



ELSEVIER

# Nanomechanics of adhesion proteins

Deborah Leckband

Recent advances in molecular force measurements have resulted in the quantification of the nanomechanical properties of single molecular bonds, and elucidated novel relationships between molecular architecture and biomolecular adhesion. The measured forces to rupture single intermolecular bonds revealed novel and unexpected ways that proteins respond to mechanical force. Measurement of the magnitude of interprotein forces and the distances over which they act further determined how protein architecture may contribute to both the stability and structural organization of adhesive junctions.

## Addresses

Department of Chemical and Biomolecular Engineering, and Department of Chemistry, Center for Biophysics and Computational Biology, University of Illinois at Urbana-Champaign, 600 South Mathews Avenue, Urbana, Illinois 61801, USA  
e-mail: leckband@uiuc.edu

**Current Opinion in Structural Biology** 2004, 14:524–530

This review comes from a themed issue on  
Biophysical methods  
Edited by Arthur G Palmer III and Randy J Read

Available online 15th September 2004

0959-440X/\$ – see front matter  
© 2004 Elsevier Ltd. All rights reserved.

DOI 10.1016/j.sbi.2004.09.002

## Abbreviations

<b>AFM</b>	atomic force microscope
<b>APC</b>	antigen-presenting cell
<b>BFP</b>	BioForce Probe
<b>CAM</b>	cell adhesion molecule
<b>FN</b>	fibronectin
<b>Ig</b>	immunoglobulin
<b>MHC</b>	major histocompatibility complex
<b>NCAM</b>	neural cell adhesion molecule
<b>NTA</b>	nitro-tri-acetic acid
<b>PSGL-1</b>	P-selectin glycoprotein ligand 1
<b>SFA</b>	surface force apparatus
<b>TCR</b>	thymus cell receptor

## Introduction

Molecular force probes are powerful tools for investigating the nanomechanical properties of cell adhesion molecules (CAMs) [1]. Force measurements reveal the links between chemistry and adhesion [2,3], and between structure and molecular mechanics, and probe macromolecular unfolding [4,5]. This review highlights recent force measurements of the mechanical properties of CAMs. These investigations elucidated new binding

mechanisms, structural models of CAM adhesion and novel responses to mechanical force.

## Principles of force measurement

Molecular forces can be quantified using a variety of approaches [1], including the surface force apparatus (SFA), the atomic force microscope (AFM), optical tweezers (OT) and the BioForce Probe (BFP). The instrumental details differ, but the principles of the force measurement, as outlined in Figure 1, are similar. (See also the Glossary of terms.)

## Atomic force microscope

The high absolute cantilever sensitivity ( $\Delta F \sim 10$  pN) of the AFM allows the measurement of single-molecule interactions between a small ( $R \sim 10$  nm) probe tip and a surface [1]. The AFM measures the relative cantilever displacement ( $\Delta D_c$ ; Figure 1) within  $\pm 1$  Å, but the absolute distance between the surfaces ( $D$ ) cannot be determined. This technique is especially well suited for measuring weak, short-range forces, such as bond strengths or molecular force-extension profiles, for which knowledge of the absolute distance between the surfaces and the local geometry is not critical.

