Published in final edited form as:

Proteins. 2009 August 1; 76(2): 271–280. doi:10.1002/prot.22332.

Modeling of Peptides Connecting the Ligand Binding and Transmembrane Domains in the GluR2 Glutamate Receptor

K. Speranskiy* and M. G. Kurnikova†

Chemistry Department Carnegie Mellon University, Pittsburgh, PA 15213

Abstract

Ligand-gated Glutamate receptors (GluR) mediate synaptic signals in the nervous system. Ionotropic GluRs of AMPA type, the subject of the present study, are tetrameric assemblies of monomer subunits, each of which is constructed in a modular fashion from functional subdomains. The extracellular ligand binding domain (LBD) changes its conformation upon binding of an agonist ligand followed by opening of a transmembrane (TM) ion channel. Peptides connecting the LBD and TM domains facilitate gating of the channel, and their structure and composition are important for the receptor functioning. In this study we used Replica Exchange Molecular Dynamics (REMD) simulations to model S1M1 and S2M3 connecting peptides of the GluR2 receptor in two implicit solvents, water and interfacial water/lipid medium characterized by lower polarity. Propensity of these peptides to form helical structures was analyzed using helicity measure derived from the free energy of the simulated ensembles of structures. The S1M1 and S2M3 connecting peptides were not helical in our simulations in both dielectric environments in the absence of the rest of the protein. The structures of the LBD fragment with known high resolution α -helical structure and of the TM3 helix were successfully predicted in the simulations, which in part validate our results. The S2M3 peptide which is important in gating formed a well-defined coil structure and salt-bridges with the S2 domain. The S1M1 peptide formed a loop structure via formation of internal salt-bridges. Potential implications of these structures on function of the receptor are discussed.

Keywords

REMD; iGluR; AMPA; peptide folding; helicity; importance sampling; ligand gated ion channel; structure-function relationship

Introduction

Ionotropic glutamate receptors (GluRs) are agonist activated cation channels that mediate fast synaptic transmission between neurons. Functioning of glutamate receptors is essential in memory and learning and plays a role in dysfunction of the central nervous system¹⁻³. Ionotropic GluRs function as homo- and/or hetero-tetrameric complexes^{4,5} in which each subunit consists of four distinct domains: an extracellular N-terminal domain, a ligand binding domain (LBD), a transmembrane domain and a C-terminal domain (see Fig. 1 for the topology of a GluR monomer). Crystal structures of LBDs have been determined for several iGluRs⁶⁻⁸. Although the structure of the transmembrane domain (TM) of the receptor is unknown its general topology is thought to be similar to that of the KcsA potassium channel^{9,10}. The prokaryotic GluR0¹¹ has a simpler topology with only two

†Corresponding author. kurnikova@cmu.edu, phone: 1-412-268-9772, fax: 1-412-268-1061.

*Present address: Physics Department, Arizona State University, Tempe, AZ

transmembrane helices and a reentrant helix, yet forms a fully functional ligand gated ion channel. GluR0 is thought to be an evolutionary intermediate between the potassium and glutamate channel families^{9,10}. An eukaryotic LBD formed by two subdomains S1 and S2 is connected to the TM domain via three short peptides of unknown secondary and tertiary structure (see Fig. 1); and the LBD of GluR0 is connected to the TM domain lacking the TM4 helix (see Fig. 1) by two linkers.

Experimental studies have demonstrated that LBD – TM domain connecting peptides are important for coupling ligand binding to channel gating. For example, mutations in these regions influence gating kinetics and desensitization in both AMPA and NMDA type receptors¹²⁻¹⁵. Understanding the structural properties of connecting peptides is therefore essential for developing models for the mechanism of iGluR functioning. However, little is yet known about the structural preferences of these peptides. In this paper we developed computational models for two connecting peptides from the GluR2 (AMPA type) receptor: the S1M1 peptide connecting the LBD S1 domain and the TM1 helix, and the S2M3 peptide connecting the LBD S2 domain and the TM3 helix (see Fig. 1); short segments of adjacent domains were also included in the simulation.

The strategy of modeling only a part of a protein sequence “extracted” from the whole, as is done in this work, is based on the understanding that GluRs are constructed in a modular fashion, such that each domain can fold and preserve its structure somewhat independently of the presence of other domains. This approach is also strongly justified by its recent success in theoretical protein folding. Namely, Ho and Dill¹⁶ performed systematic series of the Replica Exchange Molecular Dynamics (REMD) simulations¹⁷ of short peptides in continuum solvent extracted from the proteins with known tertiary structures. In these simulations, 35% of all studied peptides were structured in the same manner as in their respective proteins. Their secondary structure depended strongly on interactions with residues located in close proximity in the primary sequence and weakly on the distant residues. Based on this observation and using the REMD methodology to fold short fragments of a protein sequence in implicit solvent, Dill and co-workers¹⁸ recently reported a successful folding of several globular proteins of a priori unknown structure. The idea that amino acids adopt conformations that are mainly determined by their neighbors in the sequence has also been successfully utilized in knowledge-based strategies for modeling proteins using a database of short peptides known as I-sites¹⁹. Thus, it is reasonable to suggest that the structure of connecting peptides may also be somewhat independent of the structure of the rest of the protein.

