Supporting Information

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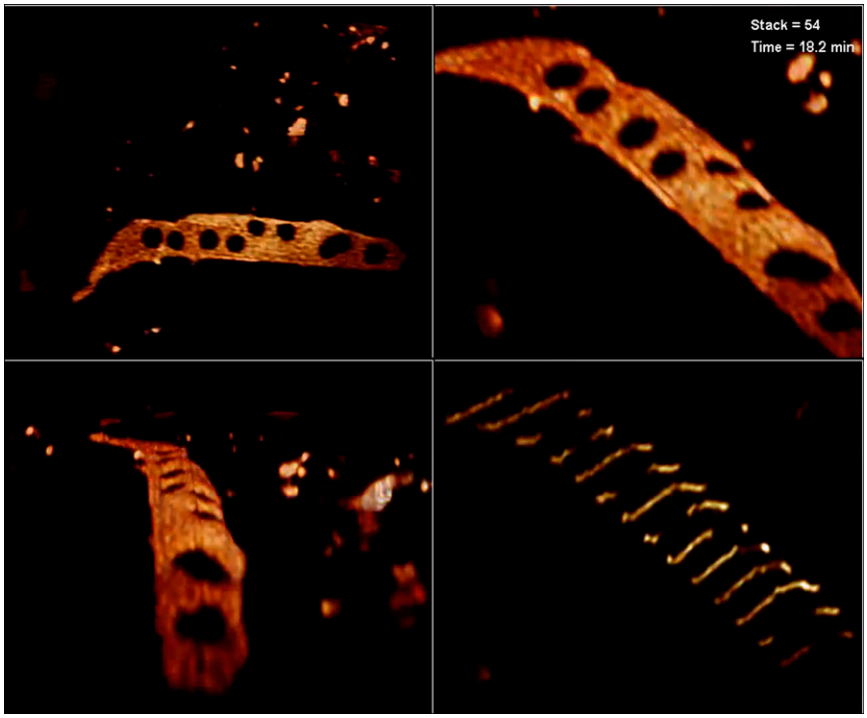
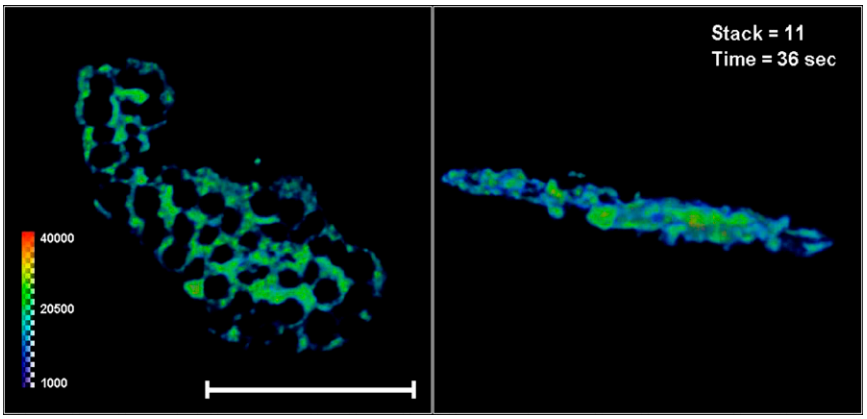


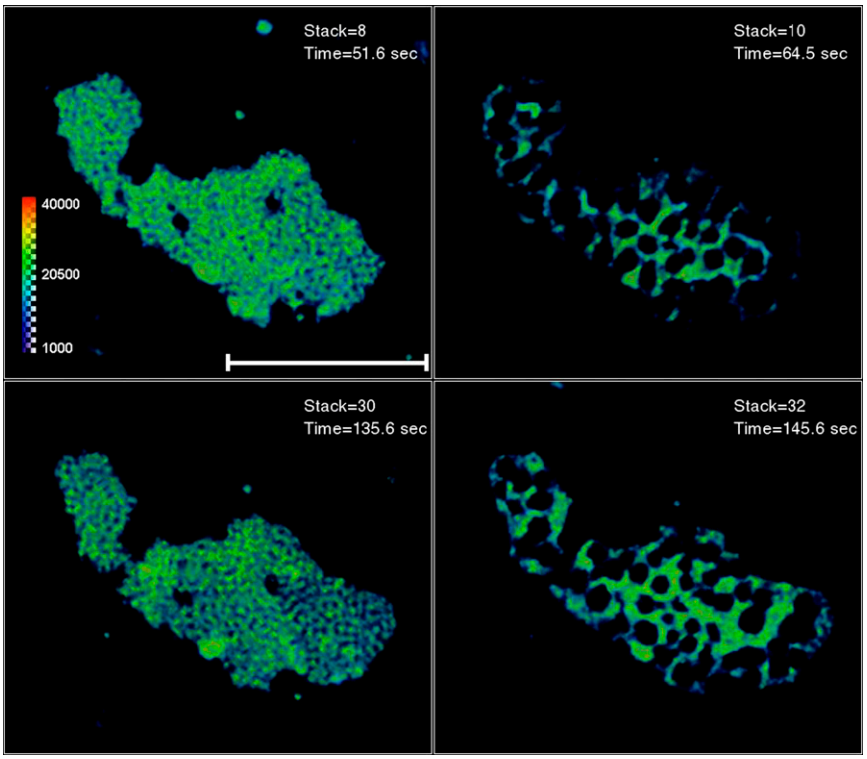
Movie S1. The formation of transient connexin-depleted regions (tCDRs) resolved by Bessel Beam plane illumination microscopy. A 3D projection of a gap junction (GJ) plaque labeled with fluorescently labeled connexin isoform 30 (Cx30-EGFP) is shown from different viewing angles. Note the presence of motile substructures clearly visualized within the GJ plaque that may represent clusters of connexin channels. The GJ plaque repeatedly responded to the application of cholera toxin (CTX) (three times) and Shiga toxin type 1 (STx1) (two times). Note the fast formation and recovery of multiple tCDRs in response to both AB5 toxins. Two hundred seven 3D image stacks were acquired every 20 s (total observation time, 69 min) and are shown in the time-lapse movie at 12 frames/s (i.e., at ~240× real speed). Selected frames also are shown in Fig. 1. Stack numbers and a time stamp are shown in the upper right corner. During the entire image acquisition only minimal fluctuations of total integrated fluorescence intensity of the 3D stack were observed (*SI Appendix*, Fig. S1*C*). However, a slow, steady decrease of total integrated fluorescence, most likely caused by photobleaching, is apparent over the course of the experiment (*SI Appendix*, Fig. S1*C*).

