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Engineering the interactions of classical cadherin cell-cell adhesion proteins

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Abstract

Classical cadherins are calcium dependent cell-cell adhesion proteins that play key roles in the formation and maintenance of tissues. Deficiencies in cadherin adhesion are hallmarks of numerous cancers. Here, we review recent biophysical studies on the regulation of cadherin structure and adhesion. We begin by reviewing distinct cadherin binding conformations, their biophysical properties, and their response to mechanical stimuli. We then describe design rules for engineering antibodies that can regulate adhesion by either stabilizing or destabilizing cadherin interactions. Finally, we review molecular mechanisms by which cytoplasmic proteins regulate the conformation of cadherin extracellular regions, from the inside-out.

Classical cadherins are essential, calcium dependent cell-cell adhesion proteins that physically connect cells in tissues and play key roles in tissue formation and in the maintenance of tissue integrity. Cadherin adhesions withstand mechanical stress and orchestrate complex cell movements during morphogenesis and wound healing (1, 2). Cadherins maintain the integrity of epithelial barriers, thereby preventing harmful agents from accessing underlying tissue. Cadherins are also expressed in a variety of leukocytes, including conventional dendritic cells, Langerhans cells, and macrophages (3). Dysregulation of cadherin adhesion results in a loss of contact inhibition and increased cell mobility, a hallmark of numerous cancers and immunodeficiencies (3, 4).

Recently, there has been much progress in understanding the biophysical mechanisms that underlie the regulation of the conformation and adhesion of cadherin extracellular domains (5). Here, we briefly review these finding. We begin by reviewing distinct cadherin adhesive conformations and their biomechanical properties. We then describe the molecular mechanisms by which antibodies can be engineered to stabilize/destabilize cadherin binding and modulate adhesion. Finally, we review the biophysical mechanisms by which cytoplasmic proteins regulate the conformation of the cadherin extracellular region from the inside-out. Our review focuses on the *trans* binding (*i.e.* binding from opposing cells) of classical cadherins such as E-cadherin, N-cadherin, P-cadherin and C-cadherin, which are among the most widely studied members of the cadherin superfamily.

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Structure and energetics of cadherin binding:

Classical cadherins are transmembrane proteins and their extracellular regions (or ectodomains), comprised of five tandemly arranged domains (EC1–5), mediate cell-cell adhesion (Figure 1a). Ectodomains from opposing cells bind in two *trans* adhesive conformations: strand-swap dimers and X-dimers (6–12). Besides binding in distinct *trans* conformations, cadherins on the same cell surface oligomerize in *cis* orientations which cluster cadherins and form robust adherens junctions (13, 14). While this review focuses on cadherin *trans* binding, cadherin *cis* association has been reviewed elsewhere (15).

Strand-swap dimers are the primary *trans* adhesive conformation and are formed when the EC1 domains of opposing cadherins symmetrically exchange their N-terminal β-strands and insert a conserved Tryptophan at position 2 (W2) into hydrophobic pockets on their adhesive partners (Figure 1a, 1b) (16–20). Structural and computational studies show that strand swapping occurs because the β-strand in cadherin monomers is under a conformational strain which is relieved by exchange of β-strands (21).

Single molecule biophysics experiments first demonstrated that prior to strand-swapping, cadherin monomers interact in a non-swapped *trans* binding conformation (6). In these studies, E-cadherins were trapped in the non-swapped ‘initial encounter complex’ by mutating the W2 residue on the N-terminal β-strands to an Alanine (W2A mutation). Structural studies showed that the W2A mutants adopted an X-dimer structure due to extensive surface interactions between the base of the EC1 domain, EC1-EC2 inter-domain linker region and the apex of the EC2 domain (Figure 1c) (22). An X-dimer structure was also adopted when N-terminal β-strands were extended by a few amino acids (23), presumably because this reduced the conformational strain that drives the formation of strand-swap dimers (21). Key interactions that stabilize the X-dimer include salt-bridges between Lysine (K14) and Aspartic acid (D138) on opposing cadherins (9, 22). The affinity for X-dimer formation in solution was measured to be significantly weaker than the affinity for strand-swap dimerization: while the K_d for strand-swap dimerization ranged from 64 μM to 97 μM (24–26), the K_d for X-dimer formation was 916 μM (22).

