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Physical-Chemical Regulation of Membrane Receptors Dynamics in Viral Invasion and Immune Defense

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Abstract

Mechanical cues dynamically regulate membrane receptors functions to trigger various physiological and pathological processes from viral invasion to immune defense. These cues mainly include various types of dynamic mechanical forces and the spatial confinement of plasma membrane. However, the molecular mechanisms of how they couple with biochemical cues in regulating membrane receptors functions still remain mysterious. Here, we review recent advances in methodologies of single-molecule biomechanical techniques and in novel biomechanical regulatory mechanisms of critical ligand recognition of viral and immune receptors including SARS-CoV-2 spike protein, T cell receptor (TCR) and other co-stimulatory immune receptors. Furthermore, we provide our perspectives of the general principle of how force-dependent kinetics determine the dynamic functions of membrane receptors and of biomechanical-mechanism-driven SARS-CoV-2 neutralizing antibody design and TCR engineering for T-cell-based therapies.

Introduction

Ligand recognition of membrane receptors triggers various crucial physiological and pathological processes from viral invasion to immunological defense. For example, beta-corona viruses (e.g. severe acute respiratory syndrome coronavirus 2, SARS-CoV-2) invasion of host cells for activating viral infections is initiated by their spike protein recognition of membrane receptors on host cells (e.g. angiotensin-converting enzyme 2, ACE2, ligand for both SARS-CoV and SARS-CoV-2), T cell receptors (TCR) expressed on T lymphocytes (e.g. cytotoxic CD8⁺ T cells or CTL) specifically recognize viral- or tumor-mutation-associated antigenic peptides presented by major histocompatibility complex class I (MHC class I) molecules, triggering a series of antigen-specific adaptive immune defense to eliminate viral infected or transformed target cells. NKG2D (Natural Killer Group 2, member D) receptors, as co-stimulatory receptors...
commonly expressed on the surface of natural killer (NK) or T cells, recognizes autologous ligands from the MIC (MHC class I chain-associated, MICA and MICB) and ULBP (UL16 binding protein, ULBP1-6) families expressed on stressed, transformed, or infected cells, to mediate the killing process of virus-infected or tumor cells. 

During these processes, mechanical cues, as essential factors, dynamically regulate receptor-ligand binding kinetics to delicately tune membrane receptor’s functions and precisely activate viral invasion or immune defenses against foreign attacks. 

With the development of single-molecule force spectroscopy techniques (SMFS) and molecular dynamics simulations, membrane receptor-ligand interactions have been resolved with high-sensitivity, high-specificity, and high spatial and temporal resolutions, revealing unprecedented dynamical biophysical regulatory mechanisms that are unable to be disclosed by qualitative biochemical analysis. Mechanical cues exerted on receptor-ligand binding complex mainly include different dynamic mechanical forces and the spatial confinement of cellular plasma membrane. Mechanical tensile or traction force generated by cytoskeletal actomyosin contraction, cell membrane bending and cell migration or shear force provided by blood flow could deform or change receptors and/or ligands’ conformations, differentially modulating the dissociation pathway of receptor-ligand interactions. The plasma membrane of cells provides a unique microenvironment that biomechanically restricts the orientation of the ectodomain of membrane receptor and the spatial diffusion or movement only within the two-dimensional (2D) membrane, thus inevitably impacting the association and dissociation processes of receptor-ligand interactions and their binding kinetics. Furthermore, mechanical regulation of receptor-ligand interactions executes crucial effects on transmembrane signaling of membrane receptors. The underlying mechanisms have been dissected by biophysical methodologies that simultaneously record binding kinetics and binding-triggered cell signaling, such as fBFP (fluorescent biomembrane force probe), BATTLES (Biomechanically-Assisted T-cell Triggering for Large-scale Exogenous-pMHC Screening), and theoretical models. 

Here, we summarize recent advances in the development of single-molecule force spectroscopy techniques and crucial functional mechanisms underlying receptor-ligand interactions for triggering viral infection and immune defense. We propose that understanding the biomechanical characteristics of how mechanical and chemical cues couple in regulation of membrane receptor-ligand interactions may help optimize the designs of neutralizing antibodies against SARS-CoVs infection and of TCR-T or neoantigen vaccines for T-cell-based immunotherapies.

## Single-molecule force spectroscopy and molecular dynamics simulations

Single-molecule force spectroscopy (SMFS) techniques, mainly containing atomic force microscopy (AFM), optical tweezers (OT), and biomembrane force probe (BFP) have revolutionized receptor-ligand binding kinetics measurements. These SMFS techniques work like soft and mechanically sensitive springs, transducing piconewton forces to the displacements of a bead or a cantilever to that can be quantitatively monitored and precisely manipulated in high spatial-temporal resolutions (for details see published reviews). Benefited from precise force manipulations on single-molecule bonds, SMFS techniques have been applied to characterize the force-dependent receptor-ligand binding kinetics under different types of dynamic piconewton forces, and to characterize force-induced conformational changes, revealing crucial functional mechanisms of membrane receptors, such as viral (e.g. SARS-CoV-2 spike), immune receptors (e.g. TCR and NKG2D) and adhesion receptors (e.g. Integrin, PSGL-1, LFA-1).

Along with the development of SMFS techniques, research on membrane receptor’s functions keep carrying forward. Taking BFP as an example, binding kinetics of receptor-ligand interactions can be derived from different methods, mainly containing dynamic force spectroscopy for measuring force-free dissociation rates, force-clamp assay for characterizing force-dependent dissociation rates, adhesion frequency assay for binding affinities and force-free association and dissociation rates, and thermal-fluctuation assay for association rates and force-free dissociation rates. With the more physiological-relevant biophysical condition, TCR-pMHC binding kinetics measurements are more matched with their ligand potencies in comparison to those by SPR measurements. Later in 2014, the integration of fluorescent spectroscopy into the BFP system enabled the recording of intracellular Ca²⁺ signaling and measuring TCR-pMHC binding kinetics simultaneously, digitalizing TCR triggering mechanisms more directly and revolutionizing canonical methodologies for studying the mechanisms of membrane receptors mechanosensing and triggering. Our group further improved the clamping force stability and accuracy of BFP in 2020, enabling the lifetime measurements of single-molecule bonds on live cells with ultra-slow dissociation kinetics, such as the interaction between anti-PD-1 mAb and PD-1 on live T cells. Thus, the rapidly developed SMFS techniques have become more efficient in...
Mechanical force

Mechanical force has been shown to be a biophysical determinant of membrane receptor-ligand interactions during viral invasion and immune defense. Mechanical force induced by cell membrane bending has been reported to be involved in cell–cell contact as well as in viral endocytosis. When virions attach to the epithelial layer of the lung airways, the bent cell membrane exerts tensile forces (e.g. 0 −30 pN) on the viral spike-ACE2 complex, regulating viral spike/ACE2 binding kinetics and accordingly mediating viral-host recognition, attachment, and invasion. A growing number of studies suggest that T cells enforce piconewton forces (e.g. 12 −19 pN for naïve CD8+ T cell) to TCR-pMHC bonds and dynamically modulate their binding kinetics, as well as their conformations to accordingly transduce signals across cell membranes. During the aforementioned processes, mechanical force regulates the dissociation rates of receptor-ligand interactions, exhibiting catch-, slip- or ideal bonds. Catch bonds slow down the dissociation of receptor-ligand interactions and prolong the bond lifetimes in a specialized force range due to newly formed interactions between residues on receptor and ligand during force-regulated dissociation pathway. In contrast, slip bonds, as Bell predicted, accelerate bond dissociation as mechanical force increases. Ideal bonds exhibit no effect on dissociation, which is independent of changes in mechanical forces. However, the detailed molecular mechanisms remain largely unknown.

Mechanical force in virus invasion

COVID-19 pandemic has caused immeasurable damage worldwide, and new variants are constantly emerging, such as beta, gamma, delta, omicron, and omicron variants (https://www.gisaid.org). The initial step of viral invasion is that the spike protein (including S1 and S2 subunit) of SARS-CoV-2 (SARS2-S) recognizes host cell receptors (targeting mainly ACE2 and also tyrosine-protein kinase receptor UFO (AXL), and immune system receptors, such as, toll-like receptors (TLR), C-lectin type receptors (CLR), neuropilin-1 (NRP1), and DPP4/CD26). Receptor-binding domain (RBD) in S1 subunit is the major domain for binding host receptor ACE2, while the S2 subunit forms fusion machinery to target host-cell plasma membrane after S1/S2 detachment. Both processes are potentially regulated by mechanical forces, and revealing the underlying mechanisms from the biomechanical angle would possibly provide novel strategies for preventing novel mutated SARS-CoV-2 strains that are able to evade therapeutic antibodies or vaccines protection.

