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Dynamics and allosteric potential of the AMPA receptor N-terminal domain

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(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 24 November 2010

Thank you for submitting your manuscript to the EMBO Journal. This submission was co-submitted with MS 76303. Your study has now been seen by three referees and their comments are provided below. As you can see while referees #1 and 3 are very supportive of the study, referee #2 is not persuaded that the advance and insights provided is sufficient to consider publication in the EMBO Journal. In particular this referee finds that the analysis remains too speculative and that there is not sufficient data provided in support of that the conformations observed are physiological relevant. The referees also indicate that too strong conclusions are made about the undefined electron density observed in the NTD cleft and suggest to tone this conclusion down unless you have good support for what its molecular nature is. Given the support provided by both referees #1 and 3, I will go with their overall recommendation and invite you to submit a suitably revised manuscript for our consideration. Please also keep in mind the specific concerns raised by referee #2 when revising your manuscript. I should remind you that it is EMBO Journal policy to allow a single round of revision only and that it is therefore important to resolve the raised concerns at this stage. When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process initiative, please visit our website: http://www.nature.com/emboj/about/process.html

Thank you for the opportunity to consider your work for publication. I look forward to your revision.

best wishes

Editor
The EMBO Journal

REFEREE REPORTS

Referee #1 (Remarks to the Author):

Sukumaran and coauthors present compelling data that the N-terminal domains of AMPA receptors are not as inflexible and static as has been inferred from previously reported crystal structures. They introduce the possibility of allosteric modulation in these domains based on new structures of the GluA3 subunit NTD as well as a sophisticated analysis of potential conformational dynamics, in part through the use of the homologous mGluR ligand-binding domain as a template. The weakest element of the study is the unresolved matter of the electron density in the cleft area of some NTD structures. The most compelling aspect of the study is the well-supported implication that this domain in AMPA receptors could have functional significance in addition to its known role in receptor oligomerization.

Major criticism
Too much is made of the mysterious electron density in the cleft. The authors do not know its molecular nature, stating that it is under investigation. The current inconclusive dataset does not provide a strong basis for concluding that the NTD is capable of "ligand binding", as is suggested by the titles of the relevant sections in the Results and Discussion sections. The observation itself seems appropriate to report, but it does not demonstrate that "in analogy to the NMDAR and like other PBPs, AMPAR NTDs have the capacity to coordinate ligand" (p. 12). An unknown electron density is not equivalent to a ligand, regardless of its provocative location in the structure.

Minor criticisms
The reference for Hubbard and Thornton in the bibliography seems incomplete.

Referee #2 (Remarks to the Author):

The current manuscript reports the structure of the amino terminal domains (NTDs) of GluA2 and GluA3 AMPA receptor subunits and suggests that NTD proteins may undergo large conformational change. This contrasts with suggestions made in previous studies on GluA2 and GluK2 NTDs that non-NMDA receptor NTDs have restricted motions. The authors also observe electron density at the cleft of the bilobe structure of GluA2 NTD, which they think may represent an NTD binding compound analogous to ifenprodil and Zn2+ in NMDA receptors. The content of the paper may lead to an interesting finding that AMPA receptor NTD may play a role in modulating the ion channel activity upon binding to NTD binding compounds, a function analogous to NMDA receptors containing GluN2A and GluN2B. However, the message conveyed by this manuscript is highly speculative and data presented here is no way near sufficient to conclude that such mechanism exists in AMPA receptors. How do authors know different conformations observed in crystals represent physiological conformations? How do they know that the conformational changes are coupled to functions while there has been absolutely no evidence for NTD-mediated functional regulation in the field?

Also, contrary to the authors' message on the importance of heterodimerization of NTD in the other paper submitted, the current manuscript exclusively talks about the NTD homo-dimers. The back-to-back presentation of these manuscripts does not make sense. Overall, the content of the manuscript is highly speculative, and the strong tone of the manuscript with such a limited set of data is very misleading to the field.