X-dimers have been proposed to serve as an intermediate during the formation and rupture of strand-swap dimers (6, 22, 27). Mutating K14 in the cadherin X-dimer binding interface to Glutamic acid (K14E), which abolishes X-dimer formation, reduced both association and dissociation rates of strand-swap dimerization by factors of ~10^4, but did not change the affinity and the structure of the strand-swap dimer (22, 28). This suggests that X-dimers serve as non-obligatory, but crucial intermediates in the formation of strand-swap dimers. In epithelial cells, inactivation of X dimers result in extraordinarily stable cell-cell junctions suggesting that X-dimers are also an intermediate in the pathway to dissociation of strand-swap dimers (27). Computational studies and single molecule Atomic Force Microscopy (AFM) measurements also show that cadherins tune adhesion by interconverting between X-dimers and strand-swap dimer structures (29). Recent single molecule AFM measurements in live cells demonstrate that approximately 70% of E-cadherins on the surface of epithelial Madin-Darby canine kidney (MDCK) cells form strand-swap dimers while the remaining cadherins form X-dimers (30). This presumably provides cells with two cadherin pools

with different adhesive properties. However, recent cryo-EM experiments (31), NMR (28) and high-speed AFM imaging experiments (32), show that X-dimers exist stably alongside monomers and strand-swap dimers, indicating that the X-dimer conformation is not just an intermediate, but is a stable adhesive structure.

Cadherins can interact in alternate structures:

While X-dimers and strand-swap dimers are the primary cadherin binding conformations, they are not the only *trans* adhesive structures that classical cadherins adopt. Biophysical studies show that wild type E-cadherin ectodomains in solution can interconvert between X-dimer and strand-swap dimer conformations via a metastable intermediate state (28, 29, 32). This intermediate conformation resembles an X-dimer, but with both W2 residues swapped (28, 29). Furthermore, recent biophysical experiments show that cadherins can also form *trans* dimers without the symmetric involvement of two W2s or of two K14-D138 salt-bridges (33). In this conformation, opposing cadherin ectodomains interact asymmetrically swapping just one W2, while simultaneously forming just one K14-D138 salt bridge (33).

Early biophysical studies showed the classical cadherins interact in three alternate conformations that involved interactions along the length of the ectodomain (34–36). In agreement with this finding, recent cryo-EM structures report a novel EC4-mediated E-cadherin *trans* dimer (31). Recently, high speed-AFM imaging of dimers formed by full-length ectodomains of E-cadherin revealed the existence of a novel S-shaped *trans* dimer structure formed by membrane-distal E-cadherin domains interacting via a broad binding interface. Imaging the conversion between X-dimers and strand-swap dimers showed that the formation of S-dimers precedes formation of X- and strand-swap dimers (32). Similar S-shaped dimers have previously been observed in the interaction of non-classical, desmosomal cadherins (37, 38). Taken together, these novel binding conformations, that are distinct from X-dimers and strand-swap dimers, indicate that the biophysics of classical cadherin interactions are still not completely understood and that cadherins can adopt a more diverse range of adhesive structures that need to be further explored.

Given the similarity in their sequence and structure, it is not surprising that different classical cadherins heterophilically interact with each other. Indeed, heterophilic binding between N-cadherin, E-cadherin and C-cadherin have been measured using ensemble force measurements (39). Kinetic measurements demonstrate that N-cadherin/E-cadherin heterophilic binding affinity lies in between the homophilic affinities of N-cadherin and E-cadherin respectively (25). Similarly, interactions between E-cadherin and P-cadherin have been detected using proximity labeling and AFM (40). However the structure of these classical cadherin heterotypic dimers are still unknown.

Biomechanics of cadherin *trans* dimers:

Besides their structural differences, X-dimers and strand-swap dimers can also be distinguished by their distinct responses to mechanical forces (29, 41, 42). Single molecule AFM measurements and computer simulations show that when X-dimers are pulled, the interacting protomers reorient which results in the formation of seven force-induced

hydrogen bonds that lock the X-dimer into tighter contact (42). Consequently, the lifetime of an X-dimer initially increases with force before subsequently decreasing (Figure1 e) (41). These biphasic interactions, which are known as catch bonds, strengthen X-dimers in the presence of a pulling force (41). Biophysical experiments also show that X-dimer catch bonds are Ca^{2+} dependent (42). In contrast to X-dimers, single molecule AFM experiments show that cadherin strand-swap dimers form more conventional slip bonds that weaken upon pulling (Figure 1d) (41, 42).