Studies have shown that mechanical forces generated by cell membrane bending boost ACE2-dependent SARS-CoV-2 invasion. Firstly, in the viral recognition stage, mechanical force exerted on a single spike/ACE2 bond is approximately between 0 and 30 pN according to theoretical calculation. Compared with SARS-S-RBD (~6% amino acid sequence difference compared to SARS2-S-RBD, the interactions of SARS2-S-RBD binding to ACE2 have stronger mechanical stability to resist stretching force through inducing a more pronounced catch-slip bond behavior with much longer force-dependent bond lifetimes (Figure 1(A)), which is positively correlated with their infectivity. Mechanistically, SARS2-S-RBD is more prone to adopt an open conformation under mechanical force loading, thereby promoting the formation of more H-bonds and hydrophobic interactions on the SARS2-S-RBD/ACE2 binding surface, compared to that in SARS-S-RBD/ACE2 binding (Figure 1(C)). Secondly in the fusion stage, mechanical force accelerates S1/S2 detachment to promote S2 structural rearrangement and fusion machinery formation. In this regard, our group for the first time revealed that mechanical force dramatically speeds up SARS2-S S1/S2 detachment by up to ~103 times faster than that in the force-free condition (Figure 1(B)).

These mechanical regulations during viral invasion were further validated by the more infectious D614G mutation of SARS2-S. D614G mutation, located outside of the RBD of the S1 subunit, causes more fatality and is inherited in Alpha, Beta, Delta and recent Omicron SARS-CoV-2 virus. A recent study showed that D614G converts the S1 protein conformation
(RBD and N-terminal domains of S1 subunit) to an ACE2-binding fusion-competent state and thus may increase viral infectivity. Our group further found that mechanical force extent almost four-time longer lifetimes of SARS2-S-D614G binding with ACE2 receptors and 35 times faster force-induced S1/S2 detachment than those of wild-type SARS2-S (Figure 1A, B). Thus, the more plausible molecular mechanism by which SARS2-S D614G mutation causes higher infection rate from biophysical standpoint is that D614G mutation fosters stronger mechanical stability of SARS2-S/ACE2 complex and better couples with mechanical force to induce much faster S1/S2 detachment. Currently, new sub-variants (e.g., omicron BA.1, BA.2, BA.2.12.1) are constantly emerging, we speculate that mechanical force are also essential for entry of other virus variants and the biophysical mechanism will be a common feature of viral invasion.

The widely adopted treatment strategy to prevent viral invasion is to block the engagement of the spike-RBD and ACE2 interactions with neutralizing antibodies. However, due to the high mutagenicity of SARS2-S1 (especially RBD), it is very likely that SARS2 evades neutralizing antibodies blockade through abolishing these mAb bindings with RBD or that SARS2 may adopt other entry pathways by binding with other host membrane proteins (e.g. AXL) to maintain viral infectivity, which are the intrinsic limitations for these RBD-blockade designs of neutralizing antibodies. We speculate that the aforementioned mechanical regulatory mechanisms could provide another novel intervention strategy to prevent virus infection, that is, mechanically locking the S1

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**Figure 1. Mechanical forces strengthen the spike/ACE2 binding and accelerate S1/S2 detachment, providing novel intervention strategy.** (A) Schematics of force-dependent bond lifetimes of the interactions of host ACE2 receptors interacting with SARS2-S-D614G (red), SARS2-S-WT (blue) and SARS-S (gray), respectively. (B) S1/S2 detachment rate versus force curves of SARS2-S-D614G (red), SARS2-S-WT (blue) and SARS2-S-WT in the presence of neutralizing antibody targeting S1/S2 (purple), respectively. (C) The dynamic structural model of force-regulated SARS2-S/ACE2 binding and conformational change of SARS2-S, force-induced S1/S2 detachment, and S1/S2-locking antibodies to impede SARS2-S/ACE2 binding.
subunit to S2 subunit to impede their detachment (Figure 1(C)). In this way, revealing the underlying biophysical mechanisms of viral invasion, especially biomechanical activation of viral spikes, would potentially optimize the design of therapeutic antibodies.

Mechanical regulation in immune defense

Force-regulated conformational changes of immune receptors (e.g. TCR) or their ligands (e.g. pMHC, MICA) have been reported to uncover the detailed molecular mechanisms of receptor-ligand interactions during immune defense in recent studies. Single-molecule force spectroscopy revealed that mechanical force strengthens agonistic pMHC-TCR (canonical αβTCR, not reverse docking αγTCR and γδTCR) interactions through catch bonds (Figure 2(A)). In contrast, antagonistic pMHC-TCR interactions only exhibit slip bonds under mechanical force (Figure 2(B)). To allosterically activate TCR-pMHC catch bonds, mechanical force induces conformational changes in pMHC to promote new contacts (hydrogen bonds) at the peptide-TCR or MHC-TCR binding interface. Mechanical stretching of single pMHC molecule results in an about 13 nm extension, and locking such extension attenuates TCR-pMHC catch bonds, demonstrating that force-induced conformational changes in pMHC contribute to the catch bonds formation. Mechanical force mainly drives three sequential steps of conformational changes in an agonistic pMHC/TCR complex to activate catch bonds. First, TCR exploits its CDRβ (complementarity determining region) to establish a physical contact with the functional hotspot (e.g. the fourth or sixth residue in the FGG loop in the constant region of the TCR) that would potentially form/impede new H-bonds and/or salt bridge interactions at the TCR-pMHC binding surface under force, instead of mutating the residues that directly contact with pMHC, to ensure their affinities with catch bond enhancement/inhibition, potentially boosting T-cell responses/avoiding excessive T-cell cross-reactivity, respectively (Figure 2(E)). As a validation of the newly developed strategy, they selected a tumor-associated MAGE-A3-specific TCR (A3A-TCR) that was previously used in clinical trials with high binding affinities to its antigens and with severe off-target toxicity due to high cross-reactivity. With the catch bonds engineering strategy, engineered analogs of A3A-TCR maintain binding affinity in the natural physiological range and high-efficiency activation and reduce TCR’s cross-reactivity. It is the milestone that catch bond is utilized to optimize TCR for immunotherapy, providing a new biophysical angle to revolutionize T cell-mediated adoptive cell therapy. On the other hand, cancer-associated somatic mutations in pHLA-A2 were found to limit conformational extension in agonistic pHLA and impede TCR-pMHC catch bonds (Figure 2(D)), which is also a promising target for the catch bond engineering strategy.

The biophysical regulatory mechanisms described above also prompt researches of other immune receptors in the innate immune system (e.g. NKG2D on lymphocyte cells). NK cells are another type of cytotoxic cells in the immune defense, which can rapidly respond to kill virus-infected or tumor cells. NK cells exert tensile forces through NKG2D receptors, which interact dynamically with their ligands (e.g., MICA, MICB, ULBP1, and ULBP3), converting mechanical stimuli into biochemical signals. MICA is the most pronounced ligand to exploit mechanical force and
has the longest bond lifetimes with NKG2D at the optimal force of ~10 pN compared with MICB, ULBP1, and ULBP3 ligands. At a low-force regime (<10 pN), mechanical force induces the catch bonds formation, while increasing mechanical force beyond 10 pN form the slip bonds to shorten the bond lifetimes for NKG2D-MICA interactions. Other ligands bound to NKG2D exhibit only slip bonds under full force spectrum. Thus, mechanical force aids NKG2D the power to discriminate different...
ligands, similar to but less potent than TCRs, through differential force-induced ligand conformational change of NKG2D/ligand complex. Collectively, we believe that revealing mechanical regulations on other immune receptors, as well as the popular immune checkpoint receptors (e.g. PD-1 and CTLA4), would also promote the optimization of immunotherapies strategy from a biomechanical angle to treat not only infectious diseases, tumors but also autoimmune diseases.

**Spatial confinement of cell plasma membranes**

In addition to the effects of mechanical force, the spatial confinement by plasma membrane could also enforce significant impacts on the association and dissociation processes, especially their kinetic rates of receptor-ligand interactions and accordingly regulate membrane receptors functions. The association rates ($k_{on}$) characterize how fast the receptor-ligand bond forms, while the dissociation rates characterize how long the bond lasts ($k_{off}$), and binding affinity ($K_a$) is defined by the ratio of $k_{on}/k_{off}$, reflecting receptor-ligand binding strength at equilibrium states. In this section, we summarize recent advances about how spatial confinement of cell plasma membranes modulates the binding kinetics of receptor-ligand interactions and how SNPs (Single Nucleotide Polymorphisms) in transmembrane (TM) regions of membrane receptors exert allosteric regulatory effects on their binding kinetics under membrane confinement.

