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## Authors
Priest, Andrew Vae
Koirala, Ramesh
Sivasankar, Sanjeevi

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# Single-molecule studies of classical and desmosomal cadherin adhesion

**Andrew Vae Priest**[1,2,#], **Ramesh Koirala**[1,2,#], **Sanjeevi Sivasankar**[1,*]

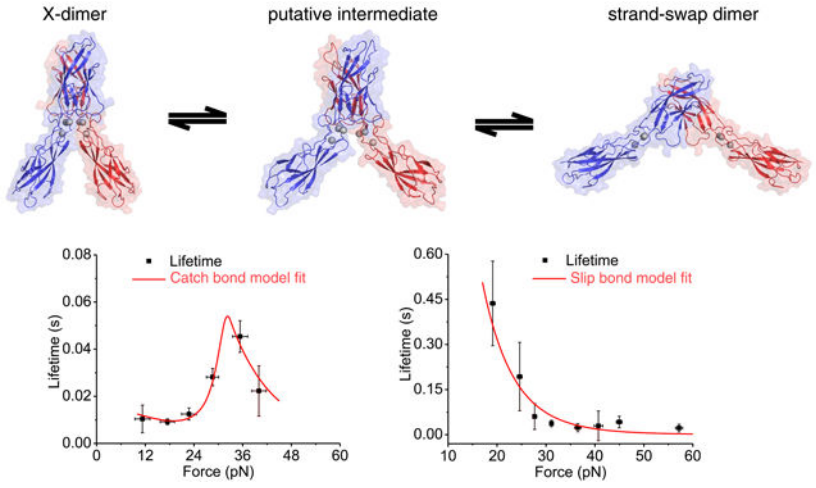
[1]Department of Biomedical Engineering, University of California, Davis, CA 95616.

[2]Department of Physics and Astronomy, Iowa State University, Ames, IA 50011.

## Abstract

Classical cadherin and desmosomal cadherin cell-cell adhesion proteins play essential roles in tissue morphogenesis and in maintaining tissue integrity. Deficiencies in cadherin adhesion are hallmarks of diseases like cancers, skin diseases and cardiomyopathies. Structural studies and single molecule biophysical measurements have revealed critical similarities and surprising differences between these key adhesion proteins. This review summarizes our current understanding of the biophysics of classical and desmosomal cadherin adhesion and the molecular basis for their cross-talk. We focus on recent single molecule measurements, highlight key insights into the adhesion of cadherin extracellular regions and their relation to associated diseases, and identify major open questions in this exciting area of research.

## Graphical Abstract




*Corresponding author: ssivasankar@ucdavis.edu.
#Equal Contribution.



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## 1. INTRODUCTION

Tissue formation and integrity relies on the ability of cells to sense, transmit and respond to mechanical forces; cadherin cell-cell adhesion proteins are integral to these functions. While the extracellular region of cadherins form load-bearing complexes that mechanically link cells to each other, their intracellular regions interact with the cytoskeleton and regulatory proteins [Figure 1]. The cadherin superfamily of cell-cell adhesion proteins are composed of four major subfamilies: classical cadherins, desmosomal cadherins, protocadherins and atypical cadherins [1]. Of these proteins, classical and desmosomal cadherins are essential for the maintenance of tissue integrity. While classical cadherins mediate cell-cell adhesion in all soft tissue and play critical roles in tissue morphogenesis, desmosomal cadherins mediate robust cell-cell adhesion in tissues like the epidermis and heart that are exposed to significant levels of mechanical stress [1, 2]. Mutations in classical cadherins and down regulation of their expression have been implicated in several cancers [3]. Similarly desmosomal cadherin mutations and autoantibodies that target desmosomal proteins result in arrhythmogenic cardiomyopathies and skin blistering diseases, respectively [2]. Recently, single molecule biophysical measurements have resolved the mechanistic links between classical and desmosomal cadherin structure and adhesion; these studies have revealed several critical similarities and a few surprising differences between these essential adhesion proteins. We critically review these measurements and discuss major open questions and future directions in this exciting area of research.

