SUPPLEMENTAL EXPERIMENTAL PROCEDURES

CONSERVED PROPERTIES FOR POTRA DOMAINS FROM THE STRUCTURE OF THE PERIPLASMIC REGION OF A CYANOBACTERIAL OMP85 PROTEIN

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Experimental Phasing – Experimental phasing of the tetragonal crystal form was carried out using selenomethionine modified proteins. However, this phasing strategy was initially not accessible as the expression construct contained no internal methionines. Thus, the three hydrophobic amino acids F291, L345 and L373 were exchanged with methionine, using site directed mutagenesis. Selenomethionine protein was expressed using a metabolic inhibition protocol (1). Of the three selenomethionine labeled variants, *ana*Omp85-POTRA F291M crystallized from the crystallization buffer of the tetragonal crystal from, containing additionally 0.24 M Na thiocyanate and 0.01 M NaAcetate, pH 4.6. SAD data were collected at a wavelength of 0.934Å (high remote) at a fixed wavelength beamline. HKL2MAP/ShelxD (2,3) was used to determine the selenomethionine substructure, followed by phasing and automatic model building using SOLVE/RESOLVE (4,5). The initial model compromised 173 amino acids, 147 of which were assigned to alanine.

Molecular dynamics simulation – Molecular dynamics simulations were performed with GROMACS v4 (6) and the GROMOS96 united-atom force field 53a6 (7). The MD system setup and MD simulations were performed as described earlier (8) if not stated otherwise. In brief: the simulation box contained 62,796 water molecules, 5 sodium counter ions, and 2485 atoms from the protein; 190,878 atoms in total. The protein was at least 2.25 nm away from the boundaries of the rhombic-dodecahedron-shaped simulation box. Short-range Coulomb interactions were cut off at 1.0 nm and van-der-Waals forces at 1.4 nm. Long-range electrostatic interactions were handled by PME (9). Temperature coupling was performed with GROMACS' v-rescale method (10). The protein was subjected to a steepest descents energy minimization, followed by 100 ps of MDS with the non-hydrogen atoms fixed to their starting positions. MD simulations of four replica of this system were set up with different random starting velocities with a targeted simulation time of 100 ns each.

Angle calculation between POTRA domains – Swing and twist angles of POTRA domains as shown in Fig. 2 were calculated with a Yasara macro (www.yasara.org). For swing angle calculation the central β-strand of the β-sheet of each POTRA domain is defined as major axis. The first amino acid in the major axis is defined as hinge of the respective POTRA domain. A reference point for twist angle calculation is defined by the α-helix α2. The orientation of the protein is set to (0,0,0) for the Euler-like angles α, β and γ. The following procedure is repeated iteratively for each POTRA domain in the direction from N to C terminus: 1. center on the hinge, 2. calculate the direction of the major axis, 3. the swing angle is the arc cosine between the direction vector and the X-axis. 4. rotate major axis of current POTRA domain and the POTRA domains C terminal to the current one into the X-axis, 5. twist angle is the arc tangent between the Y and Z component of the reference point of α-helix α2, 6. rotate the current and following POTRA domains around the X-axis by the twist angle. In order to deal with the flexibility of the secondary structure elements used to define the major axis and the reference point (see above) during the MD simulations, we reduced the "noise" introduced by these structural fluctuations by superimposing each of

the POTRA domains in a simulation frame with the respective POTRA domain from the starting structure of the simulations. These superimposed POTRA domains were joined to form a single object, which was used for the angle calculations described above.

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