Specific points:

Pg6 The entire section "Inter-protomer rearrangements in the GluA3 NTD"

Figure 2 is just impossible to interpret. The surface presentation of the dimeric structures does not tell much. How are dimeric arrangements in dimerI, II, and III different from one another? Do

different dimeric arrangement create completely different dimer interface? If so, it needs to be presented in the paper. Are lower lobes (LL) separated in the similar manner in dimer II and III to that observed in dimer I (as in Fig. 1D)? A crucial question is whether or not these subunit arrangements mean anything physiological. How do authors know the observation is not a crystallographic artifact? Further experiments have to be conducted to make any conclusion out of this. Perhaps, inter-subunit disulphide cross-linking experiments in the context of full-length receptors would help.

Pg6 "Although new contacts ....thus more heterogeneous interface (Table I; Supplementary Figure S4)."

It is not clear why authors think dimer II and III are less stable. This needs much more explanation.

Fig. 3 panel B.

The authors should show superposition of their GluA2 NTD structures with others previously published, perhaps as a supplementary figure. They should clearly show what kind of structural differences they observe and discuss what they mean.

Fig. S4.

Is there any reason for not calculating LD for dimer III? Also, the orders of supplementary figures do not make sense. For example Fig. S4 comes earlier in the manuscript than Fig. S3.

Pg11 "Ligand density in the GluA2 NTD binding cleft"

Does it take 2.0 sigma contour level to observe the continuous density at the cleft of the bilobe structure? It seems likely that the density will be broken into several pieces when contoured at more reasonable level, such as 3-4. Could a part of the density just represent a weak binding site of a phosphate molecule included in the crystallization condition?

Referee #3 (Remarks to the Author):

This paper presents three sets of novel data:
- The first X-ray crystal structure of the AMPA receptor subunit GluA3 N-terminal domain (NTD), a large extracellular domain that precedes the glutamate-binding domain and that plays a central role in receptor assembly. In fact, three different GluA3 NTD dimer structures are described. While the individual GluA3 NTD displays the typical (and expected) clamshell-like structure, the dimeric structures show unanticipated structural features differing from the previously published structures of the GluA2 NTD dimer (Jin et al., EMBOJ, 2009) and the kainate receptor subunit GluK2 NTD dimer (Kumar et al., NSMB, 2009). The most striking difference is a repositioning of the lower lobes (LLs), which in GluA3 NTD dimer (form I) are physically separated, while in GluK2 NTD, and to a lesser extent GluA2 NTD, these are tightly packed together with an extensive dimer interface. The authors show that the separation of the lower lobes in GluA3 likely arises from electrostatic repulsion between facing LL arginine residues.
- The AMPA receptor NTDs of GluA3, but also of GluA2, are flexible and can undergo structural rearrangements, similar to those seen in other members of the LIVBP-like family, including the agonist-binging domain of the metabotropic glutamate receptor mGluR1. Motions are intra-protomer, clamshell-like closures and openings, or inter-protomers, counter-rotations of the two NTDs. This conclusion is reached first, by comparing the three GluA3 NTD dimer forms (I, II and III) and second, using normal mode analysis on GluA2 and GluA3 NTDs. The most dynamic NTD region is the lower lobe, a region that directly connects to the glutamate-binding domain.
- GluA2 NTDs have in their central interlobe cleft a distinct electron density suggesting the presence of a small ligand. AMPA receptor NTDs may thus have a ligand-binding capacity similar to NMDA receptor NTDs which are known to bind subunit-specific allosteric modulators. The density remains unassigned yet.

This is an important paper that reveals an unsuspected level of structural flexibility of AMPA receptor N-terminal domains, in particular through motions of unconstrained clamshell lower lobes.

The results presented thus contradict the prevailing view that AMPA (and kainate) receptor NTDs are rigid bodies that are incapable of conformational changes. Even if the present study does not provide direct functional evidence that NTD motions can influence receptor activity, the extensive and thorough structural analysis performed by the authors provides strong evidence that a NTD-driven modulation of AMPA receptor function, similar to what has been described for NMDA receptors, is a serious possibility. Undoubtedly, this work will have a significant impact in the field of glutamate receptors and neurotransmission in general by suggesting future structural, pharmacological, and possibly physiological, studies.