It is possible that switching between X-dimer and strand-swap dimer conformations enable cells to tune their adhesive properties (29, 30). This ability to modulate adhesion may be important in phenomenon such as collective cell migration, which serves to keep tissue intact during morphogenesis, wound repair and cancer metastasis (43, 44). However, the biological roles of E-cadherin catch and slip bonds still remains to be determined.

Regulating cadherin adhesion using antibodies:

Classical cadherins are essential regulators of tissue homeostasis, and disruption of cadherin adhesion signals disease progression (45). E-cadherin acts as a tumor suppressor and deficiencies in E-cadherin adhesion are associated with the metastasis of breast cancer (46), colorectal cancer (47), gastric cancer (48), and lung cancer (49). Similarly, reduced expression of neuronal N-cadherin, strongly correlates with metastasis in neuroblastoma (50). Conversely, re-expression of E-cadherin in cadherin deficient cancer cells can prevent tumor progression and invasion (51). Due to their tumor suppressive properties, there is intense interest in developing antibodies that activate or strengthen E-cadherin adhesion for potential applications in reducing cancer metastasis.

Several monoclonal antibodies (mAbs) have been identified, which target the E-cadherin ectodomain and enhance cell-cell adhesion (52). These mAbs strongly activate adhesion in Colo 205 cells, a non-adhesive cell-line with a full but inactive complement of E-cadherin and its cytoplasmic binders. Activation of adhesion also induced dephosphorylation of specific residues in p120-catenin (52), an cadherin cytoplasmic binding partner that stabilizes the cadherin complex by preventing its internalization and degradation (53). Mutating the phosphorylation sites on p120-catenin to either phosphomimetic or non-phosphorylatable amino acids confirmed that p120-catenin phosphorylation regulates cadherin-dependent cell aggregation (52).

One of these mAbs, 19A11, was shown to prevent the metastatic invasion of mouse lung cancer cells expressing human E-cadherin (54, 55), enhance epithelial barrier function and limit progression of inflammatory bowel disease (56). Intercellular adhesion frequency measurements showed that either treating Colo 205 cells with 19A11, or dephosphorylating p120-catenin increased the homophilic binding affinity of E-cadherin (57). These results suggest that conformational changes in the E-cadherin ectodomain induced by 19A11 binding, allosterically correlate with p120-catenin associated changes across the cell membrane.

Recently, the structure of 19A11 bound to the EC1–2 domains of E-cadherin has been determined using X-ray crystallography and the molecular mechanism by which 19A11 strengthens adhesion has been identified using biophysical methods (58). This structure demonstrates that 19A11 binds to the EC1 domain of E-cadherin, between the swapped β-strand and the pocket region – two regions that are known to be key energetic determinants of strand-swap dimer stability (Figure 2) (21, 59). Computer simulations and single molecule AFM measurements show that 19A11 forms two key salt bridge interactions with E-cadherin which stabilize both the swapped β-strand and the pocket region which houses a W2 from its binding partner. To strengthen E-cadherin adhesion, at least one of these salt bridges needs to be formed between both E-cadherins in the *trans* dimer and their bound 19A11. Abolishing these salt-bridges eliminates adhesion strengthening (58).

Simultaneously, structures of three activating mAbs (19A11, 66E8 and 59D2) bound to the full ectodomain of E-cadherin were resolved using X-ray crystallography and cryo-Electron Microscopy (Figure 2) (31). These structures revealed that mAb binding resulted in a distinctive twisted strand-swap dimer conformation caused by an outward shift in the N-terminal β-strands that may represent a strengthened adhesive state (31). All three activating antibodies bound at or near the anchor points of the swapped β-strands, again suggesting that stabilization of this strand is the dominant mechanism by which 19A11 strengthens E-cadherin adhesion (Figure 2) (31). These structural and biophysical studies outline mAb ‘design principles’ that can be exploited to strengthen strand-swap dimer adhesion. These studies demonstrate that selective stabilization of the swapped β-strand and its complementary binding pocket are sufficient to strengthen E-cadherin adhesion. Importantly, the activating mAbs do not inhibit access to the *cis*-binding interfaces (31), suggesting that these mAbs do not impact normal cadherin clustering. It remains to be seen if these design principles can be applied to develop new classes of mAbs that strengthen the adhesion of other classical cadherins as well.

