Cryo-EM structure of an amyloid fibril formed by full-length human SOD1 reveals its conformational conversion

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease. Misfolded Cu, Zn-superoxide dismutase (SOD1) has been linked to both familial and sporadic ALS. SOD1 fibrils formed in vitro share toxic properties with ALS inclusions. Here we produced cytotoxic amyloid fibrils from full-length apo human SOD1 under reducing conditions and determined the atomic structure using cryo-EM. The SOD1 fibril consists of a single protofibril with a left-handed helix. The fibril core exhibits a serpentine fold comprising N-terminal segment (residues 3–55) and C-terminal segment (residues 86–153) with an intrinsic disordered segment. The two segments are zipped up by three salt bridge pairs. By comparison with the structure of apo SOD1 dimer, we propose that eight β-strands (to form a β-barrel) and one α-helix in the subunit of apo SOD1 convert into thirteen β-strands stabilized by five hydrophobic cavities in the SOD1 fibril. Our data provide insights into how SOD1 converts between structurally and functionally distinct states.

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myotrophic lateral sclerosis (ALS), also called Lou Gehrig’s disease, is a progressive, fatal neurodegenerative disease that involves the loss of upper and lower motor neurons. Ninety percent of ALS cases are sporadic and little is known about the origin, while ten percent of ALS cases are inherited familial ALS. The sod1 gene, serving as a major antioxidant gene, was the first to be linked to the familial form of ALS and other genes associated with genetic ALS include those encoding TDP-43 and FUS. The misfolding of human Cu, Zn-superoxide dismutase (SOD1) in motor neuron cells plays a crucial role in the etiology of the disease. SOD1 is a 32-kDa homo-dimeric metalloenzyme; each subunit consists of 153 amino acids and contains one copper ion and one zinc ion. The SOD1 structure in each subunit features an antiparallel β-barrel composed of eight β-strands and two α-helices, which is stabilized by a disulfide bond between Cys57 and Cys146. In sharp contrast, the high-resolution structures of SOD1 amyloid fibrils are not available so far. Therefore, it is unclear for the conformational conversion of SOD1 from its immature form with no post-translational modifications into an aggregated form during the pathogenesis of ALS.

The mature form of SOD1 is exceptionally stable and it is very unlikely that the mature, metalated, dimeric, and disulfide-intact form ever converts into the aggregated form. It has been proposed that immature forms of SOD1, which lack copper and zinc ions and the disulfide bond in the structure, are the origin for cytotoxic misfolded conformations. Previous studies have shown that the amyloid-like aggregates isolated from ALS transgenic mice or cells expressing ALS-causing SOD1 mutations contain metal-deficient and disulfide-reduced SOD1, suggesting their pathogenic potential. Substantial experiments have demonstrated that the full-length apo SOD1 can convert into amyloid fibrils by incubation with reducing agents in vitro. Importantly, SOD1 fibrils formed in vitro are able to incorporate into cells and transmit intercellularly. Moreover, SOD1 fibrils produced under reducing conditions also share pathological properties with ALS inclusions, such as the ability to induce mitochondria damage, cause neuroinflammation and activate microglial cells triggering neurodegeneration in ALS. Thus, structural determination of the SOD1 fibril is of importance for understanding the pathogenic mechanism of SOD1 in both familial and sporadic ALS.

Here we prepared homogeneous cytotoxic amyloid fibrils from recombinant, full-length apo human SOD1 under reducing conditions and determined the atomic structure by using cryo-EM. Our findings provide structural insights into the conversion of SOD1 between physiological and fibrillar states.

Cryo-EM structure of SOD1 fibril. We next determined the atomic structure of the cytotoxic SOD1 amyloid fibrils by cryo-EM (Table 1 and Figs. 1 and 2). The cryo-EM micrographs, two-dimensional (2D) class average images, and atomic force microscopy (AFM) images show that the SOD1 fibril is composed of a single protofibril with a left-handed helical twist (Fig. 1a, Supplementary Fig. 3a, and Supplementary Fig. 4a–e). The helical pitch is 144 ± 5 nm (Fig. 1a) or 146 ± 5 nm (Supplementary Fig. 4a–e). The SOD1 subunit within the protofibril is arranged in a staggered manner (Supplementary Fig. 3b). The fibrils are morphologically homogeneous, showing a fibril full width of 12.3 ± 0.7 nm (Fig. 1a) and Supplementary Fig. 3a) or 12.9 ± 1.0 nm (Supplementary Fig. 4a–e).

