Supporting information for article:

Small-angle neutron scattering studies on the AMPA receptor GluA2 in the resting, AMPA and GYKI-53655 bound states

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Table S1  Information about the samples, the SANS measurements and the software used for the data analysis.

| Sample details | GluA2, Apo, in dDDM. | GluA2, AMPA-bound state, in dDDM, neutral pH. | GluA2, AMPA-bound state, in dDDM, acidic pH. | GluA2, GYKI-53655 bound state, in dDDM. |
|----------------|----------------------|-----------------------------------------------|-----------------------------------------------|------------------------------------------|
| Uniprot ID     | P19491 (GRIA2_RAT)   |                                               |                                               |                                          |
| Organism       | Rattus Norvegicus     |                                               |                                               |                                          |
| Ligands        | None                 | 1 mM AMPA                                     | 10 mM AMPA                                    | 1 mM GYKI-53655                          |
| Buffer         | 20 mM Tris/DCI, 100 mM NaCl, 0.5 mM dDDM, pH 7.5 | 20 mM Tris/DCI, 100 mM NaCl, 0.5 mM dDDM, pH 7.5 | 20 mM Tris/DCI, 100 mM NaCl, 0.5 mM dDDM, pH 5.5 | 20 mM Tris/DCI, 100 mM NaCl, 0.5 mM dDDM, pH 7.5 |
| Extinction coefficient* [M⁻¹ cm⁻¹] | | | 519100 | |
| Density* [g/ml] | | | 1.37 | |
| Molecular weight* [kDa] | | | 367.7 | |
| Mean scattering length density* of protein in D₂O [10⁻⁶ Å²] | | | 3.0 | |
| Mean scattering length density* of DDM tail groups in D₂O [10⁻⁶ Å²] | | | 6.4 | |
| Mean scattering length density* of DDM head groups in D₂O [10⁻⁶ Å²] | | | 6.4 | |
| Mean scattering length density* of solvent (D₂O) [10⁻⁶ Å²] | | | 6.4 | |
| Protein concentration* [mg/ml] | | | 0.20 mg/ml | 0.17 mg/ml |
| | | | 0.54 µM | 0.46 µM |
| | | | 0.31 mg/ml | 0.31 mg/ml |
| | | | 0.84 µM | 0.84 µM |

SANS data collection details

| Instrument | KWS1@FRM2 (https://www.mlz-garching.de/kws-1) |
|------------|-----------------------------------------------|
| Date for data collection | 19/09 2017 8/12 2016 19/09 2017 8/12 2016 |
| Wavelength ($\lambda_{\text{mean}}, \Delta\lambda/\lambda$) | 5.0 Å, 10 % (FWHM) |
| Beam dimensions | Rectangular beam, 6x10 mm$^2$ (at sample), 30x30 mm$^2$ (first pinhole) |
| Resolution effects | Width of the resolution function $\Delta q(q)$ was calculated by the beamline software and given in the 4th column in data, which was used in WillItFit. |
| Settings (Sample-detector/Collimation) | 1.5m/4.0m, 4.0m/4.0m, 8.0m/8.0m |
| Measured $q$-range | 0.006-0.3 Å$^{-1}$ |
| Absolute calibration | By plexiglass |
| Exposure time (total for all 3 settings) | ~ 2.5 hours | ~ 4.5 hours | ~ 4.5 hours | ~ 4.0 hours |
| Temperature | 10 °C |

**Software employed**

| Indirect Fourier transformations to obtain $p(r)$ | BayesApp$^{R1,R2}$ (www.bayesapp.org) |
| Calculation of theoretical $p(r)$ | CaPP (https://github.com/Niels-Bohr-Institute-XNS-StructBiophys/CaPP) |
| Addition of water layer to protein | |
| Fitting of data with combined analytical and atomistic models | WillItFit$^{R3}$ (https://sourceforge.net/projects/willitfit) |
| Fischer/Petoukhov $M_w$ determination | Own implementation in MATLAB (Table S3) |
| Missing sequence modelling | MODELLER$^{R4}$ (https://salilab.org?modeller) |
| Graphic model visualization | PyMOL |
| Guinier analysis | Own implementation in MATLAB (Fig. S6) |
| Ab initio dummy bead modelling | DAMMIF$^{R5}$ (https://www.embl-hamburg.de/biosaxs/dammif.html) |
| Note: DATGNOM$^{R6,R7}$ was used to generate the $p(r)$ function needed as input to DAMMIF |