Movie S1



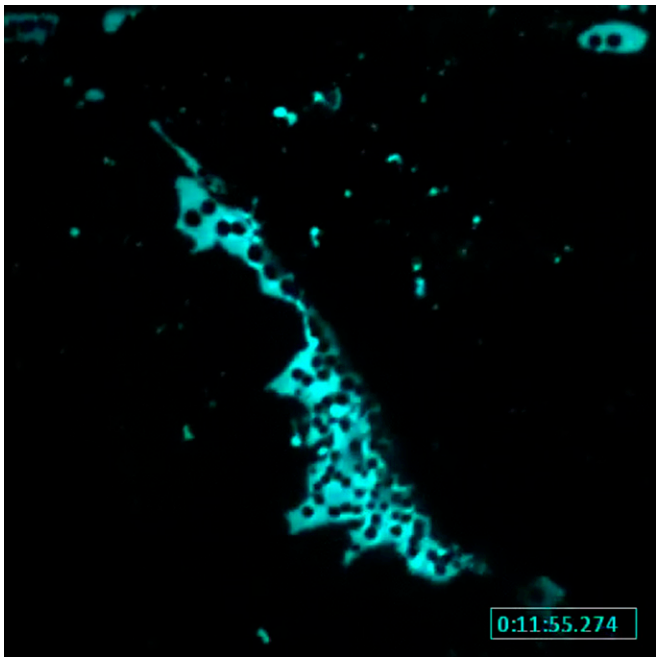
Movie S2. Dynamic 3D resolution of tCDR formation in a Cx30-EGFP–labeled GJ plaque induced by Shiga toxin. (Selected frames from this movie are shown in Fig. 2.) The left panel shows an *en face* view of the GJ plaque, and the right panel shows a side view of the GJ plaque from the 4D stack. Stationary CDRs (which in Fig. 2 *B* and *C* are indicated by pink arrows) recover toward the end of the movie. Note that the 3D outline, geometry, and size of the GJ plaque remain largely unchanged during tCDR formation and recovery and that both the rapid formation and slower disappearance of tCDRs occur much faster than the movement of structures containing the Cx30-EGFP membrane (*Right*). For this movie, 134 3D image stacks were acquired at 5-s intervals by two-photon mode Bessel beam plane illumination microscopy. The total time sequence represents 442 s and is displayed at 11 frames/s (36× real speed).

Movie S2



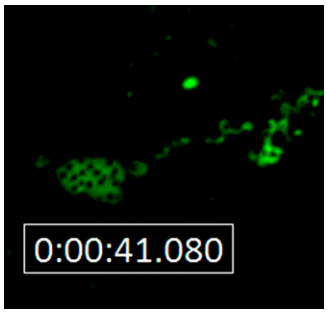
Movie S3. Rotation of a 3D rendering of image stacks 8, 10, 30, and 32 taken from Movie S2, showing the same GJ plaque before and after tCDR formation. Image stacks 8 and 10 and image stacks 30 and 32 document the tCDR response of the same GJ plaque to two pulses of STx1. Note two stationary CDRs in stacks 8 and 30 at the same positions before and after tCDR recovery. Also note that tCDR formation in response to the two pulses of STx1 occurs at different positions within the GJ plaque. Times are indicated in seconds.

Movie S3



Movie S4. Formation of connexin-depleted regions (CDRs) in a Cx30-CFP–labeled GJ plaque imaged using spinning disk confocal microscopy. Note the repeated, reversible tCDR formation in response to two pulses of CTX (added at time points 10 min and 24 min, respectively) and one pulse of Shiga toxin (added at 23 min). This movie corresponds to panels *A–E*, *G–K*, and *N–R* in Fig. 3 in the main text. Also note the smooth fluorescence of the GJ plaque (i.e., the absence of any visible substructures) in contrast to Movies S1–S3, which were acquired using Bessel beam plane illumination microscopy. The total time sequence shown corresponds to 6 h, 9 min. Time-lapse images were taken every15 s and are shown at 200× real speed. At time point 32 min (i.e., after tCDR recovery from three pulses of AB5 toxins), irreversible structural alterations in the GJ plaque were beginning and were accompanied by slow internalization of the GJ plaque over the next 5.5 h of observation. During the last 4 h of the movie, the acquisition rate was slower, one frame/2 min. Times are indicated in the time stamp box as h:min:s:ms.

Movie S4



Movie S5. The kinetics of tCDR formation recorded using a fast, free-run image acquisition with an Andor EM-CCD camera (with ~376 ms per frame). Note the very fast appearance of tCDRs within a time interval of 700–800 ms. The movie corresponds to the selected frames shown in *SI Appendix*, Fig. S2 *A–I* but represents a larger region of interest than shown in *SI Appendix*, Fig. S2 *D–I*.

Movie S5

Other Supporting Information Files

SI Appendix (PDF)