis of integrin dissociation (which is difficult to analyze biochemically in cells with many adhesive bonds) and to the role of mechanics and forces in the regulation of integrin function.

To understand how Chen et al. (2012) visualized and analyzed the binding properties of integrin using biophysical approaches, it is necessary to describe their basic experimental strategy. The authors used a Bioforce probe that consists of two micropipettes, one holding the cell expressing the integrin, the other holding a red blood cell (RBC) to which is attached a bead

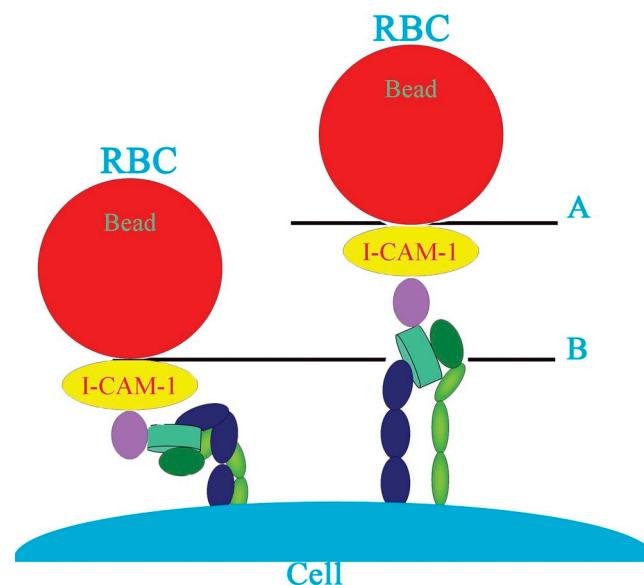


Figure 1. Measuring integrin conformational transitions using the Bioforce probe. Bonds between the αA domain (purple) of integrin $\alpha\beta 2$ and its ligand I-CAM-1 attached to a bead are formed by bringing the two into contact. Bonds can form with either the bent conformation (left) or the extended conformation (right). Bonds formed in the bent conformation can switch to the extended conformation without dissociation. This would increase bond stability (and hence affinity by slowing the dissociation rate). Bonds formed in the extended form can switch to the bent form without dissociation, but this will reduce their stability and increase the dissociation rate. The conformational switches are followed by the position of the bead. Lines A and B mark the displacement between the two conformations. The RBC (top) and the cell (bottom) would be attached to the Bioforce probe micropipettes.

coated with the ligand (see Video 1 in Chen et al., 2012). A video camera monitors the position of the bead with high precision (3 nm). A micromanipulator moves the cell micropipette until the cell touches the bead (with a force of 20 pN for 100 ms) and then is retracted a set distance and held. The objective of this is to allow a single integrin–ligand bond to form; the retraction prevents additional bonds from forming. If a bond forms, the bead will follow the retraction because it is attached through a ligand to a cell surface integrin. The RBC, which acts as a spring, will be stretched. After a time, the bond will dissociate and the RBC will retract the bead. This allows the measurement of the lifetime of single integrin–ligand bonds. The new insight comes when the movement of the bead is followed during the lifetime of the bond. The force tracings show two distinct events: a displacement away from the cell membrane and a reciprocal displacement toward the cell membrane. The mean magnitude of these displacements was similar to that predicted from the x-ray diffraction data for the bent and extended forms of the integrin (~ 17 nm). This interpretation was reinforced through the measurement of bond stiffness. More variation in the displacement of the bead indicated a weaker bond when it was in the bent state and a shorter bond lifetime for the bond in the bent state, which also indicates a weaker bond. Thus, force differences (the displacement of the bead held by the RBC/spring) allow us to see integrin conformational shifts in real time.