The measurement of receptor-ligand interactions in a two-dimensional (2D) manner (e.g., micropipette assay), where the binding kinetics are detected based on two apposing cell membranes that reconstitute the physiological conditions of membrane proteins, is significantly different from those in three-dimensional (3D) manners (e.g. surface plasmon resonance, SPR) using purified ligands in soluble states. The existing regulatory mechanisms of how spatial confinement of plasma membrane regulate receptor-ligand interactions can be roughly divided into two categories. Firstly, 2D cell plasma membrane physically restricts the orientation of membrane receptors and/or ligands, affecting 2D binding affinities through changing association rates, such as interactions between human Fcγ receptor III (CD16, stimulatory receptor expressed mainly on NK cells as well as neutrophils, monocytes, macrophages, and T cells) or FcγRIIB (inhibitory receptor expressed on B lymphocytes and their IgG ligands). The molecule orientation changes arise primarily from differences in length or inclination of the TM regions of membrane receptors inside the membrane, altering their membrane-confined lateral mobility. Secondly, the cell plasma membrane influences the spatial diffusion or movement of surface molecules with long ectodomains (e.g. CD45), regulating the binding kinetics of receptor-ligand interactions at cell–cell interfaces. When the receptor diffuses on the plasma membrane, both the distance and the local concentration of membrane receptors and/or ligands would change in the contact zone. The diffusion or movement within 2D cell plasma membrane can be driven by many possible mechanisms according to kinetic segregation model, such as: molecule length changes, spatial reorganization, or functional clustering. However, the mechanism by which molecular diffusion facilitates or prevents receptor-ligand interactions is still undefined. Collectively, the physical regulation of the spatial confinement of cell plasma membranes reveals a novel mechanism for the regulation of physical–chemical coupling on receptor-ligand interactions.

Single nucleotide polymorphisms (SNPs) in transmembrane regions of membrane receptors can allosterically regulate receptor-ligand interactions through coupling the TM-lipid bilayer biochemical interaction with the spatial confinement of cell plasma membrane. An SNP variant of FcγRIIB, I232T, in systemic lupus erythematosus (SLE), impedes its two-dimensional binding affinities through association rates with its ligands (IgG1, IgG2, and IgG3) and suppresses immune cells activation. Considering that the I232T variant is located in the transmembrane regions of the FcγRIIB receptor, and that the tilted transmembrane regions lead to a bent ectodomain conformation that impedes ligands association, SNP potentially couples with membrane confinement to affect the receptor-ligand recognition and signaling functions. SNP mutations have also been identified in immune checkpoint molecules, such as LAG-3 (Lymphocyte-activation gene 3) and T cells, (Figure 3). First, force-induced conformational changes in the extracellular domains of membrane receptors (e.g. TCR) sequentially propagates across the cell membrane,
inducing structural changes in the cytoplasmic domains and downstream signaling (Figure 3).117 In details, when a TCR recognizes an agonistic pMHC, TCR’s extracellular domains (e.g. FG loop) probably transmit forces to the TCR transmembrane (TM) regions that form a compact and precisely organized structure with the CD3 subunits (ed, ec, ff) within the membrane.64,116,117 The juxtamembrane region of the CD3 ff signaling module likely acts as a mechanical pivot connecting TCR a chain, thereby transmitting biochemical and biomechanical information across the cell membrane.6 Second, accumulation of dynamic catch bonds between TCR and pMHC triggers T cell signaling.18 By simultaneously measuring the TCR-pMHC force-dependent binding kinetics and binding-triggered intracellular signaling, Zhu and his colleagues demonstrated that the accumulative bond lifetimes within the initial 60 seconds between TCR and agonist pMHC are the most relevant to intracellular signaling (e.g. Ca2+ flux), rather than the other parameters (e.g. binding affinities, peak bond lifetimes, average lifetimes, or longest lifetimes).18 On the contrary, for antagonistic pMHC ligands and TCR interactions, the accumulative bond lifetimes are very short, and the Ca2+ signals are not induced within the specific time window.18 This indicates that the synchronization between extracellular receptor-ligand interactions and intracellular kinase activation is critical for triggering T cells. Third, mechanosensing through membrane receptors induces a mechanical feedback loop that affects T cell activation. Mechanical-induced “dynamic catch” in trimolecular or multi-molecular interactions (e.g. TCR-pMHC-CD8-PD-1136), regulate the linkage and cis-interactions of intracellular signaling molecules (e.g. linking CD8 and TCR-CD3 via lymphocyte-specific protein tyrosine kinase, Lck) to form a mechanotransduction loop, also known as inside-out signaling, further amplifying the ligand discriminative ability of membrane receptors.57 In addition, PD-1 specifically blocks the mechanotransduction loop during T-cell antigen recognition.136 However, the detailed mechanisms about how mechanical forces coordinate the mechanical signaling from these co-receptor, co-stimulatory, and co-inhibitory molecules to delicately tune the intracellular signaling cascades remain unclear.

Collectively, biomechanical parameters are key determinants of membrane receptors transmembrane signaling, providing novel physical transmission mechanisms for receptor’s ligand recognition and signal triggering.

**Perspectives**

**Force fluctuations**

Force fluctuations are functionally significant in numerous biological contexts, such as embryonic lineage sorting,137 cell migration,138 and signal transduction.5 During embryonic development, primitive endoderm (PrE, founder of the yolk sac) and ectoderm (EPI, founder of the fetus) need to be physically separated. Dynamic cell surface fluctuations, rather than static cell surface parameters, robustly ensure physical lineage sorting.137 During cell adhesion, the integrin-based focal adhesions (FAs) exhibit dynamic fluctuating traction through...
extracellular matrix cytoskeleton motion. Force exerted on receptor-ligand bonds in vivo potentially act in a cyclically fluctuating manner and often repeat with multiple cycles, depending on cytoskeletal actin velocity at the immune synapse or membrane fluctuations. It is known that active actin cytoskeleton modulates the binding kinetics of receptor-ligand bonds in situ, but the cyclically fluctuating regulatory mechanisms to activate membrane receptors remain unclear. Membrane fluctuations are also influenced by the movement of the actin cytoskeleton and other factors, such as membrane microvilli structure, that affect receptor-ligand binding strength. Besides, the frequency and amplitude of force fluctuations play critical roles in determining receptor-ligand binding and membrane receptors functions. Different frequencies, amplitudes, and directions of dynamic force control different signal patterns and determine cell fates. Therefore, gaining insights into how dynamic force of different frequencies, amplitudes and directions are functionally crucial to membrane receptors functions, worth for deeper exploring.

Membrane receptors dynamics buffer force fluctuations

The biophysical parameters of receptor-ligand interactions play critical effects on buffering force fluctuations. When the frequency of oscillatory force is sufficiently rapid, the signals exerted on receptor-ligand bonds may be unstable and easily disturbed. Despite this interference, organisms could provide specific mechanisms and exhibit extraordinary robustness to reduce noise. Thus, membrane receptors are trying to exploit the oscillatory forces to optimize their functions (e.g. Integrin or TCR). Studies have shown that cyclical force could reinforce cell adhesion by prolonging much longer-lived bond lifetimes of integrin and its ligand interactions. TCR exploits the cyclical forces exerted by actin movement significantly increase T cell signaling strength (Ca²⁺ flux), providing a novel cyclic mechanical reinforcement possibility of T cell triggering. When the cells are treated with Latrunculin A (LatA), which inhibits actin polymerization, the cyclical force could compensate the Ca²⁺ signals. But how TCR exploits force fluctuations to convert into biochemical signals is unknown. Mechanical-induced dynamic catch bonds of TCR-pMHC interactions may be easier to buffer force fluctuations than slip bonds when the bonds experience fluctuating forces. The detailed molecular mechanism needs to be further explored. We thought that mechanical regulation under cyclic forces may differ from constant force regulation, which may enable the cell to fine-tune its mechanotransduction through membrane receptors. Here, we propose two possible explanations for how membrane receptors buffer force fluctuations. First, force fluctuations may accelerate catch bonds formation and increase the number of the long-lived bond lifetimes, helping membrane receptors to filter the noise interference and amplifying the amount of signal the cell can receive. Second, force fluctuations may greatly enhance the rebinding and unbinding rate of receptor-ligand binding by inducing more open conformation, accelerating the process of kinetic proofreading and leading to a more stable mechano-feedback loop to reinforce the receptor-ligand interactions. Thus, the regulatory mechanisms by which biomechanical factors (cycling force) regulate membrane receptors signaling transduction are worthy of further exploration, and we speculate these mechanisms could be shared by other mechanical-sensing membrane receptors (e.g. integrins, NKG2D, and PD-1).

Conclusions and future directions

Over the past decade, a conceptual framework of physical–chemical regulation of membrane receptor dynamics has emerged to address fundamental scientific questions of viral invasion and immune defense. These findings provide novel mechanisms by which mechanical cues modulate the conformation of membrane receptors or ligands, determining the receptor-ligand binding kinetics and signaling transduction. These dynamic mechanisms will very likely inspire non-canonical thoughts for developing novel clinically relevant immunotherapeutic treatment [e.g. immunotherapeutic antibodies, or TCR-T]. However, multiple outstanding questions remain to be answered:

1. What are the oscillating patterns of forces on receptor-ligand bonds under physiological conditions?
2. Whether and how viral exploit oscillatory force to boost viral invasion?
3. How do force fluctuations dynamically modulate the catch-bond behavior of receptor-ligand interactions?
4. Whether dynamic mechanical stimulation can accelerate the process of kinetic proofreading?