## 2. CLASSICAL CADHERINS

Classical cadherins are single-pass transmembrane proteins that mediate the integrity of all soft tissue, play essential roles in tissue morphogenesis and wound healing. Deficiencies in classical cadherin adhesion correlate with several cancers. Classical cadherins have extracellular regions (or ectodomains) that are comprised of five tandemly repeated extracellular (EC1–5) domains with three calcium binding sites in each interdomain linker [4]. Cadherins from opposing cells mediate adhesion by binding in ‘*trans*’ conformations [Figure 2A] while cadherins on the same cell surface associate laterally in a ‘*cis*’ orientation [5, 6]. Here, we only focus on the *trans* binding between cadherin ectodomains; *cis* dimerization has been reviewed elsewhere [4].

### 2.1 CLASSICAL CADHERIN *TRANS* INTERACTIONS

Classical cadherins from opposing cells bind in two $Ca^{2+}$ dependent *trans* adhesive conformations: strand-swap dimers [Figure 2B] and X-dimers [Figure 2C]. Strand-swap dimers are the primary cadherin adhesive conformation and are formed by the exchange of a conserved Tryptophan (W2) residue between the outermost (EC1) domains of opposing proteins [5, 7] [Figure 2B]. Single-molecule force [8] and Förster resonance energy transfer (FRET) [9, 10] experiments have mapped the domains of classical cadherin involved in strand-swap dimer formation and the $Ca^{2+}$ dependence of the dimerization process. While strand swap dimers are crucial for robust cell-cell adhesion, mutating the conserved W2 to alanine (W2A) does not completely abolish adhesion [11, 12]. Single molecule FRET and atomic force microscopy (AFM) measurements show that the W2A mutant forms a weak

complex that does not involve strand swapping [10]. Structural studies show that this mutant is trapped in an X-dimer conformation, which is formed by extensive surface interactions between the outer two (EC1–2) domains [13] [Figure 2C]. Mutating Lys14 to Glutamic acid (K14E), which compromises X-dimer formation, slows W2 strand-swapping but does not affect the dimerization affinity of the swapped dimer [13]. These studies demonstrate that X-dimers serve as an intermediate during the formation and rupture of strand-swap dimers [10, 13, 14].

Single-molecule AFM experiments have also elucidated the force-induced changes in the binding kinetics of X-dimers and strand-swap dimers formed by E-cadherin– a classical cadherin found in epithelial cells. W2A mutants, which are trapped in an X-dimer conformation, exhibit a catch bond relationship between lifetime and force: the bond lifetimes initially increases with force before decreasing [Figure 2E] [15]. This data can be fit to a sliding-rebinding model [16], where interacting ectodomains slide past each other in the presence of a force and form noncovalent interactions which strengthens the bond. Indeed, AFM, Molecular Dynamics (MD) and Steered MD (SMD) simulations have shown that X-dimer catch bonds are $Ca^{2+}$ dependent and are formed by the force-induced rearrangement of X-dimers, which results in the formation of several *de-novo* hydrogen bonds [Figure 2G, H] that lock the X-dimer into tighter contact [17]. In contrast to X-dimers, K14E strand-swap dimers, show slip bond behavior; their bond lifetimes decreased with increasing tensile force [Figure 2F] [15]. Importantly, consistent with X-dimers being a weaker adhesive structure than strand-swap dimers, X-dimer catch bonds are shorter lived than strand-swap dimer slip bonds [Figure 2E, F]. Recent single molecule AFM measurements and computer simulations show that wild type E-cadherin interconverts between X-dimers and strand-swap dimers; along the transition pathway for interconversion, the proteins adopt a metastable intermediate state [Figure 2D] [18]. Consequently, by switching between strand-swap and X-dimer conformations, E-cadherins are able to strengthen or weaken their adhesion.

