Supporting Information

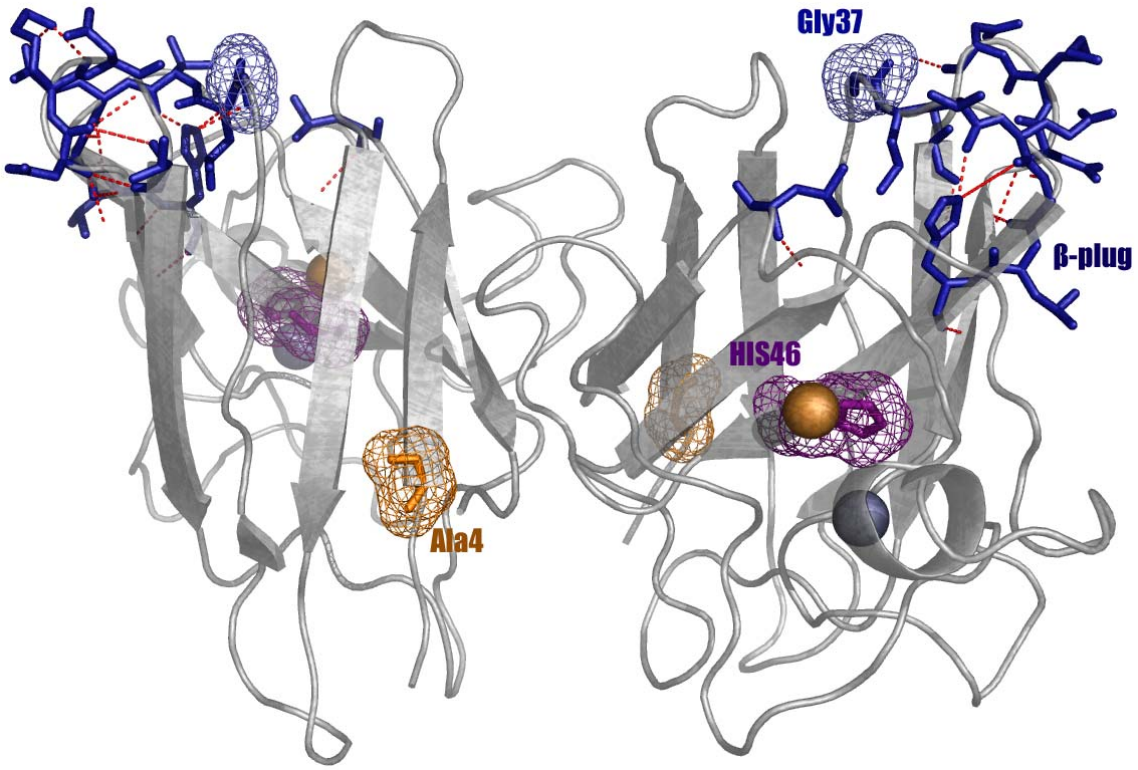

Fig. 5. The structure of wild-type SOD1. The structure of wild-type SOD1 and the location of familial amyotrophic lateral sclerosis (FALS)-associated mutants A4V, H46R, and G37R. The β-plug is denoted by the blue color.

