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Suppressed concentration fluctuations in rat basophilic leukemia cell synapse: (indirect) evidence for large signaling complexes RACHEL DRAWBOND, BioFrontiers Center and Department of Physics and Energy Science, University of Colorado at Colorado Springs, JAMES THOMAS, Department of Physics and Astronomy, University of New Mexico, KATHRIN SPENDIER, BioFrontiers Center and Department of Physics and Energy Science, University of Colorado at Colorado Springs — The spatial extent of membrane receptor signaling complexes can be difficult to determine. Using a concentration fluctuation signature, we show an indirect way of determining the size of the IgE-FcεRI receptor signaling complex (IgE-RC) in rat basophilic leukemia (RBL) cells. This approach applies the concept that at high IgE-RC area fractions, randomly placed complexes cannot obey Poisson statistics, due to excluded area. When IgE-loaded RBL cells interact with a supported lipid bilayer (SLB) presenting binding ligands, IgE-RCs coalesce to form a large central patch called the mast cell synapse. RBL cells labeled with varying concentrations of fluorescent and dark anti-DNP IgE settled onto SLBs with 25 mol% DNP-lipid. Using TIRF microscopy, synaptic patches were imaged. At high fractions of fluorescent IgE, the spatial variance of the fluorescence fluctuations was observed to be suppressed, compared to the variance expected from Poisson statistics. Comparison of experiment to computer models suggest that the actual size of IgE-RC is at least two times larger than reported in literature, indicating that additional cytosolic or membrane proteins may associate with IgE-RCs.

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