Deriving the Open State Structure of Glutamate Receptor through MapSGLD Flexible Fitting into Cryo-Electron Microscopy Maps

Xiongwu Wu and Bernard R. Brooks

***Abstract*—Cryo-electron microscopy and other imaging technique opened a new window to the analysis of large biomolecular assemblies under biologically relevant conditions. In most cases, electron microscopy maps have low resolution and high noises. Such low-resolution maps do not have enough information to uniquely determine atomic structures of macromolecular systems. The map-restrained self-guided Langevin dynamics (MapSGLD) method we developed previously can utilize structural information embedded in a force field to flexibly fit macromolecular systems into low resolution maps to obtain energetically favored atomic structures that satisfy the maps. Using glutamate receptor as an example, we describe how to perform flexible fitting with MapSGLD to obtain atomic structures from EM maps. The open state atomic structure of the glutamate receptor shows the LBD in the clamshell closed conformation that agrees with the LBD x-ray structure. Most importantly, our MapSGLD flexible fitting structure captures the open state ion channel, which has not been observed so far in x-ray structures.**

***Index Terms*—electron microscopy, flexible fitting, glutamate receptor, open state, molecular structure, map, conformational search, molecular simulation, self-guided Langevin dynamics**

I. Introduction

Atomic structures of macromolecular systems are essential to understand their functional mechanism. X-ray and NMR are conventional approaches to obtain macromolecule structures. But many systems or functional states are not accessible by these approaches because they are unable to form crystal, or their molecular sizes are too big. The advance of cryo-electron microscopy (EM) opens a new window to the analysis of large biomolecular assemblies under biologically relevant conditions. Even though EM images are low in resolution, they have been used to produce complex structures based on individual protein structures obtained from X-ray or NMR methods, often through rigid fitting [1-8].

Proteins often adopt different conformations in different states, such as in bound and unbound states. In addition, proteins have certain conformational flexibility and can adapt to different environmental conditions. To accommodate the conformational change, a process called flexible fitting is used to change structures from X-ray or NMR to match electron microscopy images. A series of methods have been developed to perform flexible fitting[9-16]. The map-restrained self-guided Langevin dynamics (MapSGLD) simulation method[17-19] we developed previously is a simulation based method, which means that the method samples the conformational space according to their distribution probabilities. A molecular simulation is regarded as a computer experiment. Ideally, if the force field is accurate and the simulation system represents the real experimental system, the conformation with the highest distribution probability from a simulation would be the experiment observed structure of the studied system. However, many factors affect the simulation results, such as inaccuracy in the force field, simplification in the simulation system setup, short length of a simulation. These defects prevent a simulation to identify the correct structure. This problem can be solved by incorporating experimental information to make the experiment observed structure the global minimum. One typical example is NMR NOEs, which specifies atom pairs in short distances and have been used extensively to determine protein structures. Cryo-EM maps provide electron distribution information and can be used to derive atomic structures of macromolecular systems. To force a simulation, converge to the experimental observed structure, MapSGLD adds map-restraints into the energy landscape to bias the conformational search toward structures resemble the EM maps. In addition, MapSGLD uses self-guided Langevin dynamics (SGLD) algorithm[20-24] to search the conformational space, which enables large scale conformational change to reach the global minimum. This method has been applied successfully in EM study of protein structure and functions[25-27].

Ionotropic glutamate receptors (iGluRs) are cation channels that mediate signal transmission in the central nervous system and are the targets of extensive research efforts[28-36]. To

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understand the structural activation mechanism of the ion channel, we need atomic structures of both the close and open states. Even though there are x-ray structures obtained in conditions for both the close state and the open state, the ion channel stayed close due to the crystallization and other reasons[33, 34]. The EM maps in both states have been obtained. [28-30, 32] However, the low resolution, especially in the transmembrane domain (TMD) prevents obtaining atomic structure of the TMD in the open state. Because of the low resolution, people often rely on rigid fitting to obtain atomic structures[32]. While rigid fitting can provide structural information between rigid structures, little information can be extracted from within rigid structures. Here, as an example to demonstrate the application of MapSGLD, we performed flexible fitting of the open state EM map obtained by Meyerson et al. [32] to derive the open state structure, from which we can investigate the structural mechanism of glutamate receptor activation.