CRediT authorship contribution statement

Rui Qin: Formal analysis, Writing - original draft, Writing - review & editing. Chenyi An: Funding acquisition, Supervision, Writing - review & editing. Wei Chen: Conceptualization, Formal analysis, Funding acquisition, Supervision, Writing - review & editing.

DATA AVAILABILITY

No data was used for the research described in the article.
Acknowledgment

We are thankful to all the Chen lab members. The review is grateful for the support of National Natural Science Foundation of China [31971237 to W.C. and 12102389 to C.Y.A.] and China Postdoctoral Science Foundation of China [31971237 to W.C. and 31971236 to R.Q.] and Science Foundation [2020M681834 to C.Y.A.].

Declaration of Interest

Authors declare that they have no competing interests.

Received 16 June 2022; Accepted 18 August 2022; Available online 22 August 2022

**Keywords:**
- viral invasion
- immune defense
- mechanical force
- spatial confinement of plasma membrane
- membrane receptors dynamics

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References

1. Yin, H., Flynn, A.D., (2015). Drugging Membrane Protein Interactions. *Annu Rev Biomed Eng* 18, 1–26. https://doi.org/10.1146/annurev-bioeng-092115-025322.

2. Hu, W., Zhang, Y., Fei, P., Zhang, T., Yao, D., Gao, Y., Liu, J., Chen, H., et al., (2021). Mechanical activation of spike fosters SARS-CoV-2 viral infection. *Cell Res*, 1–14. https://doi.org/10.1038/s41422-021-00558-x.

3. Zamoyska, R., (1998). CD4 and CD8: modulators of T-cell receptor recognition of antigen and of immune responses? *Curr Opin Immunol* 10, 82–87. https://doi.org/10.1016/s0952-7915(98)80036-8.

4. Merch, A.M., Bálint, S., Santos, A.M., Davis, S.J., Dustin, M.L., (2020). Coreceptors and TCR Signaling – the Strong and the Weak of It. *Front Cell Dev Biol*, 1–13. https://www.frontiersin.org.

5. He, J., Xiong, X., Yang, H., Li, D., Liu, X., Li, S., Liao, S., Chen, S., et al., (2022). Defined tumor antigen-specific T cells potentiate personalized TCR-T cell therapy and prediction of immunotherapy response. *Cell Res*, 1–13. https://doi.org/10.1038/s41422-022-00627-9.

6. Gudipati, V., Rydzek, J., Doel-Perez, I., Alves, V.D.R.G., Scharf, L., Nigsberger, S.K., Lobner, E., Kunert, R., et al., (2020). Inefficient CAR-proximal signaling blunts antigen sensitivity. *Nature Immunol*, 1–21. https://doi.org/10.1038/s41590-020-0719-0.

7. Siller-Farán, J.A., Dushek, O., (2018). Molecular mechanisms of T cell sensitivity to antigen. *Immunological. Review*, 1–12. https://doi.org/10.1111/imr.12690.

8. de Andrade, L.F., Tay, R.E., Pan, D., Luoma, A.M., Ito, Y., Badrinath, S., Tsoucas, D., Franz, B., et al., (2018). Antibody-mediated inhibition of MICA and MICB shedding promotes NK cell–driven tumor immunity. *Science* 359, 1537–1542. https://doi.org/10.1126/science.aag0505.

9. Fan, J., Shi, J., Zhang, Y., Liu, J., An, C., Zhu, H., Wu, P., Hu, W., et al., (2021). NKG2D discriminates diverse ligands through selectively mecano-regulated ligand conformational changes. *Embo J*, e107739. https://doi.org/10.15252/embj.2021107739.

10. Raulet, D.H., (2003). Roles of the NKG2D immunoreceptor and its ligands. *Nature Rev Immunol* 3, 781–790. https://doi.org/10.1038/nri1199.

11. Zhu, C., (2000). Kinetics and mechanics of cell adhesion. *J Biomech* 33, 23–33. https://doi.org/10.1016/s0021-9290(99)00163-3.

12. Zhu, C., Chen, Y., Ju, L.A., (2019). Dynamic bonds and their roles in mechanosensing. *Curr Opin Chem Biol* 53, 88–97. https://doi.org/10.1016/j.cob.2019.08.005.

13. Huse, M., (2017). Mechanical forces in the immune system. *Nature Rev Immunol* 17, 679–690. https://doi.org/10.1038/nri.2017.74.

14. Chen, W., Zhu, C., (2013). Mechanical regulation of T-cell functions. *Immunol Rev* 256, 160–176. https://doi.org/10.1111/imr.12122.

15. Zhu, C., Chen, W., Lou, J., Rittase, W., Li, K., (2019). Mechanosensing through immunoreceptors. *Nature Immunol* 20, 1269–1278. https://doi.org/10.1038/s41590-019-0491-1.

16. Liu, B., Chen, W., Zhu, C., (2015). Molecular Force Spectroscopy on Cells. *Annu Rev Phys Chem* 66, 427–451. https://doi.org/10.1146/annurev-physchem-040214-121742.

17. An, C., Hu, W., Chen, W., (2020). Ultra-stable Biomembrane Force Probe for Accurately Determining Slow Dissociation Kinetics of PD-1 Blockade Antibodies on Single Living Cells. *Nano Lett*, 1–8. https://doi.org/10.1021/acs.nanolett.0c01360.

18. Liu, B., Chen, W., Evavold, B.D., Zhu, C., (2014). Accumulation of Dynamic Catch Bonds between TCR and Agonist Peptide-MHC Triggers T Cell Signaling. *Cell*, 357–368. https://doi.org/10.1016/j.cell.2014.02.053.

19. Kong, F., Li, Z., Parks, W.M., Dumbauld, D.W., García, A. J., Mould, A.P., Humphries, M.J., Zhu, C., (2013). Cyclic Mechanical Reinforcement of Integrin-Ligand Interactions. *Mol Cell*, 1060–1068. https://doi.org/10.1016/j.molcel.2013.01.015.

20. Chen, W., Zamitsyna, V.I., Sarangapani, K.K., Huang, J., Zhu, C., (2008). Measuring Receptor-Ligand Binding Kinetics on Cell Surfaces: From Adhesion Frequency to Thermal Fluctuation Methods. *Cell Mol Bioeng*, 1, 276. https://doi.org/10.1007/s12195-008-0024-8.

21. Hu, K.H., Butte, M.J., (2016). T cell activation requires force generation. *J Cell Biol*, 1–11. https://doi.org/10.1083/jcb.201511053&domain-pdf.

22. Feng, Y., Reinhzer, E.L., Lang, M.J., (2018). αβ T Cell Receptor Mechanosensing Forces out Serial Engagement. *Trends Immunol* 39, 596–609. https://doi.org/10.1016/j.it.2018.05.005.

23. Chen, W., Lou, J., Evans, E.A., Zhu, C., (2012). Observing force-regulated conformational changes and ligand dissociation from a single integrin on cells. *J Cell Biol* 198, 497–512. https://doi.org/10.1083/jcb.201201091.

24. Charras, G., Yap, A.S., (2018). Tensile Forces and Mechanotransduction at Cell-Cell Junctions. *Curr Biol* 28, R445–R457. https://doi.org/10.1016/j.cub.2018.02.003.
25. Chesla, S.E., Selvaraj, P., Zhu, C., (1998). Measuring Two-Dimensional Receptor-Ligand Binding Kinetics by Micropipette. *Biophys J* **75**, 1553–1572. https://doi.org/10.1016/s0006-3495(98)74074-3.

26. Chen, W., Evans, E.A., McEver, R.P., Zhu, C., (2008). Monitoring Receptor-Ligand Interactions between Surfaces by Thermal Fluctuations. *Biophys J* **94**, 694–701. https://doi.org/10.1016/j.bpj.2007.11.030.

27. Chesla, S.E., Li, P., Nagarajan, S., Selvaraj, P., Zhu, C., (2000). The Membrane Anchor Influences Ligand Binding Two-dimensional Kinetic Rates and Three-dimensional Affinity of Fc/RII (CD16)∗. *J Biol Chem* **275**, 10235–10246. https://doi.org/10.1074/jbc.275.14.10235.

28. Liu, B., Chen, W., Natarajan, K., Li, Z., Margulies, D.H., Zhu, C., (2015). The cellular environment regulates in situ kinetics of T-cell receptor interaction with peptide major histocompatibility complex. *Eur J Immunol* **45**, 2099–2110. https://doi.org/10.1002/eji.201445358.