REMD is a physics-based simulation method that has been previously successfully utilized for *in silico* folding of peptides and proteins in solution^{16,18,20-23}, in lipid bilayers^{24,25} and at the membrane interface²⁶. Implicit solvent REMD chosen in this work is an efficient methodology to sample conformational space of relatively short peptides. Significant improvements have been achieved in the amino acid force field parameters and implicit solvent representation. Extensive comparisons of the peptide structure prediction using a variety of force fields and solvation models have been reported in literature^{27,28}. Recent versions of Cornell *et al.* force field²⁹ have been successfully used for folding of both helical and beta-structured peptides^{21,30,31}. One of the more recent parameterizations of the Cornell *et al.* force field, parm03, has been chosen in this study³². Recent simulations reported by several groups estimated simulation times and protocols needed for convergence of a simulation. A range between 1-200 ns of required simulation times was reported for a variety of peptides^{20,33}. While implicit solvent simulations can be reliably performed to full convergence, it is easy to estimate that simulation length and number of replicas required to fully converge an explicit solvent simulation are several orders of magnitude higher than the implicit solvent simulation due to the solvent viscosity and multiple configurations of the

solvent molecules. In cases where such simulations were reported, the requirements of simulation times needed for convergence remain an area of active research applicable only to well characterized test systems^{34,35}.

Results of the REMD simulations reported in this study were analyzed using free energy landscapes projected on the principal component vectors of the conformational space derived from the simulated ensembles of peptide secondary structures. The secondary structure of the simulated peptides was also characterized using a measure of helicity, which is introduced and described in this work.

This paper is structured as follows. Details of the peptide compositions used in the simulations as well as choices of the modeling parameters and protocols for the REMD simulations and trajectory analysis methods are described in the **Models and Methods** section. The **Results** section presents a technical report on simulations performed and structural analysis of the simulated peptides. The brief **Discussion and Conclusion** section presents a discussion of our results in context of currently available experimental structural and functional data.

Models and Methods

Compositions of Modeled Peptides

The lengths of the GluR2 LBD - TM connecting peptides and their margins in the protein primary sequence were previously discussed and determined using bioinformatics tools and biochemical experiments. We have used Memsat³⁶ and TMHMM³⁷ bioinformatics tools to determine lengths and margins of the S1M1 and the S2M3 peptides. Memsat predicted that the TM1 helix spans sequential positions 525 - 543; TMHMM predicted positions 521-543. In the experimental study¹³ the TM1 helix was determined to be at positions 526-543, which agrees with the Memsat prediction. Hence we consider the connecting peptide S1M1 spanning the positions 506-524 with the sequence KPQKSKPGVFSFLDPLAYE (see also Fig. 1). The S1M1 peptide was modeled in two different compositions termed S1M1_{long} and S1M1_{short}. The S1M1_{long} consists of 19 residues 506-524, the S1M1_{short} consists of 13 residues 506-519.

Both bioinformatics servers predicted location of the TM3 helix at positions 604-626. In experimental studies the TM3 was identified to span positions 600-623 placing the beginning of S2M3 at position 624¹³. However, the longest S2M3 sequence was reported positions 619-631³⁸. We modeled S2M3 peptide in three different compositions: 1) the TM3+S2M3 peptide consisting of 13 residues that include a short fragment of the TM3 domain spanning 619-623 (NLAAF) and the S2M3 itself spanning positions 624-631 (LTVERMVS); 2) the TM3_{long}+S2M3_{short} sequence included a longer fragment of the TM3 domain 613-623 (ISSYTANLAAF)(and only a LTV fragment of the S2M3 peptide); and 3) the TM3+S2M3+S2 sequence that included the TM3+S2M3 sequence with the addition of eight residues from the S2 domain (PIESAEDL) that form a fragment of an α -helix in the LBD crystal structure.

Replica Exchange Simulations

To sample conformational space of the peptides we utilized replica exchange molecular dynamics algorithm (REMD)¹⁷. REMD, an enhanced sampling method, has been widely used to model folding of small proteins^{18,22}. In REMD multiple copies of a system are simulated in parallel at different temperatures. Periodically, neighboring replicas attempt to exchange their temperatures using the Metropolis acceptance criterion³⁹. Thus, REMD allows replicas to escape from local minima on a rugged potential energy landscape and fully sample conformational space of a peptide. In addition, REMD provides correct

thermodynamic ensemble sampling at each temperature; hence, free energy profile of a system at given temperature can be deduced from such simulations using an appropriate unbiasing technique, e.g. the weighted histogram analysis method (WHAM)^{40,41}.