Using helical reconstruction in RELION3.1 (ref. 36), we determined a density map of the ordered core of SOD1 fibril, with an overall resolution of 2.95 Å, which features well-resolved side-chain densities and clearly separated β-strands along the fibril axis (Fig. 1b and Supplementary Fig. 5). Cross-sectional view of the 3D map of the SOD1 fibril and top view of the density map show a protofibril comprising the N- and the C-terminal segments, with an unstructured flexible region in between (Fig. 1b, e). The 3D map showed a single protofibril in the SOD1 fibril with a left-handed helix, and the left-handed structure of the fibril is supported by AFM images (Supplementary Fig. 4a–e). The half-helical pitch is 73.1 nm (Fig. 1c). The SOD1 subunit within the protofibril stacks along the fibril axis with a helical rise of 4.82 Å and a twist of −1.187° (Fig. 1d).

We unambiguously built a structure model of SOD1 fibril comprising the N-terminal segment (residues 3 to 55) and the C-terminal segment (residues 86 to 153) at 2.95 Å (Fig. 2). The density of an intrinsic disordered segment comprising residues 56 to 85 is invisible due to high flexibility (Fig. 2a–c), which is reminiscent of the internal disordered segments observed in the structures of patient-derived amyloid fibrils from systemic AL amyloidosis. The presence of the internal disordered segment represents an interesting structural feature of SOD1 fibril formed under reducing conditions.

Side chains for the residues in the SOD1 fibril core can be well accommodated into the density map (Fig. 2a). The exterior of the SOD1 fibril core is mostly hydrophilic, whereas side chains of most hydrophobic residues are located in the interior of the SOD1 fibril fold (Fig. 2b–g and Supplementary Fig. 6). Five hydrophobic cavities (Supplementary Fig. 6a and Fig. 2g), four hydrogen bonds (Supplementary Fig. 7a), and a very compact...
β-strand architecture (Fig. 2b, d). Six β-strands (β1 to β6) and seven β-strands (β7 to β13) are present in the N- and C-terminal segments of the SOD1 fibril core structure, respectively (Fig. 2b–d). The height of one layer along the helical axis is 15.82 Å, which is the distance between the highest point in the loop between β4 and β5 and the lowest point in the loop between β8 and β9 (Fig. 2c).

The SOD1 fibril contains a long intramolecular interface comprising residues 36 to 48 in the N-terminal half and residues 98 to 109 in the C-terminal half (Fig. 3a). Three pairs of intramolecular salt bridges formed by Lys36 and Asp109, His43 and Asp101, and His46 and Glu100 (with distances <4 Å; Fig. 3b–e) are identified to stabilize the intramolecular L-shaped interface between the N- and C-terminal parts of SOD1 fibril (Figs. 2a, b, f, g, and 3b–e). Side chains of most residues (Lys36, Thr39, His43, His46, His48, Ser98, Glu100, Asp101, Ser105, Ser107, and Asp109) in the interior of the intramolecular L-shaped interface are hydrophilic (Figs. 2b, g, and 3a). The presence of a mostly hydrophilic intramolecular interface represents another interesting structural feature of SOD1 fibril formed under reducing conditions.