The following points require further attention:
- For comparison purposes, it would be interesting to extend the normal mode analysis to the GluK2 NTD dimer, which supposedly, displays little conformation mobility.
- Figure 2: for clarity, UL and LL labels (for upper and lower lobes) should be added. Labelling of the helices in panels B and C is also required. Panel D: the differences in structure between the three dimers are hard to see. A side view, with some degree of transparency, might be more appropriate.
- Regarding the undefined electron density in the NTD cleft, the authors should discuss the fact that in the apo NMDA GluN2B NTD structure, unexpected densities corresponding to ions (present at high concentration in the crystallization buffer) have also been observed (Karakas et al., 2009)
- Figure 4B: legend of Y-axis refers to Mode 1 but aren't all modes represented in this Figure?
- Page 6, 2nd paragraph: it is stated that going from dimer I to dimer II involves a 'translation along the 2-fold axis of symmetry" but p9, "counter-rotations about the central axis" are mentioned regarding the sampling of dimer I-III conformations. Please clarify.
- Abstract. The last sentence is a little bit pushy. Adding the word "potential" before "novel target" is recommended.
- The Clayton et al., 2009 JMB paper deserves better than just a single citation in the Introduction. In particular, the conservation of the interlobe cleft residues within the AMPA receptor family (described p 12) has already been described by Clayton and coll.
- p5, end of 2nd paragraph: Figure S2C instead of S2B
- Fig 1D: adding the sequences of GluK1-3 subunits would be valuable
- Fig 1B, dimer I instead of dimer A
- Legend Fig S6 refers to Fig S4A: should be S3A.
- Legends of Figs 3, 4 & 5: the term "NTD" is missing

1st Revision - authors' response 03 December 2010

Response to reviewers EMBOJ-2010-76305 (Sukumaran et al.)

Reviewer 1.

We thank the reviewer for the overall positive response.

The reviewer queries the electron-density in the GluA2 NTD interlobe cleft.
We absolutely agree that drawing firm conclusions regarding the precise nature of AMPAR NTD ligands is still premature and was not intended in the current paper. We want to stress nevertheless that density was consistently observed after refinement in various high-resolution data sets (better than 2 Å resolution) from different crystallographic conditions, which has not been reported previously. We feel therefore that “ligand-binding capacity” is not an overstatement. In an attempt to not over-rate the cleft density at the current stage we have now:

1. altered (‘toned down’) the abstract section referring to the ligand.
2. removed one of the Figures demonstrating density (Figure 5C).
3. contoured the ligand density in Figure 5B at 2 *and* 3 sigma (as also suggested by reviewer 2).
4. ‘toned down’ the text discussing ligand density in the last paragraph of the ‘Introduction’ and in both the ‘Results’ and ‘Discussion’ sections of the revised text.
5. reworded the title in the 1st discussion section from “Ligand-binding capacity for the AMPAR NTD” to “Electron density in the AMPAR NTD cleft”, as suggested by the reviewer.

We have also fixed the Hubbard and Thornton reference.

Reviewer 2.
Overall the reviewer feels that our data are too speculative and do not support the notion that AMPAR NTDs encode allosteric potential.

1. *"How do the authors know different conformations observed in the crystals represent physiological conformations"?*

This is undoubtedly a key question, a question one often faces when interpreting crystal structures. We present 3 different GluA3 NTD dimers (dimers I – III). Dimer I is consistently observed in multiple crystallographic data sets and overall resembles currently available GluA2 NTD and mGluR LBC structures when comparing contacts across the upper lobes (Figure 3A and Suppl. Figure S1A). Dimer I is therefore most likely the energetically favored state of the isolated GluA3 NTD. Dimer II diverges most from this state. In this dimer form, a Met residue in the lower lobe (LL) at position 150 comes to lie at the 2-fold axis of symmetry. The M150 positions are separated by 5.8Å Cb distance across the dimer II interface, whereas in dimer I the distance is 15.6 Å; we have capitalized on this and have generated the M150C mutant, which when assessed on non-reducing SDS-PAGE indeed produces a greater proportion of the dimeric band. Therefore, the M150C forms an inter-dimer disulfide bridge confirming that position 150 can come within close proximity in solution, supporting the conformational relevance of dimer II thus lowering the probability that the different observed dimer forms are crystallographic artifacts. These new results are included in the revised text in Suppl. Figure S4B.

Furthermore, our simulations using normal mode analysis showed that the different dimer conformations are mutually accessible relying only on the intrinsic fluctuations present from the dimeric topology; i.e the different dimer conformations "represent snapshots along a readily accessible mode of motion," as we stated on page 7 of the manuscript. Normal mode analysis is a well-established method for correlating structural fluctuations predicted computationally with functional changes and allosteric mechanisms measured experimentally (Bahar 2010 review ref).