The REMD was utilized as implemented in AMBER⁴². The topology and coordinates were prepared using the LEAP module of AMBER⁴². All simulations were initialized starting from an extended conformation of a peptide. 12 replicas were exponentially spaced in the temperature range from 280K to 450K. An acceptance probability of the exchange attempts was approximately 30%. Exchanges were attempted every 1 ps. The time step of the simulations was set to 2 fs. Bonds containing hydrogen atoms were constrained via SHAKE algorithm⁴³. The non-polar surface penalty constant was set to be 0.005 kcal/mol·Å². Snapshots were saved every 1 ps for further analysis. The total time of each simulation was 15 ns per replica; the last 10 ns of each simulation were used in analysis. The parm03 force field was used in this study³². The terminal ends were neutral in all simulations. The solvent was implicitly represented using the generalized Born/solvent accessible surface (GB/SA) model of Tsui and Case⁴⁴. The S1M1_{long} and TM3+S2M3 peptides were simulated using two dielectric environments: *i*) $\epsilon = 80$ to represent bulk water and *ii*) $\epsilon = 10$ to represent the head group region and water interface of the lipid bilayer⁴⁵.

Principal Component Analysis of the Covariance Matrix

Data from each simulation were combined and unbiased using the weighted histogram analysis method (WHAM)^{40,41,46}. WHAM calculates an estimate of the density of states from which one can calculate the free energy of a system projected onto specific reaction coordinates. In this study we chose a two-dimensional projection of free energy on the first two principal components of the multidimensional conformational space of a simulated peptide, characterized via the covariance matrix. A $3N \times 3N$ covariance matrix R (N is the number of the C_α atoms in the system) was constructed using K snapshots from an MD trajectory⁴⁷:

$$R = \frac{1}{K} DD^T,$$

where D is the $3N \times K$ matrix of deviations $D_{ia} = X_i(t_a) - \langle X_i \rangle$ of the mass-weighted coordinates $X_i(t_a)$ for each C_α atom $i = 1 \dots N$ at a time t_a ($a = 1 \dots K$), from their time average positions $\langle X_i \rangle$. In principal component analysis (PCA) the covariance matrix is diagonalized to determine its eigenvalues and corresponding eigenvectors. Projection of the simulated data onto the first two principal components with the largest eigenvalues makes it suitable for visualization of distinct clusters of the simulated structures^{**}.

Helicity measure

While projection of the free energy onto principal components of the covariance matrix helps to search for generically similar structures, a more direct search for the elements of

^{**} Note that the standard PCA may poorly characterize conformational space of the flexible unstructured peptides⁴⁸. The reason is that for a flexible molecule which adopts a variety of structures it is difficult to distinguish between global and internal degrees of freedom. To remedy this problem several modifications of PCA have been proposed, e.g. the isotropic reorientation eigenmode dynamics (IRED)^{48, 49}, in which no *a priori* separation of the overall global and the internal motions is required. When a single dominant structure is present in an analyzed dataset both the PCA and IRED methodologies will find a small subset of components that describe the majority of configurations. Both methods will also predict that in the absence of a dominant structure many components contribute significantly to the description of a conformational space of a simulated system. Thus, considering that we only need to distinguish between structured and unstructured datasets rather than to analyze the nature of eigenmodes in detail, either method is adequate for our study.

known secondary structure is also useful for characterization of a peptide structure. The S2M3 peptide modeled in this paper is flanked by α -helical sub-domains, therefore, here we are especially interested in determining whether it has propensity to form helical structures. The A peptide segment is typically considered to be α -helical if at least three consecutive residues have their ϕ and ψ angles lying in the α -helical region of the Ramachandran plot⁵⁰, namely: $-100^\circ \leq \phi \leq -30^\circ$, $-80^\circ \leq \psi \leq -5^\circ$. To characterize the degree of helicity found in the connecting peptides we introduce a measure of helicity of the peptide backbone. We define the helicity for a sequence of three residues as follows:

$$helicity = \sum_{i=1}^3 a_i, \text{ where } a_i = \begin{cases} 1, & -100^\circ \leq \phi \leq -30^\circ, -80^\circ \leq \psi \leq -5^\circ \\ 0, & \text{elsewhere} \end{cases},$$

where i is the residue counter in a sequential triplet. Free energy is calculated using the REMD simulated ensemble as a function of the helicity value of each sequential triplet. Figure 2 shows the free energy extracted from one REMD simulation for one triplet of residues (shown in the figure) as a function of a helicity value. We define helicity measure of a triplet as a value of its helicity at the minimum of its free energy. For example, in Fig. 2 helicity measure of the triplet is one, thus, in the corresponding structure only one residue is helical. The structure of a peptide is locally helical only when its helicity measure equals three. The helicity of the whole peptide is assessed by calculating the helicity measure of each sequential triplet of residues.