**Structural parameters**

| Guinier analysis |
| $I(0)$ [cm$^{-1}$] | 0.043 ± 0.002 | 0.108 ± 0.004 | 0.052 ± 0.07$^{74}$ | 0.109 ± 0.004 |
| Mw from $I(0)$ [kDa] (ratio to expected) | 220 (0.60) | 347 (0.94) | 240 (0.66) | 347 (0.94) |
| $R_g$ [Å] | 60.8 ± 4.3 | 62.2 ± 3.0 | 79.1 ± 11.5$^{14}$ | 62.8 ± 3.3 |
| Minimum $q$ used [Å$^{-1}$] | 0.0069 | 0.0078 | 0.0104 | 0.0103 |
| Maximum $q \cdot R_g$ | 1.26 | 1.24 | 1.29 | 1.30 |

$p(r)$ analysis
| Property                        | Combined analytical and atomistic model | Ab initio dummy bead modelling | SASBDB IDs for data and models |
|--------------------------------|-----------------------------------------|-------------------------------|--------------------------------|
|                                | [0.006,0.3]                            | [0.006,0.3]                   | SASBDB ID                     |
|                                | 3.3                                     | 5.0                           | SASDDY5                        |
|                                | Reduced $\chi^2$ (best fit)             |                               | SASDDZ5                        |
|                                |                                         |                               | SASDD26                        |
|                                |                                         |                               | SASDD36                        |
|                                | $q$-range for fitting [Å⁻¹]             |                               |                                |
|                                | [0.006,0.3]                            | [0.006,0.3]                   |                                |
|                                | 3.3                                     | 5.0                           |                                |
|                                | Reduced $\chi^2$ (best fit)             |                               |                                |
|                                | 3.3                                     | 5.0                           |                                |
|                                | Number of calculations                  | 10                            | SASBDB ID                      |
|                                | Symmetry                                | P1, none                      | SASBDB ID                      |
|                                | NSD                                     | 1.5 ± 0.1                     | SASBDB ID                      |
|                                | Resolution (from SASRES R8) [Å]         | 56 ± 4                        | SASBDB ID                      |
|                                | Filtered volume [nm³]                   | 380                           | SASBDB ID                      |
|                                | Mw from filtered volume                 | 238 kDa                       | SASBDB ID                      |
|                                | (ratio to expected)                     | (0.92)                        | SASBDB ID                      |
| Fischer $M_W$ determination    |                                       |                               |                                |
| Molecular weight [kDa]         | 396 ± 52                                | 379 ± 49                      | SASBDB ID                      |
| (ratio to expected)            | (1.08)                                  | (1.03)                        | SASBDB ID                      |
| Number of good parameters      | 5.2                                     | 4.2                           | SASBDB ID                      |
| Number of Shannon channels     | 11.8                                    | 11.0                          | SASBDB ID                      |
| Number of error calculations   | 260                                     | 759                           | SASBDB ID                      |
| Regularization parameter log($\alpha$) | 14.3                                 | 14.7                          | SASBDB ID                      |
| Reduced $\chi^2$               | 2.15                                    | 6.84                          | SASBDB ID                      |
| Number of calculations         |                                         |                               | SASBDB ID                      |
| Symmetry                       | P1, none                                |                               | SASBDB ID                      |
| NSD                            | 1.5 ± 0.1                               |                               | SASBDB ID                      |
| Resolution (from SASRES R8) [Å]| 56 ± 4                                  |                               | SASBDB ID                      |
| Filtered volume [nm³]          | 380                                     |                               | SASBDB ID                      |
| Mw from filtered volume        | 238 kDa                                 |                               | SASBDB ID                      |
| (ratio to expected)            | (0.92)                                  |                               | SASBDB ID                      |

Footnotes and references

*1 Calculated with Expasy Protparam (https://web.expasy.org/protparam/).
*2 Calculated with Biomolecular Scattering Length Density Calculator (http://pssl.s.isis.rl.ac.uk/Psldc).
*3 Protein concentration determined by UV280 absorption for GluA2 AMPA-bound at pH 7.5 and GluA2 GYKI-bound. Determined with BCA assay for GluA in the resting state and GluA2 AMPA-bound at pH 5.5.