The two distinct *trans* conformations adopted by classical cadherins may play important roles in phenomenon such as collective cell migration, which is a hallmark of tissue remodeling, morphogenesis and wound repair [19]. While collectively migrating cells use cytoskeletal protrusions and guidance cues that are similar to those used by single migratory cells, the presence of cadherin cell-cell adhesion junctions result in ‘supra-cellular’ properties, such as collective polarization and force generation, and, eventually, complex tissue organization [19]. An intriguing possibility is that strand-swap dimers interactions may be formed by leader cells that require more stable cell-cell contacts with follower cells. In contrast, the cells deep within the cell monolayer, which require more dynamic contacts, may form X-dimers. However, the molecular mechanisms by which formation of X-dimers and strand-swap dimers are regulated by cells, and the physiological roles of these conformations in collective cell migration, is still unknown.

### 2.2 ROLE OF CYTOPLASMIC ADAPTOR PROTEINS IN REGULATING CADHERIN ADHESION

The cytoplasmic tail of classical cadherins associate with intracellular p120-catenin and β-catenin which in turn recruit the actin binding and bundling protein α-catenin [Figure 1A]. Binding of p120-catenin to the cadherin cytoplasmic domain stabilizes the cadherin complex by preventing cadherin internalization and degradation [20]. The cadherin-catenin complex links to filamentous actin either by the direct binding of α-catenin and F-actin or by indirect association of α-catenin and F-actin via vinculin [Figure 1A] [21]. Adhesive forces transmitted across intercellular junctions by cadherins, induce conformational changes in α-catenin [22] which recruits vinculin to the site of force application [23]. These force-induced changes modulate adhesion by mediating cytoskeletal rearrangements [24]. Additionally, recent studies show that β-catenin directly binds to vinculin [25] and forms an alternate bypass connection from cadherin to the actin cytoskeleton.

Recent studies demonstrate that E-cadherin adhesion is regulated by the phosphorylation state of p120-catenin: dephosphorylation of p120-catenin strengthens adhesion while p120-catenin phosphorylation weakens E-cadherin binding [26, 27]. Biophysical studies also show that binding of α-catenin [28, 29] and vinculin [30] is obligatory in strengthening E-cadherin adhesion. These findings suggest that association of p120-catenin, α-catenin and vinculin to the cadherin cytoplasmic tail, allosterically regulate ectodomain conformation, and consequently cadherin adhesion. However, the ‘inside out’ molecular mechanisms by which regulatory events within the cell allosterically control the conformation of the extracellular region is still a mystery.

### 2.3 CLASSICAL CADHERINS IN CANCER

Experimental studies show that E-cadherin acts as a tumor suppressor – re-expression of E-cadherin in cadherin deficient cancer cells can prevent tumor progression and invasion [31]. Similarly, reduced expression of N-cadherin— a classical cadherin found in neurons, strongly correlates with metastasis in neuroblastoma [32] . The role of classical cadherins, as a cancer marker has been reviewed recently [3]. However, several studies show that E-cadherin is often expressed in metastatic tumor cells [33, 34] indicating that E-cadherin expressing tumor cells can downregulate adhesion without affecting E-cadherin expression levels on the cell surface. A recent study showed that dephosphorylation of p120-catenin (which is known to strengthen adhesion) significantly reduced the number of cells metastasized from the mammary gland to the lung [35]. Furthermore, several point mutations in the E-cadherin ectodomain that are prevalent in hereditary diffuse gastric cancer, selectively interfere with the inside-out regulation of E-cadherin adhesion, rather than the ability of E-cadherin to adhere [35]. This suggests that inside-out regulation of E-cadherin plays an important role in cancer progression. However, the conformational states adopted by E-cadherin in these mutations, is still an open question.