Results

The first modeled peptide TM3_{long}+S2M3_{short} is composed of a long fraction of the TM3 domain and only a short fraction of the S2M3 peptide immediately adjacent to TM3 as shown in Fig. 3a. No high resolution structure has been determined for this domain but there exists strong evidence that the TM3 domain is α -helical⁵¹. The purpose of modeling the TM3_{long}+S2M3_{short} is to determine how strong is its propensity to the helix formation, and determine whether this sequence can form a secondary structure in the absence of the rest of the protein. To some degree this simulation also serves as a test case for the REMD methodology as applied in this study. The low polarity environment of the simulation mimics polarizability of the water/lipid interface⁴⁵ where the connecting peptides reside in the whole receptor. The calculated free energy of the TM3_{long}+S2M3_{short} peptide is shown in Fig. 3b projected onto the space of the first two principle components of the covariance matrix of the structural ensemble generated using REMD simulations and re-weighted using WHAM algorithm as described in **Models and Methods**. Such representation of a configurational manifold of the simulated structures exposes structural commonalities present in an ensemble. Namely, when similar structures dominate in the simulated ensemble its two-dimensional free energy plot features few well pronounced minima. Indeed, the free energy profile in Fig. 3b shows two deep closely spaced minima. Two representative structures corresponding to these two free energy minima are also shown in Fig 3b. By visual inspection both structures form regular or nearly regular α -helices. To quantify this observation, we further characterized conformational space of the peptide in terms of helicity measure of the sequential triplets of residues (as described in detail in the **Models and Methods** section). In this analysis we introduce a helicity measure of a triplet of residues by finding location of its free energy minimum in the triplet helicity axis. A triplet is helical only if its helicity measure equals three. The helicity measure graph shown in Fig. 3c further corroborates definite α -helical structure for this sequence up to the last triplet included in the calculation (AFL). Note, that the first triplet of a sequence starts with the second residue of the sequence as described in Fig. 2 caption. L624 residue is currently

categorized as a part of the S2M3 connecting peptide rather than the TM3 helix, however it is possible that in the full receptor the AFL triplet is capping the TM3 helix.

The TM3+S2M3 peptide (Fig. 4a) containing the full sequence of the S2M3 peptide and five residues of the TM3 domain has been modeled in both water and low dielectric in order to determine how sensitive is its structure to the local environment. As seen in Figs. 4b and 4c showing the helicity measure plots for both simulations, no helical structures were formed by this peptide in either environment. However, the same pattern of helicity is observed for triplets 4 through 9, which include mainly the S2M3 sequence itself indicating that structural preference of this peptide is influenced little by the solvent polarity. The AFL triplet however exhibits one helical turn in water simulation indicating its propensity to helicity, thus further supporting the observation derived from the simulation of the TM3_{long}+S2M3_{short} sequence above. Free energy maps for TM3+S2M3 peptide (not shown) did not reveal any specific structural propensity for this peptide strongly suggesting that it is naturally unstructured in the absence of the whole protein.

The TM3+S2M3+S2 peptide (see Fig. 5a) contains the S2M3 connecting peptide sequence as well as fragments of both adjacent domains: the LBD (S2) and the TM3. The presence of the structured domains flanking the peptide strongly biases its environment towards native-like environment in the whole protein¹⁶. Indeed, the free energy map (see Fig. 5b) obtained for this peptide in water exhibits deep global minimum indicating a well defined structure. A representative structure is shown in Fig. 6a. This structure is dominated by two helices. The helicity plot for the whole TM3+S2M3+S2 peptide is shown in Fig. 5c. The first helix is formed by eight residues PIESAEDL of the S2 domain (see Fig. 5a). The structure of this fragment known with high resolution⁶ is correctly predicted in the simulation. The root mean squared deviation (RMSD) of the C α atoms of the helical turn formed by the SAEDL peptide is only 0.8Å from the x-ray structure⁶. Excellent agreement of the modeled structure with its known template further validates the results presented in this work. The second helix is formed by the short fragment of the TM domain and the helix capping residues AFL in agreement with simulations described above. The S2M3 connecting peptide itself formed a coiled structure.

Comparing the helicity measure of all simulations of the S2M3 containing peptides, shown in Figs. 3c, 4b, 4c, and 5c, a similar or identical pattern of helicity measure emerges for the S2M3 peptide indicating its natural propensity to form a coil structure independently of an environment and composition of adjacent sequences. The S2M3 peptide showed no α -helical propensity in all our simulations despite being located between two α -helical domains. In the simulated structures the residue R628 of the S2M3 peptide formed stable hydrogen bonds with the D638 and E634 of the S2 LBD helix as shown in Figs 6b and 6c respectively. This persistent hydrogen bond network of interactions between the LBD and S2M3 connecting peptide may be present in the whole receptor and contribute to gating. It has been shown that mutations of residues R628 and E627 strongly affect gating kinetics of the receptor¹⁴, however no relation of such functional study to the structural determinants of the domain interactions has been thus far possible.

Free energy maps for the S1M1_{long} peptide (see Fig. 7a) simulated in implicit water and in low dielectric are shown in Fig. 7 (b) and (c) respectively. Both plots feature multiple shallow minima separated by low energy barriers. Moreover, structures residing within single free energy basin significantly differ from each other indicating an absence of a well defined structure in this peptide. Additional evidence of an absence of a structure in both simulations stems from the fact that only 40% of the total structural variability of the simulated ensemble is accounted for by the first two principal components of the covariance

matrix. Furthermore, extending the number of principle components to five did not reveal any significant clusterization in the higher dimensional space.