*4 It was not possible to obtain fully linear region at $qR_G < 1.3$ (Fig. S6C), so the the values may be incorrect.

*5 $M_W$ determined with the Fischer method (Fischer et al., 2011) with parameters given in Table S3.

*6 The dummy atom model for GluA2 apo is approximate, since aggregation was not taken into account. For the same reason, a dummy atom model was only generated for the GluA2 apo sample, where the aggregation scattering contribution was very minor.

R1 Hansen, S. (2000). *J. Appl. Cryst.* **33**, 1415-1421.

R2 Hansen, S. (2014). *J. Appl. Cryst.* **47**, 1469-1471.

R3 Pedersen, M. C., Arleth, L. & Mortensen, K. (2013). *J. Appl. Cryst.* **46**, 1894-1898.

R4 Fiser, A., Do, R.K. & Sali, A. (2000). *Protein Sci.* **9**, 1753-1773.

R5 Franke, D. & Svergun, D. I. (2009). *J. Appl. Cryst.* **42**, 342-346.

R6 Konarev, P. V., Volkov, V. V., Sokolova, A. V., Koch, M. H. J. & Svergun, D. I. (2003). *J. Appl. Cryst.* **36**, 1277-1282.

R7 Petoukhov, M. V., Konarev, P. V., Kikhney, A. G. & Svergun, D. I. (2007). *J. Appl. Cryst.* **40**, 223-228.

R8 Tuukkanen, A. T., Kleywegt, G. J. & Svergun, D. I. (2016). *IUCrJ* **3**, 440-447.
Table S2  Fischer and Petoukhov $M_W$ determination (Petoukhov et al., 2012; Fisher et al., 2010), where $M_W$ is determined via. the scattering “invariant” $Q$ (Porod, 1982)

The upper integration limit $q_m$ used to determine $Q$ was $8/R_g$ (Petoukhov et al., 2012). $V_{\text{app}}$ is the apparent volume, and is the same for the two methods. In the Fischer method, linear coefficients $A$ and $B$ given in the table are used to convert $V_{\text{app}}$ to the Porod volume $V_p$, and the weight-to-volume conversion constant of 0.83 kDa/nm$^3$ to obtain $M_W^F$. In the Petoukhov method, $M_W^P$ is determined directly from the $V_{\text{app}}$ using the conversion constant 0.625 kDa/nm$^3$. The constant subtracted backgrounds $K$ were used to assure a constant plateau in the Porod plots (Fig. S2) and the data sets were extrapolated to $q = 0$ by simple linear extrapolation. An implementation in MATLAB of the methods was used. The value for $M_W$ obtained with the Fisher method is given in Table S1 and used in the paper, since this method takes the size of the particle into account, which adds an important correction for large proteins such as GluA2. Values of $R_g$ and $I(0)$ from the $p(r)$ analysis were used (Table S1).

| Fischer/Petoukhov $M_W$ determination | Resting | AMPA pH 7.5$^*$ | AMPA pH 5.5 | GYKI-53655 |
|--------------------------------------|---------|----------------|-------------|------------|
| $V_{\text{app}}$ [nm$^3$]            | 871.7   | 833.4          | 970.1       | 820.4      |
| $M_W^F$ [kDa] (ratio to expected)     | 396 ± 52| 379 ± 49       | 442 ± 57    | 373 ± 48   |
| $\Delta M_W/\sigma^*$                | 0.56    | 0.22           | 1.3         | 0.1        |
| $M_W^P$ [kDa] (ratio to expected)     | 545 ± 109| 521 ± 104     | 606 ± 121   | 513 ± 103  |
| $\Delta M_W/\sigma$                  | 1.5     | 1.5            | 2.8         | 1.4        |
| $K$ [10$^{-3}$cm$^{-1}$]              | 0.65    | 1.30           | 0.61        | 1.30       |
| $q_m = 8/R_g$ [Å$^{-1}$]              | 0.133   | 0.131          | 0.127       | 0.131      |
| $A$ [Å$^3$]                          | -10500  | -10500         | -10500      | -10500     |
| $B$                                  | 0.56    | 0.56           | 0.56        | 0.56       |

$^*$ Assuming a 13% uncertainty on $M_W^F$ (Fischer et al., 2010, p. 106), and a 20% uncertainty on $M_W^P$ (Petoukhov et al., 2012, p. 344).