Discussion
SOD1 is involved in the pathogenesis of the motor neuron disease ALS where it is observed to form intracellular fibrillar inclusions37,39,40. These proteinaceous inclusions are also observed in cases of Parkinson’s disease and aged individuals41,42. Here, we presented cryo-EM structure of a human SOD1 fibril and compared the structures of apo SOD1 dimer and SOD1 fibril produced under reducing conditions (Fig. 4). Notably, the SOD1 molecule adopts largely distinctive secondary, tertiary, and quaternary structures in two different states of SOD1, highlighting the high structural polymorphs and phenotypic diversity of SOD1 in physiological and fibrillar states. The apo human SOD1 dimer contains eight β-strands (to form an antiparallel β-barrel), two α-helices, and a single disulfide bond between Cys57 in α1 and Cys146 in β8 in each subunit as well as an intermolecular interface involving strong hydrophobic interactions and hydrogen bonding from Gly51 and Gly114 of one molecule to Ile151 of the other10 (Fig. 4a, b). In contrast, once folding into cytotoxic, mitochondrial dysfunction-inducing fibril structure, SOD1 molecules form six β-strands (β1 to β6) by its N-terminal segment (residues 3 to 55) and seven β-strands (β7 to β13) by its C-terminal segment (residues 86 to 153), exhibiting an in-register intramolecular β-strand architecture (Fig. 4a, c). Moreover, the cytotoxic SOD1 fibril structure features a long, mostly hydrophilic intramolecular L-shaped interface and an intrinsic disordered segment comprising residues 56 to 85 (Fig. 4a, c). Once apo SOD1 dimer converts to its fibrillar form, the SOD1 molecule undergoes a completely conformational rearrangement, with the antiparallel β-barrel of apo SOD1 converted to β1–β5, β7, β8, β11, and β13, the loop between β4’ and α1 converted to β6, α1 and the loop between α1 and β5’ converted to the internal disordered segment, the loop between β6’ and β7’ converted to β9 and β10, and α2 of apo SOD1 converted to β12 in the SOD1 fibril (Fig. 4b, c). Of note, the pathological relevance of the SOD1 fibril reported here remains unknown, although treatment of cells with the fibrils disrupts mitochondrial membrane permeability and integrity33, causes severe mitochondrial impairment (this work) and inflammation32, and activates microglia33. It is still unclear whether the fibrils are pathogenic agents in ALS9,13.

This work builds on previous extensive studies of SOD1 fibril formation12,14,19,23–30,43. Valentine and colleagues extensively investigated SOD1 fibrils and used EM and AFM to characterize the fibrils formed by SOD1 and its mutants33,26. Shaw and co-

Table 1 Cryo-EM data collection, refinement, and validation statistics.

| Table 1 Cryo-EM data collection, refinement, and validation statistics. |
|---------------------------------------------------------------|
| **SOD1 fibril (EMD-32227, PDB 7VZF)**                         |
| **Data collection and processing**                             |
| Magnification                                                 | 130,000                                      |
| Voltage (kV)                                                   | 300                                          |
| Camera                                                       | K2 summit (Titan Krios)                       |
| Frame exposure time (s)                                       | 0.16                                         |
| Movie frames (n)                                              | 40                                           |
| Electron exposure (e-/Å²)                                     | 60                                           |
| Defocus range (μm)                                            | −2.0 to −1.2                                 |
| Pixel size (Å)                                                | 1.04                                         |
| Symmetry imposed                                             | C1                                           |
| Box size (pixel)                                              | 320                                          |
| Inter-box distance (Å)                                        | 33.3                                         |
| Micrographs collected (n)                                     | 2931                                         |
| Segments extracted (n)                                        | 288,744                                      |
| Segments after Class2D (n)                                    | 147,525                                      |
| Segments after Class3D (n)                                    | 70,067                                       |
| Map resolution (Å)                                            | 2.95                                         |
| FSC threshold                                                | 0.143                                        |
| Map resolution range (Å)                                      | 2.30–5.01                                    |
| **Refinement**                                                |                                              |
| Initial model used                                           | De novo                                      |
| Model resolution (Å)                                          | 2.95                                         |
| FSC threshold                                                | 0.143                                        |
| Model resolution range (Å)                                    | 2.95                                         |
| Map sharpening B factor (Å²)                                  | −77.93                                       |
| **Model composition**                                         |                                              |
| Nonhydrogen atoms                                            | 2,628                                        |
| Protein residues                                              | 363                                          |
| Ligands                                                      | 0                                            |
| B factors (Å²)                                                | 70.90                                        |
| R.m.s. deviations                                             |                                              |
| Bond lengths (Å)                                              | 0.009                                        |
| Bond angles (°)                                               | 1.060                                        |
| Validation                                                   |                                              |
| MolProbity score                                             | 2.86                                         |
| Clashscore                                                   | 37.29                                        |
| Poor rotamers (%)                                             | 0                                            |
| Ramachandran plot                                            |                                              |
| Favored (%)                                                   | 73.50                                        |
| Allowed (%)                                                   | 26.50                                        |
| Disallowed (%)                                                | 0                                            |
Previous studies proposed two alternative models of SOD1 fibril based on protease digestion experiments and mass spectrometric analyses\(^5,25,26\). The so-called “three key region model” predicts that the SOD1 fibril core contains one N-terminal segment comprising residues 1 to 30 and two C-terminal segments comprising residues 90–120 and 135–153 (ref. 23). This is in good agreement with our model, wherein β1 and β2, β8–β11, and β13 would correspond to the first, second, and third segments in the three key region model\(^23\). The other N-terminal core model predicts that the SOD1 fibril core contains the first 63 residues of the N terminus of the protein\(^29\). This is partly compatible with our model, wherein the six β strands (β1 to β6) present in the N-terminal segment would correspond to the minimal protease-resistant core region comprising residues 1–63 in the N-terminal core model\(^26\). In all three models, SOD1 fibrils are produced from the immature form of the protein under reducing conditions. Previous work had shown that amyloid fibril formation is initiated by the immature, disulfide-reduced, apo form of SOD1\(^{21}\). In our SOD1 fibril model, Cys57, Cys111, and Cys146 are all in disulfide-reduced conformations with free thiol groups, and misfolded and aggregated SOD1 evolves from a pool of immature SOD1. Intriguingly, the side chains of those Cys residues appear exposed to quite crowded areas of the cytosolic cavity (Fig. 2a, b, and Supplementary Fig. 6b, f). Strikingly, among two hundred and sixteen genetic mutations of SOD1 identified from different familial ALS\(^1,5,8,17–22,25,26,29,44–50\) (https://alsod.iop.kcl.ac.uk/), one hundred and eighty-two clinically identified mutations are located within the SOD1 fibril core structure determined in this study, in which one hundred and five representative genetic ALS-related mutations are listed in Fig. 4a. Notably, residues forming strong salt bridges (His43, His46, Glu100, Asp101, and Asp109) that contribute to the stabilization of the intramolecular L-shaped interface between the N- and C-terminal parts of SOD1 fibril (Figs. 2a, b, f, and g) or hydrogen bonds (Val14 and Asp125) that contribute to the maintenance of the SOD1 fibril structure (Supplementary Fig. 7b–d) are also ALS-associated mutation sites\(^1,5,8,17–22,25,26,29,44–50\). Based on the cryo-EM fibril structure, the disease mutations, such as H43R, H46R, H46D, E100G, E100K, D101G, D101H, D101N, D101Y, D109Y, D109N, and D125H (Fig. 4a, salt bridge mutations, and hydrogen bond mutations), may disrupt important interactions in the cytosolic SOD1 fibril structure. This suggests that the different mutations may induce SOD1 to form fibrils with structures and cytotoxicity distinct from the one presented here, which might be
**Fig. 2 Atomic structure of SOD1 fibril.**