While the PCA analysis of the free energy of the S1M1_{long} peptide indicated absence of a general secondary structure for the whole peptide, it is possible that elements of typical secondary structures may be formed by several residues within a peptide. To assess possible partial folding of peptides in the form of a helix we applied the helicity analysis of the simulated peptide structure. The helicity measures for the S1M1_{long} peptide in both solvents, shown in Fig. 7 (d) and (e) show that only two sequential residues were found in helical conformations demonstrating the absence of helical propensity for this peptide.

One common feature of multiple structures of the S1M1_{long} peptide is formation of a loop formed by hydrogen bonds and salt bridges between positively charged K506, K509, and K511 located at the S1 adjacent end of the peptide, and the negatively charged D519 and E524 at the TM1 adjacent end of the peptide. Several representative structures of the peptide with the salt bridges present are shown in Fig. 7b and 7c. However, we have to consider a possibility that in the whole receptor in the presence of entire LBD and TM domains geometric restrictions may prevent formation of these salt bridges. To investigate this peptide propensity to form structure in the absence of the salt bridges we introduced a cropped version of the S1M1_{long} peptide S1M1_{short}, which lacks the end aspartate and glutamate capable of forming salt-bridges with the positively charged lysine residues. The helicity measure pattern of the S1M1_{short} peptide (not shown) was identical to that of the S1M1_{long} peptide, indicative of no helical propensity of this sequence.

Uncertainties in all free energy calculations presented in this work were small. All errors in estimating free energy of residue triplets to calculate helical propensity of a sequence were within 10%. See, e.g. the error bars in Fig. 2, which shows a representative plot of a free energy *versus* helicity of a residue triplet. In all cases a state with all three residues in helical conformation was well separated from other non-helical states.

Discussion and Conclusions

In this paper we presented the results of the first computational modeling of the structure of the GluR2 LBT –TM domain connecting peptides S1M1 and S2M3. The peptides were modeled using REMD technique in implicit water and low dielectric solvent in absence of the rest of the protein and in presence of small fragments of the adjacent LBD and TM domains with known structure. In particular, we were interested in determining whether their primary sequences exhibit propensity towards formation of helical structures. In order to quantify such analysis we introduced a helicity measure.

The simulations of the TM3_{long}+S2M3_{short} peptide confirmed the α -helical structure of the TM3 helix deduced previously in the experiments⁵¹. While the general agreement exists that the TM3 has helical structure, the structure of the highly conserved helix capping motif SYTANLAAF is still under debate. Studies of the water accessibility of the cysteine substituted residues in this motif in the AMPA GluR1 subunit suggest the α -helical secondary structure for this highly conserved motif of TM3⁵¹. At the same time cysteine substitutions in this motif in the NR1 subunits of the NMDA receptor showed no clear helical pattern of water accessibility¹³. In our simulations in low dielectric environment the TM3_{long}+S2M3_{short} peptide which included this motif assumed α -helical conformation. The simulations of the TM3+S2M3 and TM3+S2M3+S2 peptides in water also showed formation of a helical turn in residues AFL, suggestive of strong conformational bias of this triplet towards α -helical structure. Thus, this motif located at the border between the S2M3

peptide and the TM3 helix showed helical propensity indicating that it may cap the TM3 helix.

The connecting peptide S2M3 itself formed a coil structure in the presence of the fragments of the adjacent α -helical domains. The model of the TM3+S2M3+S2 peptide predicted both the TM3 helical fragment and the LBD S2 α -helix known from the LBD crystal structure in excellent agreement with experiments.

The side chain of the R628 of the S2M3 peptide has been identified experimentally as strongly influencing gating and desensitization properties of the receptor¹⁴. In our model R628 formed persistent salt-bridges with anionic residues of the LBD S2 helix. Such structural arrangement may provide the important coupling between the LBD and the TM domains. It is possible that mutations at this site and the adjacent site E627 known to strongly affect gating of the receptor influence the stability of this coupling via increasing or decreasing the number of hydrogen bonds and salt bridges between the S2 and S2M3 peptide. Clearly, further studies are required to test the plausibility of this suggestion.

The S1M1 peptide did not form helical turns in any of the simulations. Both length variations of this peptide had a maximum of two helical residues among all triplets. This result can be rationalized based on the fact that the S1M1 sequence contains three proline residues that are typically distorting helices. One of the proline residues, P520, is located only four residues apart from a presumed membrane-water interface. It has been observed recently⁵² that prolines near a water/lipid interface regions in membrane proteins cause helix breaking. Thus, it seems reasonable to suggest that the TM1 helix stops at the beginning of the S1M1 sequence and S1M1 is not helical as resulted from our simulations. The salt bridges formed between cationic lysine residues K506, K509, and K511 located at one end of the peptide, and negatively charged aspartate D519 and glutamate E524 at the other end of the peptide were responsible for observed persistent closed loop structure of the peptide. It has been reported that in the experiments substitutions in the S1M1 sequence affected gating of the receptor¹⁵, however no single site substitutions has been reported for this peptide. We suggest that the salt bridges observed in the simulations may play functional role in this protein. Such hypothesis can be tested experimentally by mutagenesis of specific residues that would disrupt a presumed salt bridge or by designing crosslinks that could covalently lock the ends of a peptide. Further investigation is required to test this hypothesis.

