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Computational simulations of TNF receptor oligomerization on plasma membrane

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Abstract

The interactions between tumor necrosis factors (TNFs) and their corresponding receptors (TNFRs) play a pivotal role in inflammatory responses. Upon ligand binding, TNFR receptors were found to form oligomers on cell surfaces. However, the underlying mechanism of oligomerization is not fully understood. In order to tackle this problem, molecular dynamics (MD) simulations have been applied to the complex between TNF receptor-1 (TNFR1) and its ligand TNF- α as a specific test system. The simulations on both all-atom (AA) and coarse-grained (CG) levels achieved the similar results that the extracellular domains of TNFR1 can undergo large fluctuations on plasma membrane, while the dynamics of TNF α -TNFR1 complex is much more constrained. Using the CG model with the Martini force field, we are able to simulate the systems that contain multiple TNF α -TNFR1 complexes with the timescale of micro-seconds. We found that complexes can aggregate into oligomers on the plasma membrane through the lateral interactions between receptors at the end of the CG simulations. We suggest that this spatial organization is essential to the efficiency of signal transduction for ligands that belong to the TNF superfamily. We further show that the aggregation of two complexes is initiated by the association between the N-terminal domains of TNFR1 receptors. Interestingly, the *cis*-interfaces between N-terminal regions of two TNF receptors have been observed in the previous X-ray crystallographic experiment. Therefore, we provide supportive evidence that *cis*-interface is of functional importance in triggering the receptor oligomerization. Taken together, our study brings insights to understand the molecular mechanism of TNF signaling.

1 | INTRODUCTION

During inflammation, cytokines are released from injured cells.¹ They recruit leukocytes to reach the site of injury and remove the foreign pathogens.² Proteins in the superfamily of tumor necrosis factors (TNFs) are one major class of these cytokines.³ They bind to the cell surface proteins called TNF receptors.⁴ The binding between TNF and TNF receptors triggers the intracellular signaling pathways, such as NF- κ B pathway that is an essential

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regulator of cell survival.^{5–8} Understanding the dynamics of TNF receptors on cell surfaces after ligand binding has therapeutic impacts on patients with autoimmunity and various infectious diseases.⁹ The quaternary structure of most members in the TNF superfamily appears as a noncovalent homotrimer. Each subunit in the complex possesses of eight β -strands that are spatially organized into a classic “jelly roll” structural topology.¹⁰ On the other hand, most receptors that bind to ligands of TNF superfamily contains repeating cysteine-rich domains, resulting in a rod-like shape.¹¹ Consequently, a trimeric TNF ligand can simultaneously bind to three receptors, with one receptor monomer binding at the interface between two consecutive subunits of the corresponding ligand. This large ligand-receptor complex (Figure 1), which is also called a signaling complex, forms the basic unit on the cell surface to activate the intracellular signaling pathways.¹² Moreover, recent experiments using high-resolution microscopy showed that TNF receptors on the plasma membrane of intact cells can further cluster into oligomers after ligand binding.¹³ In a previously solved X-ray structure, it was found that TNF receptors can form a parallel dimer connected by a so called “*cis*-binding interface.”¹⁴ The *cis*-binding and ligand-binding interfaces in a receptor are not overlapped with each other, which provide the possibility that the signaling complexes can aggregate through the *cis*-interactions and form higher-order oligomers. In spite of the available structural and imaging information, however, the molecular mechanism of TNF receptor oligomerization still remains poorly understood.

Molecular dynamics (MD) simulation is a computational technique that is able to trace the dynamic process with atomic details of any given biomolecular system.^{15–17} Therefore, it has been used to study the functions of many cell surface proteins and membrane receptors.^{18–26} However, current applications of all-atom (AA) MD simulations to more complicated behaviors of cell surface proteins in their membrane environments such as oligomerization are not allowed due to the intense consumption for computational resources. As a result, coarse-grained (CG) force fields have been developed to probe the dynamics of larger molecular systems with longer timescales by sacrificing the information of interactions on the atomic level.^{27–36} Among a large variety of CG approaches, the Martini force field was constructed by extensively calibrating the nonbonded interactions between CG building blocks of basic chemical units against the experimental data.³⁷ The CG simulations with the Martini force field were successfully applied to reproduce the dynamic processes of lipid systems such as self-assembly of lipid bilayers.³⁸ Furthermore, the Martini force field has recently been extended to protein systems and shown its power to simulate membrane proteins embedded in their lipid environments.³⁹ For instance, the clustering of outer membrane protein in *Escherichia coli* plasma membrane was obtained by the method.⁴⁰ In another example, G-protein-coupled receptors were found to form high-order oligomers at the end of the Martini simulations.⁴¹ These computational studies suggest that the spatial organization of membrane proteins could be a general mechanism to carry out their functions in their cellular environments.