a) Cryo-EM map of an amyloid fibril from recombinant, full-length apo human SOD1 with the atomic model overlaid. The SOD1 fibril core comprises the N-terminal segment (residues 3-55) and the C-terminal segment (residues 86 to 153) colored green and purple, respectively, with an internal disordered segment.

b) Schematic view of the SOD1 fibril core. Residues are colored as follows: white, hydrophobic; green, polar; red and blue, negatively and positively charged, respectively; and magenta, glycine. β strands are indicated with bold lines. Side chains of most hydrophobic residues are located in the interior of the SOD1 fibril fold.

c) Sequence of the fibril core comprising residues 3-55 and 86-153 from full-length human SOD1 (1 to 153) with the observed six β strands colored violet (β1), blue (β2), light blue (β3), cyan (β4), light cyan (β5), and green (β6) in the N-terminal region and the observed seven β strands colored light green (β7), yellow (β8), gold (β9), orange (β10), pink (β11), magenta (β12), and light magenta (β13) in the C-terminal region. The dotted lines correspond to residues 1-2 and residues 56-85 not modeled in the cryo-EM density.

d) Ribbon representation of the structure of an SOD1 fibril core containing five molecular layers and two segments. e) As in d, but viewed perpendicular to the helical axis, revealing that the height of one layer along the helical axis is 15.82 Å.