Acknowledgments

This work was partially supported by the Research Corporation and NIH (grant GM067962). MD simulations were carried out at the Pittsburgh Supercomputer Center (under NSF-PACI grant CHE030007P).

Reference List

1. Hollmann M, Heinemann S. Cloned glutamate receptors. *Annu Rev Neurosci.* 1994; 17(1):31. [PubMed: 8210177]
2. Dingledine R, Borges K, Bowie D, Traynelis SF. The glutamate receptor ion channels. *Pharmrev.* 1999; 51(1):7–61.
3. Mayer ML. Glutamate receptors at atomic resolution. *Nature.* 2006; 440(7083):456–462. [PubMed: 16554805]
4. Laube B, Kuhse J, Betz H. Evidence for a tetrameric structure of recombinant NMDA receptors. *J Neurosci.* 1998; 18(8):2954–2961. [PubMed: 9526012]
5. Rosenmund C, Stern-Bach Y, Stevens CF. The tetrameric structure of a glutamate receptor channel. *Science.* 1998; 280(5369):1596–1599. [PubMed: 9616121]

6. Armstrong N, Gouaux E. Mechanisms for activation and antagonism of an AMPA-Sensitive glutamate receptor: Crystal structures of the GluR2 ligand binding core. *Neuron*. 2000; 28(1):165–181. [PubMed: 11086992]
7. Mayer ML, Olson R, Gouaux E. Mechanisms for ligand binding to GluR0 ion channels: Crystal structures of the glutamate and serine complexes and a closed apo state. *J Mol Biol*. 2001
8. Mayer ML. Crystal structures of the GluR5 and GluR6 ligand binding cores: Molecular mechanisms underlying kainate receptor selectivity. *Neuron*. 2005; 45(4):539–552. [PubMed: 15721240]
9. Panchenko VA, Glasser CR, Mayer ML. Structural similarities between glutamate receptor channels and K⁺ channels examined by scanning mutagenesis. *J Gen Physiol*. 2001; 117(4):345–359. [PubMed: 11279254]
10. Kuner T, Seeburg PH, Guy HR. A common architecture for K⁺ channels and ionotropic glutamate receptors? *Trends Neurosci*. 2003; 26(1):27–33. [PubMed: 12495860]
11. Chen GQ, Cui C, Mayer ML, Gouaux E. Functional characterization of a potassium-selective prokaryotic glutamate receptor. *Nature*. 1999; 402(6763):817–821. [PubMed: 10617203]
12. Villarreal A, Regalado PM, Lerma J. Glycine-Independent NMDA Receptor Desensitization: Localization of Structural Determinants. *Neuron*. 1998; 20(2):329–339. [PubMed: 9491993]
13. Beck C, Wollmuth LP, Seeburg PH, Sakmann B, Kuner T. NMDAR channel segments forming the extracellular vestibule inferred from the accessibility of substituted cysteines. *Neuron*. 1999; 22(3):559–570. [PubMed: 10197535]
14. Yelshansky MV, Sobolevsky AI, Jatzke C, Wollmuth LP. Block of AMPA receptor desensitization by a point mutation outside the ligand-binding domain. *J Neurosci*. 2004; 24(20):4728–4736. [PubMed: 15152033]
15. Schmid SM, Korber C, Herrmann S, Werner M, Hollmann M. A domain linking the AMPA receptor agonist binding site to the ion pore controls Gating and causes lurcher properties when mutated. *J Neurosci*. 2007; 27(45):12230–12241. [PubMed: 17989289]
16. Ho BK, Dill KA. Folding very short peptides using molecular dynamics. *PLoS Comp Biol*. 2006; 2(4):228–237.
17. Sugita Y, Okamoto Y. Replica-exchange molecular dynamics method for protein folding. *Chem Phys Lett*. 1999; 314(1-2):141–151.
18. Ozkan SB, Wu GA, Chodera JD, Dill KA. Protein folding by zipping and assembly. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104(29):11987–11992. [PubMed: 17620603]
19. Bystroff C, Garde S. Helix propensities of short peptides: Molecular dynamics versus bioinformatics. *Proteins: Struct Funct Genet*. 2003; 50(4):552–562. [PubMed: 12577261]
20. Lei HX, Wu C, Liu HG, Duan Y. Folding free-energy landscape of villin headpiece subdomain from molecular dynamics simulations. *Proc Natl Acad Sci U S A*. 2007; 104(12):4925–4930. [PubMed: 17360390]
21. Roe DR, Hornak V, Simmerling C. Folding cooperativity in a three-stranded beta-sheet model. *J Mol Biol*. 2005; 352(2):370–381. [PubMed: 16095612]
22. Nymeyer H, Gnanakaran S, Garcia AE. Atomic simulations of protein folding, using the replica exchange algorithm. *Numerical Computer Methods, Pt D. Methods in Enzymology*. 2004; 383:119–149. [PubMed: 15063649]
23. Simmerling C, Strockbine B, Roitberg AE. All-atom structure prediction and folding simulations of a stable protein. *J Am Chem Soc*. 2002; 124(38):11258–11259. [PubMed: 12236726]
24. Im W, Feig M, Brooks CL. An implicit membrane generalized Born theory for the study of structure, stability, and interactions of membrane proteins. *Biophys J*. 2003; 85(5):2900–2918. [PubMed: 14581194]
25. Bu LT, Im W, Charles LI. Membrane assembly of simple helix homo-oligomers studied via molecular dynamics simulations. *Biophys J*. 2007; 92(3):854–863. [PubMed: 17085501]
26. Im W, Brooks CL. Interfacial folding and membrane insertion of designed peptides studied by molecular dynamics simulations. *Proc Natl Acad Sci U S A*. 2005; 102(19):6771–6776. [PubMed: 15860587]

