based on an incomplete version of the $\alpha_6\beta_2$ ring seems more plausible (**Fig. 3**).

Finally, how has the pattern of activity regulation been conserved throughout evolution? The class III enzymes dimerize very differently and interact with a different type of β_2 subunit (**Fig. 1**). Moreover, the radical is generated on α_2 and can be used for many catalytic cycles before having to be regenerated[18], unlike class Ia enzymes, whose subunits have to reassociate on each cycle. Does the ATP cone affect subunit interactions or act on the α_2 subunit itself?

Given that $\alpha_4\beta_4$ oligomers of *E. coli* RNR, the first sign of higher-order association, were first observed by Brown and Reichard more than 40 years ago[19], it is exciting that the circle is now beginning to close on the structural basis for RNR activity regulation. The window that has just been opened is also a window of opportunity for future studies.

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Glutamate receptor ion channels: where do all the calories go?

Mark L Mayer

Glutamate receptor ion channels use the free energy of ligand binding to trigger ion channel activation and desensitization. In this issue, an analysis of all-atom molecular dynamics simulations dissects the binding process, reveals a substantial gain in free energy produced by domain closure for agonists and reports unique energy landscapes for individual ligands.

Glutamate receptor ion channels (iGluRs) are complex allosteric proteins that have generated enormous interest in the neuroscience community because of their role in synaptic transmission, learning, memory formation and brain development[1]. The emergence of a comprehensive iGluR pharmacology in the 1980s, combined with the cloning of 18 iGluR genes 10 years later, led to the identification of numerous subtype-selective ligands and allosteric modulators. Current medicinal chemistry research programs in the pharmaceutical industry continue to develop new iGluR ligands, several of which have therapeutic potential for a range of neurological and psychiatric diseases. Questions not answered by these studies include what the precise mechanisms underlying the affinity of ligand binding may be and why 'partial agonists' produce less activation than 'full agonists' such as the neurotransmitter glutamate[2,3].

On page 283 of this issue, Lau and Roux[4] use a comprehensive set of all-atom molecular dynamics simulations for a panel of nine iGluR agonists, partial agonists and antagonists to address these and other important problems in the binding-gating conundrum for ligand-activated ion channels. Using this approach, they dissect the energetics of binding into components resulting from ligand interactions with the protein, on the one hand, from the free-energy changes resulting from ligand-induced conformational modifications on the other. Surprisingly, they find that the binding of glutamate is energetically unfavorable when it is docked into the ligand-binding domain (LBD), but this is compensated for by a large free-energy change produced by closure of the LBD (**Fig. 1**). They find that the energy resulting from LBD closure is less for partial agonists than for full agonists, but there are unique landscapes for each of the nine ligands studied, highlighting the complexity of mechanisms that underlie the binding of small molecules to proteins. Such a comprehensive analysis of the energetics of ligand binding to a neurotransmitter receptor has not been attempted before. It will undoubtedly be applied to other systems when crystal structures have been solved to provide the necessary molecular templates for molecular dynamics simulations.

Lau and Roux study the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor GluA2, a model system for the receptor family that mediates fast synaptic transmission throughout the brain, and the only iGluR for which a full-length structure has been solved[5]. By contrast, during the past decade, numerous biochemical, crystallographic and NMR studies have focused on the LBDs of iGluRs, which can be genetically isolated and expressed as soluble recombinant proteins. GluA2, in particular, has been studied extensively, and more than 80 ligand complexes have been solved by X-ray crystallography[6]. These studies have generated models for activation[7] and desensitization[8] that have been amply substantiated by experimental tests[9–12] and have also identified binding sites for allosteric modulators[8,13,14]. Despite these impressive advances, our understanding of the

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thermodynamic principles that underlie binding of glutamate receptor ligands has remained primitive. Although molecular dynamics simulations have previously been done for the LBDs of iGluRs[15,16], these have largely ignored the energetics of ligand binding. The results of Lau and Roux represent a radical departure from this and suggest that the trajectory from the open to the closed conformation of the LBD differs for individual ligands, even when the end states are similar; the trajectory for binding of glutamate reveals at least two intermediate structures (**Fig. 1**). Also striking is the difference in conformational stability of the 6,7-dinitroquinoxaline-2,3-dione (CNQX) and 6-cyano-7-nitroquinoxaline-2,3-dione (DNQX) complexes, antagonists that are generally considered as having nearly identical binding mechanisms.