Besides adhesion strengthening, inhibition of cadherin adhesion also has potential cancer therapeutic applications. Classical cadherins like P-cadherin act as tumor enhancers and overexpression of P-cadherin is associated with various types of cancer progression (60, 61). Consequently, there has been much recent effort in developing strategies to inhibit P-cadherin adhesion. An inhibiting antibody, TSP7, was developed that binds to the P-cadherin EC1 domain (Figure 2); steric hindrance between two bound TSP7s was shown to prevent the formation of X-dimers thus kinetically reducing strand-swap dimer formation and disrupting P-cadherin mediated cell adhesion (62). Similarly, a small chemical fragment that binds to a cavity in between the EC1 and EC2 domains of P-cadherin, was shown to prevent the formation of hydrogen bonds that are crucial for X-dimer formation (63). These experiments outline a plausible strategy for inhibiting cadherin adhesion by preventing X-dimer formation. However, this strategy may not be applicable to E-cadherin since E-cadherin strengthening mAbs 19A11, 66E8 and 59D2 all prevent X-dimer formation but still strengthen cell adhesion (31, 58). This suggests that X-dimers are necessary intermediates in P-cadherin strand-swap dimer formation, but may be non-obligatory in E-cadherin binding. These structural insights illustrate that differential biophysical targeting of each type of classical cadherin may be necessary for designing specific antibodies to tune adhesion.

Furthermore, studies indicate that there are several strategies to design antibodies that inhibit classical cadherin adhesion. E-cadherin inhibiting antibodies, namely 52F9, 67G8 and DECMA1, have been reported to recognize the EC5 domain and inhibit E-cadherin mediated cell adhesion (Figure 2) (31, 52, 64). Since the inhibiting antibody epitope is located distant from the E-cadherin *trans* binding site, it is unlikely that these inhibiting antibodies destabilize or inhibit *trans* binding conformations. Rather, binding to the EC5 domain may activate cytoplasmic signaling pathways which may in turn inhibit adhesion. Indeed, E-cadherin inhibition induced by the binding of DECMA1 has been reported to be associated with the activation of Epidermal Growth Factor Receptor 1/2 (HER1/2) and their downstream signaling pathways, which also has the potential to suppress the HER2 positive breast cancer (64). Recent data also suggests that DECMA1 functionally disrupts cell-cell adhesion by promoting proteolysis of E-cadherin (65).

Regulation of cadherin adhesion by cytoplasmic proteins:

The cytoplasmic region of classical cadherins associate with the catenin family of proteins: namely p120-catenin, β-catenin, γ-catenin/plakoglobin and α-catenin. The cadherin-catenin complex, in turn, links to filamentous actin (F-actin) either by the direct binding of α-catenin and F-actin or by the indirect association of α-catenin and F-actin via vinculin (Figure 2) (66). Additionally, recent studies show that β-catenin can directly bind to vinculin (67) and form an alternate bypass connection from cadherin to the actin cytoskeleton. These linkages between cadherin and the actin cytoskeleton are not static. Single molecule AFM and optical tweezer measurements in live cells reveal that on the apical region of MDCK cells, only ~50% of E-cadherin are linked to the cytoskeleton (30, 68).

Many of the cytoplasmic linkages between E-cadherin and the actin cytoskeleton are mechanosensitive – the binding of both α-catenin and vinculin to F-actin have been shown to display catch bond behaviors (69–71). Adhesive forces transmitted across intercellular junctions by cadherin, induce conformational changes in α-catenin (72, 73), strengthen F-actin binding (69) and recruit vinculin to the sites of force application (74, 75). These force-induced changes mediate cytoskeletal rearrangements and recruit myosin to cell-cell junctions (76–78). Cadherin coupling to α-catenin, vinculin, and the actin cytoskeleton is also regulated by the phosphorylation of p120-catenin (79) which decouples cadherin from vinculin and F-actin (80).