II. PROTOCOL

MapSGLD has been implemented in CHARMM (version c38 and later) and AMBER (version 12 and later) which are freely available from www.charmm.org (CHARMM) and www.ambermd.org (AMBER). Below we list the steps to derive the open state atomic structure of the glutamate receptor from cryo-EM maps using MapSGLD.

1. Collect experiment data. Download the open state Cryo-EM map emd-2684 from EMDB[37]

2. Build Initial structure of the glutamate receptor

2.1) Obtain protein sequence P19491 from protein information database[38].

2.2) homology modeling glutamate receptor using each of the four chains in 3KG2 as template through SWISS-MODEL[39-42].

2.3) Assemble the models of the four chains according to 3KG2 to form a tetramer. The tetramer homology model is saved to a pdb file: glua2_model.pdb.

3. MapSGLD simulation. Here we use CHARMM input scripts to show the flexible fitting process. "CHARMM>" is the prompt for input. An exclamation mark "!" at the first position of input defines a comment line.

3.1) define charmm36 force field[43] and SCPISM solvation model[44]. These force field input files come with the CHARMM package.

```
CHARMM>! Read in topology of building blocks
CHARMM>OPEN READ FORM UNIT 11 NAME data/top_all36_prot.inp
CHARMM>READ RTF CARD UNIT 11
CHARMM>close unit 11
CHARMM>! Read in force field parameters
CHARMM>OPEN READ FORM UNIT 12 NAME data/par_all36_prot.inp
CHARMM>READ PARAM CARD UNIT 12
CHARMM>close unit 12
CHARMM>! activate SCPISM solvation model
CHARMM>open read unit 14 card name data/scpism.inp
CHARMM>SCPI UISM 14
```

3.2) Construct the simulation system from the homology model of the tetramer.

```
CHARMM>! Readin the pdb file
CHARMM> open read form unit 12 name glua2_model.pdb
CHARMM> read segid p unit 12 pdb build setup
CHARMM>! Generate internal coordinates
CHARMM> autogen angle dihe
CHARMM> ! generate missing coordinates
CHARMM> ic para
CHARMM> ic build
CHARMM>! Minimization to get rid of atom clashes
CHARMM> mini abnr nstep 200
```

3.3) Rigid fitting of the initial structure into open state cryo-EM map emd-2684

```
CHARMM>!Read in the map file
CHARMM>emap read map0 name "emd-2684.map" format ccp4
CHARMM>!Generate map object from structure
CHARMM>emap gene map1 sele all end
CHARMM>!Define rigid domain
CHARMM>emap assign map1 as rig1 sele all end
CHARMM>! Perform GTMC rigid fitting
CHARMM>emap dock gtmc mapid map0 rigid rig1 -
        ntra 2 nrot 2 ncyc 10 nstep 100
CHARMM>! Get the final coordinates of the system
CHARMM>emap proj rig1 sele all end
CHARMM>! Save coordinates for later analysis
CHARMM>coor copy comp
```

3.4) MapSGLD simulation to flexibly fit the structure into emd-2684

```
CHARMM>!define map restraint atoms
CHARMM>emap reference map0 select all end
CHARMM>!define map restraint strength
CHARMM>emap assign map0 as rig0
CHARMM>emap cons 0.02 rig0
CHARMM>! apply restraint to maintain two-fold symmetry
CHARMM>cons harm relative force 0.05 –
select iseg 1:2 end select iseg 3:4 end
CHARMM>! Constrain all bonds with hydrogens
CHARMM>SHAKE BONH PARA

CHARMM>! Set collision frequency for SGLD
CHARMM>SCAL FBETA SET 1.0

CHARMM>! Perform SGLD simulation
CHARMM>DYNA LEAP LANG STRT NSTEP 1000000 TIME 0.001 -
        SGLD TSGAVG 0.2 SGFT 1.0 TBATH 300 FIRST 300
```