In this study, we present a computational model to understand the molecular mechanism of TNF receptor oligomerization. The complex formed between TNF receptor-1 (TNFR1) and its ligand TNF- α is used as a specific test system. MD simulations were carried out on both atomic and CG levels. Although AA simulations were performed on systems containing only one receptor and one signaling complex, CG models were constructed for the systems

in which multiple receptors or signaling complexes were placed on lipid bilayers. We found that the conformational fluctuations of receptor and signaling complex derived from CG simulations are comparable with AA simulations, indicating the accuracy of using CG force field to capture the dynamics of the simulation systems. We further show that at the end of the CG simulations, TNF signaling complexes can aggregate into oligomers on plasma membrane through the lateral interactions between receptors. Different from the system that only contains TNF receptors, the oligomerization of ligand-receptor complexes localizes the transmembrane regions of receptors, thus leading to a more regular distribution on cell surfaces. This difference in spatial organization due to ligand binding has functional implication to the intracellular signal transduction of TNF receptor. Finally, the analysis of CG simulations suggests that the aggregation of two signaling complexes is initiated by the association between the N-terminal domains of receptors. Interestingly, the *cis*-binding interface of receptors observed in the crystal structure is also located at the N-terminal region. We thus provide supportive evidence that the *cis*-interactions between receptors are functionally important. Taken together, our study brings mechanistic understanding to the oligomerization of TNF signaling complexes in their cellular environments.

2 | MODELS AND METHODS

2.1 | Construction of simulation systems

The current study uses the complex formed between TNFR1 and its ligand TNF- α as a model system. We first built an atomic model of TNFR1 on the plasma membrane. The model contains the ectodomain of the receptor, the transmembrane helix, and a linker region that connect these two segments. The transmembrane helix is inserted into the lipid bilayer. A model of TNF α -TNFR1 signaling complex on the plasma membrane was further constructed comprising a trimeric ligand and three bound receptors (Figure 1A) with their transmembrane regions inserted into the lipid bilayer. Both AA and CG MD simulations were carried out for systems that contain one or more signaling complexes, as well as systems that contain one or more receptors alone. An overview of the simulation systems in this study is found in Table 1.

Specifically, the initial extracellular conformation of TNFR1 was adopted from the crystal structure with PDB id 1EXT.⁴² For the TNF α -TNFR1 complex, no experimental structure is available. Therefore, the initial conformation of its extracellular region was adopted from the computational model that was previously built by Xie's group.⁴³ In brief, the crystal structure of TNF- α bound with TNFR2 (3ALQ)⁴⁴ was used as a basis to guide the superposition of TNFR1 onto the Ca atoms of TNFR2. The interface between single receptor and ligand monomer is highlighted in Figure 1B. The atomic coordinates of the complex can be downloaded from http://www.cbligand.org/downloads/TNF_TNFR1.pdb. The transmembrane domains of TNFR1 for both receptor alone and ligand-receptor complex systems were built as standard α -helices, and the linkers between transmembrane and extracellular regions of TNFR1 were modeled by the online server, ModLoop.⁴⁵

2.2 | Protocol of AA MD simulation

The AA simulations were performed on two specific systems: one contains a single TNFR1 receptor, and the other contains a single TNF α -TNFR1 complex. The single receptor was inserted into a lipid bilayer that contains 442 POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine) lipids, while the complex was inserted into a lipid bilayer that contains 554 POPC lipids (Table 1). Counterions (Na⁺, Cl⁻) were added to neutralize the net charge of the simulation box and to maintain an appropriate ionic strength (0.1 M). For both systems, simulations were carried out using GROMACS with the CHARMM36m force field and the TIP3P water. The system was equilibrated at 310 K and 1 atm to remove unrealistic contacts. After equilibration, we performed a production run. A uniform integration step of 2 fs was used for all types of interactions, throughout all simulations. A cutoff of 13 Å was used for van der Waals interactions, and electrostatic interactions were calculated with the particle mesh technique for Ewald summations, also with a cutoff of 13 Å. Temperature and pressure are controlled using the v-rescale thermostat and the Parrinello-Rahman barostat, respectively. Finally, a 300 ns trajectory was attained for the receptor alone, and a 200 ns trajectory was attained for the ligand-receptor complex.