Studies show that α-catenin and vinculin play important roles in strengthening and stabilizing cadherin adhesion: bead twisting experiments show force-induced stiffening of E-cadherin-based junctions and cell doublet stretching experiments demonstrate reinforcement of cell-cell adhesion in vinculin and α-catenin dependent manners (81–83). AFM measurements with α-catenin knockdown cells also show that reducing the amount of cytoplasmic α-catenin decreases unbinding force of E-cadherin ectodomains (84, 85). Intercellular adhesion frequency measurements also provide biophysical evidence for the allosteric regulation of E-cadherin binding by the phosphorylation status of p120-catenin (57). However, the molecular mechanism for the ‘inside-out’ regulation of cadherin extracellular conformation and adhesion is only now beginning to be resolved.

Recent live-cell, single molecule AFM measurements show that the association of vinculin with the E-cadherin cytoplasmic tail allosterically drives the conversion of X-dimers to strand-swap dimers (30). These measurements demonstrate that while E-cadherins bound to vinculin form robust strand-swap dimers, E-cadherins are trapped in a weaker X-dimer conformation when vinculin is knocked-out or when vinculin binding to α-catenin is disrupted. AFM experiments and computer simulations show that vinculin binding to the E-cadherin cytoplasmic tail recruits myosin II to the sites of cell-cell contact. Forces due to actomyosin contractility propagates to the E-cadherin ectodomain and promotes the conversion of weak X-dimers to stronger strand-swap dimers (30).

However, the molecular mechanisms by which other cytoplasmic proteins (besides vinculin) regulate E-cadherin adhesion remain to be clarified. For instance, the biophysical mechanisms by which p120-catenin allosterically alters the adhesive properties of E-cadherin needs to be worked out. Similarly, it is unclear if α-catenin solely remodels cell-cell junctions or if it also allosterically alters the adhesive properties of individual E-cadherins.

Besides catenins, diverse signaling molecules are found at cell-cell contacts and many of these molecules are activated in a cadherin-dependent manner (86). For instance, cadherin binding sites are major locations for protein tyrosine phosphorylation, including both receptor tyrosine kinases and cytoplasmic kinases. One example is the Src-family kinases (SFKs), which are cytoplasmic tyrosine kinases, often found at cadherin-based cell–cell contacts (87, 88). Both E-cadherin and P-cadherin mediated cell adhesions have been shown to signal the activation of Src kinases at cell-cell contacts, and inhibiting Src kinases signaling is known to impair the functions of cadherins (89, 90). However, the molecular connections between Src kinases and cadherin adhesion is poorly understood and it is unclear if Src kinase activation affects cadherin ectodomain binding conformation.

Conclusions:

The distinct mechanical signatures of X-dimers and strand-swap dimers (41, 42) suggest different functional roles for these conformations in processes like collective cell migration, tissue formation and wound healing. However, the biological roles of X-dimer catch bonds and strand-swap dimer slip bonds remains to be elucidated. Determining this would likely require the development of conformation-specific antibodies that can distinguish between these structures. Similarly, new biophysical studies continue to reveal novel ectodomain binding conformations that are distinct from X-dimers and strand-swap dimers (29, 31–33). The biophysical properties of these conformations and their mechanistic roles in cadherin adhesion remain to be resolved.

Furthermore, how these distinct cadherin conformations are regulated in the context of cell function are unknown. Analogous to the case of integrins where adhesion is regulated from the inside-out (91), classical cadherin adhesion is also regulated by cytoplasmic proteins. However, the molecular mechanisms by which cytoplasmic effectors such as p120-catenin, α-catenin, and Src kinases regulate cadherin ectodomain conformation remains to be clarified.

While several mAbs that strengthen E-cadherin adhesion have been developed (52), only the biophysical mechanism by which 19A11 strengthens adhesion has been resolved in molecular detail (31, 57, 58). Determining the activating mechanisms for the remaining mAbs will provide a more holistic picture of how mAbs enhance cadherin adhesion. Additionally, although several antibodies have been found to inhibit cadherin function, the conclusive molecular mechanisms by which they act is unclear. Furthermore, several other classical cadherins are implicated in cancer metastasis including P-cadherin and N-cadherin (43, 60, 92, 93). The molecular mechanism of E-cadherin activating antibodies such as 19A11 (31, 58) may prove useful in designing mAbs that regulate the adhesion of other classical cadherins.