29. Blumenthal, D., Burkhardt, J.K., (2020). Multiple actin networks coordinate mechanotransduction at the immunological synapse. *J Cell Biol* **219**, 301–312. https://doi.org/10.1083/jcb.201911058.

30. Ma, Z., Disher, D.E., Finkel, T.H., (2012). Mechanical Force in T Cell Receptor Signal Initiation. *Front Immunol* **3**, 217. https://doi.org/10.3389/fimmu.2012.00217.

31. Wang, J., Reinherz, E.L., (2012). The structural basis of αβ T lineage immune recognition: TCR docking topologies, mechanotransduction, and co-receptor function. *Immuno Rev* **250**, 102–119. https://doi.org/10.1111/j.1600-065x.2012.01161.x.

32. Chen, Y., Lu, A., Zhou, F., Liao, J., Xue, L., Su, O.P., Jin, D., Yuan, Y., et al., (2019). An integrin αlβ3 intermediate affinity state mediates biomechanical platelet aggregation. *Nature Mater* **18**, 760–769. https://doi.org/10.1038/s41563-019-0323-6.

33. Feng, Y., Zhao, X., White, A.K., Garcia, K.C., Fordyce, P. M., (2021). Structure-activity mapping of the peptide- and molecule force spectroscopy for dissecting biophysical regulation of membrane receptors functions on live cells. *Biophys Rep* **7**, 377–383. https://doi.org/10.1026/2021.210022.

34. Kinoshita, K., Leung, A., Simon, S., Evans, E., (2010). Long-Lived, High-Strength States of ICAM-1 Bonds to β2 Integrin, II: Lifetimes of LFA-1 Bonds Under Force in Leukocyte Signaling. *Biophys J* **98**, 1467–1475. https://doi.org/10.1016/j.bpj.2009.12.4316.

35. Wu, P., Zhang, T., Liu, B., Fei, P., Cui, L., Qin, R., Zhu, H., Yao, D., et al., (2019). Mechano-regulation of Peptide-MHC Class I Conformations Determines TCR Antigen Recognition. *Mol Cell* **73**, 1015–1027.e7. https://doi.org/10.1016/j.molcel.2018.12.018.

36. Huang, J., Zarnitsyna, V.I., Liu, B., Edwards, L.D., Li, P., Nagarajan, S., Zhu, C., (2010). The kinetics of two-dimensional TCR and pMHC interactions determine T-cell responsiveness. *Nature* **464**, 932–936. https://doi.org/10.1038/nature08944.

37. Hospital, A., Goñi, J.R., Orozco, M., Gelpí, J.L., (2015). Molecular dynamics simulations: advances and applications. *Adv Appl Bioinform Chem Aabc* **8**, 37–47. https://doi.org/10.2147/aabc.s70333.

38. Humphrey, W., Dalke, A., Schulten, K., (1996). VMD: Visual molecular dynamics. *J Mol Graphics* **14**, 33–38. https://doi.org/10.1016/0267-8591(96)00018-5.

39. Pette, E., Huhn, A., Abu-Shah, E., Utzov, M., Wilson, D.B., Dustin, M.L., Davis, S.J., van der Merwe, P.A., et al., (2021). The discriminatory power of the T cell receptor. *Elife* **10**, e44119. https://doi.org/10.7554/​elife.44119.

40. Goyette, J., Depoil, D., Yang, Z., Isaacson, S.A., Allard, J., van der Merwe, P.A., Gaus, K., Dustin, M.L., et al., (2022). Diphosphorylation accelerates the dissociation of ZAP70 from the T cell receptor e2116815119 *Proc Natl Acad Sci* **119** https://doi.org/10.1073/pnas.2116815119.

41. Neuman, K.C., Nagy, A., (2008). Single-molecule force spectroscopy: optical tweezers, magnetic tweezers and atomic force microscopy. *Nature Methods* **5**, 491–505. https://doi.org/10.1038/nmeth.1218.

42. Bauer, M.S., Gruber, S., Hausch, A., Gomes, P.S.F.C., Millies, L.F., Nicolaus, T., Schendel, L.C., Navajas, P.L., et al., (2022). A tethered ligand assay to probe SARS-CoV-2-ACE2 interactions e2114397119 *Proc Natl Acad Sci* **119** https://doi.org/10.1073/pnas.2114397119.

43. Tian, F., Tong, B., Sun, L., Shi, S., Zheng, B., Wang, Z., Dong, X., Zheng, P., (2021). N501Y mutation of spike protein in SARS-CoV-2 strengthens its binding to receptor ACE2. *Elife* **10**, e69091. https://doi.org/10.7554/​elife.69091.

44. Koelew, M., Ray, A., Moreira, R.A., Juniku, B., Poma, A. B., Alsteens, D., (2021). Molecular insights into receptor binding energetics and neutralization of SARS-CoV-2 variants. *Nature Commun* **12**, 6877. https://doi.org/10.1038/s41467-021-27325-1.

45. Reinherz, E.L., (2019). The structure of a T-cell membrane sensor. *Nature Rev Immunol* **20**, 502–504. https://doi.org/10.1038/s41563-019-02646-w.

46. Marshall, B.T., Long, M., Piper, J.W., Yag, T., McEver, R.P., Zhu, C., (2003). Direct observation of catch bonds involving cell-adhesion molecules. *Nature* **423**, 190–193. https://doi.org/10.1038/nature01605.

47. Chen, W., Lou, J., Zhu, C., (2010). Forcing Switch from Short- to Intermediate- and Long-lived States of the ΑζA Domain Generates LFA-1/ICAM-1 Catch Bonds*. *J Biol Chem* **285**, 35967–35978. https://doi.org/10.1074/jbc.m110.155770.

48. Chenyi, A., Wei, C., (2021). Multiplexed single-molecule force spectroscopy for dissecting biophysical regulation of membrane receptors functions on live cells. *Biophys Rep* **7**, 377–383. https://doi.org/10.52601/bpr.2021.210022.

49. Dong, X., Zheng, P., (2021). N501Y mutation of spike protein in SARS-CoV-2 strengthens its binding to receptor ACE2. *Elife* **10**, e69091. https://doi.org/10.7554/​elife.69091.
55. Park, S., Shi, Y., Kim, B.C., Jo, M.H., Cruz, L.O., Gou, Z., Ha, T., Lu, L.-F., et al., (2020). Force-dependent trans-endocytosis by breast cancer cells depletes costimulatory receptor CD80 and attenuates T cell activation. Biosens Bioelectron 165, 112389. https://doi.org/10.1016/j.bios.2020.112389.

56. Smythe, E., Warren, G., (1991). The mechanism of receptor-mediated endocytosis. Eur J Biochem 202, 689–699. https://doi.org/10.1111/j.1432-1033.1991.tb16424.x.

57. Riggi, M., Bourgoint, C., Macchione, M., Matile, S., Loewith, R., Roux, A., (2019). TORC2 controls endocytosis through plasma membrane tension. J Cell Biol 218, 2265–2276. https://doi.org/10.1083/jcb.201910096.

58. Zhang, Y., Ge, C., Zhu, C., Salaita, K., (2014). DNA-based digital tension probes reveal integrin forces during early cell adhesion. Nature Commun 5, 1–10. https://doi.org/10.1038/ncomms6167.

59. Liu, Y., Blanchfield, L., Ma, V.-P.-Y., Andargachew, R., Gallor, K., Liu, Z., Evavold, B., Salaita, K., (2016). DNA-based nanoparticle tension sensors reveal that T-cell receptors transmit defined pN forces to their antigens for enhanced fidelity. Proc Natl Acad Sci 113, 5610–5615. https://doi.org/10.1073/pnas.1600163113.

60. Ma, V.-P.-Y., Hu, Y., Kellner, A.V., Brockman, J.M., Velusamy, A., Blanchard, A.T., Evavold, B.D., Alon, R., et al., (2022). The magnitude of LFA-1/ICAM-1 forces fine-tune TCR-triggered T cell activation. Sci Adv 8, eabd4485. https://doi.org/10.1126/sciadv.abd4485.

61. Hong, J., Ge, C., Jothishukumar, P., Yuan, Z., Liu, B., Bai, K., Li, K., Rittase, W., et al., (2018). A TCR mechanotransduction signaling loop induces negative selection in the thymus. Nature Immunol 19, 1379–1390. https://doi.org/10.1038/s41590-018-0259-z.

62. Yi, J., Wu, X.S., CitteS, T., Hammer, J.A., (2012). Actin retrograde flow and actomyosin II arc contraction drive receptor cluster dynamics at the immunological synapse in Jurkat T cells. Mol Biol Cell 23, 834–852. https://doi.org/10.1091/mbc.e11-08-0731.

63. McEver, R.P., Zhu, C., (2007). A catch to integrin activation. Nature Immunol 8, 1035–1037. https://doi.org/10.1038/nm1007-1035.