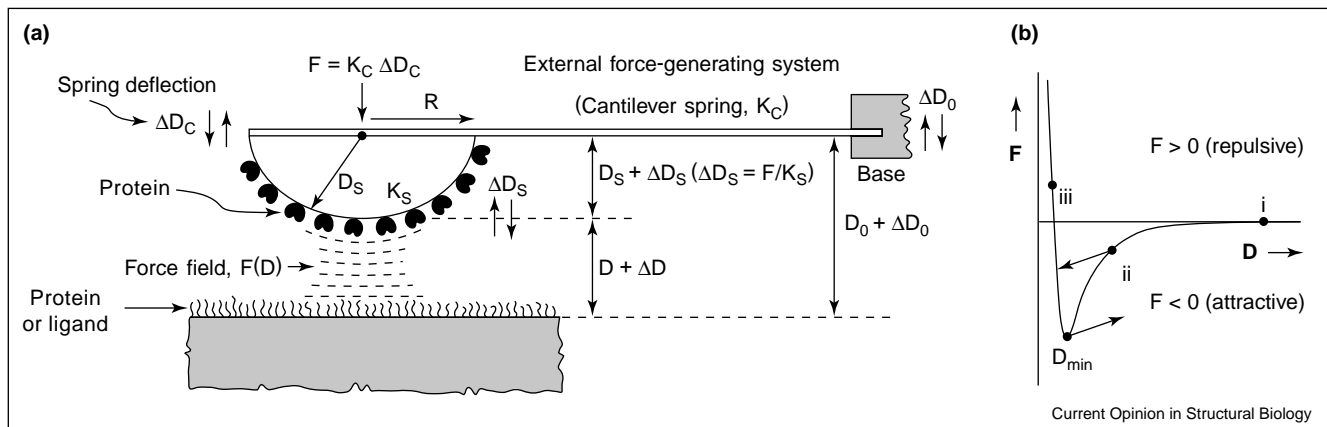
## Surface force apparatus

The SFA measures the force ( $F$ ) and absolute surface separation ( $D$ ) between macroscopic surfaces, as well as the local geometry ( $R$ ) [1]. The absolute intersurface distances ( $D$ ; c.f. Figure 1) are determined within  $\pm 1$  Å by interferometry. The SFA measures the force between macroscopic curved surfaces, where  $R = 1\text{--}2$  cm. A spring quantifies the total integrated force between the surfaces with an absolute force sensitivity ( $\Delta F$ ) of  $\pm 10$  nN. This sensitivity is lower than that of the AFM, but forces between macroscopic surfaces scale with the surface dimensions (e.g. with  $R$ ; c.f. Figure 1) [6]. The normalized force sensitivity of the SFA is  $\Delta F_c(D)/R = \pm 10^{-5}$  N/m<sup>2</sup>, whereas that of the AFM is  $10$  pN/10 nm  $\pm 10^{-3}$  N/m<sup>2</sup>. Thus, accurate normalized force-distance profiles can be measured between large surfaces without high absolute force sensitivity [1]. The SFA is therefore the method of choice for accurately quantifying force-distance profiles between surfaces.

## Functional significance of the modular cell adhesion molecule architecture

The molecular architectures of the extracellular regions of a large fraction of CAMs comprise multiple, tandemly repeated domains [7,8] (Figure 2). The atomic-level structures of only a few of these proteins have been determined, because of challenges inherent to structure

Figure 1



Principles of force measurement. **(a)** Schematic showing the sample geometry and parameters relevant to force measurement with the SFA and AFM. The curved probe is supported on a cantilever spring with force constant  $K_C$ . The cantilever deflection,  $\Delta D_C$ , quantifies the intersurface force at each separation. The movement of the external position control determines the probe displacement ( $\Delta D_0$ ) and  $\Delta D_C$ . The absolute surface separation ( $D$ ) is determined by  $\Delta D_0$ ,  $\Delta D_C$  and substrate (tip) deformations ( $\Delta D_S$ ). The AFM tips ( $R = 100\text{--}1000 \text{ \AA}$ ) are smaller than the SFA probe radius ( $R = 1\text{--}2 \text{ cm}$ ). **(b)** Hypothetical force-distance profile between two surfaces. At large distances (i), there is no intersurface force. Closer in, the force becomes attractive ( $F < 0$ ) and reaches its maximum value at the minimum in the curve ( $D_{\min}$ ). At (ii)  $D > D_{\min}$ , the gradient in the force exceeds the spring constant  $K_C$  and the surfaces jump into contact (arrow). The depth of the minimum, relative to  $F = 0$ , determines the intersurface adhesion. At  $D < D_{\min}$  (iii), the force increases due to a repulsive force ( $F > 0$ ). If  $D$  again increases, the force decreases to  $D_{\min}$  and the surfaces jump out of contact from  $D_{\min}$  (arrow) [1].

determination of large molecules. Of primary interest are the relationship between the modular architecture and adhesive function, the identities of the functional modules and atomic-level details of the adhesive interface(s). SFA measurements tested hypotheses concerning the regulation of intermembrane space by protein architecture. Studies focused on the binding of CD2 and CD48 (or CD58), which may play both structural and adhesive roles in organizing immunological synapses [9,10]. CD2 is expressed on T cells and its ligand, CD48 (in rats) or CD58 (in humans), is expressed on antigen-presenting cells (APCs) [11]. The extracellular regions of CD2 and CD48 comprise two tandemly linked immunoglobulin (Ig) domains, and are approximately 7.5 nm in total length (Figure 2).

Biochemical evidence suggests that CD2 binds its ligands in a head-to-head configuration spanning  $\sim 13.5 \text{ nm}$  (Figure 3a) [11,12]. Importantly, this  $\sim 13.5 \text{ nm}$  gap

matches the dimensions of the complex between major histocompatibility complex (MHC) and thymus cell receptor (TCR), which triggers the immune response. CD2 is postulated to control intermembrane spacing,

