Supplementary Materials and Methods

Energy perturbation calculations

Molecular dynamics (MD) simulations were started from the x-ray structure of the neuronal SNARE complex (chains A-D from PDB code: 1SFC (Sutton et al., 1998)). The simulation system contained 3,001 protein atoms, 8,275 SPC water molecules (Berendsen et al., 1981), and 16 sodium ions, resulting in a system size of 27,842 atoms.

MD simulations were carried out using the GROMACS simulation suite (Lindahl et al., 2001). Shake and Settle (Ryckaert et al., 1977; Miyamoto and Kollman, 1992) were applied to constrain covalent bond lengths, allowing an integration step of 2 fs. The temperature was kept constant by separately coupling (tau = 0.1 ps) the protein and solvent to an external temperature bath (Berendsen et al., 1984). The pressure was kept at 1 bar by weak coupling (tau = 1.0 ps) to a pressure bath (Berendsen et al., 1984). The GROMACS force field was applied, which is the GROMOS 87 force field (van Gunsteren and Berendsen, 1987) with slight modifications (Van Buuren et al., 1993) and explicit hydrogens on the aromatic side chains. Polar hydrogens were attached to the protein using the ADDHYD routine implemented in the WHAT IF package (Vriend, 1990). To equilibrate the system, 5 ns of conventional molecular dynamics were performed. At a distance smaller than 1.0 nm, electrostatic interactions were calculated explicitly, long-range electrostatic interactions were calculated by particle-mesh Ewald summation (Darden et al., 1993).

The free energy changes ΔG associated with the mutations (see Table 1) were calculated using free energy perturbation (FEP) simulations. Using a thermodynamic cycle, the difference in stability between the WT and mutant protein was estimated from difference $\Delta\Delta G$ in the free energy changes ΔG for the mutation in the folded protein and in a model of the unfolded state (modeled as a tripeptide in solution). All FEP simulations were carried out using the method of slow growth (Bash et al., 1987, Ossig et al., 2000), i.e. by gradually introducing the mutation into the simulated system (using soft-core parameters α=0 (resulting in linear interpolation of the non-bonded interactions) and σ=0.3 nm) during a simulation period of 1 ns. For both the folded and unfolded state (tripeptide), forward and backward mutations were simulated to ensure reversibility and to quantify hysteresis effects for error estimation.

All FEP simulations started from the equilibrated structure, using a time step of 1 fs and cut-off radii of 1.0 and 1.4 nm for the van der Waals and electrostatic forces, respectively.

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