## 3. DESMOSOMAL CADHERINS

Desmosomal cadherins form the desmosome, a vital intercellular adhesive junction found in the skin and heart that links to the intermediate filament (IF) cytoskeleton and mechanically

couples cells [Figure 1B]. There are two types of desmosomal cadherins: desmoglein (Dsg) and desmocollin (Dsc), which are organized into four Dsg isoforms (Dsg1–4) and three Dsc isoforms (Dsc1–3) that show tissue and differentiation specific expression patterns [2]. Desmosome linkage to intermediate filaments is facilitated by binding of desmosomal cadherins to armadillo proteins plakoglobin (PG) and plakophilin (PKP) which in turn are linked to IFs by desmoplakin (DP) [Figure 1B].

### 3.1 DESMOSOMAL CADHERIN *TRANS* INTERACTIONS

The extracellular region of desmosomal cadherins contain four cadherin homology repeats and an anchor domain [36]. While co-expression of both Dsg and Dsc are needed for intercellular adhesion [2, 37], the types of *trans* interactions that occur between Dsg and Dsc are only partially understood. Single molecule AFM experiments demonstrate that Dsg2 and Dsc2 interact to form robust heterophilic (Dsc2:Dsg2) dimers while Dsc2 also forms weak homophilic (Dsc2:Dsc2) dimers [38, 39]. These findings are supported by solution binding and structural assays, which demonstrate that while all isoforms of Dsg and Dsc form heterophilic dimers, homophilic interactions are either weak or non-existent [36]. Analysis of surface charges of residues along the interaction interface reveal that Dsc and Dsg have complementary charges suggesting an electrostatic mechanism for preferential heterophilic binding [36]. Affinities for the heterodimers match their role and expression levels in stratified epithelia -Dsg1:Dsc1 and Dsg4:Dsc1, which are found in the most differentiated or outermost layers of human skin, have the highest affinities while Dsg3:Dsc3, which are found in the basal layers of the epidermis, are the weakest binding pairs [36]. However, other single molecule AFM studies show homophilic interactions between Dsc3 and between Dsg1–3 [40, 41]. An AFM study performed on live cells also suggests that Dsg3 primarily forms homodimers on keratinocytes [41]. Consequently, the precise molecular mechanisms and roles that homo- and heterodimers play in desmosome adhesion remain unresolved.

### 3.2 DESMOSOMAL CADHERINS IN SKIN AND HEART DISEASE

Autoimmune skin blistering diseases pemphigus vulgaris (PV) and pemphigus foliaceus (PF) result from autoantibodies developed primarily against Dsg1 and Dsg3 [2]. These antibodies impair normal adhesion causing painful blisters to form on the skin and mucous membranes [2, 42, 43]. Pemphigus pathogenesis has been studied using single molecule AFM in cell-free and keratinocyte monolayer conditions, where it was show that PV and PF autoantibodies inhibit Dsg3 binding but do not affect Dsg1 adhesion [44]. These AFM studies along with cell biological assays show that intracellular signaling affected by pemphigus autoantibodies regulates desmosome adhesion and may also play a role in pemphigus pathogenesis [44].

Dsg2 and Dsc2 are the only desmosomal cadherins found in cardiac tissue and mutations in Dsg2 and Dsc2 have been associated with arrhythmogenic right ventricular cardiomyopathy (ARVC) [45, 46]. This disease is marked by fatty deposits and fibrosis in the right ventricle and leads to ventricular arrhythmias and sudden cardiac failure [2]. Recently, two mutations in Dsg2 ectodomains, associated with ARVC were studied with single molecule AFM and cell dissociation assays [40]. These studies showed that the binding kinetics of homophilic

Dsg2 interactions were significantly different between wild type Dsg2 and the two ARVC mutants. Both mutants had a lower off-rate but a higher $K_D$ than wild type, suggesting that these ARVC mutations may affect desmosomal integrity [40]. However, since the AFM measurements tested only homophilic Dsg2 interactions, how these mutations affect adhesion, and consequently ARVC, in desmosomes —where heterophilic binding likely occurs— remains to be seen. A comprehensive review of desmosomes and their diseases can be found elsewhere [2].