Therefore, we conclude that the different dimer forms are physiologically relevant based on the concordance of our structural, biochemical, and computational results all taken together.

The reviewer states that "there is absolutely no evidence for NTD-mediated functional regulation in the field".
This is true; apart from subtle changes in desensitization kinetics in the GluA4 NTD-deletion construct (Kuusinen et al. JBC 274, 1999), little is known about the functional role of the AMPAR NTD. However, to our knowledge, this specific question has not been assessed directly, i.e. no study to date has generated structure-guided NTD mutants at strategic positions (e.g. within the cleft, in the lower lobes interface) and rigorously tested their effect(s) on the functional spectrum of the AMPAR. This needs to be accomplished in future experiments. GluA3, based on the structural properties presented in the current paper, will be a strong candidate to address this open question. We hope the present study provides a strong motivation for this rather large undertaking.

The reviewer questions why this paper "exclusively talks about NTD homo-dimers", while the co-submission (Rossmann et al) assesses AMPAR heteromers.
In the two papers we address two distinct functions of the NTD – its role in heteromerization (Rossmann et al.) and its potential allosteric function (Sukumaran et al.). In the latter paper we analyze a new structure, the GluA3 NTD and its derivatives – this structure features unexpected properties indicative of allosteric potential (including a partly-zipped open dimer interface resembling metabotropic glutamate receptors). Together with labile dimer contacts, these features imply flexibility and thus allosteric potential, which is independently assessed via normal mode analysis. The analysis of the novel homodimer is a natural first step; AMPAR NTD heterodimer structures will undoubtedly follow soon and will serve as a substrate for further analysis.

The reviewer questions the clarity of Figure 2 (this point is also raised by reviewer 3). We have made the following changes to this figure:
Panel D (top-view dimers) have been moved up (now panel A) and also show front views of the 3 dimers to more clearly demonstrate their differences in packing (also suggested by reviewer 3) and orient the viewer as to the overall dimeric conformations. We have also labeled the figure more extensively including assignment of lobes and of all secondary structure elements.

'How do the dimeric arrangements differ and are completely new dimer interfaces created'?
The differences are shown in Figure 2A-C (of the original submission); Figure 2B provides a direct view onto the GluA3 dimer interface and quantifies the displacements of structural segments of dimers II and III relative to dimer I (values are indicated in Figure 2B).
These differences were revealed by superimposing one of the protomers from each of the three dimers (colored in grey). The resulting interfaces are indeed very different; the atomic contacts and contact densities were calculated from structural alignments using the program PINQ (Lesk AM 1986. Integrated access to sequence and structural data. In Biosequences: perspectives and user services in Europe, pp 23-28. Bruxelles, Belgium). We also computed other interface parameters such as hydrophobicity, conservation and residue propensity (Bahadur et al. JMB 336, 2004); the results are presented in Suppl. Figure 4 and in Supplementary Table II.

The physiological relevance of the GluA3 dimers is being questioned. It is suggested to assess this via disulfide-crosslinking experiments in the full-length receptor.
Labile interfaces have been described by others, e.g. Mueser et al. Biochemistry 39, 2000: "Interface sliding as illustrated by the multiple quaternary stuctures of liganded hemoglobin.", and in Cheung and Hendrickson, Curr Opin. Microbiol. 13, 2010. As described above, we have been able to crosslink the M150C mutant dimer. Proximity between M150 residues across the dimer interface is seen in dimer II (as described above). This was done for the isolated NTD (to match the context of the crystallography experiment). The results are now included in Supplementary Figure S4.

"It is not clear why the authors think dimers II and III are less stable".
This comes from structure analysis of the sort described for the GluA2 dimer interface (Table I) in the 3rd 'Results' section: 'The GluA2 LLs are not tightly packed and exhibit structural variabilities'. Details of the methods are listed in the supplementary 'Materials and Methods' under 'Structure analysis computations', as discussed above. The results of this analysis are listed in Supplementary Table II for GluA3 dimers I and II (the resolution of dimer III, 4.2 Å, was too low to conduct atomic-level analysis) and are documented in Suppl. Figure 4. In particular, the LD (or local density index), a measure for the packing density of interfacing atoms and a metric to discriminate specific vs. non-specific interfaces (Bahadur et al JMB 336, 2004), was ~ 30 for dimer II UL and LL interfaces, which classifies these interfaces as less stable and 'non-specific' (average LD value for non-specific interfaces as computed by Bahadur et al. was 32). Tighter interfaces have values > 40, which is seen for the upper lobe dimer interfaces of GluA2 and the GluA3 dimer I (Table 1).