$^*$ $\Delta M_W/\sigma$ is the normalized residual molecular weight, i.e. the difference between the experimentally determined value and the expected molecular weight in units of the experimental error. If $M_W/\sigma < 2$ then the null-hypothesis (tetrameric state) cannot be rejected, given a significance level of 5%.
### Table S3  
*Rg* of fractal oligomers and the amount of oligomers in the fitted models.

| Data                        | Apo            | AMPA pH 7.5     | AMPA pH 5.5     | GYKI-53655   |
|-----------------------------|----------------|----------------|----------------|--------------|
| Model                       | X-ray, rest. + frac. olig. | X-ray, rest + frac. olig. | EM, act. + frac. olig. | EM, des. + frac. olig. | EM, class3 + frac. olig. | EM, GYKI + frac. olig. |
| *Rg* for fractal oligomers [Å] | 126 ± 250     | 190 ± 177      | 145 ± 135      | 116 ± 62     | 175 ± 166     | 240 ± 254     |
| Fraction in oligomeric form, *γ* [%] | 0.9 ± 6.5      | 0.4 ± 1.0      | 0.7 ± 2.1      | 2.7 ± 5.3    | 1.0 ± 3.0     | 0.2 ± 0.6     |
Figure S1  Guinier plots and residual plots for GluA2 in the resting state (A), in the AMPA bound state at pH 7.5 (B), in the AMPA bound state at pH 5.5 (C) and in the GYKI-53655 bound state (D). Residuals show the difference between log(I) and the fit, weighted with the errors on log(I). Resulting values for I(0) and R_g are given in Table S1. The AMPA bound state at pH 5.5 (panel C) does not have a fully linear Guinier region at qR_g < 1.3, meaning that the values for I(0) and R_g may be wrong. The values of I(0) and R_g from the p(r) funciton was therefore used for M_W determination.
Figure S2  Porod plots (black) for GluA2 in the resting state (A), in the AMPA bound state at pH 7.5 (B), in the AMPA bound state at pH 5.5 (C) and in the GYKI-53655 bound state (D). Additional constant backgrounds were subtracted to give a constant behavior at high-q (red). The constants are listed in Table S2.
Figure S3  Kratky plots for GluA2 in the resting state (A), in the AMPA bound state at pH 7.5 (B), in the AMPA bound state at pH 5.5 (C) and in the GYKI-53655 bound state (D). Constant backgrounds were subtracted, and listed in Table S2.
3KG2: NSIQIGGLFPFRGADQSFRQSMVQFSSTERLTHIDNLEVANSFVATNAPCSQFSGR   60
4U2P: NSIQIGGLFPFRGADQSFRQSMVQFSSTERLTHIDNLEVANSFVATNAPCSQFSGR   60
5WEO: NSIQIGGLFPFRGADQSFRQSMVQFSSTERLTHIDNLEVANSFVATNAPCSQFSGR   60
5VHZ: NSIQIGGLFPFRGADQSFRQSMVQFSSTERLTHIDNLEVANSFVATNAPCSQFSGR   60
5L1H: NSIQIGGLFPFRGADQSFRQSMVQFSSTERLTHIDNLEVANSFVATNAPCSQFSGR   60