3.5) Minimization to remove structure fluctuations.

```
CHARMM>! apply Adopted Basis Newton-Raphson minimization
```

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CHARMM>minimization abnr nstep 1000

4. Output final conformation for further analysis

4.1) Save the final conformation in PDB format
CHARMM>open writ form unit 16 name glua2_mapsgld.pdb
CHARMM>writ coor pdb unit 16

4.2) Superimpose the ATD in A and B chains to examine conformational changes in the ATD dimers
CHARMM>coor orien rms sele iseg 1:2 and resid 4:377 end

4.3) Superimpose the LBD in A and D chains to examine the clamshell closing in the LBD dimers
CHARMM>coor orien rms sele (iseg 1 .or. iseg 4) -
.and. (resid 397:504 .or. resid 635:770) end

4.4) Superimpose the TMD four M3 helices to examine the opening of the ion channel.
CHARMM>coor orien rms sele resid 600:630 end

4.5) Superimpose the tetramer as a whole to examine conformational changes in tetramer organization.
CHARMM>coor orien rms sele all end

III. REPRESENTATIVE RESULTS

The procedure to derive atomic structures from EM maps is demonstrated with the open state glutamate receptor. The open state map was downloaded from EMDB, emd-2684, and is shown in Fig.1(a). The initial structure of the glutamate receptor is homology modeled using 3KG2's A, B, C, D chains as templates. These four models form a tetramer and was rigid fit into the map using the core-weighted grid threading Monte Carlo method[2] as shown in Fig.1(b). As can be seen, the initial structure overlaps with the map very well. It is difficult to tell from the surface contour how well a structure fit into a map. Instead, the distribution of electron density is what matters for a correct fit. In other words, rigid fitting misses detailed internal structure information. MapSGLD utilizes the match in electron density to guide the conformational sampling. After the MapSGLD flexible fitting, the structure fit very well in the map, as shown in Fig.1(c).

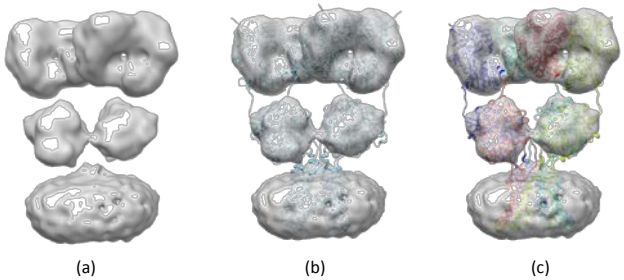

Fig. 1. The emd-2684 map (a), rigid fitting structure (b), and MapSGLD flexible fitting structure (c). The map is shown as grey surface; the initial structure is shown as cyan ribbons; and the MapSGLD result is shown as blue, green, yellow, and red ribbons for chain A, B, C, and D, respectively.