2.3 | Protocol of CG MD simulation

The CG simulations were performed on six various systems. As shown in Table 1, two of these systems contain a single receptor and a single complex, while simulations contains multiple receptors (12) and multiple signaling complex (4) were performed on two different temperatures (310 K and 347 K). For all systems, simulations were performed using the Martini 2.2 force field and the GROMACS 5.0.6 simulation package. The CG representations of the simulated proteins were derived from the conformation in their corresponding atomistic simulation at 100 ns. The derived CG models were then embedded into POPC bilayers using the “insane.py” script.⁴⁶ The ElnDyn elastic network⁴⁷ was applied with a cutoff of 0.9 nm and a force constant of 500 kJ/mol nm⁻². We further used a scaling factor α to adjust the strength of protein-protein interactions. As described in previous studies,^{48,49} the van der Waals parameters for interactions between different proteins are multiplied by this scaling factor α . In detail, the scaled well-depth e_{scaled} between two MARTINI beads is calculated as $e_{\text{scaled}} = 2.0 + \alpha(e_{\text{original}} - 2.0)$, where e_{original} is the original value of well-depth between two MARTINI beads, and 2.0 kJ/mol is the lower limit of well-depth. As a result, $\alpha = 1$ gives the original MARTINI bead-bead interactions, while $\alpha = 0$ gives the weakest MARTINI bead-bead interactions. The most commonly used scaling factors in the literature are 0.2⁴⁹ and 0.8,⁴⁸ which were applied in this study.

In the CG simulations, each system was equilibrated for 5 ns using the Berendsen thermostat and barostat before a production run. The production run was performed with a time step of 20 fs. The short-range cutoff for both the nonbonded and electrostatic interactions was 1.1 nm. The Lennard-Jones potential was cut off by the potential shift Verlet scheme. The reaction field method was applied for the electrostatics, with dielectric constants of 15. The nonbonded neighbor lists were updated every 20 steps. Temperature and pressure are controlled using the v-rescale thermostat and the Parrinello-Rahman barostat, respectively. Finally, 10 μ s trajectories were attained for all systems. The CG model of the ligand-receptor

complex is shown in Figure 1C by a snapshot taken from the simulations. Due to the simplified nature of the CG model, molecules can only be clearly viewed by the surface representation.

3 | RESULTS

3.1 | Compare the dynamics of TNFR1 receptor with complex in AA and CG simulations

Before we apply CG simulations to study oligomerization of ligand-receptor complexes, which is a very large-scale dynamic phenomenon, we first validated if this CG model and Martini force field are able to capture the very basic conformational fluctuations of membrane-bound free receptors and the difference when they stay in their ligand-bound state. We did the validation by comparing the results between CG and AA simulations. In the cellular environment of a cell surface receptor, the transmembrane helix is confined in the plasma membrane, while its ectodomains are tethered by the linker region and fluctuate in the extracellular area above the lipid bilayer. As a result, we defined a conformational angle θ , which serves as a good variable to quantify the basic dynamic property of TNFR1 on the plasma membrane. The angle is formed between the vector that points from the membrane proximal domain (domain 4) to the N-terminal domain (domain 1) of TNFR1's extracellular region and the vector that points to the membrane surface normal, as shown in Figure 2A. We calculate the distribution of θ for the TNFR1 from the 300-ns AA MD simulation. The results are plotted as the black solid curve in Figure 2C. This figure indicates that the conformational angle of the receptor ranges between 20° and 120° and approximately forms a normal distribution, which is centered at 75° . This suggests that overall the extracellular domains of TNFR1 undergo large fluctuations, but follow a relatively simple dynamics of bending motions that lead to the normal distribution of θ . This is largely due to fact that the extracellular domains are tethered to the membrane surface by a single linker.

We further delineate the dynamics of the entire TNF α -TNFR1 signaling complex by defining another conformational angle θ . The angle is formed between the vector that points along the central axis of symmetry in the ligand trimer and the vector that points to the membrane surface normal, as shown in Figure 2B. We calculate the distribution of θ for the signaling complex from the 200-ns AA MD simulation. The results are plotted as the black dashed curve in Figure 2C. Comparing to the system that only contains the TNFR1 receptor, the distribution of the complex is shifted toward the left, indicating that the complex appeared more upright on plasma membrane along the simulations. Moreover, the conformational angle of the complex ranges between 0° and 60° , which is much narrower than the distribution of TNFR1 receptor alone. This can be explained by the fact that all three receptors in the signaling complex are tethered to the membrane, and thus the complex is much less flexible. As a result, multiple peaks are observed in the distribution of the complex, instead of the normal distribution in the system, which contains a single receptor. These multiple peaks could be due to the fact that the AA simulation of complex needs longer timescale to be fully converged. It could also be caused by the complicated dynamics originated from the multiple degrees of confinement between three receptors in the signaling complex.

CG simulations can reach the timescale, which is not accessible for AA simulations. As a tradeoff, the details of atomic interactions are simplified by coarse-graining the atoms into specific groups. In order to check if the CG model can still capture the similar conformational dynamics in the AA model, CG simulations were generated for the above two systems using the Martini force field. The same conformational angles were used to quantify the fluctuations in the extracellular domains of TNFR1 receptor and the entire signaling complex in the CG simulations. Consequently, the distribution of θ for the receptor from the 10 μ s CG MD simulation is plotted as red solid curve, while the distribution of θ for the signaling complex from the 10 μ s CG MD simulation is plotted as the red dashed curve in Figure 2C. Interestingly, both distributions of receptor and complex under CG simulations are overlapped very well with AA simulations. In specific, the distribution of CG simulation for receptor is centered at 75° and forms a normal distribution. In contrast, the distribution of CG simulation for complex is shifted toward left, ranges between 0° and 60° , and does not follow normal distributions. Specifically, there is an additional lower peak around 45° in the complex distribution. This is also consistent with the AA simulation of complex, in which distribution of θ is extended to this interval with multiple peaks. Instead of free fluctuations in the single receptor, which leads to the normal distribution of θ , we suggest that the additional peaks in the ligand-bound system are caused by the interference of fluctuations between difference receptors in the complex.