"The authors should show superposition of their GluA2 structure with others previously published" "...they should clearly show what kind of structural differences they observe and discuss what they mean".
This has essentially been accomplished in Figure 3B of the current manuscript, where 7 GluA2 chains were superimposed and displacements computed with the program PINQ. The structural differences are shown in red. To clarify this further, we have now included a direct superposition as an inset, showing the two most divergent chains for each segment, as suggested by the reviewer (Figure 3B inset).

"Does it take 2.0 sigma contour level to observe the continuous density in the cleft"?
This is a good point; to answer this query we now present both 2.0 and 3.0 sigma contour level in the revised version. The density is still substantial at 3.0 sigma (magenta mesh in Figure 5B).

Reviewer 3:

We thank the reviewer for the overall positive response. The reviewer is supportive of our work; mainly minor issues are raised, which we address point-by-point:

1. *The reviewer suggests to include Normal Mode Analysis for the GluK2 NTD.*
   This is a good point that was on our agenda and is now included in Figure 4B of the revision. GluK2 (PDB 3H6G) is structurally more similar to GluA2 than GluA3 based on RMS deviations between the structures. At the protomer level, GluK2 undergoes similar clamshell like motions as seen in GluA2 and GluA3. At the level of the dimer, the anti-correlated motions between the protomers seen in the softest mode of GluA2 and GluA3 can be seen in the softest mode of GluK2 as well. The distribution of motions (among residues), i.e. the mode shape, is strikingly similar between the 3 NTDs; all three potentially access similar types of motion, which can be seen from the similarities of the curves (now included in Figure 4C of the revised paper). In terms of the absolute sizes of motions however, which are reflected by the eigenvalues, GluK2 and GluA2 are significantly stiffer than GluA3 (respective eigenvalues of 0.19, 0.19 and 0.11, as correctly hypothesized by the reviewer. An analysis of the overall motion of the protein, based on the first 10 modes of GNM, re-iterates that the lower-lobe is more mobile than the upper-lobe (this information is not part of the paper but is included for inspection by the editor and the reviewers). It is shown that the mobility of the lower-lobe in GluK2 is more comparable to GluA2, with GluA3 having the highest mobility in the lower-lobe region because of lesser interfacial contacts (see lower panel in the attached figure).

2. We have amended Figure 2 as suggested.

3. *The authors should discuss that the apo density in the NR2B NMDAR NTD features electron density*
   A discussion regarding the apo NMDA GluN2B has now been added to the 1st paragraph in the 'Discussion'.

4. *Are all modes represented in Figure 4B?*
   Figure 4B does show mode 1 only, this is the 'softest, most likely mode of motion.'

5. *It is stated that going from dimer I to dimer II involves a 'translation along the 2-fod axis' whereas on p 9 'counter-rotations about the central axis' are mentioned, please clarify*
   In page 6, the comparison is purely structural, based on the structures of the two dimers. Since the most collective motion of GluA3 dimers is a counter-rotation of the two protomers; the sentence mentioning 'translation' has now been removed to avoid confusion.

6. We have amended the abstract as suggested by the reviewer.

7. The Clayton et al. 2009 reference is now featuring more prominently as suggested; the paper is discussed in the 'Discussion' section.

8. Figure S2C in-text citation on p5 has been fixed

9. *Adding the GluK1-3 sequences to Figure 1D would be valuable*
   A GluK1-3 alignment has been generated and is shown in Figure 1A of the revised text.

10. -12. Dimer I instead of dimer A has been fixed**;** all figure legends have been rectified, as suggested.

2nd Editorial Decision 22 December 2010

Thank you for submitting your revised manuscript to the EMBO Journal. I asked the original referees #2 and 3 to review the revised version and I have now received the comments back. Referee #3 is satisfied with introduced changes, while referee #2 has a few remaining comments. I would like to give you the opportunity to respond to those either in the point-by-point response or in the manuscript text should you feel that is necessary. Once we receive the revision, we will go aheard and accept the paper for publication. If you have any further questions please do not hesitate to

contact me.