64. Krshnan, L., Park, S., Im, W., Call, M.J., Call, M.E., (2016). A conserved $\alpha$1 transmembrane interface forms the core of a compact T-cell receptor–CD3 structure within the membrane. Proc Natl Acad Sci 113, E6649–E6658. https://doi.org/10.1073/pnas.1611445113.

65. Li, Z., Kong, F., Zhu, C., (2016). A model for cyclic mechanical reinforcement. Sci Rep-Uk 6, 35954. https://doi.org/10.1038/srep35954.

66. Gee, M.H., Han, A., Loftgren, S.M., Beausang, J.F., Mendozza, J.L., Birnbaum, M.E., Bethune, M.T., Fischer, S., et al., (2018). Antigen Identification for Orphan T Cell Receptors Expressed on Tumor-Inflicting Lymphocytes. Cell 172, 549–556.e16. https://doi.org/10.1016/j.cell.2017.11.043.

67. Dushek, O., Dustin, M.L., (2018). CD8 helps TCR catch slippery self pMHC. Nature Immunol 19, 1280–1281. https://doi.org/10.1038/s41590-018-0261-5.

68. Lou, J., Zhu, C., (2007). A Structure-Based Sliding-Rebinding Mechanism for Catch Bonds. Biophys J 92, 1471–1485. https://doi.org/10.1529/biophysj.106.097048.

69. Bell, G.I., (1978). Models for the Specific Adhesion of Cells to Cells. Science 200, 618–627. https://doi.org/10.1126/science.347575.

70. Andreakos, E., Abel, L., Vinh, D.C., Kaja, E., Drolet, B.A., Zhang, Q., O’Farrelly, C., Novelli, G., et al., (2022). A global effort to dissect the human genetic basis of resistance to SARS-CoV-2 infection. Nature Immunol 23, 159–164. https://doi.org/10.1038/s41590-021-01030-z.

71. Hoffmann, M., Klein-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., Schiergens, T.S., Herrler, G., et al., (2020). SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell 181, 271–280.e8. https://doi.org/10.1016/j.cell.2020.02.052.

72. Gadanece, L.K., McSweeney, K.R., Qaradakhi, T., Ali, B., Zulli, A., Apostolopoulos, V., (2021). Can SARS-CoV-2 Virus Use Multiple Receptors to Enter Host Cells? Int J Mol Sci 22, 992. https://doi.org/10.3390/ijms22030992.

73. Solerte, S.B., Sabatinio, A.D., Galli, M., Fiorina, P., (2020). Dipetidyl peptidase-4 (DPP4) inhibition in COVID-19. Acta Diabetol 57, 779–783.https://doi.org/10.1007/s00592-020-01539-9.

74. Daly, J.L., Simonetti, B., Klein, K., Chen, K.-E., Williamson, M.K., Antón-Plágaro, C., Shoemark, D.K., Simón-Gracia, L., et al., (2020). Neurupin-1 is a host factor for SARS-CoV-2 infection. Science 370, 861–865. https://doi.org/10.1126/science.abd3072.

75. Huang, X., Ye, Q., Chen, M., Li, A., Mi, W., Fang, Y., Zaytseva, Y.Y., O’Connor, K.L., et al., (2019). N-glycosylation-defective splice variants of neurupin-1 promote metastasis by activating endosomal signals. Nature Commun 10, 3708. https://doi.org/10.1038/s41467-019-11580-4.

76. Wang, S., Qiou, Z., Hou, Y., Deng, X., Xu, W., Zheng, T., Wu, P., Xie, S., et al., (2021). AXL is a candidate receptor for SARS-CoV-2 that promotes infection of pulmonary and bronchial epithelial cells. Cell Res 31, 126–140. https://doi.org/10.1038/s41422-020-00460-y.

77. Chou, J., Thomas, P.G., Randolph, A.G., (2022). Immunology of SARS-CoV-2 infection in children. Nature Immunol 23, 177–185. https://doi.org/10.1038/s41590-021-01123-9.

78. Moss, P., (2022). The T cell immune response against SARS-CoV-2. Nature Immunol 23, 186–193. https://doi.org/10.1038/s41590-021-01122-w.

79. Diamond, M.S., Kanneganti, T.-D., (2022). Innate immunity: the first line of defense against SARS-CoV-2. Nature Immunol 23, 165–176. https://doi.org/10.1038/s41590-021-01091-0.

80. Wang, J., (2021). Force boosts SARS-CoV-2 infection. Nature Cell Biol 23, 1051. https://doi.org/10.1038/s41556-021-00772-0.

81. Gorbalenya, A.E., Baker, S.C., Baric, R.S., De Groot, R. J.; Drosten, C., Gulyaeva, A.A., Haagmans, B.L., Lauber, C., et al., (2020). Severe acute respiratory syndrome-related coronavirus: The species and its viruses – a statement of the Coronavirus Study Group. Biorxiv. https://doi.org/10.1101/2020.02.07.937862. 2020.02.07.937862.

82. Volz, E., Hill, V., McCrone, J.T., Price, A., Thomson, E.C., Rambaut, A., Connor, T.R., (2021). Evaluating the Effects of SARS-CoV-2 Spike Mutation D614G on...
Transmissibility and Pathogenicity. *Cell* **184**, 64–75.e11. https://doi.org/10.1016/j.cell.2020.11.020.

83. Hou, Y.J., Chiba, S., Halfmann, P., Ehre, C., Kuroda, M., DinnonIII, K.H., Leist, S.R., Schäfer, A., et al., (2020). SARS-CoV-2 spike protein binds efficiently to cell receptors ex vivo and transmission in vivo. *Science* **370**, 1464–1468. https://doi.org/10.1126/science.abe6499.

84. Zhou, B., Thao, T.T.N., Hoffmann, D., Taddeo, A., Ebert, N., Labroussea, F., Pohlmann, A., King, J., et al., (2021). SARS-CoV-2 spike protein D614G change enhances replication and transmission. *Nature* **592**, 122–127. https://doi.org/10.1038/s41586-021-03361-1.

85. Plante, J.A., Liu, Y., Liu, J., Xia, H., Johnson, B.A., Lokugamage, K.G., Zhang, X., Murouato, A.E., et al., (2021). Spike mutation D614G alters SARS-CoV-2 fitness. *Nature* **592**, 116–121. https://doi.org/10.1038/s41586-020-2995-3.

86. Shi, A.C., Xie, X., (2021). Making sense of spike D614G in SARS-CoV-2 transmission. *Science China Life Sci* **64**, 1062–1067. https://doi.org/10.1007/s11427-020-1893-9.

87. He, X., Hong, W., Pan, X., Lu, G., Wei, X., (2021). SARS-CoV-2 Omicron variant: Characteristics and prevention. *MedComm* **2** https://doi.org/10.1002/mcc2.1110. 10.1002/mcc2.1110.

88. Hewins, B., Rahman, M., Bermejo-Martin, J.F., Kelvin, A. A., Richardson, C.D., Rubino, S., Kumar, A., Ndishimeye, P., et al., (2022). Alpha, Beta, Delta, Omicron, and SARS-CoV-2 Breakthrough Cases: Defining Immunological Mechanisms for Vaccine Waning and Vaccine-Variant Mismatch. *Front Virol* **2**, 849936. https://doi.org/10.3389/fviro.2022.849936.

89. Yuan, M., Liu, H., Wu, N.C., Lee, C.-C.-D., Zhu, X., Zhao, F., Huang, D., Yu, W., et al., (2020). Structural basis of a shared antibody response to SARS-CoV-2. *Science* **369**, 1119–1123. https://doi.org/10.1126/science.abb2321.

90. Giron, C.C., Laaksonen, A., da Silva, F.L.B., (2022). Differences between Omicron SARS-CoV-2 RBD and other variants in their ability to interact with cell receptors and monoclonal antibodies. *Biorxiv* https://doi.org/10.1101/2022.01.29.20248640.

91. Cao, Y., Su, B., Guo, X., Sun, W., Deng, Y., Bao, L., Zhu, Q., Zhang, X., et al., (2020). Potent Neutralizing Antibodies against SARS-CoV-2 Identified by High-Throughput Single-Cell Sequencing of Convalescent Patients’ B Cells. *Cell* **182**, 73–84.e16. https://doi.org/10.1016/j.cell.2020.05.025.

92. Dejirirattisai, W., Hoo, J., Zhou, D., Schreiber, G., Stuart, D.I., Sreearom, G.R., (2022). SARS-CoV-2 Omicron-B.1.1.529 leads to widespread escape from neutralizing antibody responses. *Cell* **185**, 467–484.e15. https://doi.org/10.1016/j.cell.2021.12.046.

93. Lan, J., Ge, J., Yu, J., Shan, S., Zhou, H., Fan, S., Zhang, Q., Shi, X., et al., (2020). Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature Publishing Group*, pp. 1–16. https://doi.org/10.1038/s41586-020-2180-5.