The comparison between CG and AA simulations suggests that with ligands bound, the TNF receptors in the complex are more straight-up and possess multiple degrees of confinement, while they are more flexible and tilted toward the membrane without ligands. Therefore, this comparison indicates that the CG simulation and Martini force field are able to reflect the basic differences in conformational fluctuations between ligand-bound and ligand-unbound systems of TNF receptors. It is worth mentioning that comparing with the AA simulations, the distributions derived from the CG simulations are narrower and smoother. This is probably because of the reason that the conformational space was sampled more thoroughly in the CG simulations. Another difference between CG and AA simulations is that the three transmembrane helices of receptors were associated together in the CG simulations (Figure 1C), but not in the AA simulations. This could also be due to the reason that the timescale of AA simulation is not long enough.

In summary, our study shows that the extracellular domains of TNFR1 can undergo large fluctuations. Without the presence of ligand, the receptor tends to lean toward the plasma membrane, while the conformation of the TNF α -TNFR1 complex is more perpendicular to the surface of plasma membrane. Comparing with the AA simulations, we are able to simulate the systems with much longer timescale using the CG model, at the meantime maintain the similar conformational dynamics. Therefore, in the following study, we further applied the CG model to the systems, which contain multiple copies of TNF α -TNFR1 complexes and simulate the process of their oligomerization.

3.2 | Characterize the oligomerization of signaling complexes on plasma membrane

Based on the validation of the CG model, we extended it to a larger system, which contains multiple signaling complexes. In detail, four TNF α -TNFR1 complexes were uniformly

distributed on a square surface of lipid bilayer of 30 nm length as an initial conformation of the CG simulation (Figure 3A). Simulation with 10 μ s long was carried out at the standard temperature of 310 K. Some representative snapshots are plotted in Figure 3 from the trajectory. Following the uniform distribution at the beginning of the simulation, complexes undergo random diffusions on the plasma membrane. As shown in Figure 3B, the complex with the orange and cyan receptors first encountered when the simulation reached 1 μ s. When the simulation reached 4.6 μ s, we found that the other two complexes with the purple and pink receptors also formed a lateral interaction (Figure 3C). These two small clusters were finally merged together when the simulation reached 6.3 μ s and formed a larger oligomer on the plasma membrane. The final oligomer is shown in Figure 3D, which is appeared as a configuration with the linear interactions of four signaling complexes. Moreover, the figure indicates that the binding interfaces between complexes in the oligomer are all located at the surfaces of the TNFR1 receptors. These surfaces are on the opposite side of the ligand-receptor binding interfaces in the complexes. Therefore, our simulations demonstrate that the TNF α -TNFR1 complexes are able to form oligomer on the plasma membrane. In addition to the ligand-receptor binding interface, we suggest that the oligomerization of signaling complexes is mediated by this lateral binding interface in the TNFR1 receptor.

In order to provide more detailed analysis on the lateral interactions between receptors in the oligomer, we calculated the probability of being on the lateral interface for each residue of a receptor. In specific, given a configuration during the simulation, we enumerated all pairs of residues between two receptors that belong to different complexes. For a specific pair of residues, if the distance between their C α atoms was less than 1 nm, we assumed that the corresponding residues were located on the lateral interface. After we scanned receptors between all pairs of four complexes along the entire simulation trajectory, the probability of being on the lateral interface for a specific residue was derived by counting how likely this residue was observed on the interface. Because the probability was averaged over all pairs of receptors along the timescale of 10 μ s, it only gives a statistical overview about which parts of a receptor are more often to be involved in the lateral interactions. As a result, the distribution of our calculated interface probability is plotted in Figure 4A as a function of residue number, while the secondary structure index is shown in the bottom. We also projected the values of probability onto the structure of the receptor as a heat map. As shown in Figure 4B, the red color indicates the regions that have the highest probability of being on the lateral interface. The figure confirmed that the lateral interface between receptors is located on the opposite side of the ligand-receptor binding interfaces. Interestingly, the peaks in the probability distribution that are highlighted by red arrows in Figure 4A are well overlapped with the “*cis*-interface” existing in the X-ray structure (PDB 1NCF). This provides the supporting evidence that this “*cis*-interface” might be biologically functional to regulate the oligomerization of TNG ligand-receptor complexes.