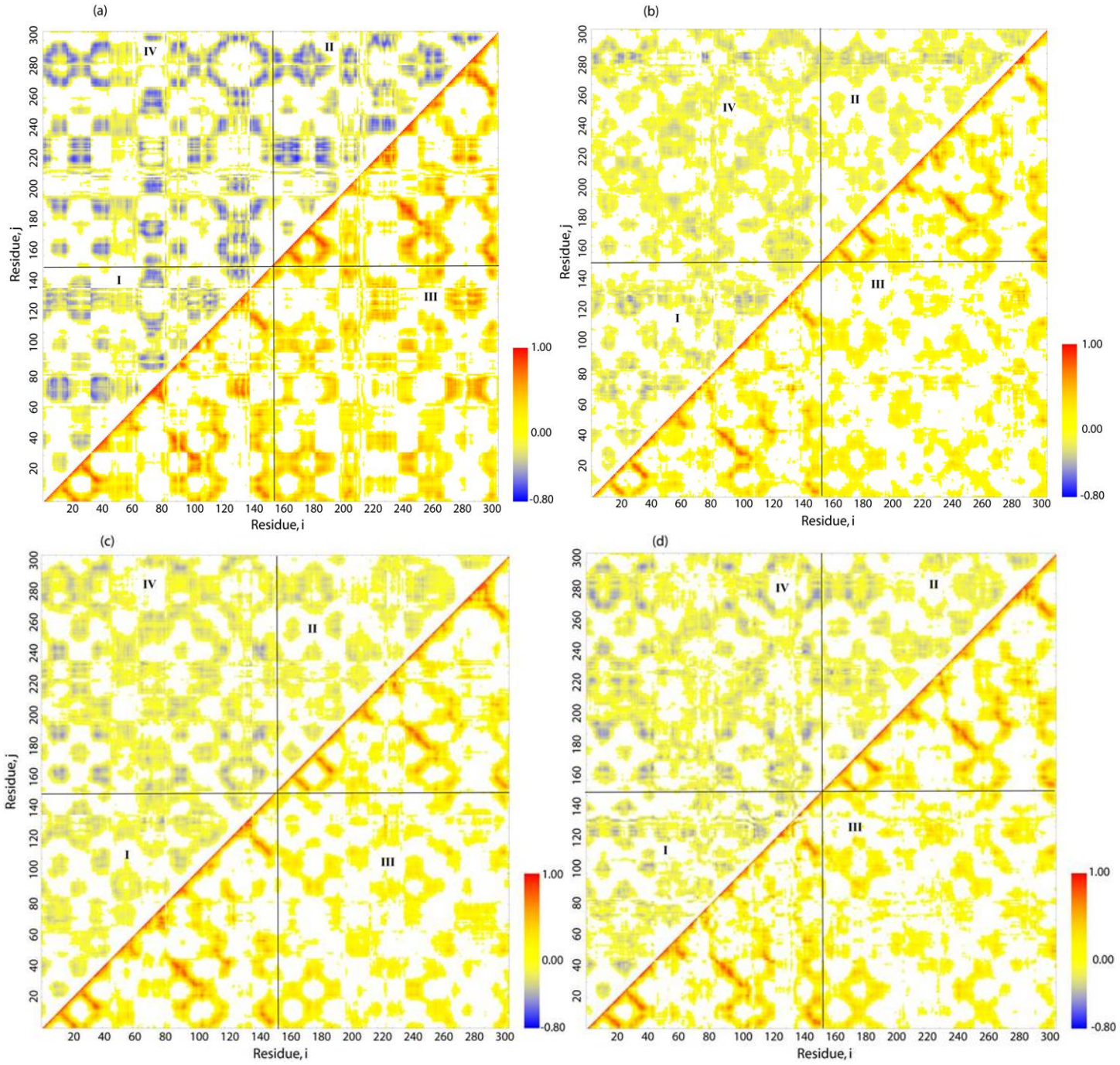

Fig. 6. Covariance matrices of wild type and A4V, H46R, and G37R mutants. (*a*) Wild-type dimer. (*b*) A4V dimer. (*c*) H46R dimer. (*d*) G37R dimer. Shown are 5-ns MD trajectories at 300 K. Positive correlations (red) are below the main diagonal, whereas anticorrelations (blue) are above the main diagonal. Squares I and II represent the correlations within the two monomers, and III and IV represent the correlations and anticorrelations between the monomers, respectively.