94. McCallum, M., Czudnochowski, N., Rosen, L.E., Zepeda, S.K., Bowen, J.E., Walts, A.C., Hauser, K., Joshi, A., et al., (2022). Structural basis of SARS-CoV-2 Omicron immune evasion and receptor engagement. *Science* **375**, 864–868. https://doi.org/10.1126/science.abn6652.

95. Tegally, H., Hufeisen, E., Giovannetti, M., Iranzadeh, A., Fonseca, V., Gandhari, J., Doolabh, D., Pillay, S., et al., (2020). Emergence and rapid spread of a new severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) lineage with multiple spike mutations in South Africa. *Medrxiv* https://doi.org/10.1101/2020.12.21.20248640. 2020.12.21.20248640.

96. Shi, R., Chan, S., Duan, X., Chen, Z., Liu, P., Song, J., Song, T., Bi, X., et al., (2020). A human neutralizing antibody targets the receptor-binding site of SARS-CoV-2. *Nature* **584**, 120–124. https://doi.org/10.1038/s41586-020-2381-y.

97. Iketani, S., Liu, L., Guo, Y., Liu, L., Chan, J.-F.-W., Huang, Y., Wang, M., Luo, Y., et al., (2022). Antibody evasion properties of SARS-CoV-2 Omicron sublineages. *Nature* **604**, 553–556. https://doi.org/10.1038/s41586-022-04594-4.

98. Costello, S.M., Shoemaker, S.R., Hobbs, H.T., Nguyen, A.W., Hsieh, C.-L., Maynard, J.A.; McClellan, J.S., Pak, J. E., et al., (2022). The SARS-CoV-2 Spike protein reversibly samples an open-trimer conformation exposing novel epitopes. *Nature Struct Mol Biol* **29**, 229–238. https://doi.org/10.1038/s41594-022-00735-5.

99. Yuan, M., Wu, N.C., Zhu, X., Lee, C.-C.-D., So, R.T.Y., Lv, H., Mok, C.K.P., Wilson, I.A., (2020). A highly conserved cryptic epitope in the receptor binding domains of SARS-CoV-2 and SARS-CoV. *Sci New York N Y* **368**, 630–633. https://doi.org/10.1126/science.abb2769.

100. Sui, J., Li, W., Murakami, A., Tamin, A., Matthews, L.J., Kong, S.K., Bowen, J.E., Walls, A.C., Hauser, K., Joshi, A., et al., (2020). Robust neutralizing antibodies to SARS-CoV-2. *Sci New York N Y* **369**, 956–963. https://doi.org/10.1126/science.abb7520.

101. Kim, C., Ryu, D.-K., Lee, J., Kim, Y.-I., Seo, J.-M., Kim, Y.-G., Jeong, J.-H., Kim, M., et al., (2021). A therapeutic neutralizing antibody targeting receptor binding domain of SARS-CoV-2 spike protein. *Nature Comm* **12**, 288. https://doi.org/10.1038/s41477-021-6060-5.

102. Weisblum, Y., Schmidt, F., Zhang, F., DaSilva, J., Poston, D., Lorenzi, J.C., Muecksch, F., Rutkowska, M., et al., (2020). Escape from neutralizing antibodies by SARS-CoV-2 spike protein variants. *Elife* **9**, e61312. https://doi.org/10.7554/elifе.61312.

103. Chi, Y., Yan, R., Zhang, J., Zhang, G., Zhang, Y., Hao, M., Zhang, Z., Fan, P., et al., (2020). A neutralizing human antibody binds to the N-terminal domain of the Spike protein of SARS-CoV-2. *Sci New York N Y* **369**, 650–655. https://doi.org/10.1126/science.abc6952.

104. Rogers, T.F., Zhao, F., Huang, D., Beutler, N., Burns, A., He, W., Limbo, O., Smith, C., et al., (2020). Isolation of potent SARS-CoV-2 neutralizing antibodies and protection from disease in a small animal model. *Science* **369**, 956–963. https://doi.org/10.1126/science.abc7520.

105. Wajnberg, A., Amanat, F., Firpo, A., Altman, D.R., Bailey, M.J., Mansour, M., McMahon, M., Meade, P., et al., (2020). Robust neutralizing antibodies to SARS-CoV-2 infection persist for months. *Sci New York N Y* **370**, 1227–1230. https://doi.org/10.1126/science.abb7728.

106. Liu, L., Wang, P., Nair, M.S., Yu, J., Rapp, M., Wang, Q., Luo, Y., Chan, J.-F.-W., et al., (2020). Potent neutralizing antibodies against multiple epitopes on SARS-CoV-2 spike. *Nature* **584**, 450–456. https://doi.org/10.1038/s41586-020-2571-7.
107. Zhang, T., Hu, W., Chen, W., (2021). Plasma Membrane Integrates Biophysical and Biochemical Regulation to Trigger Immune Receptor Functions. *Front Immunol* **12**, 613185. https://doi.org/10.3389/fimmu.2021.613185.

108. Adams, E.J., Chien, Y.-H., Garcia, K.C., (2005). Structure of a γδ T Cell Receptor in Complex with the Nonclassical MHC T22. *Science* **308**, 227–231. https://doi.org/10.1126/science.1106885.

109. Zareie, P., Szeto, C., Farenc, C., Gunasinghe, S.D., Kolawole, E.M., Nguyen, A., Blyth, C., Snq, Y.X.Y., et al., (2021). Canonical T cell receptor docking on peptide–MHC is essential for T cell signaling. *Science* **372**, eabe9124. https://doi.org/10.1126/science.abe9124.

110. Luoma, A.M., Castro, C.D., Adams, E.J., (2014). γδ T cell surveillance via CD1 molecules. *Trends Immunol* **35**, 613–621. https://doi.org/10.1016/j.it.2014.09.003.

111. Mallis, R.J., Duke-Cohan, J.S., Das, D.K., Akitsu, A., Luoma, A.M., Banik, D., Stephens, H.M., Tetteh, P.W., et al., (2021). Molecular design of the γδ T cell receptor ectodomain encodes biologically fit ligand recognition in the absence of mechanosensing. e2023050118 *Proc Natl Acad Sci* **118**, https://doi.org/10.1073/pnas.2023050118.

112. Sibener, L.V., Fernandes, R.A., Kolawole, E.M., Carbone, C.B., Liu, F., McAffee, D., Birnbaum, M.E., Yang, X., et al., (2018). Isolation of a Structural Mechanism for Uncoupling T Cell Receptor Signaling from Peptide-MHC Binding. *Cell* **174**, 672–687.e27. https://doi.org/10.1016/j.cell.2018.06.017.

113. Bowerman, N.A., Colf, L.A., Garcia, K.C., Kranz, D.M., (2009). Different Strategies Adopted by Kb and Ld to Generate T Cell Specificity Directed against Their Respective Bound Peptides. *J Biol Chem* **284**, 32551–32561. https://doi.org/10.1074/jbc.m109.045001.

114. Rammensee, H.-G., Bachmann, J., Emmerich, N.P.N., Bachor, O.A., Stevanovic, S., (1999). SYFPEITHI: database for MHC ligands and peptide motifs. *Immunogenetics* **50**, 213–219. https://doi.org/10.1007/s002510005095.

115. Burrows, S.R., Rossjohn, J., McCluskey, J., (2006). Have we cut ourselves too short in mapping CTL epitopes? *Trends Immunol* **27**, 11–16. https://doi.org/10.1016/j.it.2005.11.001.

116. Hwang, W., Mallis, R.J., Lang, M.J., Reinherz, E.L., (2020). The γδTICR mechanosensor exploits dynamic ectodomain allostery to optimize its ligand recognition site. *PNAS* **117**, 21336–21345. https://doi.org/10.1073/pnas.2005899117.

117. Das, D.K., Feng, Y., Mallis, R.J., Li, X., Keskin, D.B., Hussey, R.E., Brady, S.K., Wang, J., et al., (2015). Force-dependent transition in the T-cell receptor β-subunit allosterically regulates peptide discrimination and pMHC bond lifetime. *Proc Natl Acad Sci* **112**, 1517–1522. https://doi.org/10.1073/pnas.1424829112.

118. Holler, P.D., Chlewicki, L.K., Kranz, D.M., (2002). TCRs with high affinity for foreign pMHC show self-reactivity. *Nature Immunol* **4**, 55–62. https://doi.org/10.1038/nn8363.

119. Chlewicki, L.K., Holler, P.D., Monti, B.C., Clutter, M.R., Kranz, D.M., (2005). High-affinity, Peptide-specific T Cell Receptors can be Generated by Mutations in CDR1, CDR2 or CDR3. *J Mol Biol* **346**, 223–239. https://doi.org/10.1016/j.jmb.2004.11.057.

