Movie Legends

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Single-Molecule Microscopy Reveals Plasma Membrane Microdomains Created by Protein-Protein Networks that Exclude or Trap Signaling Molecules in T Cells

Supplemental Data for Douglass et al.

Movie S1. Single Lck-GFP Diffusing in the Plasma Membrane of a Jurkat T Cell (MPG 1727 kb)

A video-rate sequence was acquired with laser illumination total internal reflection microscopy and an intensified cooled ccd camera (see Experimental Procedures). Duration, 6.67 s.

Movie S2. Dual-Color Imaging of LAT-GFP (Green) Relative to CD2-mRFP Clusters (Red) (MPG 3383 kb)

A population-level image of CD2-mRFP was captured using TIRF at low laser intensity and low camera gain, and then single molecules of LAT-GFP were imaged in the same cell at higher laser illumination. Each frame of the video-rate GFP sequence was then overlaid onto the CD2-mRFP image. Duration, 5.13 s.

Movie S3. Dual-Color Imaging of Lck-GFP (Green) Relative to CD2-mRFP Clusters (Red) in the Cell Shown in Figure 6A. (MPG 1763 kb)

Imaging was performed as in Movie S2, except that an ImageJ fast Fourier transform bandpass filter was applied to the CD2-mRFP image. Image brightness and contrast were then adjusted manually to maximize the intensity difference between clustered and nonclustered regions of the cell surface. Duration, 6.67 s.

Movie S4. Representation of Single-Molecule Trajectories among CD2 Clusters (MPG 2420 kb)

Imaging and adjustment of the CD2 image were performed as in Movie S2. For clear depiction of single-molecule trajectories, a scheme was devised in which a Gaussian spot was drawn at each particle centroid over time and superimposed upon the CD2 background image. The results of this method for a single-molecule of LAT-GFP are shown in the left panel of the movie, while the raw video sequence is shown at right. This clip also clearly demonstrates diffusional trapping of LAT-GFP within CD2 zones. Duration, 4.77 s.

Movie S5. CD2 Clusters Limit the Free Diffusion of Single Molecules (MPG 424 kb)

The centroid positions (green dot) of single molecules of CD45-GFP (Movie S5) or LAT(Y-F) (Movie S6) were tracked at video rate, and the images were superimposed. Movies show that these molecules are deflected from CD2 cluster boundaries and tend to diffuse in the space between clusters. Duration, 4.87 s.

Movie S6. CD2 Clusters Limit the Free Diffusion of Single Molecules (MPG 380 kb)

See Movie S5 legend. Duration, 2.47 s.

Movie S7. Diffusional Trapping of Single LAT-GFP Molecules in CD2 Clusters (MPG 493 kb)

Imaging was as described for Movie S4. A video-rate movie shows that restricted diffusion of LAT-GFP occurs when the centroid of the single molecule superimposes with the CD2-enriched zone. Duration, 6.03 s. See also the LAT-GFP trajectory in Movie S4.

Movie S8. Diffusional Trapping of Single Lck-GFP Molecules in CD2 Clusters (MPG 279 kb)

Video-rate movie shows that restricted diffusion of Lck-GFP occurs when the centroid of the single molecule superimposes with the CD2-enriched zone. Duration, 2.80 s.

Movie S9. Diffusional Trapping of Single Lck-GFP Molecules in CD2 Clusters (MPG 512 kb)

See Movie S8 legend. Duration, 4.57 s.

Movie S10. A Model of Protein-Protein Interaction-Mediated Diffusional Trapping and Exclusion as the Basis for Signaling Domain Formation in T Cells (MPG 7567 kb)