3KG2: VYAIFGFYDSDKVSVNTTSCGTLHVSFITPSFPTDGTHFVIQMPDLKAGALSLIEYYQ   120
4U2P: VYAIFGFYDSDKVSVNTTSCGTLHVSFITPSFPTDGTHFVIQMPDLKAGALSLIEYYQ   120
5WEO: VYAIFGFYDSDKVSVNTTSCGTLHVSFITPSFPTDGTHFVIQMPDLKAGALSLIEYYQ   120
5VHZ: VYAIFGFYDSDKVSVNTTSCGTLHVSFITPSFPTDGTHFVIQMPDLKAGALSLIEYYQ   120
5L1H: VYAIFGFYDSDKVSVNTTSCGTLHVSFITPSFPTDGTHFVIQMPDLKAGALSLIEYYQ   120

3KG2: WDKFAYLYDSDRGLSTLQAIALQVEQVGSKYVFTMLLNLKLQ樯QGDSKEDMVLKAG 180
4U2P: WDKFAYLYDSDRGLSTLQAIALQVEQVGSKYVFTMLLNLKLQ樯QGDSKEDMVLKAG 180
5WEO: WDKFAYLYDSDRGLSTLQAIALQVEQVGSKYVFTMLLNLKLQ樯QGDSKEDMVLKAG 180
5VHZ: WDKFAYLYDSDRGLSTLQAIALQVEQVGSKYVFTMLLNLKLQ樯QGDSKEDMVLKAG 180
5L1H: WDKFAYLYDSDRGLSTLQAIALQVEQVGSKYVFTMLLNLKLQ樯QGDSKEDMVLKAG 180

3KG2: RRVILDCERDKVNDIVDQVITIGKHVKYHYIIANLGFTDGDLLKIQFGAEGSFQIJD 240
4U2P: RRVILDCERDKVNDIVDQVITIGKHVKYHYIIANLGFTDGDLLKIQFGAEGSFQIJD 240
5WEO: RRVILDCERDKVNDIVDQVITIGKHVKYHYIIANLGFTDGDLLKIQFGAEGSFQIJD 240
5VHZ: RRVILDCERDKVNDIVDQVITIGKHVKYHYIIANLGFTDGDLLKIQFGAEGSFQIJD 240
5L1H: RRVILDCERDKVNDIVDQVITIGKHVKYHYIIANLGFTDGDLLKIQFGAEGSFQIJD 240

3KG2: YDDSLVKFIERWSLEKEYPAHTATIKYTSLTYDADVQVTAFRNLKQRIEISRR   300
4U2P: YDDSLVKFIERWSLEKEYPAHTATIKYTSLTYDADVQVTAFRNLKQRIEISRR   300
5WEO: YDDSLVKFIERWSLEKEYPAHTATIKYTSLTYDADVQVTAFRNLKQRIEISRR   300
5VHZ: YDDSLVKFIERWSLEKEYPAHTATIKYTSLTYDADVQVTAFRNLKQRIEISRR   300
5L1H: YDDSLVKFIERWSLEKEYPAHTATIKYTSLTYDADVQVTAFRNLKQRIEISRR   300

3KG2: GNAGDCLANAVPWQGGEIERALKQVQVELSGNIFQDGNNKRNITYINIMELKNTGPR 360
4U2P: GNAGDCLANAVPWQGGEIERALKQVQVELSGNIFQDGNNKRNITYINIMELKNTGPR 360
5WEO: GNAGDCLANAVPWQGGEIERALKQVQVELSGNIFQDGNNKRNITYINIMELKNTGPR 360
5VHZ: GNAGDCLANAVPWQGGEIERALKQVQVELSGNIFQDGNNKRNITYINIMELKNTGPR 360
5L1H: GNAGDCLANAVPWQGGEIERALKQVQVELSGNIFQDGNNKRNITYINIMELKNTGPR 360