A control system was constructed in which no ligands were included. In detail, the CG model of 12 TNFR1, the same number of receptors as in the system of four complexes, was uniformly distributed on a square surface of lipid bilayer with 30 nm in length as an initial conformation. Simulation of 10 μ s was carried out at 310 K and the final configuration from the trajectory is plotted in Figure 5A. We compare the final configuration with the

simulation of four complexes in which a linear oligomer was formed (Figure 5B). It clearly shows that a much more irregular spatial pattern is observed in the system without TNF α ligands. In specific, receptors are randomly distributed on the plasma membrane. When the ligands are not presented, the ligand-receptor binding interfaces become available in the extracellular domains of TNFR1 receptors. This leads to a more homogeneous organization of nonspecific interactions between some of these receptors. As a result, different from the system of ligand-bound complexes in which the transmembrane domains of receptors are localized with ordered spatial separation, the transmembrane domains of ligand-unbound receptors are scattered in the lipid bilayer with a much higher stochasticity. We suggest that the ordered spatial patterns formed by TNF α -TNFR1 complexes play an important functional role in regulating their intracellular signaling pathways, as described in the discussions.

It has been shown that the protein-protein interactions are over-estimated in the Martini force field.⁴⁸ In order to address this issue, simulations were carried out at a higher temperature (347 K) to reduce the binding affinity by increasing the entropic fluctuations.^{50,51} As described in the Methods section, we also introduced a scaling factor α to directly shield the relative binding effect between molecules in the system. The final configurations from the simulations of receptors (Figure 5C) and complexes (Figure 5D) at high temperature, as well as from the simulations of receptors (Figure 5E) and complexes (Figure 5F) with reduced intermolecular interactions ($\alpha = 0.8$), show the similar results as the standard temperature and unscaled interactions. In order to analyze the process of oligomerization on a more quantitatively level, we calculated the minimal distance between all pairs of complexes as a function of time during the simulation. The minimal distance between a given complex pair was determined by calculating the distances from all the CG beads in one complex to the CG beads in the other complex in the pair. The changes of minimal distances for all pairs of complexes along simulation time are plotted in Figure 6A for the standard temperature, Figure 6B for the higher temperature, and Figure 6C for interactions with scaled factor $\alpha = 0.8$. As indicated by the black curve in Figure 6A, the initial lateral interaction at the standard temperature occurred at 1 μ s when the first two complexes interacted with each other, corresponding to the snapshot in Figure 3B. At the temperature of 310 K, the oligomer was finally formed after 6 μ s, when the last complex aggregated into the cluster, as indicated by the red curve in Figure 6A. In contrast, we found that the process of oligomerization was greatly accelerated at 347 K, although the strength of interactions between complexes was effectively reduced by the increase of simulation temperature. As shown in Figure 6B, all four complexes were clustered together before 1 μ s of the simulation. We compared the basic properties of lipid bilayers in simulations between normal and higher temperatures (Table 2). The results show that the lipids in the simulation of higher temperature diffuse faster. We therefore suggest that the acceleration of oligomerization can be explained by the faster diffusions of complexes on plasma membrane at high temperature. As a result, it takes shorter amount of time for complexes to encounter with each other. When we reduced the intermolecular interactions by α that equals 0.8, Figure 6C shows that the first three complexes (complexes 1, 2, and 4) formed a cluster before 2 μ s, leaving the last complex (complex 3) diffusing around in the box. As indicated by the blue curve in the figure, complex 3 finally joined the cluster until the simulation

reached 8 μ s. As a result, our results suggest that ligand-receptor complexes still tend to form lateral oligomers even when the force field in the simulations was considerably scaled down. This demonstrated that the oligomerization of TNF receptors after ligand binding is not simply an artificial effect due to the overestimation of Martini force field. Finally, it is worth mentioning that when we further reduced the intermolecular interactions with a lower scaling factor ($\alpha = 0.2$), dissociations of TNF ligands from the receptors were observed at the very beginning of the simulations.

3.3 | Understanding the kinetic mechanism of association between two signaling complexes

In order to decipher the detailed mechanism of how two signaling complexes associate together, for a given *cis*-interaction between two receptors from a pair of complexes, we further calculated the minimal distances from each of the four extracellular domains in the receptor of one complex to their corresponding domains in the receptor of the other complex. These minimal distances between a specific pair of domains were determined by calculating the distances from all the CG beads in one domain to the CG beads in the other domain. They are plotted in Figure 7 as a function of time under the simulation at 347 K. In specific, the curves of minimal distance for two complexes which formed the first lateral interaction are shown in Figure 7A, while Figure 7B,C shows the minimal distances between complexes that formed the next two lateral interactions in the oligomer, respectively. In all the three figures, the minimal distances between the N-terminal domains (domain 1) of two corresponding receptors are plotted as black curves. Similarly, the red, green, and blue curves in the figures are the minimal distances between the next three domains (domains 2–4) of two corresponding receptors.