Figure 2

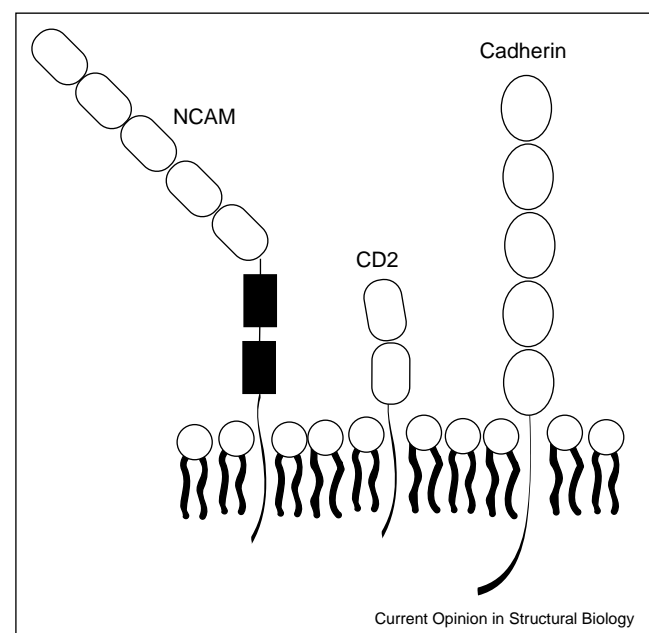
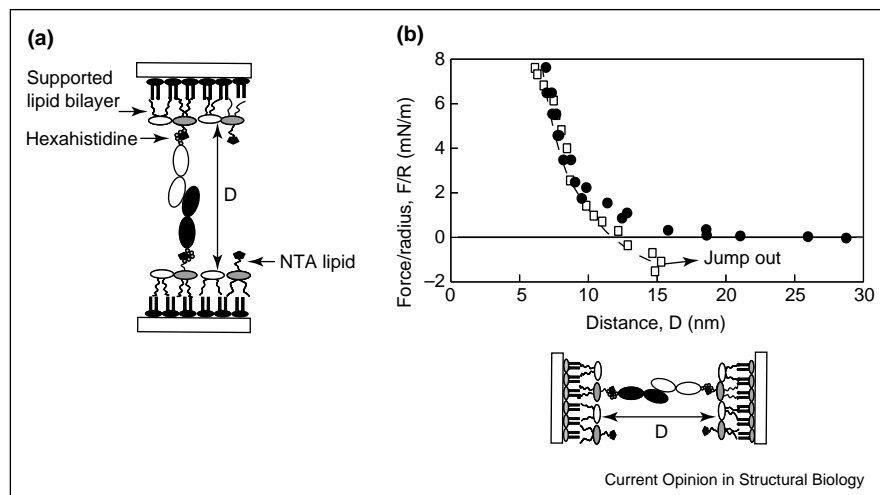


Illustration of the modular architecture of adhesion proteins. The circles represent cadherin domains, ellipses represent Ig-type domains and the rectangles are FN type III domains.

#### Glossary of terms

<b>D</b>	absolute distance between surfaces
<b><math>D_{\min}</math></b>	position of the minimum in the force curve
<b><math>\Delta D_C</math></b>	deflection in the cantilever spring
<b><math>\Delta D_S</math></b>	deformation in a soft substrate or probe tip
<b><math>E_f</math></b>	energy per area between two parallel flat surfaces
<b>F</b>	force
<b><math>F_c</math></b>	force between curved surfaces
<b><math>K_C</math></b>	bending modulus of the 'cantilever' spring
<b><math>K_S</math></b>	bending modulus of a soft deformable substrate
<b>R</b>	radius of curved or spherical probe tip

Figure 3



Molecular mechanism of CD2-CD48 adhesion. **(a)** Proposed CD2-CD48 binding configuration (ellipses represent Ig domains). **(b)** Normalized force-distance profile between supported bilayers displaying CD2 and CD48. The proteins are immobilized via hexahistidine binding to NTA-modified lipids. Upon approach (filled circles), the proteins repel ( $F > 0$ ) at  $D < 16$  nm. Upon separation (open squares), the proteins adhere ( $F < 0$ ) and the bonds fail at  $D_{\min} = 15.3 \pm 0.5$  nm (arrow).

thereby correctly positioning the opposing MHC and TCR proteins [11]. The dimensions of the complex are also of more general interest because protein size segregation may facilitate protein organization at adhesive junctions [9,10,13\*\*].