The Bioforce probe has allowed us to observe movements of single molecular domains, which, remarkably, correspond to movements predicted in the classical model for integrin

activation. Because those experiments were performed using intact cells, they provide strong evidence for the existence of both the extended and bent conformations on the cell surface and for the generation of increased affinity by integrin extension. In the classical model for integrin activation, the focus has been on the observed conformational shift between extended and bent forms that can occur with purified integrins (Ye et al., 2010). This switch is generally observed with the integrin in an unbound state. The Bioforce probe sees the other side of the coin. Binding to the ligand occurred to either the bent (inactive) or extended (active) form, and the switching between the two states occurred while the ligand was bound. This distinction is important because each model points to different control mechanisms. The classical model points to a regulation of the binding rate to the ligand, which is governed by the energy of activation, the collision frequency, and the frequency in which collisions lead to bond formation. The Bioforce probe analysis points to mechanisms that affect the rate of dissociation, which involves stability of the bond and can be modulated by force as well as chemistry. The biochemical bias of methods that support the classical model are not adept at analyzing the postbinding changes in bond stability. In contrast, Bioforce probe experiments use direct physical manipulation to form the bond, and hence the natural events of bond formation are not observable. The analysis contains the elements that affect bond dissociation but are missing elements of bond association events. In each case, the experimental analysis biases the conclusion. Because the classical and the Bioforce probe approaches complement each other, we have a better basis for generating a more accurate model of integrin regulation.

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References

- Chen, W., J. Lou, E.A. Evans, and C. Zhu. 2012. Observing force-regulated conformational changes and ligand dissociation from a single integrin on cells. *J. Cell Biol.* 199:497–512. <http://dx.doi.org/10.1083/jcb.201201091>
- Frelinger, A.L. III, X.P. Du, E.F. Plow, and M.H. Ginsberg. 1991. Monoclonal antibodies to ligand-occupied conformers of integrin alpha IIb beta 3 (glycoprotein IIb-IIIa) alter receptor affinity, specificity, and function. *J. Biol. Chem.* 266:17106–17111.
- Friedland, J.C., M.H. Lee, and D. Boettiger. 2009. Mechanically activated integrin switch controls alpha β 1 function. *Science*. 323:642–644. <http://dx.doi.org/10.1126/science.1168441>
- Kong, F., A.J. García, A.P. Mould, M.J. Humphries, and C. Zhu. 2009. Demonstration of catch bonds between an integrin and its ligand. *J. Cell Biol.* 185:1275–1284. <http://dx.doi.org/10.1083/jcb.200810002>
- Menko, A.S., and D. Boettiger. 1987. Occupation of the extracellular matrix receptor, integrin, is a control point for myogenic differentiation. *Cell*. 51:51–57. [http://dx.doi.org/10.1016/0092-8674\(87\)90009-2](http://dx.doi.org/10.1016/0092-8674(87)90009-2)
- Pavalko, F.M., C.A. Otey, K.O. Simon, and K. Burridge. 1991. α -actinin: a direct link between actin and integrins. *Biochem. Soc. Trans.* 19:1065–1069.
- Takagi, J., B.M. Petre, T. Walz, and T.A. Springer. 2002. Global conformational rearrangements in integrin extracellular domains in outside-in and inside-out signaling. *Cell*. 110:599–511. [http://dx.doi.org/10.1016/S0092-8674\(02\)00935-2](http://dx.doi.org/10.1016/S0092-8674(02)00935-2)
- Xiong, J.P., T. Stehle, B. Diefenbach, R. Zhang, R. Dunker, D.L. Scott, A. Joachimiak, S.L. Goodman, and M.A. Arnalout. 2001. Crystal structure of the extracellular segment of integrin alpha Vbeta3. *Science*. 294:339–345. <http://dx.doi.org/10.1126/science.1064535>
- Ye, F., G. Hu, D. Taylor, B. Ratnikov, A.A. Bobkov, M.A. McLean, S.G. Sligar, K.A. Taylor, and M.H. Ginsberg. 2010. Recreation of the terminal events in physiological integrin activation. *J. Cell Biol.* 188:157–173. <http://dx.doi.org/10.1083/jcb.200908045>