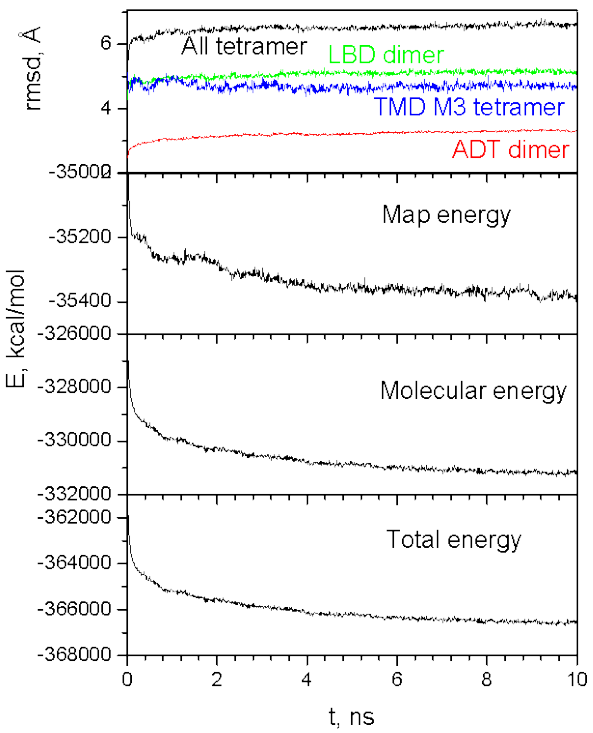

Fig. 2. MapSGLD simulation profiles. The lower 3 panels show the map restraint energy, the molecular energy, and total energies during the flexible fitting simulations. The top panel shows the root-mean-square deviations of the conformations from the initial conformation. The black, red, green, and blue lines represent the rmsd of the whole tetramer, the ADT dimer, the LBD dimer, and the ion channel formed by TMD four M3 helices.

The flexible fitting process can be better understood from the simulation profiles. Fig.2 shows the energies and the root-mean-square deviations (rmsd) during the MapSGLD flexible fitting. The rmsd is calculated against the initial conformation. As can be seen, the map restraint energy went down throughout the simulation, indicating the conformation changed to better match the EM map. The molecular energy also went gown significantly, indicating the tetramer became more stable after the flexible fitting. The overall energy of course went to lower value after the simulation, indicating the tetramer final structure is more probable than the initial one. The top panel of Fig.2 shows the rmsd of different parts of

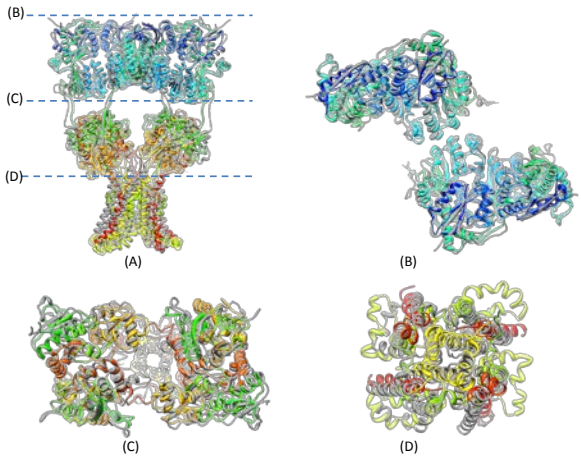

Fig. 3. Conformations before (grey) and after (spectrum colors by sequence) flexible fitting into the open state map. (A) shows the side view of the two conformations superimposed together; (B) shows the top view of the ADT tetramer; (C) shows the top view of the LBD tetramer; and (D) shows the top view of the TMD tetramer.

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the tetramer. On average, the flexible fitting resulted in a 6.6 Å conformational change. A major part of the change is the domain rearrangement in the tetramer, because each individual domain has smaller rmsd. The ADT dimer has the smallest change with a rmsd of 3.2 Å. The LBD has the largest change with a rmsd of 5.1 Å. The ion channel formed by four M3 helices has the large change with a rmsd of 4.6 Å. Therefore, the major conformational change happened in the LBD and TMD.

The overall differences between the initial conformational and the final conformation are shown in Fig.3. The overall architectures of the two conformations are similar. The ADT tetramer shows little change. Large differences are observed in the LBD and TMD tetramers. These observations agree with the plot shown in Fig.2.

Fig.4 examines the ADT dimers before and after the flexible fitting to the open state map. The dimer becomes slightly more compact after the simulation.

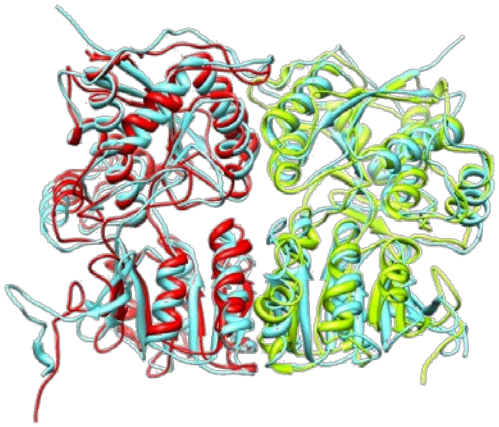

Fig. 4. ADT dimer conformations comparison. Initial (cyan) and flexible fitting into the open state map (red for chain A and green for chain B).