f) Electrostatic surface representation of the structure of an SOD1 fibril core containing five molecular layers and two segments. g) Hydrophobic surface representation of the structure of an SOD1 fibril core as in d, f, g. Three pairs of amino acids (Lys36 and Asp109; His43 and Asp101; and His46 and Glu100) form three salt bridges at the intramolecular interface between the N- and C-terminal regions of SOD1 fibril. The surface of two regions of the SOD1 fibril core is shown according to the electrostatic properties (red, negatively charged; blue, positively charged) (f) or the hydrophobicity (yellow, hydrophobic; blue, hydrophilic) (g) of the residues.
related to the structural diversity of SOD1 fibrils, strains, and phenotypic diversity of SOD1 in pathological state. Thus, we plan to collect structural data on various SOD1 mutations including metal-binding region mutants H46R, H46D, G85R, and D125H and wild-type-like mutants A4V, D90A, and G93A in the near future. Interestingly, as for the ALS-associated residues including His43, His46, Glu100, Asp101, and Asp109, His43 forms a hydrogen bond with Thr39, His46 forms a strong salt bridge with Asp124, and Asp101 forms a strong salt bridge with Arg79 in the subunit to stabilize the structure of apo SOD1 dimer (Supplementary Fig. 8a–d), whereas His43 and Asp101 form a strong salt bridge and His46 forms a strong salt bridge with Glu100 in the SOD1 fibril to stabilize the intramolecular L-shaped interface (Fig. 3b–d). This indicates that reorganization of salt bridges may occur for these ALS-associated mutated SOD1 during their conformational conversion from apo into fibrillar form.

In summary, we revealed by cryo-EM that the full-length human SOD1 displays a novel amyloid fibril structure. The SOD1 fibril displays a very compact fold with an internal disordered segment, which contains thirteen β-strands stabilized by five hydrophobic cavities and four hydrogen bonds, and a long, mostly hydrophilic intramolecular L-shaped interface stabilized by three strong salt bridges. The comparison of the structures of apo SOD1 dimer and SOD1 fibril reveals the substantial conformational conversion from a β-sheet-rich (correspond to the antiparallel β-barrel structure), immature form of SOD1 to a totally distinct β-sheet-rich (correspond to an in-register intramolecular β-strand architecture), fibrillar form of SOD1 during pathogenesis of ALS. The fibril structure will be valuable in regard to understanding the structural basis underlying SOD1 misfolding and inspiring future research on the structural polymorphism of SOD1 strains and their relationship to ALS.
Methods

Protein purification. A plasmid-encoding, full-length human SOD1 (1–153) was a gift from Dr. Thomas O’Halloran (Chemistry of Life Processed Institute, Northwestern University). The sequence for SOD1 1–153 was expressed from the vector pET-3d, which was transformed into E. coli BL21 (DE3) cells (Novagen, Merck, Darmstadt, Germany). SOD1 protein was purified to homogeneity by Q-Sepharose chromatography as described by Chattopadhyay et al.23 and Xu et al.9. After purification, recombinant wild-type SOD1 was demetallated by dialysis in 10 mM EDTA and 10 mM NaAc buffer (pH 3.8) five times as described by Chattopadhyay et al.23 and Xu et al.9. In all, 10 mM NaAc buffer (pH 3.8) and 20 mM tris-HCl buffer (pH 7.4) were used for further dialysis. The apo SOD1 was then concentrated, filtered, and stored at −80 °C. A Analyst-800 atomic absorption spectrometer (PerkinElmer) was used to quantify metal content of SOD1 samples. Samples of wild-type SOD1 contained <5% of residual metal ions, indicating that the samples were indeed in the apo state. SDS-PAGE and mass spectrometry were used to confirm that the purified apo SOD1 proteins were single species with an intact disulfide bond. A NanoDrop OneC Microvolume UV-Vis Spectrophotometer (Thermo Fisher Scientific) was used to determine the concentration of apo SOD1 according to its absorbance at 214 nm with a standard calibration curve drawn by BSA.

SOD1 fibril formation. Recombinant, full-length apo human SOD1 (30 μM) were incubated in 20 mM tris-HCl buffer (pH 7.4) containing 5 mM TCEP and shaking at 37 °C for 40–48 h, and the SOD1 fibrils were collected. Large aggregates in SOD1 fibril samples were removed by centrifugation for 5000 × g at 4 °C for 10 min. The supernatants were then concentrated to ~30 μM in a centrifugal filter (Millipore). A NanoDrop OneC Microvolume UV-Vis Spectrophotometer (Thermo Fisher Scientific) was used to determine the concentrations of the SOD1 fibril according to its absorbance at 214 nm with a standard calibration curve drawn by BSA.

