

Membrane-Membrane Proximity Induced by Ca²⁺-dependent Multivalent Binding of Synaptotagmin 1 to Phospholipids

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Supplementary Methods

Protein expression and purification

Wild type and mutant C₂AB-fragments were expressed in Escherichia coli BL21 cells at 20°C overnight. The cell pellet was resuspended in 40 mM Tris (pH 8.2), 200 mM NaCl, 1% Triton X-100 buffer containing 2 mM DTT and protease inhibitors. The cells were passed 3 times through an EmulsiFlex-C5 cell disrupter (Avensin) at 14000 psi and spun at 28000 g for 30 min. The supernatant was incubated with glutathione-sepharose beads. The resin was extensively washed with at least 200 ml of 40 mM Tris (pH 8.2), 200 mM NaCl buffer followed by 200 ml of 40 mM Tris (pH 8.2), 200 mM NaCl, 50 mM CaCl₂ buffer, which was repeated 2 times. The C₂AB-fragment was cleaved from the GST moiety by thrombin (12 units) in 10 ml of thrombin cleavage buffer (50 mM Tris [pH 8.0], 200 mM NaCl, 2.5 mM CaCl₂, 1 mM DTT) at 25°C for 3 hrs. The protein was eluted from the resin with 200 mM NaP (pH 6.2), 300 mM NaCl buffer and purified by cation exchange chromatography on SourceS column (Pharmacia) in 50 mM NaAc (pH 6.2), 5 mM CaCl₂ buffer using a linear gradient from 300 mM to 600 mM NaCl in 8 column volumes. UV spectra were acquired on a Hewlett-Packard 8452A spectrophotometer to verify the lack of bacterial contaminants, which absorb at 260 nm. Proteins were stored at -80°C after adding 0.3 mM TCEP to the samples.