## 4. CROSS TALK BETWEEN CLASSICAL AND DESMOSOMAL CADHERINS

Several studies have also shown a cross-talk between classical and desmosomal cadherins suggesting that classical cadherins are needed to facilitate desmosome assembly *in vivo*. Immuno-electron micrographs demonstrate that E-cadherin localizes to the intercellular region of desmosomes [47] and that blocking E-cadherin adhesion with antibodies delays desmosome formation [48]. Studies also show that desmosome formation in keratinocytes requires junctional initiation by E- or P-cadherin, a classical cadherin expressed in the placenta and myoepithelial cells [49]. E- and P-cadherin deficient mice show defective desmosome assembly [50]. However, the precise molecular mechanisms by which classical cadherins promote desmosome formation are unknown. Recently, the role of the E-cadherin ectodomain at different stages of desmosome formation was characterized using an integrated structure/function analysis that combined single molecule force measurements with an AFM, super resolution imaging and confocal fluorescence microscopy [39]. These studies identified a novel $Ca^{2+}$- independent direct interaction between E-cadherin and Dsg2 that is mediated by a conserved Leu 175 on E-cadherin; previous structural studies have shown that L175 mediates homophilic E-cadherin *cis* dimerization [6]. The data suggests that desmosome assembly is initiated in two stages: a first stage that requires stable E-cadherin *trans*-homodimerization and a second stage characterized by the direct heterophilic binding between E-cadherin and Dsg2 that facilitates further desmosome assembly [Figure 3] [39]. The interactions between E-cadherin and Dsg2 are short-lived and as desmosomes mature, Dsg2 dissociates from E-cadherin and forms stable bonds with Dsc2 to mediate robust adhesion [Figure 3] [39].

## 5. FUTURE DIRECTIONS

While the unique conformations adopted by classical cadherins and the distinct adhesive properties of these conformations are well established, how these structures are utilized and regulated in the context of cell function are unknown. Analogous to the case of integrins where adhesion is regulated from the inside-out, classical and desmosomal cadherin adhesion is also regulated by cytoplasmic proteins. However, the biophysical mechanisms that underlie the regulation of classical and desmosomal cadherin conformation (and consequently adhesion) are still unclear. Resolving this question will likely require the development of single molecule AFM based techniques to study the biomechanics of single cadherins on live-cell surfaces.

While classical cadherin junctions are among the most investigated cell-cell adhesion complexes, desmosomes are among the most poorly understood adhesive organelle.

Consequently, several fundamental biophysical questions on desmosome structure-function remain unanswered. For instance, the force dependent kinetic properties of desmosomal cadherins and the range of conformations that these proteins can adopt in the presence of mechanical stress are unknown. Furthermore, the unique functional roles of each desmosomal cadherin isoform are not understood. Addressing these questions are essential to advancing our understanding of desmosome function in healthy and disease states.