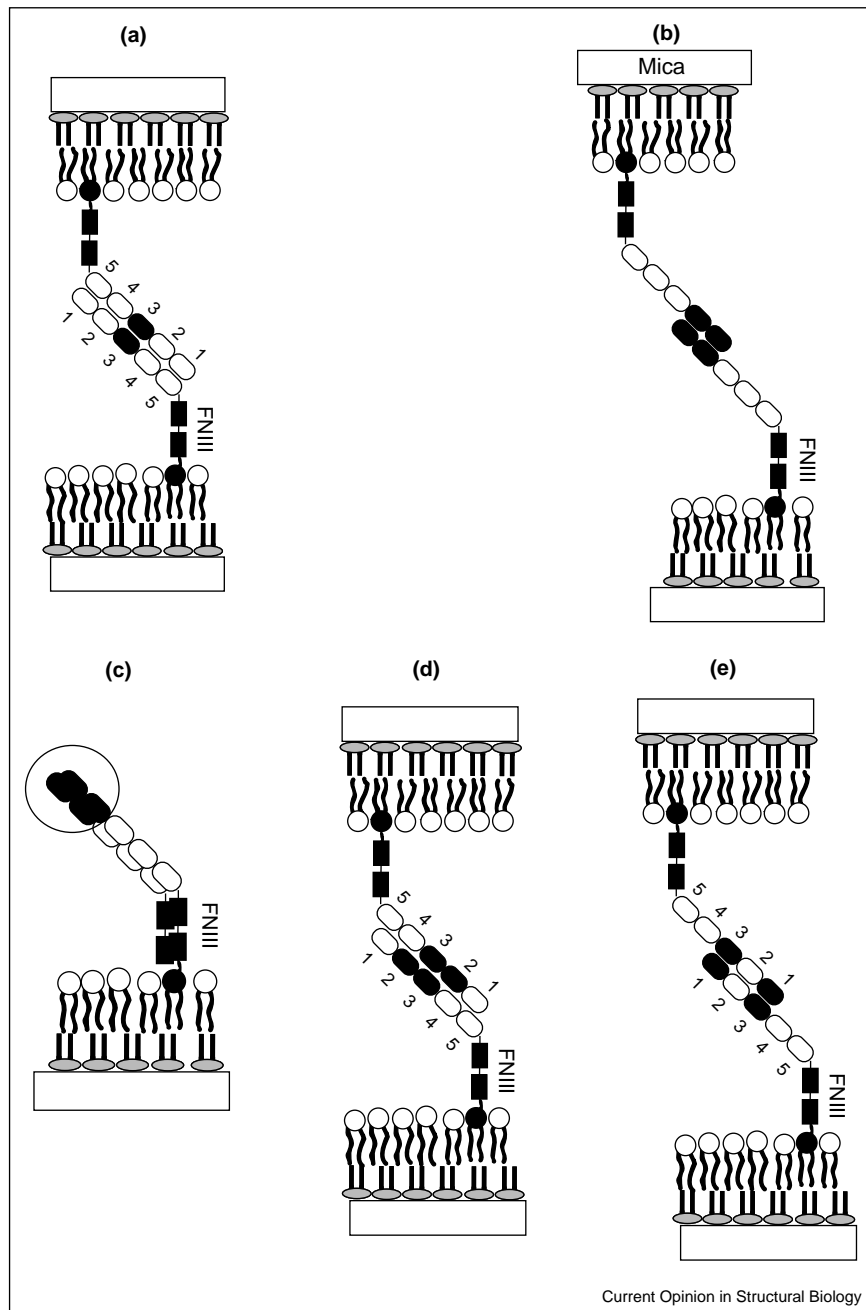
Force measurements between CD2 and CD48 on opposite membranes (Figure 3a) confirmed their head-to-head binding configuration [14\*\*]. In normalized force-distance profiles (Figure 3b), the repulsive force ( $F > 0$ ) at  $D < 16$  nm is due to steric repulsion between the proteins [14\*\*]. Upon separation, the proteins adhere ( $F < 0$ ) and bond failure occurs at the minimum in the force curve at a membrane separation of  $D_{\min} = 15.3 \pm 0.5$  nm [14\*\*]. The end-to-end complex length, defined by the position of bond failure, is  $15.3 - (2 \times 1 \text{ nm}) = 13.3$  nm, accounting for the  $\sim 1$  nm NTA (nitrilo-tri-acetic acid) anchors (Figure 3) [15]. This directly measured 13.3 nm distance agrees quantitatively with the predicted complex size [11] and is direct evidence of head-to-head binding between these proteins. These measurements also quantitatively confirmed that the CD2-CD48 and TCR-MHC complexes are dimensionally matched.

The ectodomains of many CAMs exhibit more complicated structures than the CD2 family [7,8]. This makes the identification of functional domains and binding modes difficult using standard biochemical and structural analyses. For example, the neural cell adhesion molecule (NCAM) is a multidomain member of the Ig superfamily that binds to NCAM on adjacent cells [8]. Its extracellular region contains five Ig domains and two fibronectin (FN) type III domain repeats [7] (Figure 2). There is a distinct

bend between the fifth Ig domain (Ig5) and the FN domains [16,17] (Figure 2). Three different models of NCAM adhesion are based on biochemical [18–20] and structural data [21,22,23\*]. In model 1 (Figure 4a), the five Ig domains overlap completely and isologous Ig3 contacts form the principal adhesive interface [18,19]. In model 2 (Figure 4b) [21,22], Ig domains 1 and 2 from each NCAM form antiparallel, double reciprocal bonds. Model 3 (Figure 4c–e) is based on the structure of the fragment comprising Ig domains 1–3 [23\*]. Here, Ig domains 1 and 2 are proposed to form lateral bonds. Two putative adhesive bonds involve antiparallel contacts between Ig2 and Ig3 (Figure 4d), and between Ig1 and Ig3 (Figure 4e) [23\*]. Each of these models predicts the formation of different, spatially distinct NCAM complexes. Given the accuracy of force-distance profiles measured with the SFA, this approach was used to discriminate between these three possible binding mechanisms [24\*\*].

In normalized force-distance profiles between NCAM Ig domains 1–5 (Figure 5a) [24\*\*], the fragments formed two bound states at membrane separations of  $D_{\min} = 18 \pm 0.5$  nm and  $29 \pm 0.5$  nm. The bond at  $18 \pm 0.5$  nm corresponds to model 1 (Figure 4a), in which adherent Ig1–Ig5 segments overlap fully. The position of the second bond at  $29 \pm 0.5$  nm agrees quantitatively with model 2 (Figure 4b). The full NCAM ectodomain also formed two bound states (Figure 5b), although the binding distances were shifted outward due to the additional FN domains and the bend at the FN–Ig junction [16,17]. These data support models 1 and 2, but contradict model 3.