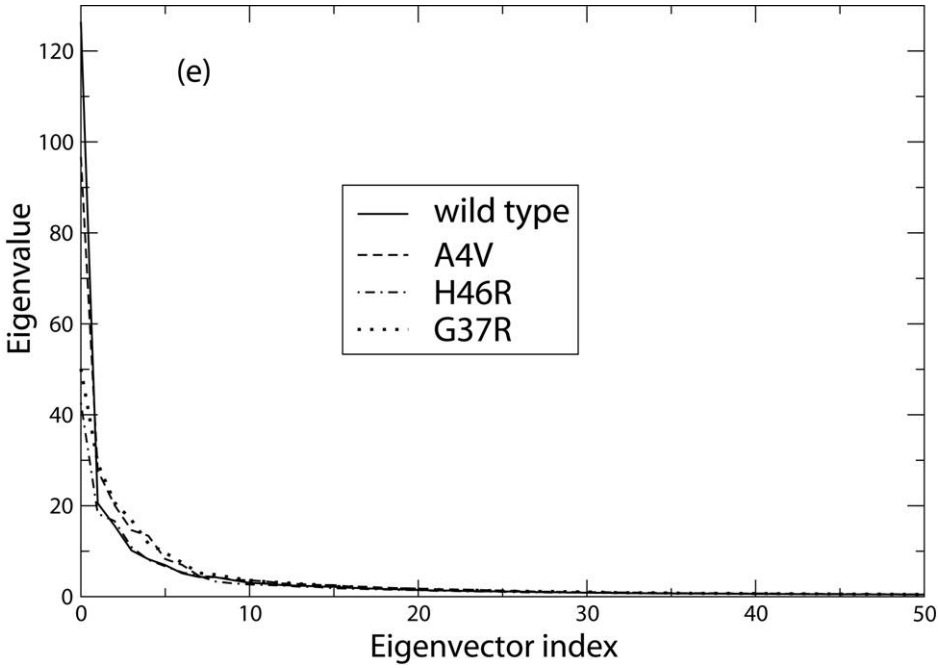

Fig. 7. The magnitude of the 50 largest eigenvalues for wild-type, A4V, H46R, and G37R dimers at 300 K.