Because the tetramer has only a 2-fold symmetry, the LBD in the four chains take two types of conformations. Fig.5 examined LBD in chain A before and after the fitting. Compared with the initial conformation, we can see if viewed toward dimer interface, the upper portion of LBD left side moves down while the lower portion of LBD left side moves up, which is termed as clamshell closure. For the LBD in chain B, the similar clamshell closure is observed (Fig.6).

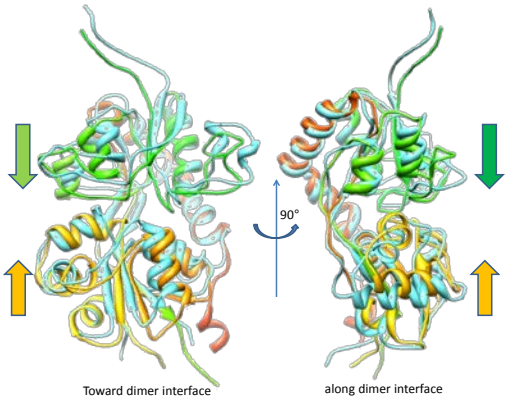

Fig. 5. LBD chain B conformations comparison. Initial (cyan) and flexible fitting into the open state map (colored as spectrum according to sequence).

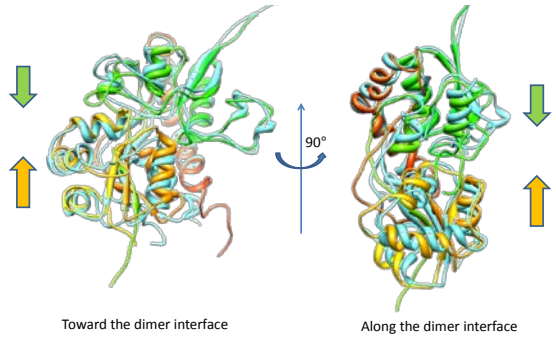

Fig. 6. LBD chain D conformations comparison. Initial (cyan) and flexible fitting into the open state map (blue, red, yellow, and green).

The key change between the close state and the open state lies in the TMD. Fig.7 shows the ion channel M3 helices in the initial conformation and in the flexible fit structure. The initial conformation was modeled with the close state structure, 3KG2, therefore, represents a close state conformation. Clearly, we can see that the mouth formed by the four helices in the flexible fit structure (the open state) opens up as compare to the initial conformation (the close state). This difference supports that the EM map, emd-2684, captures the ion channel in the open state.

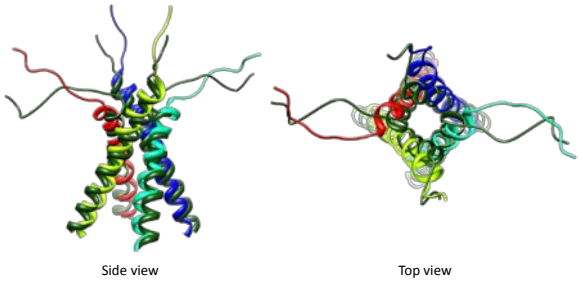

Figure 7: TMD helix M3 ion channel conformations comparison. Initial structure (dark green) and flexible fit structure (blue, red, green, and cyan for chains A, B, C, and D).

It is interesting to compare the open state structure obtained with MapSGLD flexible fit with the close state structure 3KG2 and the open state structure 4U1Y obtained from x-ray crystallography. Fig.8 compares the structures of ADT, LBD, and TMD M3 helices. As can be seen, the ADT of all the three structures are very similar, except that the fitting structure is slightly more compact than those in both x-ray structures. For the LBD, the clamshell is closed in the flexible fitting structure and in 4U1Y, while open in 3KG2. The closeness of the clamshell is a little bit more in the flexible fitting structure than in 4U1Y. The ion channel in 3KG2 and 4U1Y are similar, while in the flexible fitting structure, the ion channel opened up. Therefore, MapSGLD is able to capture the ion channel in the open state from the low-resolution map, which cannot be seen from the open-state x-ray structure, 4U1Y.