Figure 4

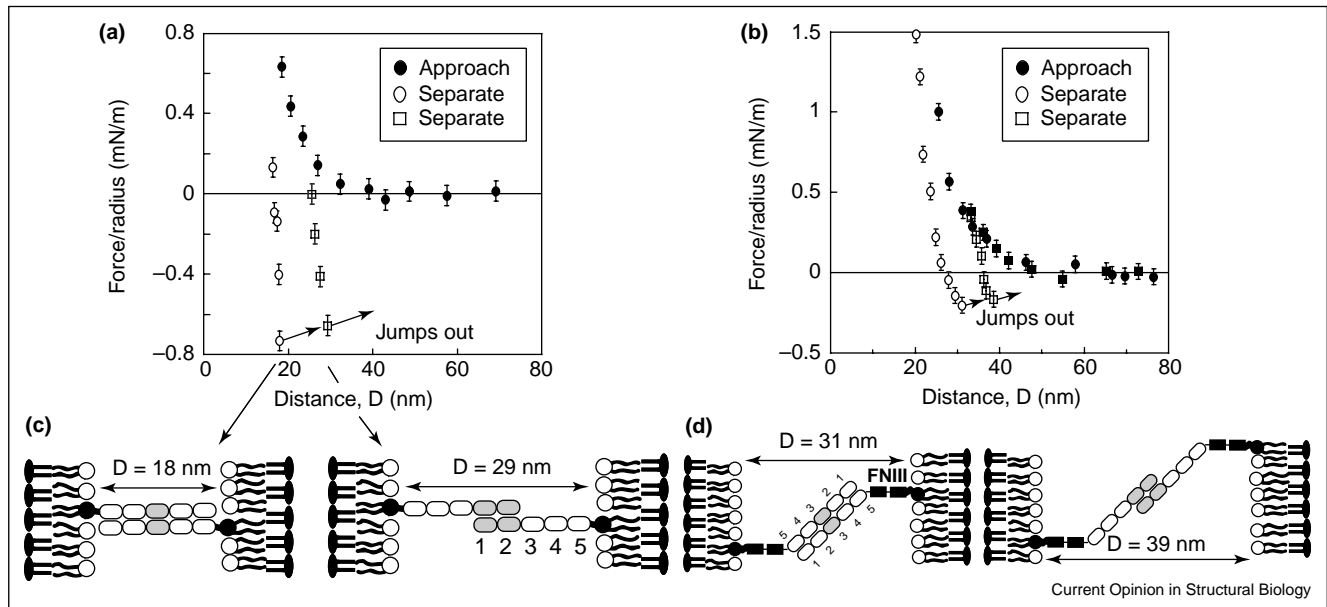


Models of NCAM adhesion. **(a)** In model 1, NCAM binds with its five Ig domains in an antiparallel configuration, mediated primarily by Ig3-Ig3 bonds. **(b)** In model 2, NCAM adheres via double reciprocal bonds between Ig domains 1 and 2. In model 3, the outer Ig domains 1 and 2 form lateral bonds **(c)**, and both antiparallel Ig2-Ig3 **(d)** and antiparallel Ig1-Ig3 **(e)** form adhesive bonds.

The interpretation of all force measurements in terms of protein structure benefits substantially from measurements with protein mutants [2<sup>••</sup>,5<sup>•</sup>,25<sup>•</sup>,26]. The adhesive domains of NCAM were thus identified from changes in the force-distance curves upon removing individual Ig domains [24<sup>••</sup>]. Johnson *et al.* [24<sup>••</sup>] thereby confirmed

that the inner bond requires Ig3, and the outer bond involves Ig1 and Ig2 (Figure 5c,d). Independent equilibrium binding measurements with the fragments further validated the proposed domain interactions (Figure 5c,d). Together, these data support the binding mechanism shown in Figure 5d.

Figure 5



Mechanism of NCAM adhesion. **(a)** Normalized force-distance profile between membranes displaying NCAM Ig1-Ig5 fragments. The surfaces repel ( $F > 0$ ) at  $D < 37$  nm (closed circles). Upon separation from  $D < 18$  nm (open circles), the proteins adhere ( $F < 0$ ), with  $D_{\min} = 18$  nm. At distances  $19$  nm  $< D < 29$  nm, the proteins bind at 29 nm (open squares). **(b)** Normalized force-distance profile between full-length NCAM. The proteins bind at  $D_{\min} = 31$  nm and 39 nm. **(c,d)** Proposed NCAM domain interactions.

### Cell adhesion molecule unfolding under force?

A proposed consequence of the multidomain architecture of CAMs is that individual domains may unfold under force and impart elasticity to adhesive junctions, as titin does in muscle [5<sup>\*</sup>]. Reported Ig domain unfolding forces typically exceed the strengths of CAM bonds [2<sup>\*\*</sup>,3,5<sup>\*</sup>]. However, protein elasticity depends on both the domain sequence and the direction of force relative to the protein axis [4<sup>\*\*</sup>,5<sup>\*</sup>]. The Ig domains of melanoma cell adhesion molecule (MelCAM), for example, do unfold at forces  $< 100$  pN [27]. Some CAM sequences may thus facilitate unfolding under weak, adhesive forces.

In SFA investigations of CD2-CD48, NCAM and cadherin, interprotein bonds abruptly ruptured at unique distances consistent with the structures of the intact proteins [14<sup>\*\*</sup>,24<sup>\*\*</sup>,25<sup>\*</sup>]. There was no apparent unfolding and measured bond strengths were low. Simulations further showed that CD2 adhesive bonds fail before the Ig domains unfold [28<sup>\*</sup>]. Although protein unfolding may indeed contribute to the mechanical properties of adhesive junctions, this has not yet been demonstrated.