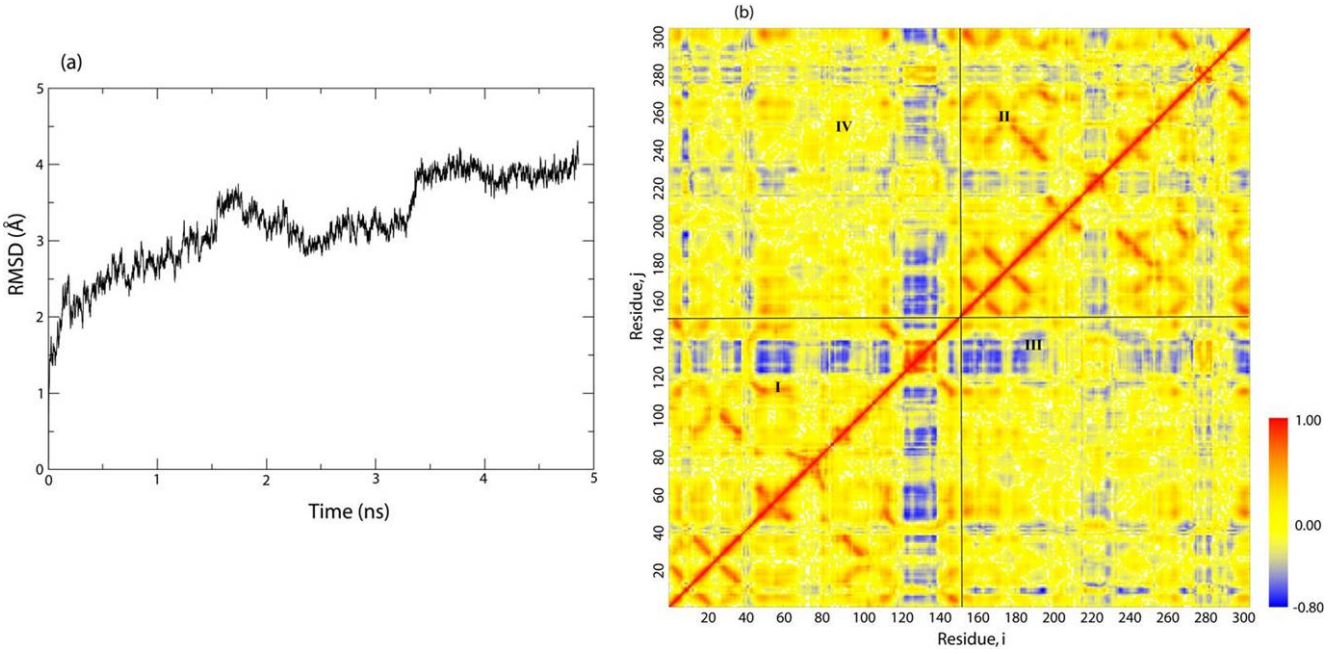

Fig. 8. (*a*) RMSD vs. time for the wild-type dimer trajectory at 400 K. (*b*) The covariance matrix for the wild-type dimer at 400 K.

