

Method S4

Detailed protocol of the docking simulation between the FHA and the tandem BRCT.

The conformation of the residue separating the FHA domain from the tandem BRCT domain is not known. Hence, to explore the potential orientation of the FHA with respect to the tandem BRCT, docking of the FHA-tandem BRCT complex was performed using the software HADDOCK1.3 (Dominguez et al., 2003) in combination with CNS (Brunger et al., 1998). The only constraint added was that at least one atom of the C-terminal residue of the FHA be in atomic contact (2 Å) contact with one atom of the N-terminal residues of the tandem BRCT. The starting structures for the docking were the 20 models of the FHA of Nbs1 and the model of the tandem BRCT of Nbs1 evaluated in this study. All the models were built using Modeler v8.2 (Sali and Blundell, 1993). In total, 2,000 rigid-body docking solutions for 20 models of the FHA were first generated by energy minimization. The driving force for the docking at this stage comes mainly from the constraint between the C- and N-termini and from van der Waals and electrostatic energy terms once the structures are within the nonbonded cut-off (8.5Å). The 200 best solutions according to intermolecular energies (Dominguez et al., 2003) were subjected to semiflexible simulated annealing in torsion angle space followed. During the simulated annealing, the amino acids at the interface (side chains and backbone) are allowed to move to optimize the interface packing. The nonbonded energies were calculated with the OPLS parameters using an 8.5Å cut-off. The electrostatic energy was calculated in vacuum with an epsilon value of 10. The analysis of the distance separating the pSer and the pThr was carried out by measuring the distance between