When you send us your revision, please include a cover letter with an itemised list of all changes made, or your rebuttal, in response to comments from review.

Sincerely

Editor
The EMBO Journal

REFEREE REPORTS

Referee #2 (Remarks to the Author):

The authors did their best to answer questions associated with physiological relevance of the dimeric orientations, which authors call dimer I, II, and III, by the following remarks: 1) Simulation using "normal mode analysis" showed mutual accessibilities to the three conformers; 2) M150C mutation at the dimer interface representing dimer II disulphide cross-links in solution (when expressed as isolated NTD proteins); and 3) there has previously been examples of "labile interfaces" as illustrated by the case of hemoglobin. What authors should have done is to incorporate M150C mutation in the full-length receptors. The relevance of dimeric arrangement observed in crystallographic studies of isolated domains has to be validated carefully in intact (or physiological) ion channels. Other groups in the field (Gouaux and Mayer) run very careful and extensive experiments to validate their structures as described in their papers: Nature. 2009 Dec 10;462(7274):745-56 and Proc Natl Acad Sci U S A. 2010 May 4;107(18):8463-8. Labile interface in hemoglobin is intriguing observation, but this does not support such a mechanism in AMPA receptors.

As reviewer 1 also pointed out, a weakness of the paper is the undefined nature of the electron density at the cleft. The more effort could be put forth to understand chemical nature. A good example is shown by a paper from Furukawa's group on GluN2B (EMBO J. 2009 Dec 16;28(24):3910-20) where they identified ion binding sites at the cleft through extensive crystallographic analysis. As pointed out in the first review, a portion of the density possibly looks like a phosphate molecule or sulphate moleculte, which may have come from the crystallization condition (NH4H2PO4) and cryo condition (200 mM ammonium sulphate), respectively. By taking advantage of relatively high x-ray diffraction quality of the crystals, the authors can at least analyze the presence or non-presence of anomalous peaks at the cleft.

Referee #3 (Remarks to the Author):

The authors have satisfactorily answered to all my points. I recommend publication.

2nd Editorial Decision 05 January 2011

EMBOJ-2010-76305R - Response to referee 2

1. The reviewer discusses the physiological relevance of GluA3 dimers I-III.
'The relevance of dimeric arrangement observed in crystallographic studies of isolated domains has to be validated carefully in intact (or physiological) ion channels. Other groups in the field (Gouaux and Mayer) run very careful and extensive experiments to validate their structures as described in their papers..'

To our knowledge, our study is the first to report alternate dimeric structures for a single iGluR NTD. Since these different dimers were observed in the context of the isolated GluA3 NTD, we feel that confirming their existence in this (minimal) context is justified. The finding that M150C in the NTD-context can crosslink in solution strongly suggests that dimer II is not a crystallographic

artefact. Probing these different dimer arrangements in the full-length receptor, while a valid and interesting question in its own right, is outside the scope of our present study, which focuses on intrinsic dynamics of the NTD.

I would also like to point out that the *entire study* by Mayer and Plested (cited by the reviewer) was conducted in order to validate that inter-subunit contacts observed in AMPARs (by the Gouaux group) are also seen in Kainate receptors. The cross-linking assay was the major tool they utilized in their study to probe these contacts, and is thus not comparable to the aim of our current work, which utilizes a broad scope of experimental and computational methods to probe NTD dynamics.

2. The reviewer questions the ligand density in the GluA2 NTD cleft.
'... a weakness of the paper is the undefined nature of the electron density at the cleft. The more effort could be put forth to understand chemical nature.'

To characterize the chemical nature of the cleft density is a major undertaking, currently in progress. We indeed looked for anomalous peaks, but all our efforts turned out negative (these included the following elements: Zn++, Pb++, Ca++, Au+++). Ligand density has to date not been described for AMPAR NTDs, yet this finding was highly reproducible for various GluA2 data sets and is absolutely novel with potential major functional consequences for AMPAR physiology. We feel that this level of description is appropriate for the scope of the current work, which describes an overall allosteric potential for AMPAR NTDs (for the first time).