27. Nymeyer H, Garcia AE. Simulation of the folding equilibrium of alpha-helical peptides: A comparison of the generalized Born approximation with explicit solvent. *Proc Natl Acad Sci U S A*. 2003; 100(24):13934–13939. [PubMed: 14617775]
28. Bursulaya BD, Brooks CL. Comparative study of the folding free energy landscape of a three-stranded beta-sheet protein with explicit and implicit solvent models. *J Phys Chem B*. 2000; 104(51):12378–12383.
29. Cornell WD, Cieplak P, Bayly CI, Gould IR, Merz KM, Ferguson DM, Spellmeyer DC, Fox T, Caldwell JW, Kollman PA. A second generation force field for the simulation of proteins, nucleic acids, and organic molecules. *J Am Chem Soc*. 1995; 117:5179–5197.
30. Pitera JW, Swope W. Understanding folding and design: Replica-exchange simulations of “Trp-cage” fly miniproteins. *Proc Natl Acad Sci U S A*. 2003; 100(13):7587–7592. [PubMed: 12808142]
31. Yang WY, Pitera JW, Swope WC, Gruebele M. Heterogeneous folding of the trpzip hairpin: Full atom simulation and experiment. *J Mol Biol*. 2004; 336(1):241–251. [PubMed: 14741219]
32. Duan Y, Wu C, Chowdhury S, Lee MC, Xiong GM, Zhang W, Yang R, Cieplak P, Luo R, Lee T, Caldwell J, Wang JM, Kollman P. A point-charge force field for molecular mechanics simulations of proteins based on condensed-phase quantum mechanical calculations. *J Comput Chem*. 2003; 24(16):1999–2012. [PubMed: 14531054]
33. Zhang W, Wu C, Duan Y. Convergence of replica exchange molecular dynamics. *J Chem Phys*. 2005; 123(15)
34. Periole X, Mark AE. Convergence and sampling efficiency in replica exchange simulations of peptide folding in explicit solvent. *J Chem Phys*. 2007; 126(1)
35. Sgourakis NG, Yan YL, McCallum SA, Wang CY, Garcia AE. The Alzheimer's peptides A beta 40 and 42 adopt distinct conformations in water: A combined MD/NMR study. *J Mol Biol*. 2007; 368(5):1448–1457. [PubMed: 17397862]
36. Jones DT, Taylor WR, Thornton JM. A model recognition approach to the prediction of all-helical membrane protein structure and topology. *Biochemistry*. 1994; 33(10):3038–3049. [PubMed: 8130217]
37. Krogh A, Larsson B, von Heijne G, Sonnhammer ELL. Predicting transmembrane protein topology with a hidden Markov model: Application to complete genomes. *J Mol Biol*. 2001; 305(3):567–580. [PubMed: 11152613]
38. Lomeli H, Wisden W, Kohler M, Keinänen K, Sommer B, Seeburg PH. High-affinity kainate and domoate receptors in rat brain. *FEBS Lett*. 1992; 307(2):139–143. [PubMed: 1322826]
39. Metropolis N, Ulam S. The Monte Carlo method. *Journal of the American Statistical Association*. 1949; 44(247):335–341. [PubMed: 18139350]
40. Ferrenberg AM, Swendsen RH. Optimized Monte-Carlo data analysis. *Phys Rev Lett*. 1989; 63(12):1195–1198. [PubMed: 10040500]
41. Kumar S, Bouzida D, Swendsen RH, Kollman PA, Rosenberg JM. The weighted histogram analysis method for free-energy calculations on biomolecules. 1. The method. *J Comput Chem*. 1992; 13(8):1011–1021.
42. Case, DA.; Darden, TA.; Cheatham, TE., III; Simmerling, CL.; Wang, J.; Duke, RE.; Luo, R.; Merz, KM.; Wang, B.; Pearlman, DA.; Crowley, M.; Brozell, S.; Tsui, V.; Gohlke, H.; Mongan, J.; Hornak, G.; Beroza, P.; Schafmeister, C.; Caldwell, JW.; Ross, WS.; Kollman, PA. AMBER 8. San Francisco: University of California; 2004.
43. Ryckaert JP, Ciccotti G, Berendsen HJC. Numerical integration of the cartesian equations of motion of a system with constraints: Molecular dynamics of n-alkanes. *J Comp Phys*. 1977; 23:327–341.
44. Tsui V, Case DA. Theory and applications of the generalized Born solvation model in macromolecular Simulations. *Biopolymers*. 2000; 56(4):275–291. [PubMed: 11754341]
45. Andersen, OS. Permeability properties of unmodified lipid bilayer membranes. In: Giebish, G., editor. *Membrane Transport and Biology*. Vol. 1. New York: Springer; 1978. p. 349-446.
46. Chodera JD, Swope WC, Pitera JW, Seok C, Dill KA. Use of the weighted histogram analysis method for the analysis of simulated and parallel tempering simulations. *J ChemTheory Comp*. 2007; 3(1):26–41.