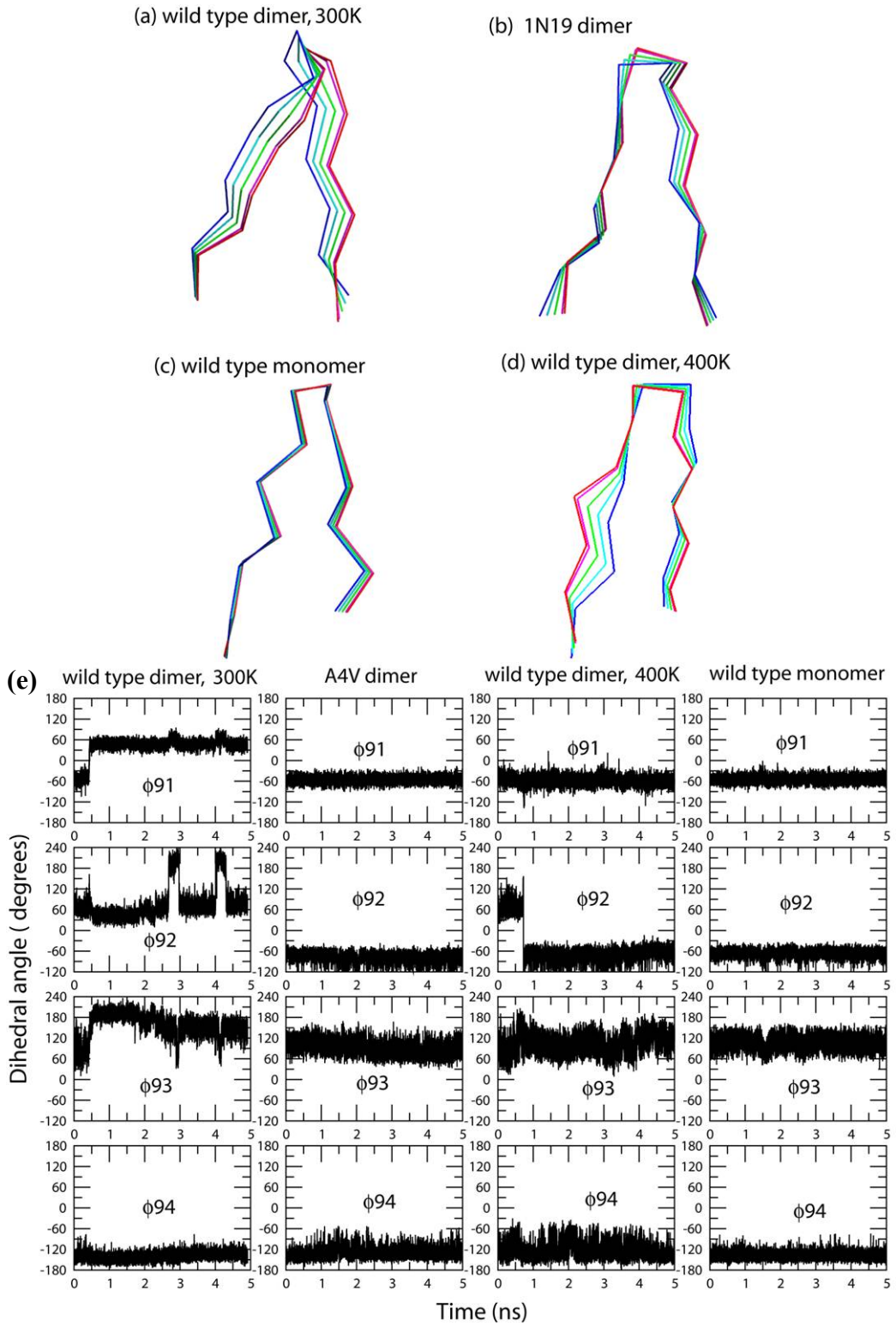

Fig. 9. The principal motion of the β-plug loop during the 5-ns MD trajectory for the wild-type dimer at 300 K (*a*), a mutant (A4V) dimer (*b*), the wild-type monomer (*c*), and the wild-type dimer at 400 K (*d*). (*e*) The time course of the dihedral angle Φ for the residues 90–94 in the β5–β6 loop, for the wild-type dimer at 300 K, the A4V dimer, the wild-type monomer, and the wild-type dimer at 400 K. Consistent with the higher

flexibility of the loop observed in *a*, the residues 90–93 undergo flipping motions in the wild-type dimer at 300 K but are rigidified in all mutant dimers, monomer, and at 400 K (data for H46R and G37 dimers not shown).