### Bond mechanics depend on the force history

Intuitively, force causes bond rupture and larger forces increase bond failure rates. This is characteristic of 'slip bonds'. However, 'catch bonds' strengthen under force,

so that the failure rate decreases with increasing force. Although catch bonds were predicted, their existence was controversial until recently [2<sup>\*\*</sup>]. Marshall *et al.* sought to resolve apparent inconsistencies in the reported lifetimes of selectin-PSGL-1 (P-selectin glycoprotein ligand 1) bonds measured by single bond pulling versus rupture by shear [2<sup>\*\*</sup>]. One difference between these approaches is that pulling measurements, as typically conducted with the AFM, increase the applied force at a constant rate until the bond fails. The dependence of the rupture force on the loading rate gives the bond lifetime [29]. By contrast, in flow chamber measurements, the shear stress on the bond instantly reaches a certain value and the lifetime is measured as a function of the (constant) shear stress [1]. These force histories differ, that is, the bond experiences a force ramp in the former case and a constant force in the latter.

Marshall *et al.* [2<sup>\*\*</sup>] successfully compared lifetime measurements from AFM and flow chamber assays, modifying the AFM measurements to mimic shear assays. They measured the bond lifetime under constant force, following an initial rapid jump in the applied force. Their measurements showed that, at low forces, the selectin-PSGL1 bond lifetime increases with force, as expected for catch bonds, whereas at high forces, it decreases with force, typical of slip bonds. The bond mechanics therefore depend on the force history. Furthermore, catch bond behavior is only observed at  $< 20$ - $50$  pN, which is

below the forces investigated in prior AFM and flow chamber studies [2\*\*].

## Conclusions

These examples illustrate the increasing prominence of mechanical measurements as powerful tools for obtaining unique information that is central to understanding adhesion protein function. The insights gained from such studies are often difficult to obtain by standard biochemical methods. More generally, the resulting discovery of physical principles relating chemistry to nanomechanics continues to open up new approaches to investigating fundamental principles of protein structure and function.

## Update

A recent report [30\*\*] has now shown that the transition between the catch bond and slip bond behavior of the selectin-PSGL-1 bond results from mechanically induced switching between two kinetic dissociation pathways. Although this study used the BFP, the physics of the measurement is similar to that of the AFM pulling experiment. When the selectin-ligand bonds are loaded at rates between 300 and 30 000 pN/s, the linear increase in the strength of the selectin bond with the loading rate indicates that bond rupture occurs via a single pathway. However, at loading rates <300 pN/s, the bond strength falls abruptly, suggesting that bond failure follows a different kinetic pathway. However, if the bonds are first tugged by an abrupt 'jump' in force to 20–30 pN, they then become much stronger, even when subjected to loading rates <300 pN/s. This behavior suggests that the selectin-PSGL-1 bond acts as a mechanochemical switch, whereby the force history causes the protein to select between two different kinetic pathways that each have different mechanical characteristics. The detection of this mechanical switching was enabled by a new 'jump/ramp' mode of force measurement, in which the application of an abrupt force jump is followed by a slower force ramp leading to bond rupture.

