Supporting Information

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SI Text

Materials and Methods. ***Expression constructs and protein purification.*** Recombinant mouse Keap1 (M1–R614) protein fused to a six-His tag at the C terminus was expressed in BL21-CodonPlus (DE3)-RIPL cells (Stratagene) and purified using Ni-NTA agarose (Qiagen). The Keap1 dimer was then isolated twice by a Superdex S-200 size exclusion column (SEC; GE Healthcare).

Transmission electron microscopy. Purified Keap1 protein at approximately 50 μg/ml was adsorbed by thin carbon film supported by copper mesh grid, rendered hydrophilic in advance by glow-discharge in low air pressure. Samples were washed with five drops of double-distilled water, negatively stained with 2% uranyl acetate solution for 30 s twice, blotted, and dried in air. Micrographs of negatively stained particles were recorded in a JEOL 100CX transmission electron microscope (EM) at a magnification of 52,100 with a 100-kV acceleration voltage. Images were recorded on SO-163 film (Eastman Kodak), developed with a D19 developer (Eastman Kodak), and digitized with a Scitex Leafscan 45 scanner (Leaf systems) at a pixel size of 1.92 Å at the specimen level.

Molecular complexes between Keap1 and the antibodies were generated by mixing purified Keap1 with an anti-mouse DC monoclonal antibody at 4° C for 4 h. After binding to the Ni-NTA agarose, excess antibodies were washed out. Bound proteins were eluted from the gel by using a buffer containing 150 mM imidazole. The Keap1/anti-DC antibody complex was separated from the unbound proteins by SEC, negatively stained and observed by EM (1). To obtain clearer images of antibody binding, protein G-gold (50 Å) conjugate (BB international) was used. Anti-Keap1 antibody/protein G-gold/Keap1 protein complexes were similarly purified by Ni-NTA agarose. The complexes were further isolated by SEC, negatively stained, and observed by EM.

Image analysis. We have developed a single particle image analysis method by using neural network (2–4) and simulated annealing (4–6) named SPINNS (6). Keap1 projections were picked up using the auto-accumulation method with simulated annealing (SA) (5). Two-hundred and sixteen particles in 200×200 pixel subframes were selected and used to train a three-layer pyramidal-type neural network (NN) (2, 3). By use of the trained NN, 12,651 particles were selected. After background subtraction, the particles selected by the NN were aligned rotationally and translationally (8, 9) by the reference free method (3). The aligned images were sorted into 250 classes by the modified growing neural gas (GNG) network method (4). Their class averages were adopted as new references (8, 9), and this cycle, from alignment to averaging, was repeated 14 times.

Euler angles of the class averages were automatically determined by the echo-correlated 3D reconstruction method with SA (6) assuming twofold symmetry and used to calculate a primary 3D structure by the simultaneous iterative reconstruction technique (SIRT) (10). The reprojections from the volume were employed as references for multi-reference alignment (MRA), and raw images in the library were aligned and further clustered to provide improved cluster averages. From these averages, a new 3D map was generated by the reconstruction method by using SA without a 3D reference.

The 3D map was further refined by projection matching (11) followed by echo-correlated reconstruction. This iteration was repeated for 10 cycles assuming twofold symmetry, followed by 4 cycles without any imposed symmetry. Particle images that correlated poorly with the 3D projections were automatically rejected by using the cross-correlation function. Final reconstruction includes 9,827 particles, 77.7% of all the selected images. The resolution of the final 3D map was assessed using the Fourier shell correlation (FSC) function (12) at a threshold of 0.5.

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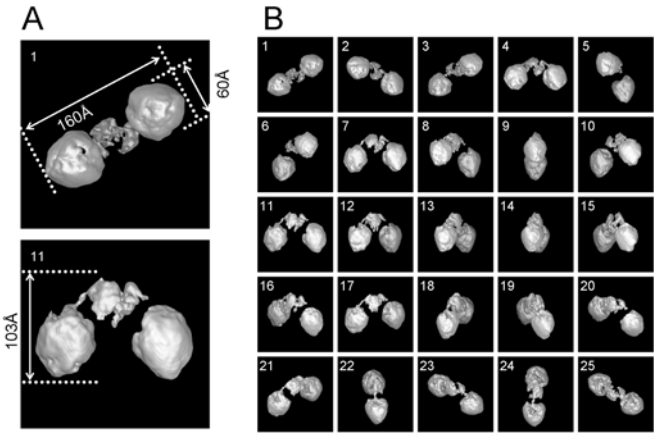

Fig. S1. Surface representation and structural features of Keap1. (*A*) Top and side views of the Keap1 protein. Keap1, presumably in its reduced form, resembles two cherries joined at the stems, with dimensions of $160 \times 60 \times 103$ Å. The molecular mass enclosed by the isosurface is 155.3 kDa, corresponding to 110.3% of the dimeric Keap1 protein. (*B*) Surface views from 25 different angles.

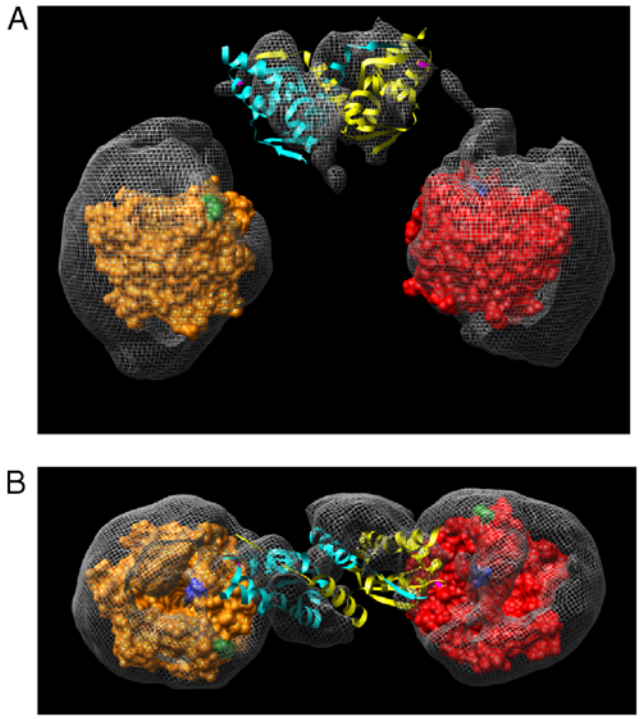

Fig. S2. Model of the entire Keap1 homodimer. (*A*) The mouse Keap1-DC x-ray model was fitted into the globular density. The x-ray model of a BTB homologue, the LRF BTB-zinc finger protein (PDB accession code 2NN2), is a near-fit with the stem-like region. Asn103 of LRF, which corresponds to Cys151 of Keap1 by sequence homology alignment, is colored *magenta* in the x-ray ribbon structural model. BTB monomer of the LRF homodimer x-ray model is colored in either *yellow* or *cyan*. Color scheme for the DC domain is the same as in Fig. 5. (*B*) Top view of the Keap1 homodimer model.