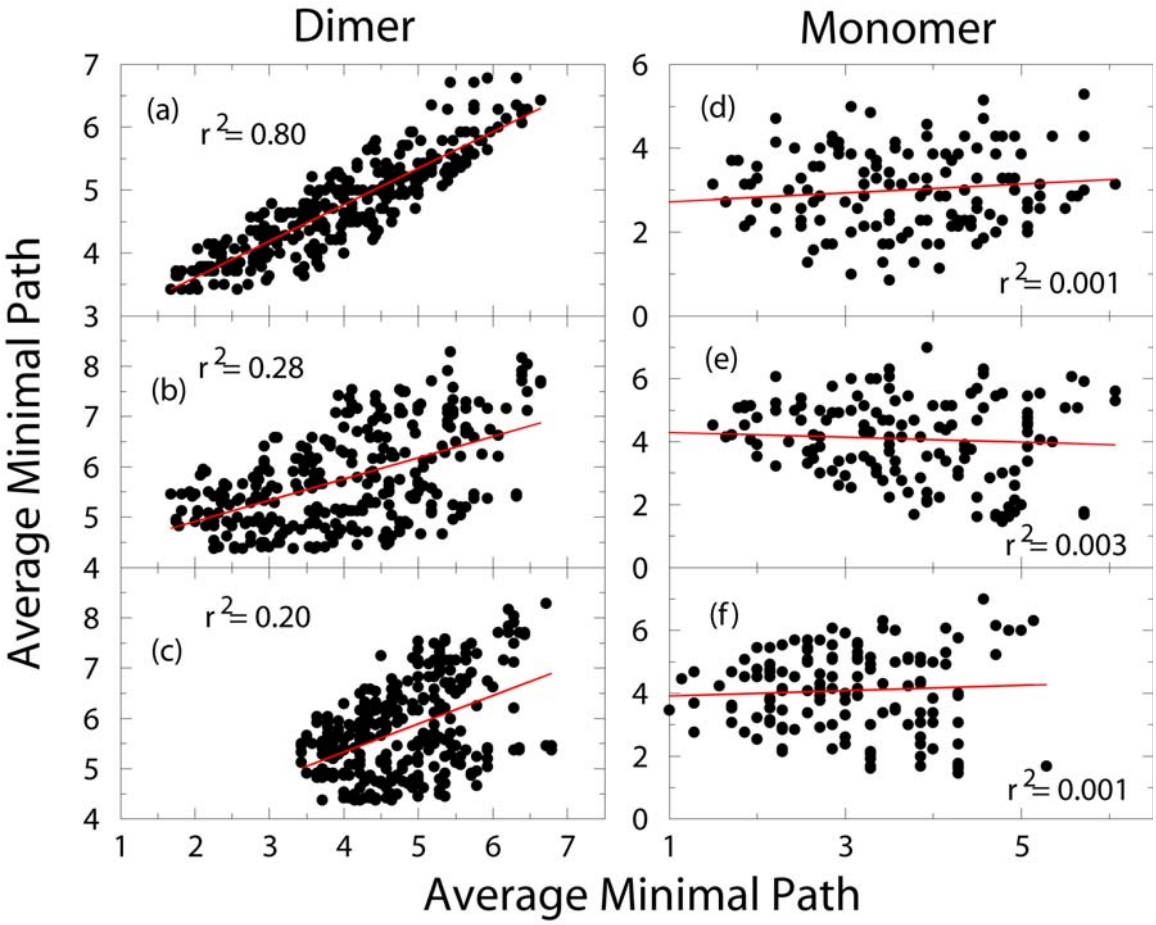

Fig. 10. We compute the average minimal paths between all residues in SOD1 dimer or monomer and three regions: the dimer interface, the metal-binding sites, and the control β-plug region. The averaging is performed over all minimal paths that connect a given residue *i* to those *j* in a specified region *R*, i.e., L_{iR} = (1/number of path)$\sum_{j \in R} L_{ij}$. (*a* and *d*) The correlation between average minimal paths from all residues in SOD1 (*a*) dimer [or monomer (*d*)] to the residues on the interface (*y* axis) and the metal-binding sites (*x* axis). As a control, the average minimal paths between all residues and the β-plug region (*y* axis in *b*–*f*) shows no correlation with that between all residues and the dimer interface (*x* axis in *b* and *e*) or the metal-binding sites (*x* axis in *c* and *f*).

Table 1. Sum of correlations and anticorrelations within and between monomers

Dimer	Within monomer 1	Within monomer 2	Intermonomer
Wild type	3,983.1 (34%)	4,880.12 (40%)	3,173.67 (26%)
A4V	1,947.60 (49%)	1,768.28 (44%)	290.62 (7%)
H46R	2,075.61 (48%)	1,734.55 (40%)	524.46 (12%)
G37R	2,240.45 (46%)	1,979.24 (40%)	724.84 (14%)

We also calculate the values of *C*inter over two nonoverlapping, 2-ns windows of the 5-ns trajectory. We find that the correlation matrices of these sub-trajectories and the obtained *C*inter values are similar to each other and to the complete 5-ns trajectory (Pearson correlation coefficient $r^2 \approx 0.8$). Therefore, we believe that the differences between mutant and wild-type correlation matrices are statistically significant.