## References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Leckband D, Israelachvili JN: **Intermolecular forces in biology.** *Q Rev Biophys* 2001, **34**:105-267.
2. Marshall BT, Long M, Piper JW, Yago T, McEver RP, Zhu C: **Direct observation of catch bonds involving cell-adhesion molecules.** *Nature* 2003, **423**:190-193.  
This study used different but complementary single-molecule force measurement techniques to show that the mechanical response of selectin-PSGL-1 bonds depends on the force history. To compare the bond characteristics determined by AFM and flow chamber assays, they modified the way they determined the bond lifetimes from AFM measurements. With this new approach, which mimics the flow chamber measurements, they showed that the lifetimes of the selectin-PSGL-1 linkages increase at low forces (<50 pN), but then decrease at high forces (>50 pN). The linkage thus exhibits biphasic rupture behavior, behaving as a catch bond at low force and a slip bond at high force. The bond mechanics clearly depend on the force history. From these results, they then predicted the lifetimes of bonds subjected to low shear stresses in subsequent flow chamber assays.
3. Evans E, Leung A, Hammer D, Simon S: **Chemically distinct transition states govern rapid dissociation of single L-selectin bonds under force.** *Proc Natl Acad Sci USA* 2001, **98**:3784-3789.
4. Carrion-Vasquez M, Li HB, Lu H, Marszalek PE, Oberhauser AF, **Fernandez JM: The mechanical stability of ubiquitin is linkage dependent.** *Nat Struct Biol* 2003, **10**:738-743.  
This study determined whether the attachment points at which force is applied to a protein determine the unfolding trajectory and hence the apparent mechanical stability of the protein. In most studies, including [5\*,27], the proteins are pulled from the N and C termini. The force vector, or the direction of the gradient in the applied mechanical potential, biases the unfolding trajectory; the activation barrier along that trajectory determines the unfolding rate and hence the domain stability. However, protein folding or unfolding in solution may proceed by several pathways, many of which could have lower activation barriers than those probed in the mechanical measurement. These authors tested this idea by changing the ubiquitin attachment points in the pulling experiment. They showed that the unfolding force was lower relative to that measured when ubiquitin was pulled along the N to C axis. They thus demonstrated that the mechanical stability (or unfolding rate) of a protein also depends on how the force is applied to the molecule.
5. Li HB, Linke WA, Oberhauser AF, Carrion-Vasquez M, Kerkvliet JG, Lu H, Marszalek PE, Fernandez JM: **Reverse engineering of the giant muscle protein titin.** *Nature* 2002, **418**:998-1002.  
In this paper, the authors showed that the elasticity of the giant muscle protein titin is determined by the composition of elastic Ig domains within the protein structure. The authors quantified the forces required to rupture the different types of Ig domains within titin and showed that the unfolding forces range from ~120 pN to ~300 pN. Some domains exhibit intermediate states in the unfolding profile. The combined properties of these different structural elements in turn determine the macroscopically observed mechanical behavior of this protein.
6. Israelachvili J: *Intermolecular and Surface Forces.* Academic Press: New York; 1991.
7. Chothia C, Jones EY: **The molecular structure of cell adhesion molecules.** *Annu Rev Biochem* 1997, **66**:823-862.
8. Walsh F, Doherty P: **Neural cell adhesion molecules of the immunoglobulin superfamily.** *Annu Rev Cell Dev Biol* 1997, **13**:425-456.
9. Lee SJE, Hori Y, Groves JT, Dustin ML, Chakraborty AK: **The synapse assembly model.** *Trends Immunol* 2002, **23**:500-502.  
This is an extensive overview of the biochemical and physical parameters underlying the formation of the immunological synapse, the specialized junction between T cells and APCs that is distinguished by the formation of distinct protein patterns at the intercellular junction. The synapse assembly model predicts experimentally observed behavior. Of particular relevance to this review, the authors identify the dimensions of the adhesive protein complexes as a principal parameter governing synapse formation.
10. Grakoui A, Bromley SK, Sumen C, Davis MM, Shaw AS, Allen PM, Dustin ML: **The immunological synapse: a molecular machine controlling T-cell activation.** *Science* 1999, **285**:221-226.
11. Davis SJ, van der Merwe PA: **The structure and ligand interactions of CD2: implications for T-cell function.** *Immunol Today* 1996, **17**:177-187.
12. Wang J, Smolyar A, Tan K, Liu J, Kim M, Sun ZJ, Wagner G, Reinherz EL: **Structure of a heterophilic adhesion complex between the human CD2 and CD58 (LFA-3) counter-receptors.** *Cell* 1999, **97**:791-803.
13. Davis SJ, Ikemizu S, Evans EJ, Fugger L, Bakker TR, **van der Merwe PA: The nature of molecular recognition by T-cells.** *Nat Immunol* 2003, **4**:217-224.  
This excellent overview of biochemical and biophysical aspects of T-cell recognition and activation describes the range of proteins that mediate T-cell recognition. The focus is on the structure and binding properties of the different proteins that mediate adhesion between T cells and APCs. In particular, they elaborate on the role of molecular architecture and protein size segregation in T-cell recognition and activation.
14. Zhu B, Davies EA, van der Merwe A, Leckband D: **Direct measurements of heterotypic adhesion between the cell**

**adhesion proteins CD2 and CD48.** *Biochemistry* 2002, **41**:12163-12170.

Direct force measurements of the distance-dependent interactions between CD2 and CD48 showed that these proteins bind in the predicted head-to-head orientation. The dimension of the complex determined by force measurement agreed quantitatively with the predicted value. The data further showed that the size of the CD2-CD48 complex is quantitatively matched to that of the TCR-MHC complex, which the CD2-CD48 complex must accommodate at adhesive junctions. This finding supports the dual adhesive and scaffolding role of CD2 and its ligand, CD48.

15. Martel L, Johnson C, Boutet S, Al-Kurdi R, Kononov O, Robinson I, Leckband D, Legrand J-F: **X-ray reflectivity investigations of two-dimensional assemblies of C-cadherin: first steps in structural and functional studies.** *J Phys IV* 2002, **12**:365-377.
16. Becker JW, Erickson HP, Hoffmann S, Cunningham BA, Edelman GM: **Topology of cell adhesion molecules.** *Proc Natl Acad Sci USA* 1989, **86**:1088-1092.
17. Hall AK, Rutishauser U: **Visualization of neural cell adhesion molecule by electron microscopy.** *J Cell Biol* 1987, **104**:1579-1586.
18. Ranheim TS, Edelman GM, Cunningham BA: **Homophilic adhesion mediated by the neural cell adhesion molecule involves multiple immunoglobulin domains.** *Proc Natl Acad Sci USA* 1996, **93**:4071-4075.
19. Rao Y, Wu X-F, Garipey J, Rutishauser U, Siu C-H: **Identification of a peptide sequence involved in homophilic binding in the neural cell adhesion molecule NCAM.** *J Cell Biol* 1992, **118**:937-949.
20. Kiselyov V, Berezin V, Maar TE, Soroka V, Edvardsen K, Schousboe A, Bock E: **The first immunoglobulin-like neural cell adhesion molecule (NCAM) domain is involved in double-reciprocal interaction with the second immunoglobulin-like NCAM domain and in heparin binding.** *J Biol Chem* 1997, **272**:10125-10134.
21. Jenson P, Soroka V, Thompson NK, Ralets I, Berezin V, Bock E, Poulsen FM: **Structure and interactions of NCAM modules 1 and 2-basic elements in neural cell adhesion.** *Nat Struct Biol* 1999, **6**:486-493.
22. Kasper C, Rasmussen H, Kastrup JS, Ikemizu S, Jones RY, Berezin V, Bock E, Larsen IK: **Structural basis of cell-cell adhesion by NCAM.** *Nat Struct Biol* 2000, **7**:389-393.
23. Soroka V, Kolkova K, Kastrup JS, Diederichs K, Breed J, Kiselyov VV, Poulsen FM, Poulsen FM, Larsen IK, Welte W *et al.*: **Structure and interactions of NCAM Ig1-2-3 suggest a novel zipper mechanism for homophilic adhesion.** *Structure* 2003, **11**:1291-1301.