Interestingly, we found that all three lateral interactions between signaling complexes were initiated by the association of their N-terminal domains. For instance, the first pair of *cis*-interaction was formed at the very beginning of the simulation. In Figure 7A, the black curve drops first and is stabilized through the rest of the simulation, indicating that the N-terminal domains from the receptors of these two complexes remained in contact after 0.2 μ s. When the simulation reached 1.5 μ s, the red and green curves are stabilized at their minimal level, indicating the interactions between domains 2 and 3. After the simulation passed 3.0 μ s, the blue curve reaches its minimal level, indicating the interaction between domains 4 was finally formed. A similar process occurred in the second lateral interaction, as shown in Figure 7B. The black curve drops before 1.0 μ s. Afterward, the red, green, and blue curves are stabilized almost at the same time at 4.0 μ s. This suggests that the second lateral interaction was also triggered by the association between the N-terminal domains of the receptors in these complexes and then was stabilized by the simultaneous formation of contacts between the rest three domains. In Figure 7C, we found that the third lateral interaction in the oligomer was initially triggered at the time very close to the formation of the second lateral interaction. The black curve in the figure drops and is stabilized before 1.0 μ s. The interaction between domains 2 is formed following the initial contact between domains 1 at 2.0 μ s. Different from the first two lateral interactions, Figure 7C suggests that domains 3 and 4 in the third lateral interaction did not form contacts by the end of the simulation. This is confirmed by the final configuration of intermolecular binding between

the complex with the pink receptors and the complex with the purple receptors, which is shown in the snapshot of Figure 5D. It is worth mentioning that throughout the simulation, all lateral interactions between complexes are regulated by the binding interfaces that are located on the surfaces of receptors. It is interesting that we did not observe the interactions that are formed between receptors in one complex and ligands in another complex in the cluster. We speculate that this is the result from the following fact. Complexes are formed through the known ligand-receptor binding interface. On the other hand, the possible binding between ligands using the rest of their vacant surfaces and the surfaces on the other side of receptors might not be energetically favored. As a result, our results give the supportive evidence that the lateral oligomerization between TNF receptors after ligand binding is maintained by an alternative binding surface only on the receptors.

Based on the analysis of the simulation data, we suggest that the membrane distal N-terminal domain of the TNFR1 receptors is an essential player to trigger the lateral interactions between signaling complexes and the follow-up oligomerization. The functional importance of the membrane distal region in TNFR receptors in regulating their assembly has previously also been documented.^{52,53} Moreover, we propose a kinetic pathway to describe the dynamics of interactions between TNF ligands and receptors on cell surfaces. The schematic of the pathway is shown in Figure 8. Specifically, TNFR1 receptors form irregular aggregation on cell surfaces without TNF α ligands (Figure 8A). Intracellular signaling is off under this disordered spatial pattern. After the presence of TNF α , the stronger interactions between receptors and ligands compete over the nonspecific interaction between receptors, so that the basic unit, called a signaling complex, comprising a trimeric ligand and three bound receptors can be formed (Figure 8B). The formation of these complexes serves as the minimal functional building block for the downstream signaling pathways. The lateral interactions are further formed between signaling complexes through a “zipper-like” mechanism, in which the membrane distal N-terminal domains from two receptors of an interacting complex pair first associate together as a kinetic intermediate (Figure 8C). The binding is then sequentially stabilized by the interactions between the rest domains in the receptors (Figure 8D). Consequently, signaling complexes are aggregated together following the same process, leading to the final oligomerization (Figure 8E). This ordered spatial organization in the oligomers of signaling complexes can facilitate the amplification of intracellular signal transduction.

4 | DISCUSSION

Proteins that belong to TNF superfamily are important cytokines to activate diverse cellular responses ranging from inflammation, apoptosis and necroptosis. The activation is triggered by the binding of these protein ligands to their target receptors on cell surfaces. The ligand-receptor interactions result in a basic signaling complex, which contains a trimeric ligand and three bound TNF receptors. Although the evidence based on structural and imaging experiments implies that TNF receptors will further form oligomers upon ligand binding, the understanding of oligomerization mechanism is incomplete due to the lack of dynamic details. Using the Martini force field and CG simulations, we demonstrated that the signaling complexes formed by TNF α and TNFR1 are able to cluster together on a lipid bilayer. The oligomerization is regulated through the interfaces between two TNF receptors.

Comparing with the ordered oligomers formed by the signaling complexes, simulations of the system that only contains TNF receptors result in much more stochastic interactions. Therefore, our results suggest that binding of TNF ligands can lead into a transition from an initial random distribution of receptors to an increase of their local concentration.