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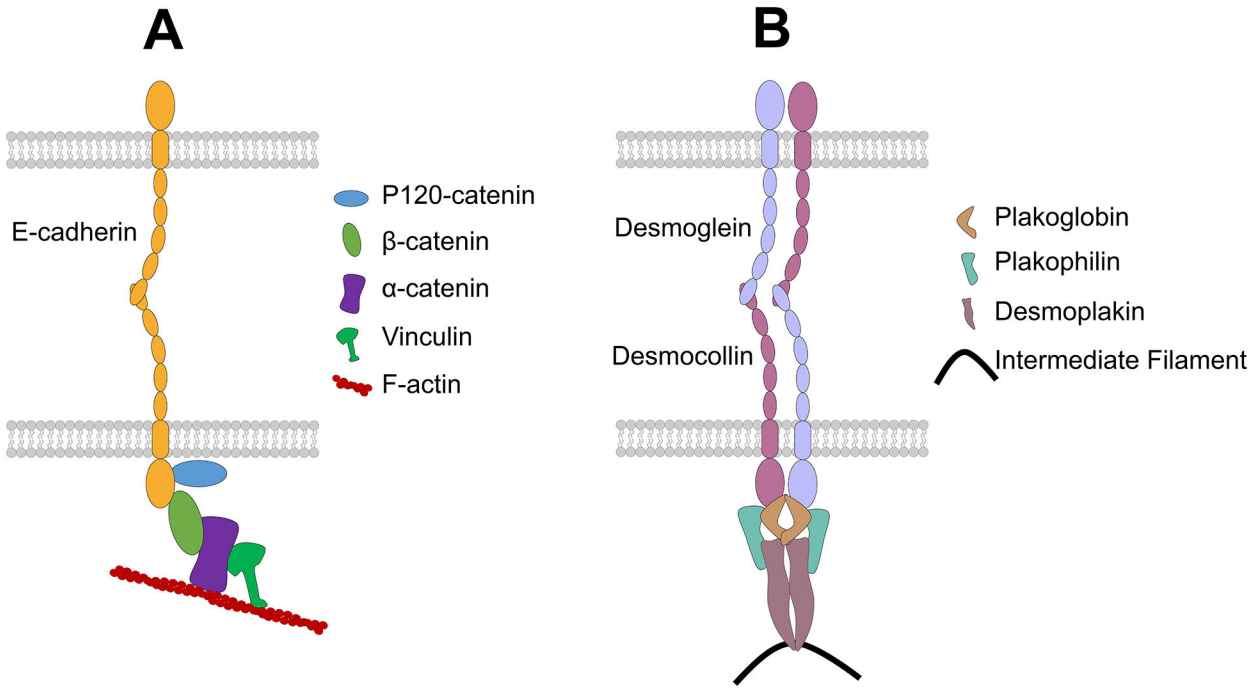


**Figure 1: Classical and desmosomal cadherin adhesive complexes.**

Cadherin ectodomains from opposing cells adhere to each other while intracellular regions interact with the cytoskeleton through effector proteins. **(A)** Classical cadherins form homodimers and associate with filamentous actin (F-actin) via β-catenin, α-catenin p120 catenin, and vinculin. **(B)** In contrast, desmosomal cadherins (Dsc and Dsg) typically form heterodimers and associate with intermediate filaments via plakoglobin, plakophilins and desmoplakin.

