A Munc13/RIM/Rab3 Tripartite Complex: From Priming to Plasticity?

Irina Dulubova[1], Xuelin Lou[2], Jun Lu[1], Iryna Huryeva[1], Amer Alam[1], Ralf Schneggenburger[2], Thomas C. Südhof[3] and Josep Rizo[1,4]

[1]*Departments of Biochemistry and Pharmacology,* [3]*Center for Basic Neuroscience, Department of Molecular Genetics, and Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, Texas 75390, USA*

[2]*Max Planck Institute for Biophysical Chemistry, AG Synaptic Dynamics & Modulation and Dept. of Membrane Biophysics, Am Fassberg 11, 37077 Germany*

[4] Corresponding author. E-mail: jose@arnie.swmed.edu. Phone: 214-645-6360. FAX: 214-645-6353

Supplementary Figure 1. Backbone superposition of the 20 structure of the RIM2α ZF domain with the lowest energies.

Methods. Resonance assignments, NOEs, and amide protection data for structure determination were obtained from a series of two-dimensional (2D) and three-dimensional (3D) experiments basically as described (Garcia et al, 2004). These experiments included 3D ^{1}H-^{15}N TOCSY-HSQC and NOESY-HSQC, HNCO, HNCACB, CBCACONH, H(C)(CO)NH-TOCSY, (H)C(CO)NH-TOCSY and HCCH-TOCSY spectra for resonance assignments (Kay et al, 1993, 1994, Zhang et al, 1994, Muhandiram and Kay, 1994), and 2D NOESY, 3D ^{1}H-^{15}N-NOESY-HSQC, and 3D ^{1}H-^{13}C NOESY-HSQC experiments (150 ms mixing times) to measure NOEs for structure determination. Aromatic proton chemical shifts were assigned via 2D DQF-COSY, TOCSY (τ_m = 38ms), and NOESY (τ_m = 150ms) spectra recorded in 99.9% D_2O. Stereospecific

assignments of valine and leucine methyl groups were obtained from a ^{1}H-^{13}C HSQC spectrum acquired on a 0.7 mM sample of 10% ^{13}C-labeled sample. Hydrogen bond restraints were set for amide protons protected from exchange with D_2O for more than 12 hr at pH6.9 with 1.5 Å < d_{NH-O} < 2.5 Å and 2.5 Å < d_{N-O} < 3.5 Å. Backbone torsion angles restraints were derived from chemical shift analysis using TALOS (Cornilescu et al, 1999). All data were processed with NMRPipe (Delaglio et al, 1995) and analyzed with NMRView (Johnson and Blevins, 1994). Aria2.0 (Linge et al., 2001) was used to calculate the final 20 structures starting from extended conformations.

References

Cornilescu, G., Delaglio, F., and Bax, A. (1999) Protein backbone angle restraints from searching a database for chemical shift and sequence homology. *J Biomol NMR*, **13**, 289-302.

Delaglio F, Grzesiek S, Vuister GW, Zhu G, Pfeifer J, Bax A (1995) NMRPipe: a multidimensional spectral processing system based on UNIX pipes, *J Biomol NMR*, **6**, 277-293.

Garcia J, Gerber SH, Sugita S, Südhof TC, Rizo J. (2004) A conformational switch in the Piccolo C2A domain regulated by alternative splicing. *Nat. Struct. Mol. Biol.*, **11**, 45-53.

Johnson BA and Blevins RA (1994) NMRView: a computer program for visualization and analysis of NMR data, *J.Biomol. NMR*, **4**, 603-614.

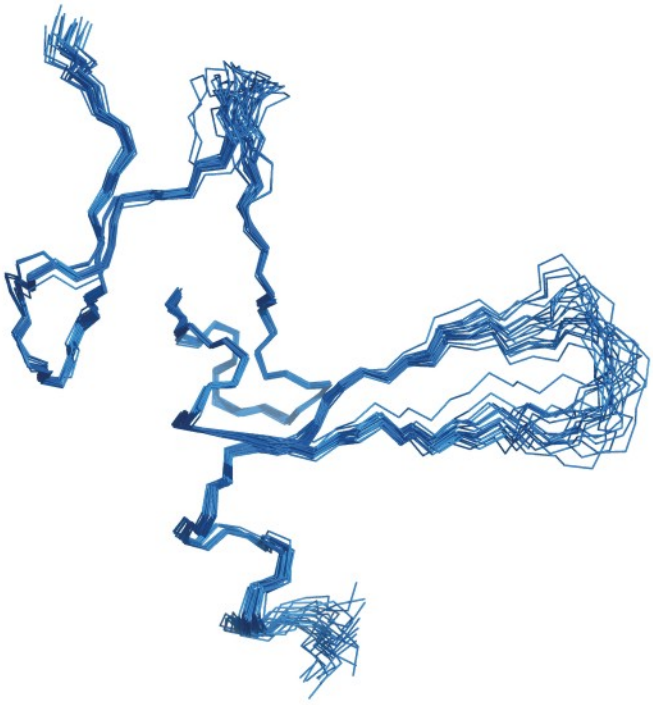
Kay LE, Xu GY, Singer AU, Muhandiram DR, Formankay JD (1993) A Gradient-Enhanced Hcch Tocsy Experiment for Recording Side-Chain H-1 and C-13 Correlations in H2O Samples of Proteins. *Journal of Magnetic Resonance Series,* **B 101**, 333-337.

Kay LE, Xu GY, and Yamazaki T. (1994) Enhanced-Sensitivity Triple-Resonance Spectroscopy with Minimal H2O Saturation. *Journal of Magnetic Resonance Series,* **A 109**, 129-133.

Linge, JP, O'Donoghue, SI and Nilges, M (2001). Automated assignment of ambiguous nuclear overhauser effects with ARIA. *Methods in Enzymology*, **339**, 71-90.

Muhandiram DR and Kay LE (1994) Gradient-Enhanced Triple-Resonance 3-Dimensional Nmr Experiments with Improved Sensitivity. *Journal of Magnetic Resonance Series,* **B 103**, 203-216.

Zhang O, Kay LE, Olivier JP, Forman-Kay JD (1994) Backbone ^{1}H and ^{15}N resonance assignments of the N-terminal SH3 domain of drk in folded and unfolded states using enhanced-sensitivity pulsed field gradient NMR techniques. *J. Biomol. NMR, **4***, 845-858.



Dulubova et al., Suppl. Fig. 1