Protein contacts in the crystal structure of the NCAM fragment comprising Ig domains 1-3 suggested three different homophilic binding interfaces, which each differed from those proposed in two previous models. The inhibition of cell adhesion by peptides derived from these interface regions appeared to support the proposed lateral and adhesive protein bonds. The data also appeared to reconcile apparent contradictions between data used to support the first two models.

24. Johnson CP, Fujimoto I, Perrin-Tricaud C, Rutishauser U, Leckband D: **Mechanism of homophilic NCAM adhesion: use of multiple domains and flexibility.** *Proc Natl Acad Sci USA* 2004, **101**:6963-6968.

Surface force measurements discriminated between three different models proposed for the mechanism of homophilic NCAM adhesion. Because the NCAM complexes in each of the three models spanned different

membrane gaps, the SFA measurements readily tested each of the proposed mechanisms. In this study, the distance resolution of the SFA resulted in the measurement of two spatially distinct adhesive bonds between NCAM ectodomains. This confirmed that NCAM has multiple adhesive domains. Additional force and equilibrium binding measurements with Ig domain deletion mutants then identified the NCAM domains that generate the two independent bound states revealed by the force measurements.

25. Zhu B, Chappuis-Flament S, Wong E, Jensen I, Gumbiner BM, Leckband DE: **Functional analysis of the structural basis of homophilic cadherin adhesion.** *Biophys J* 2003, **84**:4033-4042.  
In this SFA study of homophilic cadherin adhesion, force measurements with cadherin domain deletion mutants identified the domains responsible for each of three bound states formed between C-cadherin ectodomains. The force measurements confirmed an adhesive bond between the N-terminal domains, but also demonstrated that a second domain was essential for two of the three bound states. The precision of the distance measurement showed that the removal of non-adhesive domains only shifted the interaction potential by the lengths of the removed domains. However, the deletion of adhesive segments abrogated the bound states mediated by those regions.
26. Marzalek PE, Lu H, Li HB, Carrion-Vazquez M, Oberhauser AF, Schulten K, Fernandez JM: **Mechanical unfolding intermediates in titin modules.** *Nature* 1999, **402**:100-103.
27. Carl P, Kwok CH, Manderson G, Speicher DW, Discher DE: **Forced unfolding modulated by disulfide bonds in the Ig domains of a cell adhesion molecule.** *Proc Natl Acad Sci USA* 2001, **98**:1565-1570.
28. Bayas MV, Schulten KS, Leckband D: **Forced dissociation of the CD2-CD58 complex.** *Biophys J* 2003, **84**:2223-2233.  
Steered molecular dynamics simulations were used to gain insight into the atomic-level details determining the mechanical strength of the heterologous CD2-CD58 bond. Simulations of forced protein unbinding at different pulling rates showed that the heterophilic protein bonds break before the domains unfold, except at ultrafast pulling speeds. The simulations support the experimental data, which indicate that the complex ruptures at the protein-protein interface under loading rates achieved in AFM or SFA measurements. The simulations and experimental results together show that CD2-CD58 adhesion will not induce protein unfolding.
29. Evans E, Ritchie K: **Dynamic strength of molecular adhesion bonds.** *Biophys J* 1997, **72**:1541-1555.
30. Evans E, Leung A, Heinrich V, Zhu C: **Mechanical switching and coupling between two dissociation pathways in a P-selectin adhesion bond.** *Proc Natl Acad Sci USA* 2004, **101**:11281-11286.  
This report follows on from the findings described in [2\*\*]. These authors used the BFP [1] to quantify the dependence of the selectin-PSGL-1 bond strength on the rate of loading, or dF/dt. This approach differs from the lifetime measurement of [2\*\*], whereby the force is held constant and the lifetime is measured at each constant force. In this case, they measured the bond strength as a function of the logarithm of the constant loading rate (dF/dt). They found that the strength increases logarithmically with dF/dt at >300 pN/s. This indicates that bond rupture follows a single kinetic path. However, at dF/dt <300 pN/s, the bond strength falls abruptly, suggesting that rupture occurs by a different unbinding pathway. To determine what governs this mechanical switching, the authors altered the force history of the bonds, by first rapidly 'jumping' the force by 20-30 pN. This jump was then followed by a steady force 'ramp' dF/dt. This initial 20-30 pN tug triggered bond strengthening, even at loading rates <300 pN/s. This is another demonstration that the mechanochemical response to force depends on the force history. It also illustrates that this protein pair acts as a mechanical switch, which enables these proteins to respond in different ways to subtle changes in the mechanical stimuli encountered *in vivo*.