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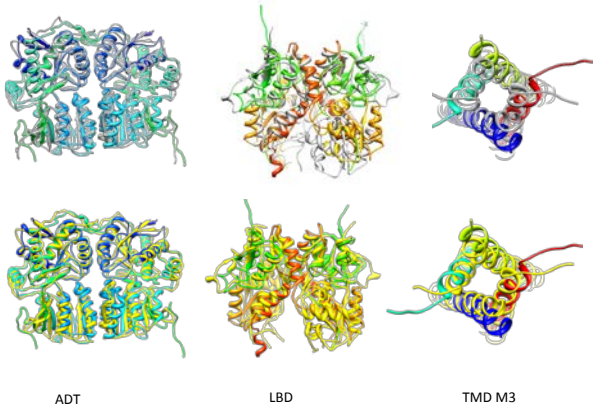

Fig. 8. The open state structure obtained with MapSGLD is compared with the closed structure 3KG2(grey) and the open state structure 4U1Y(yellow). The flexible fit structures are colored as spectrum according to sequence for ADT and LBD, and are colored blue, red, green, and cyan for chain A, B, C, and D for the TMD M3 ion channel.

IV. DISCUSSION

MapSGLD utilizes structural information from EM maps to correct the energy landscape defined by the force field so that the global minimum of the simulation system is made consistent with the experimental structure. Because the low resolution and large noises in EM maps, it is ambiguous to determine molecular structures from EM maps alone. MapSGLD is a simulation approach and does not completely rely on a map to determine all degrees of freedom, therefore, can tolerant the low resolution and high noises in EM maps. MapSGLD samples conformational space along the energy landscape defined by the force field, which contains a lot of structural information, e.g., covalent interactions including bond length and bond angles, hydrogen bonding, and hydrophobic interaction. However, due to the inaccuracy in the force field and the simplification of the simulation systems, the global free energy minimum of a simulation system may not be the experiment structure. MapSGLD combines the two types of information together to identify the structure that matches the EM map and favored in the energy landscape. Deriving the open state structure of glutamate receptor provides an excellent application example of MapSGLD.

The strength of the map potential should be chosen according to the quality of the EM map. The quality of the EM map includes the resolution and noise level. A low resolution reduces the sensitivity of the conformation. A high noise level could result in local minimums that could cause over fit. A strong map restraint may increase the map sensitivity, but also increase the noise level. Therefore, the strength of the map restraint affects the convergence of a MapSGLD simulation. Our simulations have shown that a map restraint constant of 0.01~0.05 kcal/g works well with the CHARMM force field.

The symmetry related restraints help to suppress noises in the EM map. Because noises are uncorrelated while the structural information does, the symmetry restraints can enhance the convergence of flexible fitting. For the glutamate receptor, there is a two-fold symmetry, which can be conveniently enforced by a relative harmonic restraint between A, B chains and C, D chains. CHARMM also provides the image facility that can perfectly enforce symmetries and reduce computation cost. For higher than two-fold symmetries, the image facility should be used.

Because MapSGLD simulations sample a continuous trajectory in the conformational space, all degrees of freedom deviate frequently from their optimal values. These deviations can be quickly removed by energy minimization. Therefore, energy minimization is performed after MagSGLD simulation to remove deviations from equilibrium structures.

Loop regions in molecular machinery are often less structured and difficult to see in EM maps. However, they often play important role in biological functions. MapSGLD is perfectly positioned to determine the loop conformation through sampling and structural restraints from surrounding domains. Similarly, even though the maps at the TMD region do not have high resolution enough to identify the structures, a combination with the force field lead to unambiguous flexible fitting results and reveal the difference in the close and open states. That's why low-resolution EM maps can provide useful structural information on functional mechanism. With more accurate force field and more realistic representation of the experimental systems, the simulation approach need less experimental input to identify structure and dynamic properties.

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