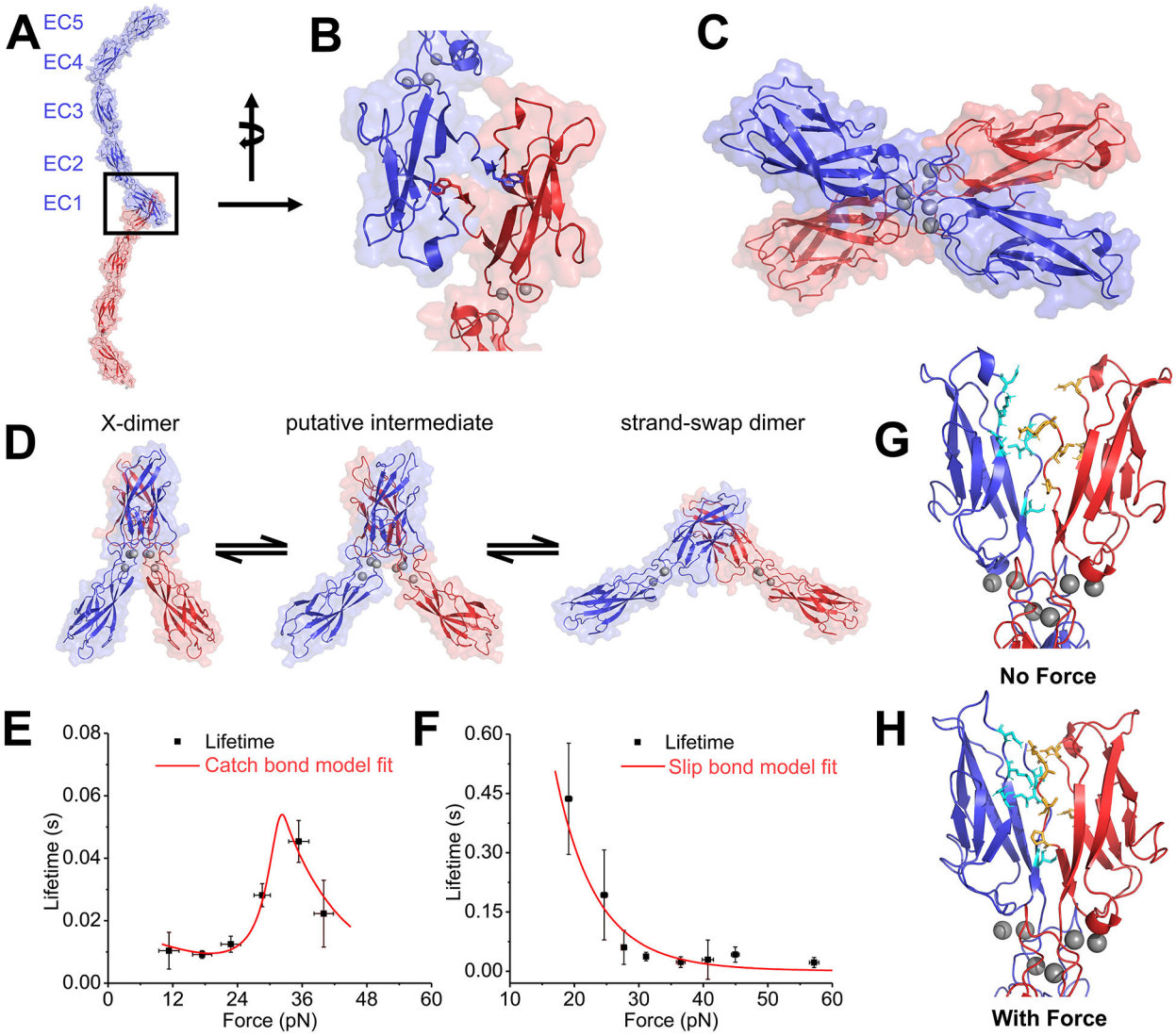


**Figure 2: Structure and biomechanics of E-cadherin *trans* dimers.**

**(A)** E-cadherin monomers from opposing cells (red and blue) bind in a strand-swap dimer conformation. **(B)** Strand-swap dimers are formed by the exchange of conserved W2 residues between opposing EC1 domains (PDB: 3Q2V). Bound $Ca^{2+}$ are denoted by gray spheres. **(C)** X-dimer interface consists of noncovalent surface interactions (hydrogen bonds and a salt bridge) between EC1 and EC2 domains near the $Ca^{2+}$ binding site (PDB: 3LNH). **(D)** E-cadherins interconvert convert between an X-dimer (left) and strand-swap dimer (right) via an intermediate state (middle; simulated structure and interconversion pathway from reference [18]). **(E)** X-dimers form catch bonds. Catch bonds initially strengthen before weakening beyond a critical pulling force. **(F)** Strand-swap dimers form slip bonds. The lifetime of slip bonds decrease rapidly with force. **(G-H)** E-cadherin X-dimer before (G) and after (H) application of force. Tensile force flexes the interacting ectodomains such that hydrogen-bond donor and acceptor amino acids (cyan residues on blue monomer and orange residues on red monomer) are brought into registry. Panels (G, H) are adapted from reference [17]. Panel (F) is adapted from reference [18].