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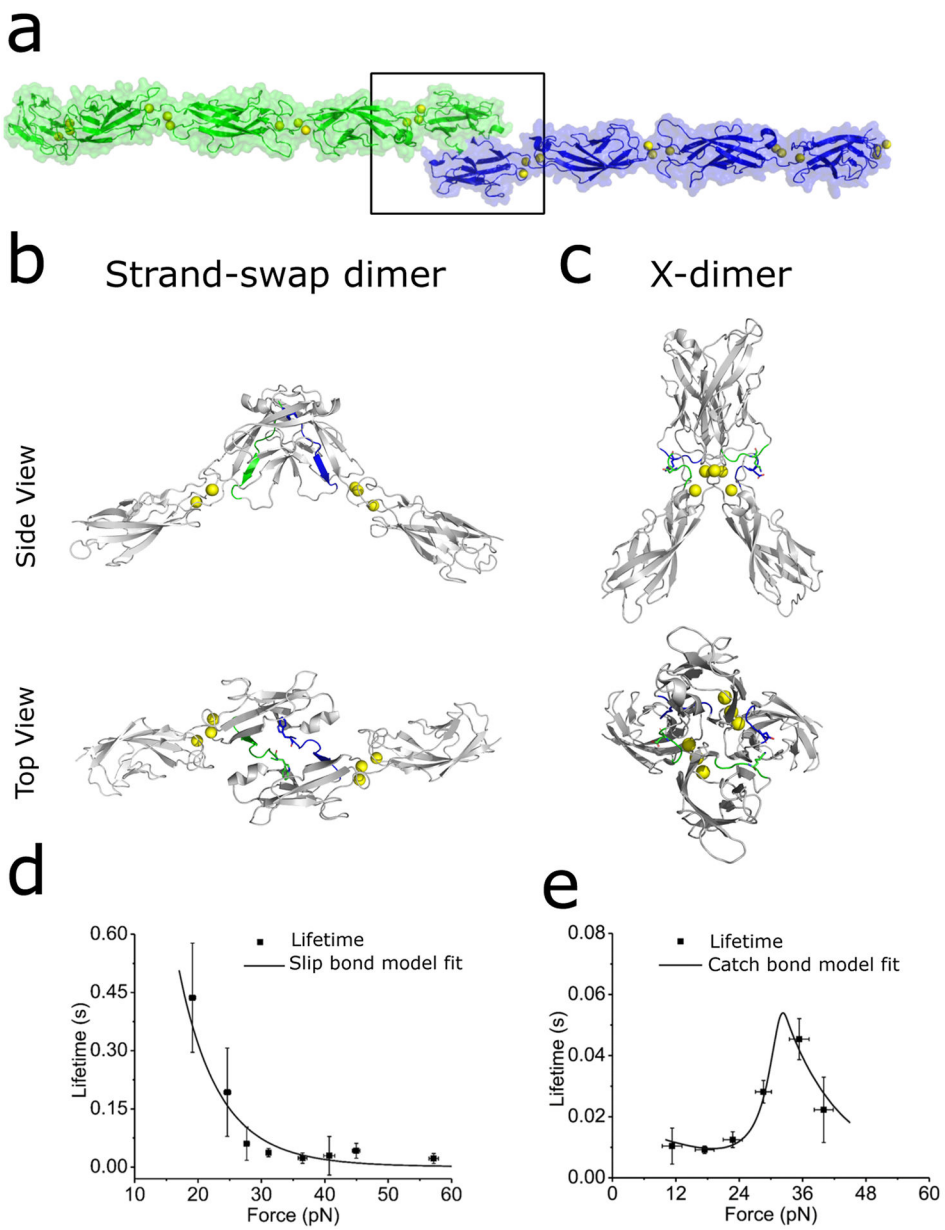

Figure 1: Structure and biomechanics of E-cadherin *trans* dimers.

(a) Strand-swap dimers formed by the interaction of full length ectodomains of E-cadherin from opposing cell surfaces (green and blue, PDB: 3Q2V). (b) Structure of the outer two domains (EC1–2) forming a strand-swap dimer (PDB: 2O72). Strand-swap dimers are formed by exchanging the N terminal β-strands (residues 1–12 from opposing ectodomains are highlighted in green and blue). (c) X-dimers (PDB: 4ZT1) are formed due to interactions between EC1–2 domains of opposing cadherins. These interactions include hydrogen bonds and a key salt bridge (K14-D138) between loops 11–15 and 135–139 which are highlighted in green and blue. (d) Strand-swap dimers form slip bonds. The lifetime of a slip bond decreases with increasing in pulling force. (e) X-dimers form catch bonds. Catch bonds

initially strengthen before weakening beyond a critical pulling force. Panel (d) is adapted from reference (29) while panel (e) is adapted from reference (42).