Lau and Roux then go on to assemble an LBD tetramer, based on the full-length GluA2 crystal structure[5], and they compare the root-mean-square (r.m.s.) deviation fluctuations for individual ligand complexes taken from their simulations with those calculated for crystal structures of the isolated LBD. One notable result is that for some partial agonists, the dimer pairs that make up a tetramer assembly undergo different degrees of movement, producing subunit nonequivalence in the gating mechanism. This is a surprising finding that has experimental precedent from work on kainate-subtype iGluRs[17]. For some ligands, notably the partial agonist kainate and the antagonist CNQX, which also acts as a very weak partial agonist in native AMPA receptors[18], there is a 4–6 Å reduction in average r.m.s. deviation in the simulated tetramer assembly, compared to the isolated LBD crystal structures; however, for the AMPA-bound, 2-amino-3-[5-*tert*-butyl-3-(phosphonomethoxy)-4-isoxazolyl]propionic acid (ATPO)-bound and apo structures, the opposite is true. These differences seem to arise from the unique conformational ensembles computed for the various ligand complexes.

Although studies like that undertaken by Lau and Roux are badly needed in order to understand in molecular detail how binding of neurotransmitters activates ligand-gated ion channels, there are caveats to the approach. The force fields for all-atom molecular dynamics simulations have now been validated—albeit imperfectly—in numerous simulations, so we can probably cross that off our list of concerns. The simulations were done using isolated LBDs, the same as used for structural and biochemical studies; however, in the intact receptor, each of the four LBDs is coupled at one end to a large N-terminal domain, which itself assembles as a dimer of dimers, and is attached at the other end by three connections to the ion-channel segment. Also different from the intact receptor, in which each of the four LBDs is packed against its partners in a dimer-of-dimers assembly, are intersubunit interactions mediated by essential structural elements directly involved in either ligand binding or the conformational change induced by agonists and partial agonists. How serious is this? One answer would be that the results shed light on the amount of energy available for ion channel gating, but in the intact receptor, the energy profile of the reaction coordinate leading from the open to the closed state may differ from that seen in the isolated LBD. Any information about how the binding energy of agonists drives the process of desensitization is also absent.

Despite these limitations, the study by Lau and Roux provides refreshing new insights into the energetics of iGluR activation, which will doubtless spur calculations for intact iGluR assemblies. Computational studies for other iGluR LBDs will be equally interesting, particularly studies of the glycine-binding subunits of NMDA receptors, for which several partial agonist crystal structures have been solved. Notably, for all of these, the extent of domain closure is nearly identical to that for full agonists[3]. This is strikingly different from what has been found for the large majority of AMPA- and kainate-receptor partial-agonist crystal structures, which reveal intermediate extents of domain closure[2,19,20].

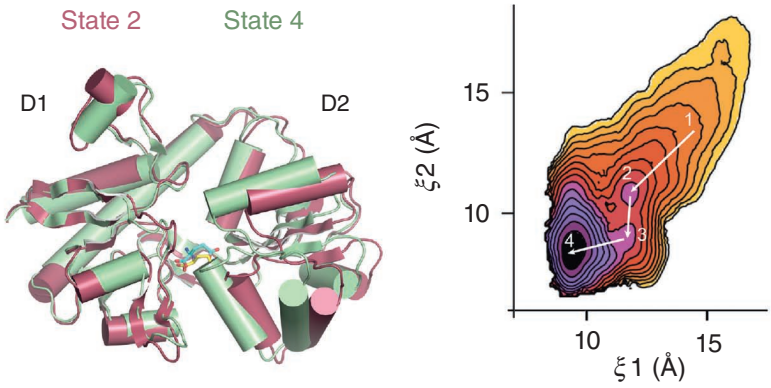

Figure 1 Free-energy changes revealed by molecular dynamics simulations for the GluA2 LBD. The left panel shows ribbon diagrams for glutamate-bound complexes corresponding to state 2, a glutamate-bound intermediate on the open-cleft-to-closed-cleft pathway, and to state 4, the final closed-cleft conformation represented by the crystal structure PDB 1FTJ (ref. 7). The structures were superimposed using domain 1 (D1) coordinates (r.m.s. deviation 0.63 Å for 122 Cα atoms); owing to partial domain closure, the r.m.s. deviation for domain 2 was 4.2 Å (107 Cα atoms), which decreased to 0.8 Å when domain 2 (D2) was independently superimposed on the crystal structure. The energy landscape corresponding to the transition from the open-cleft (state 1) to closed-cleft (state 4) glutamate-bound structures is shown in the right panel. As noted by Lau and Roux[4], the likely pathway for domain closure passes through at least two low-energy intermediates. In state 2, although the ligand makes multiple contacts with atoms in both domains 1 and 2, the pattern of the interactions differs from that in the fully closed conformation. In addition, important interdomain contacts—for example, the hydrogen bond between the side chains of Glu402 and Thr685—are not yet established in state 2. This is because Thr685 in α-helix H moves an additional 6 Å toward domain 1 in the transition from state 2 to state 4, highlighting the role of cleft closure in contributing energy to the glutamate-bound complex.

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