The spatial organization of ligand-receptor complexes will further bring functional impacts on the intracellular signal transduction. For instance, the oligomerization provides transient compartmentalization to the cytoplasmic domains of TNF receptors. The intracellular signaling processes can therefore be greatly facilitated by the spatial proximity.^{54,55} Moreover, the noises due to randomness in molecular diffusions can be greatly reduced through the formation of high-order oligomers, while the downstream signals can be effectively amplified by incorporating an overstoichiometric number of signaling molecules into the cytoplasmic platform of oligomers. The simulation results derived from our computational model of TNF receptor oligomerization thus throw light on the functional roles of spatial organization in regulating the TNF-mediated signaling pathways in cells.

TNF α is an important therapeutic target since its overexpression in various autoimmune diseases. All the biologic therapies, such as infliximab, etanercept, adalimumab, certolizumab, and golimumab,⁵⁶ were designed to block the interactions between TNF ligands and receptors so that the downstream signaling cannot be activated. Our study suggests that the signaling of TNF receptors is not only induced by the ligand binding but also modulated by their oligomerization, in which the spatial organization greatly enhances the fidelity of signal transduction. We further showed that the oligomerization is regulated by the lateral interactions between ligand-bound receptors, in which the association is triggered by their N-terminal regions. Based on these results, we propose that the level of TNF signaling can be either positively or negatively adjusted by the development of new drugs, which target the lateral binding interfaces between TNF receptors. Considering the limitation of current biologic therapies, including serious side effects, high cost, and the requirement for intravenous injections,⁵⁷ the insights from the MD simulations are beneficial to future drug design of autoimmune diseases.

The lateral interfaces between TNF receptors were found in the X-ray structure in addition to the interfaces, which are used to recognize their ligands. This *cis*-interaction drives the homodimerization of TNF receptors and further leads to their oligomerization. As more and more structural data of cell surface proteins accumulated, it has been found that the extracellular fragments in many other membrane receptors have more than one binding interfaces.^{58–62} Some of these were also identified as *cis*-binding interfaces that trigger lateral interactions between receptors on cell surfaces. Examples include, but are not limited to the crystal structures of axonin,⁵⁸ L1⁵⁹ protein and JAM-A.⁶⁰ The proposal that membrane receptors form clusters or oligomers through their *cis*-interactions has thus emerged as a general mechanism of spatial regulation of cell signaling. For instance, clustering of neural cell adhesion molecules was proposed based on the *trans* and *cis* interactions observed in its crystal structure.⁶¹ The EphA2-ephrinA5 complexes containing multiple packing interfaces were also suggested to form an elaborate assembly with alternating arrangements of parallel and antiparallel arrays.⁶² As a result, our study opens

the door to the understanding of general mechanism of cell surface receptor oligomerization and clustering in the membrane environments.

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FIGURE 1.

A structural model of the TNF α -TNFR1 signaling complex on plasma membrane was computationally built as the initial conformation for AA and CG MD simulations (A). The trimeric ligand in the complex is shown by the green cartoon representation. The three bound receptors are highlighted by different secondary structures, with the β -strands of extracellular regions in yellow and transmembrane α -helices in magenta. The complex is embedded into the lipid bilayer, which is shown in gray. A closer view is enlarged in (B) at the interface between single receptor and ligand monomer with the same color index plus the side chain of each residue. The CG model of the complex is further shown by the snapshot taken from the MARTINI simulation (C). The head groups of lipid molecules in the CG system are shown by gray beads, while the ligand trimer is shown in green by the surface representation. The extracellular regions of three receptors in the complex are shown by the same surface representation in yellow, gold, and orange, respectively, while their transmembrane helices are highlighted in blue, magenta, and purple. It can be seen from the figure that the three transmembrane helices were associated together in the CG simulation

FIGURE 2.

We defined conformational angles θ to quantify the dynamics of TNFR1 (A) and TNF α -TNFR1 complex (B) on the plasma membrane. We calculate the distribution of θ along both AA and CG MD simulations with error bars. The simulation result from AA and CG simulations are consistent, showing that the extracellular domains of TNFR1 can undergo large fluctuations on plasma membrane, while the dynamics of TNF α -TNFR1 complex is much more constrained (C)

FIGURE 3.

We applied CG model to simulate a larger system, which contains four signaling complexes. They were uniformly distributed on a square surface of lipid bilayer with 30 nm in length (A). Simulation with 10 μ s long was then carried out at the standard temperature of 310 K. The results show that the complex with the orange and cyan receptors first encountered when the simulation reached 1 μ s (B), while the other two complexes with the purple and pink receptors also formed a lateral interaction after 4.6 μ s (C). Finally, all four complexes were clustered together and formed a linear oligomer (D). We found that the lateral binding interfaces between complexes are all located at the surfaces of the TNFR1 receptors

FIGURE 4.