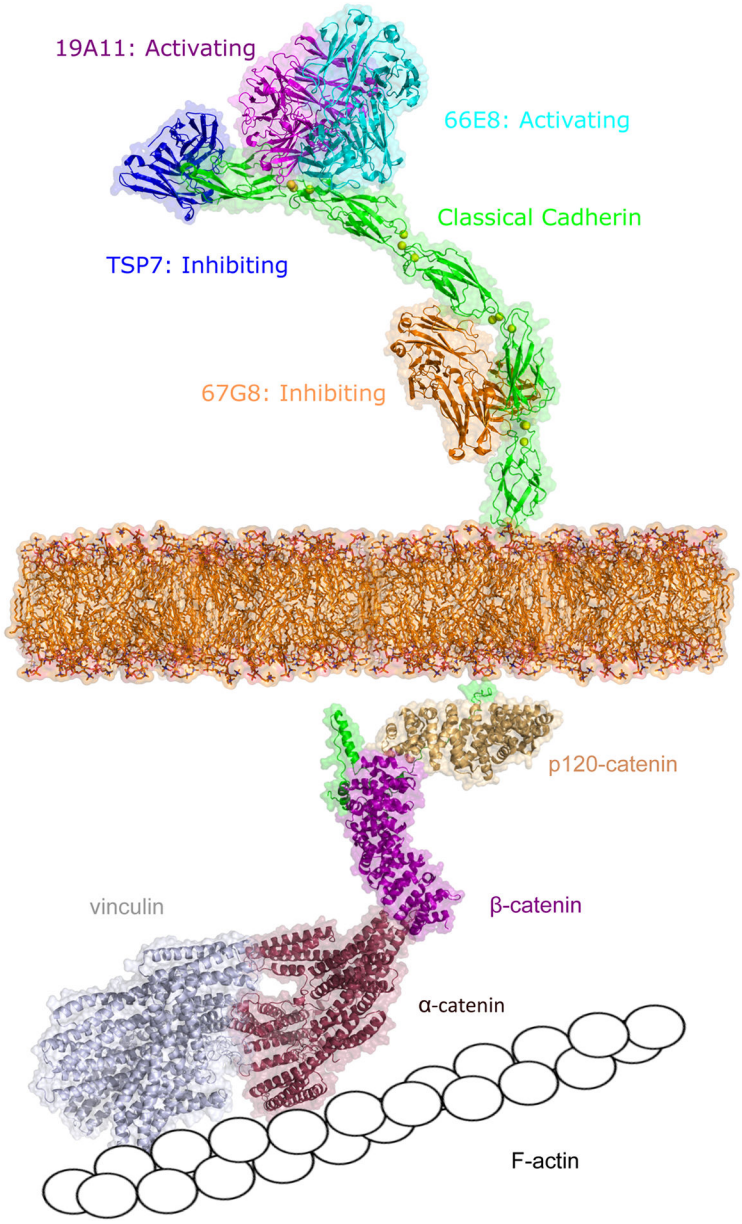

Figure 2: Regulating classical cadherin adhesion.

Cadherin adhesion can be regulating either via extracellular binding with antibodies or from the inside-out by cytoplasmic proteins. Activating antibodies 19A11 (magenta, PDB: 6CXY) and 66E8 (cyan, PDB:6VEL) recognize E-cadherin EC1 or EC2 domain (green, PDB: 3Q2V) near the cadherin *trans* binding sites. Antibody TSP7 (blue, PDB: 5JYL) inhibits P-cadherin adhesion by binding on the EC1 domain while 67G8 (orange) inhibits E-cadherin adhesion by binding on EC5 domain. The intracellular domain of cadherin (green) associates with various signaling molecules, including β-catenin (pink, PDB: 3L6X), p120-catenin (light yellow, PDB: 4R10), α-catenin (red), and vinculin (grey), which eventually link the cadherin cytoplasmic region to F-actin. The structures of α-catenin, vinculin, and E-cadherin

intracellular domain were predicted using Alphafold (94). Unless apparent from the crystal structure, all interactions were predicted using Alphafold.