Table 2. Hydrogen bonds in the β-plug region in the MD trajectories

Donor residue	Donor atom	Receptor residue	Receptor atom	Frequency intact			
				Wild type	A4V	H46R	G37R
39	O	43	HE2	0.97	0.96	0.93	0.96
90	OD1	91	H	0.60	—	—	—
90	OD2	92	H	0.31	0.79	0.76	0.81
90	O	93	H	0.89	0.93	0.95	0.85
90	O	94	H	0.90	—	—	—
93	O	37	H	0.81	0.36	0.26	—
93	O	38	H	—	0.93	0.89	0.97
94	O	90	H	0.99	—	—	—
39B	O	43B	HE2	0.97	0.97	0.97	0.96
90B	OD1	91B	H	0.28	—	0.37	—
90B	OD2	92B	H	0.79	0.51	—	0.69
90B	O	93B	H	0.99	0.97	0.96	0.94
90B	O	94B	H	0.41	—	—	—
93B	O	37B	H	0.86	0.42	—	—
93B	O	38B	H	0.44	0.90	0.96	0.95
94B	O	90B	H	0.96	0.55	—	0.31

—, frequency less than 0.2.

Supporting Methods

Protocol for Molecular Dynamics (MD) Simulations. The system was equilibrated by using the following protocol. First, the protein was fixed with positional restraints (spring constant of 500 kcal•mol^{-1}•Å^{-2}), and the solvent was energy minimized by using 500 steps of steepest descent followed by 500 steps of conjugate gradient minimization with constant volume. After solvent equilibration, the system was energy-minimized by using 2,500 steps of steepest descent without any restraints. Next, the system was heated from 0 to 300 K (or 400 K) in 20 ps of constant volume MD by using a time step of 1.5 fs, a Langevin thermostat (collision frequency of 1 ps^{-1}), weak positional constraints for the protein atoms (spring constant of 500 kcal•mol^{-1}•Å^{-2}), and SHAKE restraints for bonds with hydrogen. Then, the whole system was equilibrated without constraints with 200 ps of NPT MD simulations by using constant pressure periodic boundary with an average pressure of 1 atm (1 atm = 101.3 kPa) and a relaxation time of 2 ps. SHAKE was used for bonds with hydrogen, and the temperature was maintained at 300 K (or 400 K) through a Berendsen thermostat, by coupling to a bath with a temperature of 300 K (or 400 K) and a time constant of 0.1 ps. Finally, we performed a 5-ns MD simulation of the whole system using identical parameters as the final equilibration. A snapshot of the trajectory was stored every 0.2 ps (100 time steps). Each simulation was performed in parallel on 8-processors on a cluster of dual-processor nodes based on Athlon 2.8 GHz.

Asymmetry of Motions Between Monomers in the Dimer. The changes in the patterns of mobility upon mutation were qualitatively similar in the two monomers of the dimer, but not identical, especially in the Zn- and electrostatic-loop regions. This asymmetry in the dimer has been reported in previous shorter MD simulations on the 300-ps time scale (1), and we found that the asymmetry was preserved on the nanosecond time scale and was present in both wild-type and mutant Cu, Zn superoxide dismutase (SOD1) dimers, suggesting that the asymmetry on the picosecond–nanosecond time scale is an intrinsic

Essential Dynamics Analysis. The eigenvalues for the wild-type and A4V, H46R, and G37R dimers obtained from an essential dynamics analysis of 5-ns MD trajectories at 300 K are shown in Fig. 7. We find that approximately the first 10 modes arranged according to their eigenvalues capture ≈75% of the motions observed in the trajectories. This is in agreement with a previous essential dynamics analysis on the wild-type dimer (2).

Wild-Type SOD1 Dimer at 400 K. We performed a MD simulation of the SOD1 monomer at 400 K starting from its crystal structure (PDB entry 1SPD). The root mean square deviation (RMSD) of the protein from the minimized crystal structure increases with time as shown in Fig. 8*a*. The coupling between and within monomers is also significantly diminished (Fig. 8*b*) compared with the wild-type dimer at 300 K (Fig. 6*a*).

1. Falconi, M., Melchionna, S. & Desideri, A. (1999) *Biophys. Chem.* **81,** 197–205.

2. Chillemi, G., Falconi, M., Amadei, A., Zimatore, G., Desideri, A. & DiNola, A. (1997) *Biophys. J.* **73,** 1007–1018.