For each residue in a receptor, we calculated its probability of being on the lateral interface. The profile of this interface probability is plotted in (A) as a function of residue number, while the secondary structure index is shown in the bottom. The regions of “*cis*-interface” existing in the X-ray structure are highlighted by red arrows in the figure. We further projected these probabilities onto the structure of the receptor as a heat map (B), in which the red color indicates the regions that have the highest probability of being on the lateral interface. The TNF ligand is also included in the figure with gray surface representation. The figure shows that the ligand-receptor interface and the lateral interface between receptors are not overlapped. Our results confirmed that this lateral interaction is functionally important to regulate the oligomerization between ligand-receptor complexes on the plasma membrane

FIGURE 5.

The CG model of 12 TNFR1 receptors on plasma membrane was simulated as a control system. A much more irregular spatial pattern was observed by the end of the simulation (A), different from the linear oligomer formed by the signaling complexes (B). We also carried out CG simulations at a higher temperature (347 K) or with a scaling factor $\alpha = 0.8$ to shield the relative binding effect between molecules. The final configurations from the simulations of receptors (C) and complexes (D) at high temperature and from the simulations of receptors (E) and complexes (F) with an adjusted intermolecular force field show similar results. We therefore suggest that the ordered spatial patterns formed by TNF α -TNFR1 complexes play an important functional role in regulating their intracellular signaling pathways

FIGURE 6.

We calculated the minimal distance between all pairs of complexes as a function of time during the simulation. The changes of minimal distances for all pairs of complexes are plotted in (A) for the standard temperature of 310 K and the scaling factor $\alpha = 1$, in (B) for the high temperature of 347 K and scaling factor $\alpha = 1$, and in (C) for the standard temperature of 310 K but the scaling factor $\alpha = 0.8$. Comparing with the standard temperature, we found that the process of oligomerization was greatly accelerated at 347 K. We suggest that the acceleration of oligomerization can be explained by the faster diffusions of complexes on plasma membrane at high temperature. Moreover, we observed that the complexes can still cluster into a larger oligomer although the strength of interactions between proteins was effectively reduced by a smaller value of scaling factor

FIGURE 7.

For a given *cis*-interaction between two receptors from a pair of complexes, we calculated the minimal distances from each of the four extracellular domains in the receptor of one complex to their corresponding domains in the receptor of the other complex. They are plotted as a function of time under the simulation at 347 K. In specific, the curves of minimal distance for two complexes that formed the first lateral interaction are shown in A. The curves of minimal distance for two complexes that formed the second and third lateral interactions in the oligomer are shown in B and C, respectively. Based on the analysis of the simulation data, we suggest that the membrane distal N-terminal domain of the TNFR1 receptors is an essential player to trigger the lateral interactions between signaling complexes and the follow-up oligomerization

FIGURE 8.

The kinetic pathway of receptor oligomerization on cell surfaces is proposed based on the analysis of the simulation results. Before ligand binding, TNFR1 receptors form irregular aggregation through the nonspecific interactions (A). After the presence of TNF α , signaling complexes comprising a trimeric ligand and three bound receptors can be formed (B). The lateral interactions are formed between two signaling complexes through a “zipper-like” mechanism, initiated by the association between the N-terminal domains of two receptors from an interacting complex pair (C). The interaction is stabilized after the rest domains in the receptors form contacts (D). Finally, oligomerization of more signaling complexes can be formed following the same process (E)

TABLE 1

An overview of the simulated systems

System	Force field	Temperature (K)	Time	Number of water molecules	Number of POPC lipids	Scaling factor
TNFR1 × 1	CHARMM36m	310	300 ns	79k	442	
TNF-TNFR1 complex × 1	CHARMM36m	310	200 ns	78k	554	
TNFR1 × 1	Martini2.2	310	10 μs	203k	1347	1.0
TNF-TNFR1 complex × 1	Martini2.2	310	10 μs	200k	1291	1.0
TNFR1 × 12	Martini2.2	310	10 μs	430k	2534	1.0
TNF-TNFR1 complex × 4	Martini2.2	310	10 μs	414k	2567	1.0
TNFR1 × 12	Martini2.2	347	10 μs	430k	2534	1.0
TNF-TNFR1 complex × 4	Martini2.2	347	10 μs	414k	2567	1.0
TNFR1 × 12	Martini2.2	310	5 μs	430k	2534	0.2
TNF-TNFR1 complex × 4	Martini2.2	310	5 μs	414k	2567	0.2
TNFR1 × 12	Martini2.2	310	10 μs	430k	2534	0.8
TNF-TNFR1 complex × 4	Martini2.2	310	10 μs	414k	2567	0.8

Abbreviation: POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine.

TABLE 2

Compare basic properties of lipid bilayers in simulations of different temperatures

System temperature	Martini 310 K	Martini 347 K
Area per lipid ($\text{\AA}^2$)	65.2	69.6
Lipid diffusion coefficient ($10^{-12} \text{ m}^2/\text{s}$)	64.8	68.2