3KG2: KIGYWSEDKMV---LTDDETSLEGKTVVTVTTESPYVMKANHAALAGNERYEGCVD 418
4U2P: KIGYWSEDKMV---LTDDETSLEGKTVVTVTTESPYVMKANHAALAGNERYEGCVD 418
5WEO: KIGYWSEDKMV---LTDDETSLEGKTVVTVTTESPYVMKANHAALAGNERYEGCVD 418
5VHZ: KIGYWSEDKMV---LTDDETSLEGKTVVTVTTESPYVMKANHAALAGNERYEGCVD 418
5L1H: KIGYWSEDKMV---LTDDETSLEGKTVVTVTTESPYVMKANHAALAGNERYEGCVD 418
| ID       | Sequence                                                                 | Length |
|----------|--------------------------------------------------------------------------|--------|
| 3KG2     | LAAEIAKHCGFKYKTLTIVDGKYGARDADTKIWNGMVGEHYKADIAAPLTITLIVREE               | 478    |
| 4U2P     | LAAEIAKHCGFKYKTLTIVDGKYGARDADTKIWNGMVGEHYKADIAAPLTITLIVREE               | 480    |
| 5WEO     | LAAEIAKHCGFKYKTLTIVDGKYGARDADTKIWNGMVGEHYKADIAAPLTITLIVREE               | 478    |
| 5VHZ     | LAAEIAKHCGFKYKTLTIVDGKYGARDADTKIWNGMVGEHYKADIAAPLTITLIVREE               | 478    |
| 5L1H     | LAAEIAKHCGFKYKTLTIVDGKYGARDADTKIWNGMVGEHYKADIAAPLTITLIVREE               | 478    |
| 3KG2     | VIDFSKPFMSLGISIMIKKQPKSKPGVFSLFDPAYEIMVCIVFAYIGSVEVLFLVSRFS              | 538    |
| 4U2P     | VIDFSKPFMSLGISIMIKKQPKSKPGVFSLFDPAYEIMVCIVFAYIGSVEVLFLVSRFS              | 534    |
| 5WEO     | VIDFSKPFMSLGISIMIKKQPKSKPGVFSLFDPAYEIMVCIVFAYIGSVEVLFLVSRFS              | 538    |
| 5VHZ     | VIDFSKPFMSLGISIMIKKQPKSKPGVFSLFDPAYEIMVCIVFAYIGSVEVLFLVSRFS              | 538    |
| 5L1H     | VIDFSKPFMSLGISIMIKKQPKSKPGVFSLFDPAYEIMVCIVFAYIGSVEVLFLVSRFS              | 536    |
| 3KG2     | PYEWHTEEFEDGRETQSESSTNEFGIFNLSLWFLGAFMQQGADIISRSLISRIVGGVWF              | 598    |
| 4U2P     | PYEWHTEEFEDGRETQSESSTNEFGIFNLSLWFLGAFMQQGADIISRSLISRIVGGVWF              | 600    |
| 5WEO     | PYEWHTEEFEDGRETQSESSTNEFGIFNLSLWFLGAFMQQGADIISRSLISRIVGGVWF              | 598    |
| 5VHZ     | PYEWHTEEFEDGRETQSESSTNEFGIFNLSLWFLGAFMQQGADIISRSLISRIVGGVWF              | 598    |
| 5L1H     | -------------------------------------------------------------------------- | 579    |
| 3KG2     | FTLIIISSTYNTLANAALFTVERMVSPIESAEDLSKQTEIAYTLTDGSTKKEFRSRKIAVF            | 658    |
| 4U2P     | FTLIIISSTYNTLANAALFTVERMVSPIESAEDLSKQTEIAYTLTDGSTKKEFRSRKIAVF            | 660    |
| 5WEO     | FTLIIISSTYNTLANAALFTVERMVSPIESAEDLSKQTEIAYTLTDGSTKKEFRSRKIAVF            | 658    |
| 5VHZ     | FTLIIISSTYNTLANAALFTVERMVSPIESAEDLSKQTEIAYTLTDGSTKKEFRSRKIAVF            | 658    |
| 5L1H     | FTLIIISSTYNTLANAALFTVERMVSPIESAEDLSKQTEIAYTLTDGSTKKEFRSRKIAVF            | 659    |
| 3KG2     | DKMWTYMRSAEPSVFVRTTEGVARVRSKSKGYAYLLESTMNEIEQRKPCDTRMKVGVLN              | 718    |
| 4U2P     | DKMWTYMRSAEPSVFVRTTEGVARVRSKSKGYAYLLESTMNEIEQRKPCDTRMKVGVLN              | 720    |
| 5WEO     | DKMWTYMRSAEPSVFVRTTEGVARVRSKSKGYAYLLESTMNEIEQRKPCDTRMKVGVLN              | 718    |
| 5VHZ     | DKMWTYMRSAEPSVFVRTTEGVARVRSKSKGYAYLLESTMNEIEQRKPCDTRMKVGVLN              | 718    |
| 5L1H     | DKMWTYMRSAEPSVFVRTTEGVARVRSKSKGYAYLLESTMNEIEQRKPCDTRMKVGVLN              | 699    |