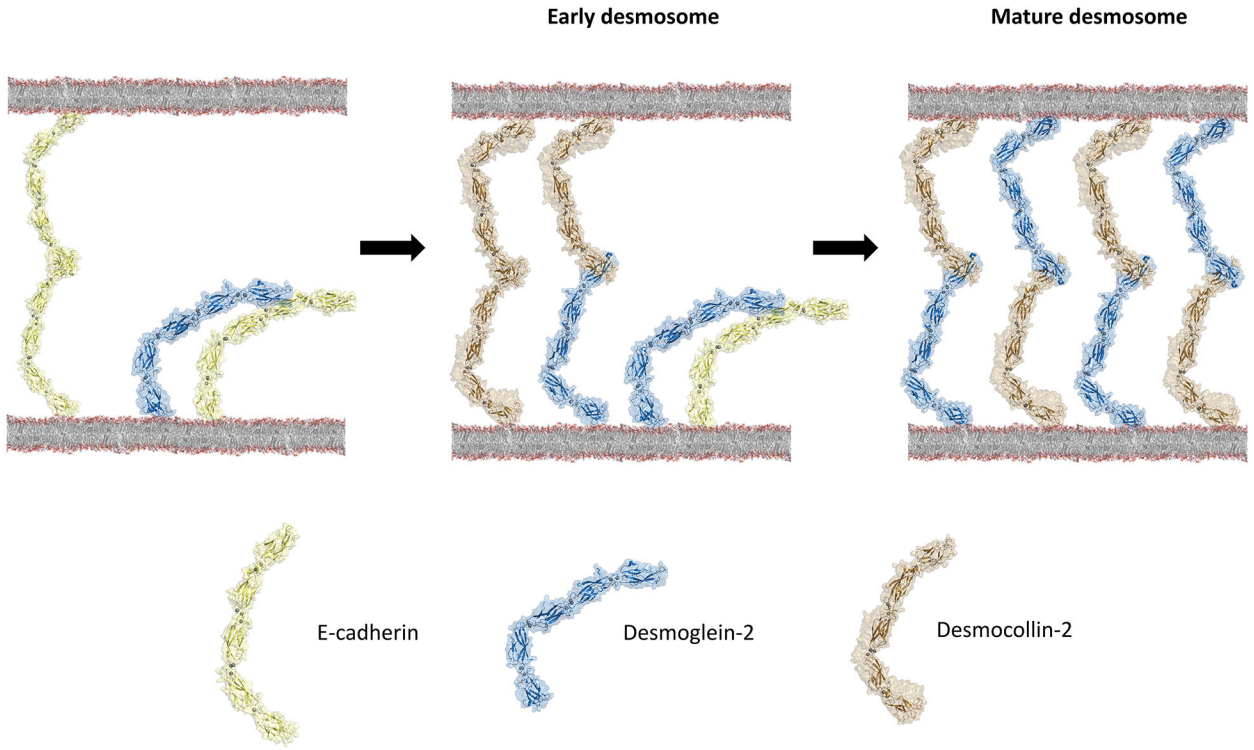


**Figure 3: Model for desmosome assembly.**

Dsg2 (PDB code: 5ERD) and Ecad (PDB code: 3Q2V) initially interact to form a low-lifetime Ecad:Dsg2 complex. This requires prior formation of Ecad *trans*-homodimers. The Ecad:Dsg2 complex is then incorporated in the nascent desmosome that contains the weak Dsc2:Dsc2 homodimer (Dsc2 was constructed from PDB codes: 5ERP and 5J5J). Ecad:Dsg2 and Dsc2:Dsc2 complexes dissociate as the desmosome matures and a robust adhesive complex of *trans* Dsg2:Dsc2 heterodimers is formed. Figure was generated by positioning Ecad, Dsg2 and Dsc2 ectodomain structures in PyMOL based on model proposed in reference [41]. Lipid bilayer coordinates were obtained from reference [51]. *Note: Only cadherin ectodomains are displayed in the figure.*