Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Electronic Supplementary information

Revealing Dynamically-Organized NMDA Receptor Ion Channel Composition in Live Cells by a Correlated Electric Recording and Super-Resolution Imaging

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Figure S1. Effect of Laplacian of Gaussian (LoG) kernel filter in the Imaging. (A) Single image of HEK-293 cell in TIRF mode shows the NMDA receptors having EYFP-GluN1a and GluN2B as fluorescent spots in the cell membrane. (B) Image observed after remove fluorescence background by convoluting a Laplacian of Gaussian (LoG) kernel filter from panal A.

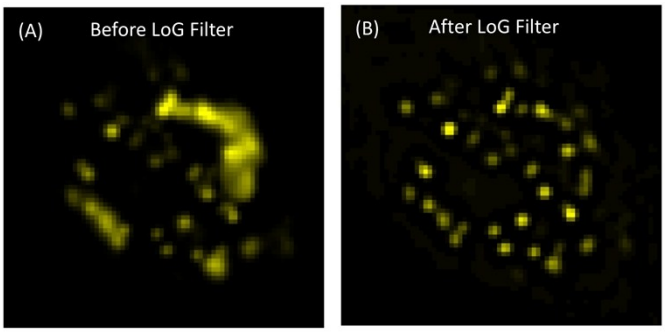

Figure S2. Some observed single-molecule bleaching-steps trajectories for NMDA receptor ion channel containing EYFP-GluN1a and Glun2B subunits. **(A-D)** Change of fluorescence intensity (black line) with time for different fluorescent spots. Red line is the fitted line for the intensity of different spots showing clear bleaching steps as 4, 5, 8 and 9 for figure A to D, respectively. For bleaching steps analysis, we have used a computational method, Progressive Idealization and Filtering (PIF), where, fluorescence background was removed by convoluting a Laplacian of Gaussian (LoG) kernel filter in the image followed by filtering the intensity traces with the Chung-Kenedy filter.

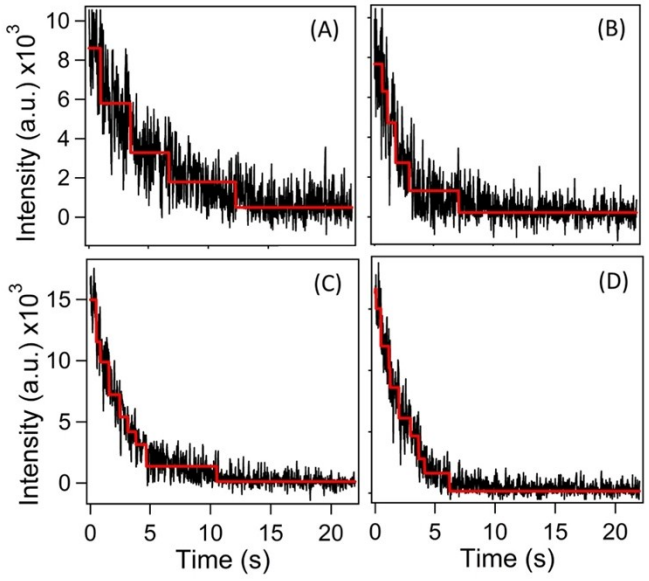

Table S1. A correlation between bleaching-steps analysis and super-resolution analysis. The size (FWHM) of super-resolved spots are computed with 2D Gaussian fitting in two directions, short and long, in case if sub-diffraction spots size is elongated; and these size shows broad distribution as shown in figure 5D and 5E1-5E3. From these distribution, we have measured the median value and interquartile range for better understanding the correlation between two single-molecule imaging methods.

Super resolution → / Bleaching Steps ↓	Median (nm)	Interquartile Range	
		Lower limit (nm)	Upper limit (nm)
1 - 2	47	39	61
3 - 6	50	38	75
7 & more	48	36	78
All	48	38	69

Movie 1 and 2. A typical movie with the fluorescence spots for NMDA receptor ion channels containing GluN1a subunit tagged with EYFP recorded in the live HEK-293 cell. In movie 1 contains all frames recored, here it can be seen that in first few seconds all the spots are looks like photo bleached; however, the later part by increasing the brightness is shown in movie 2, where some spots appear in the imaging frames, which corresponds to the molecules return to the ground state from their dark state. A detailed description of analysis for observing photo-bleaching steps as well as super-resolved image is given in experimental section of manuscript.