| 3KG2     | DSGKYGIATPKGSSLGTVPNVLKLSEQGLLKLKNKWYDKGECGAKDSGSKETKSAL                 | 778    |
| 4U2P     | DSGKYGIATPKGSSLGTVPNVLKLSEQGLLKLKNKWYDKGECGAKDSGSKETKSAL                 | 780    |
| 5WEO     | DSGKYGIATPKGSSLGTVPNVLKLSEQGLLKLKNKWYDKGECGAKDSGSKETKSAL                 | 778    |
| 5VHZ     | DSGKYGIATPKGSSLGTVPNVLKLSEQGLLKLKNKWYDKGECGAKDSGSKETKSAL                 | 778    |
| 5L1H     | DSGKYGIATPKGSSLGTVPNVLKLSEQGLLKLKNKWYDKGECGAKDSGSKETKSAL                 | 759    |
| 3KG2     | SLSNVAGFYILVGGGLAMLVALIEFCYKSRAEARAMMKGLVPRG                             | 823    |
| 4U2P     | SLSNVAGFYILVGGGLAMLVALIEFCYKSRAEARAMMKGLVPRG                             | 824    |
| 5WEO     | SLSNVAGFYILVGGGLAMLVALIEFCYKSRAEARAMMKGLVPRG                             | 817    |
| 5VHZ     | SLSNVAGFYILVGGGLAMLVALIEFCYKSRAEARAMMKGLVPRG                             | 817    |
| 5L1H     | SLSNVAGFYILVGGGLAMLVALIEFCYKSRAEARAMMKGLVPRG                             | 803    |
**Figure S4**  Sequence alignment of GluA2 structures used in present study. The alignment was made using Clustal Omega (Goujon, M., McWilliam, H., Li, W., Valentin, F., Squizzato, S., Paern, J. & Lopez, R. A new bioinformatics analysis tools framework at EMBL-EBI (2010) *Nucl. Acids Res*. W695-699). Residues in green are differing from the target sequence (3kg2). The residues marked in italics were not seen in the structures (for chain A, similar for the other chains).
Figure S5  SANS data of GluA2 in the presence of 1 mM AMPA at pH 7.5 (black) and 10 mM AMPA at pH 5.5 (red)
Figure S6  Additional fits to SANS data of GluA2 in the AMPA bound state at pH 5.5 (grey). The data were fitted with models of tetrameric GluA2 in combination with fractal oligomers. Models included GluA2 in the resting state (cyan; pdb-code 4u2p, $\chi_r^2 = 5.2$), GluA2 in the activated state (black; pdb-code 5weo; $\chi_r^2 = 4.6$), GluA2 in the desensitized state (red; pdb-code 5lhv; $\chi_r^2 = 4.8$) and the class 3 EM structure (magenta; EMD-2688; $\chi_r^2 = 1.9$).
**Figure S7**  Theoretical SANS scattering for all investigated structures. GluA2 in the resting state (X-ray; cyan; pdb-code 4u2p), in the activated state (EM; black; pdb-code 5weo), in the desensitized state (EM; pdb-code 5vhz), in the GYKI-53655 bound state (X-ray; orange; pdb-code 5l1h) and GluA2 in the class 3 state (EM; magenta; EMDB-2688). Data are normalized and a constant background of 0.01 · I(0) is subtracted (grey dashed line). The compact forms are similar, whereas the scattering curve for the more open EM class 3 structure is clearly distinguishable by eye. The compact structures differs only at high q-values, where the signal to noise ratio is low.
Figure S8  Generated structure of GluA2 in the resting state. Due to missing residues in the X-ray structure of GluA2 in the resting state (cyan; pdb-code 4u2p), a model structure was generated of GluA2 (black) using Modeller (Fiser et al., 2000), with the missing residues inserted as loops.
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