120. Cameron, B.J., Gerry, A.B., Dukes, J., Harper, J.V., Kannan, V., Bianchi, F.C., Grand, F., Brewer, J.E., et al., (2013). Identification of a Titin-Derived HLA-A1–Presented Peptide as a Cross-Reactive Target for Engineered MAGE A3–Directed T Cells. *Sci Transl Med* **5**, 197ra103. https://doi.org/10.1126/scitranslmed.3006034.

121. Arachchige, A.S.P.M., (2021). Human NK cells: From development to effector functions. *Innate Immun* **27**, 212–229. https://doi.org/10.1177/17534259211001512.

122. Deguine, J., Breat, B., Lemaître, F., Santo, J.P.D., Bousso, P., (2010). Intravital Imaging Reveals Distinct Dynamics for Natural Killer and CDB + T Cells during Tumor Regression. *Immunity* **33**, 632–644. https://doi.org/10.1016/j.immuni.2010.09.016.

123. Saux, G.L., Bar-Hanin, N., Edri, A., Hadad, U., Porgador, A., Schwartzman, M., (2019). Nanoscale Mechanosensing of Natural Killer Cells is Revealed by Antigen-Functionalized Nanowires. *Adv Mater* **31**, 1805954. https://doi.org/10.1002/adma.201805954.

124. Billaudeau, D.D., Upshaw, J.L., Schoon, R.A., Dick, C.J., Leibson, P.J., (2003). NKGD2-DAP10 triggers human NK cell–mediated killing via a Syk-independent regulatory pathway. *Nature Immunol* **4**, 557–564. https://doi.org/10.1038/ni829.

125. Georg, P., Astaburuaga-Garcia, R., Bonaguro, L., Brunel, S., Michalik, L., Lippert, L., Kostevc, T., Gäbel, C., et al., (2022). P-C-19 S Group, Complement activation induces excessive T cell cytotoxicity in severe COVID-19. *Cell* **185**, 493–512.e25. https://doi.org/10.1016/j.cell.2021.12.040.

126. Anania, J.C., Chenoweth, A.M., Wines, B.D., Hogarth, P. M., (2019). The Human FcγRll (CD32) Family of Leukocyte FcR in Health and Disease. *Front Immunol* **10**, 464. https://doi.org/10.3389/fimmu.2019.00464.

127. Hu, W., Zhang, Y., Sun, X., Zhang, T., Xu, L., Xie, H., Li, Z., Liu, W., et al., (2019). FcγRlI-B223T polymorphic change allosterically suppresses ligand binding. *Elife* **8**, e46689. https://doi.org/10.7554/elife.46689.

128. Xu, L., Xia, M., Guo, J., Sun, X., Li, H., Xu, C., Gu, X., Zhang, H., et al., (2016). Impairment on the lateral mobility induced by structural changes underlies the functional deficiency of the lupus-associated polymorphism FcγRlI-B232. *J Exp Med* **213**, 2707–2727. https://doi.org/10.1084/jem.20160528.

129. Coombs, D., Dembo, M., Wosfy, C., Goldstein, B., (2004). Equilibrium Thermodynamics of Cell-Cell Adhesion Mediated by Multiple Ligand-Receptor Pairs. *Biophys J* **86**, 1408–1423. https://doi.org/10.1016/s0006-3495(04)74211-3.

130. Allard, J.F., Dushek, O., Coombs, D., van der Merwe, P. A., (2012). Mechanical Modulation of Receptor-Ligand Interactions at Cell-Cell Interfaces. *Biophys J* **102**, 1265–1273. https://doi.org/10.1016/j.bpj.2012.02.006.

131. Rozycki, B., Lipowsky, R., Weikl, T.R., (2010). Segregation of receptor-ligand complexes in cell adhesion zones: Phase diagrams and role of thermal membrane roughness. *BioRxiv*. https://doi.org/10.1108/1367-2630/12/9/095003.

132. Razvag, Y., Neve-Oz, Y., Sajman, J., Reches, M., Sherman, E., (2018). Nanoscale kinetic segregation of TCR and CD45 in engaged microvilli facilitates early T cell activation. *Nature Commun* **9**, 732. https://doi.org/10.1038/s41467-018-03127-w.

133. Cai, E., Marchuk, K., Beemiller, P., Beppler, C., Rubashkin, M.G., Weaver, V.M., Gérard, A., Liu, T.-L., et al., (2017). Visualizing dynamic microvillar search and
stabilization during ligand detection by T cells eaal3118-12 Science 356. https://doi.org/10.1126/science.aal3118.

134. Zhang, Z., Duvefelt, K., Svensson, F., Masterman, T., Jonasdottir, G., Salter, H., Emahazion, T., Hellgren, D., et al., (2005). Two genes encoding immune-regulatory molecules (LAG3 and IL7R) confer susceptibility to multiple sclerosis. Genes Immun 6, 145–152. https://doi.org/10.1038/sj.gene.6364171.

135. Ju, L., Chen, Y., Xue, L., Du, X., Zhu, C., (2016). Cooperative unfolding of distinctive mechanoreceptor domains transduces force into signals. Elife 5, e15447. https://doi.org/10.7554/elife.15447.

136. Li, K., Yuan, Z., Lyu, J., Ahn, E., Davis, S.J., Ahmed, R., Zhu, C., (2021). PD-1 suppresses TCR-CD8 cooperativity during T-cell antigen recognition. Nature Commun, 1–13. https://doi.org/10.1038/s41467-021-22965-9.

137. Yanagida, A., Corujo-Simon, E., Revell, C.K., Sahu, P., Stirparo, G.G., Aspalter, I.M., Winkel, A.K., Peters, R., et al., (2022). Cell surface fluctuations regulate early embryonic lineage sorting. Cell 185, 777–793.e20. https://doi.org/10.1016/j.cell.2022.01.022.

138. Plotnikov, S.V., Pasapera, A.M., Sabass, B., Waterman, C.M., (2012). Force Fluctuations within Focal Adhesions Mediate ECM-Rigidity Sensing to Guide Directed Cell Migration. Cell 151, 1513–1527. https://doi.org/10.1016/j.cell.2012.11.034.

139. Colin-York, H., Javannardi, Y., Skamrah, M., Kumari, S., Chang, V.T., Khuon, S., Taylor, A., Chew, T.-L., et al., (2019). Cytoskeletal Control of Antigen-Dependent T Cell Activation. Cell Rep 26, 3369–3379.e5. https://doi.org/10.1016/j.celrep.2019.02.074.

140. Shaveitz, J.W., Fletcher, D.A., (2007). Load fluctuations drive actin network growth. Proc Natl Acad Sci 104, 15688–15692. https://doi.org/10.1073/pnas.0702061104.

141. Pullen, R.H., Abel, S.M., (2017). Catch Bonds at T Cell Interfaces: Impact of Surface Reorganization and Membrane Fluctuations. Biophys J 113, 120–131. https://doi.org/10.1016/j.bpj.2017.05.023.

142. Gov, N., (2004). Membrane Undulations Driven by Force Fluctuations of Active Proteins. Phys Rev Lett 93, 268104. https://doi.org/10.1103/physrevlett.93.268104.

143. Morgan, J., Pettmann, J., Dushek, O., Lindsay, A.E., (2021). T-cell microvilli simulations show operation near packing limit and impact on antigen recognition. Biorxiv. https://doi.org/10.1101/2021.11.24.469916. 2021.11.24.469916.

144. Al-Aghbar, M.A., Jainarayanan, A.K., Dustin, M.L., Roffler, S.R., (2022). The interplay between membrane topology and mechanical forces in regulating T cell receptor activity. Commun Biol 5, 40. https://doi.org/10.1038/s42003-021-02995-1.

145. Purvis, J.E., Lahav, G., (2013). Encoding and Decoding Cellular Information through Signaling Dynamics. Cell 152, 945–956. https://doi.org/10.1016/j.cell.2013.02.005.

146. Paulsson, J., Berg, O.G., Ehrenberg, M., (2000). Stochastic focusing: Fluctuation-enhanced sensitivity of intracellular regulation. Proc Natl Acad Sci 97, 7148–7153. https://doi.org/10.1073/pnas.110057697.

147. Feinerman, O., Veiga, J., Dorfman, J.R., Germain, R.N., Altan-Bonnet, G., (2008). Variability and Robustness in T Cell Activation from Regulated Heterogeneity in Protein Levels. Science 321, 1081–1084. https://doi.org/10.1126/science.1158013.

148. Kueh, H.Y., Charras, G.T., Mitchison, T.J., Brieher, W.M., (2008). Actin disassembly by cofilin, coronin, and Aip1 occurs in bursts and is inhibited by barbed-end cappers. J Cell Biol 182, 341–353. https://doi.org/10.1083/jcb.200801027.

149. Dushek, O., Das, R., Coombs, D., (2009). A Role for Rebinding in Rapid and Reliable T Cell Responses to Antigen. Plos Comput Biol 5, e1000578. https://doi.org/10.1371/journal.pcbi.1000578.