47. Kitao A, Hirata F, Go N. The effects of solvent on the conformation and the collective motions of protein - normal mode analysis and molecular-dynamics simulations of melittin in water and in vacuum. *Chem Phys.* 1991; 158(2-3):447–472.
48. Prompers JJ, Bruschweiler R. General framework for studying the dynamics of folded and unfolded proteins by NMR relaxation spectroscopy and MD simulation. *J Am Chem Soc.* 2002; 124(16):4522–4534. [PubMed: 11960483]
49. Prompers JJ, Bruschweiler R. Reorientational eigenmode dynamics: A combined MD/NMR relaxation analysis method for flexible parts in globular proteins. *J Am Chem Soc.* 2001; 123(30): 7305–7313. [PubMed: 11472158]
50. Daggett V, Levitt M. Molecular dynamics simulations of helix denaturation. *J Mol Biol.* 1992; 223(4):1121–1138. [PubMed: 1538392]
51. Sobolevsky AI, Yelshansky MV, Wollmuth LP. Different gating mechanisms in glutamate receptor and K⁺ channels. *J Neurosci.* 2003; 23(20):7559–7568. [PubMed: 12930794]
52. Nilsson I, Saaf A, Whitley P, Gafvelin G, Waller C, von Heijne G. Proline-induced disruption of a transmembrane alpha-helix in its natural environment. *J Mol Biol.* 1998; 284(4):1165–1175. [PubMed: 9837734]

Figure 1.

A general topology of the AMPA Glutamate receptor. The membrane is shown as a grey slab. The transmembrane helices of the transmembrane domain are marked TM1, TM2, TM3 and TM4. The ligand binding domain (LBD) is shown as two lobe domain S1 and S2. The N-terminal domain is shown as ATD. The S1M1 and S2M3 connecting peptides are shown with thick lines. The end residues of the connecting peptides are marked.

Moving window of 3 residues

K P Q K S K P G V F S F L D P L A Y E

Free energy of helical residues in the triplet

Figure 2.

Free energy as a function of a degree of helicity of the P Q K triplet of the S1M1_{long} peptide. Triplets of residues are numbered according to the number of the first residue in the triplet, i.e. triplet 1 includes residues 2, 3, 4 (since we only can define the ϕ and ψ pair starting with the second residue); triplet 2 includes residues 3, 4, 5 etc.

TM3long+S2M3short peptide:

Figure 3.

The sequence, the free energy (in kcal/mol) and the helicity measure of the TM3_{long}+S2M3_{short} peptide are shown for simulations in low dielectric: a) Sequence of peptide, the red rectangle indicates the part of the sequence that belongs to LBD, the green rectangle indicates the TM part of the sequence; b) the free energy map (kcal/mol) as a function of the two top principle components. Two representative structures from the two energy minima are also shown; c) the helicity measure plot.

TM3+S2M3 peptide:

Figure 4.

The sequence and the helicity measure of the TM3+S2M3 peptide: a) The sequence of the peptide, the red rectangle indicates the part of the LBD, the green rectangle indicates a part of the TM. The helicity measure for b) simulation in water dielectric, c) simulation in low dielectric.

TM3+S2M3+S2 peptide:

Figure 5.

The sequence, the free energy (in kcal/mol) map and the helicity measure of the TM3+S2M3+S2 peptide simulated in water: a) The sequence of the peptide, the red rectangle indicates the LBD, and the green rectangle indicates the TM; b) The free energy plotted as a function of the two top structural covariance principle components; c) the helicity measure plot.

Figure 6.

The modeled structure of the TM3+S2M3+S2 peptide simulated in water: a) The structure that corresponds to the global minimum of the free energy (see Fig.5b); Only back bone is shown; b) Formation of a salt bridge between residues R628 and D638; c) Formation of a salt bridge between residues R628, E634 and D638;

Figure 7.

The sequence, the free energy (in kcal/mol) map and the helicity measure of the S1M1_{long} peptide. a) The sequence of S1M1_{long}, the red rectangle indicates the LBD, the green rectangle indicates the TM3.; b) and c) The free energy plotted as a function of the two top structural covariance principle components: b) simulated in implicit water, c) simulated in low dielectric. Also shown are several representative structures of the peptide taken from the corresponding energy minima; d) and e) the helicity measure of S1M1_{long} as a function of the sequential triplet number advanced by one residue: d) in water dielectric, e) in low dielectric.