Congo red binding assays. SOD1 fibrils were analyzed by Congo red binding assays. A stock solution of 200 μM Congo red was prepared in phosphate-buffered saline and filtered through a filter of 0.22-μm pore size before use. In a typical assay, the SOD1 fibril sample was mixed with a solution of Congo red to yield a
final Congo red concentration of 50 µM and a final SOD1 concentration of 10 µM, and the absorbance spectrum between 400 and 700 nm was then recorded on a Cytoation 3 Cell Imaging Multi-Mode Reader (BioTek).

**TEM of SOD1 fibrils.** SOD1 fibrils were examined by TEM of negatively stained samples. Ten microliters of SOD1 fibril samples (~30 µM) were loaded on copper grids for 30 s and washed with H2O for 10 s. Samples on grids were then stained with 2% (w/v) uranyl acetate for 30 s and dried in air at 25°C. The stained samples were examined using a JEM-1400 Plus transmission electron microscope (JEOL) operating at 100 kV.

**AFM of SOD1 fibrils.** SOD1 fibrils were produced as described above. Ten microliters of SOD1 fibril samples (~30 µM) were incubated on a freshly cleaned mica surface for 2 min, followed by rinsing three times with 10 µl of pure water to remove the unbound fibrils and drying at room temperature. The fibrils on the mica surface were probed in the air by the Dimension Icon scanning probe microscope (Bruker) with ScanAsyst mode. The measurements were realized by using SCANASYST-AIR probe with a spring constant of 0.4 N/m and a resonance frequency of 70 kHz (Bruker). A fixed resolution (256 x 256 data points) of the AFM images was acquired with a scan rate of 1 Hz and analyzed by using NanoScope Analysis 2.0 software (Bruker).

**Cryo-EM of SOD1 fibril.** SOD1 fibrils were produced as described above. An aliquot of 3.5 µl of 30 µM SOD1 fibril solution was applied to glow-discharged holey carbon grids (Quantifoil Cu R1.2/1.3, 300 mesh), blotted for 3.5 s, and plunge-frozen in liquid ethane using an FEI Vitrobot Mark IV. The grids were examined using an FEI Talos F200C microscope, operated at 200 kV, and equipped with a field emission gun and an FEI Ceta camera (Thermo Fisher Scientific). The cryo-EM images were recorded using an FEI Titan Krios microscope operated at 300 kV (Thermo Fisher Scientific) and equipped with a Gatan Bio-Quantum K2 Summit camera. A total of 2931 movies were collected by beam-image shift data collection methods in super-resolution mode at a nominal magnification of x130,000 (pixel size, 1.04 Å) and a dose of 9.375 e− Å−2 s−1 (see Table 1) using SerialEM 3.8.3. An exposure time of 6.4 s was used, and the resulting videos were dose-fractionated into 40 frames. A defocus range of −1.2 to −2.0 µm was used.

**Helical reconstruction.** All 40 video frames were aligned, summed, and dose-weighted by MotionCor2-1.3.2 and further binned to a pixel size of 1.04 Å (ref. 2). Contrast transfer function estimation of aligned, dose-weighted micrographs was performed by CTFFIND4.1.8 (ref. 3). Subsequent image-processing steps, which included manual picking, particle extraction, 2D and 3D classifications, 3D refinement, and post-processing, were performed by RELION-3.1 (ref. 36).

**Atomic model building and refinement.** Local searches of symmetry in 3D classifications, particles with the same morphology were picked out. The final classification of the selected 3D classes with appropriate reference was performed to obtain the final reconstruction. The final map of SOD1 fibril was convergent with a rise of 4.82 Å and a twist angle of −1.187°. Postprocessing was performed to sharpen the map with a B factor of −77.93 Å2. On the basis of the gold standard Fourier shell correlation (FSC) = 0.143 criteria, the overall resolution was reported as 2.95 Å. The statistics of cryo-EM data collection and refinement are shown in Table 1.

**Cell viability assays.** SH-SY5Y neuroblastoma cells (catalog number GDC0210) and HEK-293T cells (catalog number GDC0187) were obtained from China Center for Type Culture Collection (CCTCC, Wuhan, China) and cultured in minimum essential media and in Dulbecco’s modified Eagle’s medium ( Gibco Invitrogen), supplemented with 10% (v/v) fetal bovine serum (Gibco), 100 U/ml penicillin, and 100 U/ml penicillin in 5% CO2 at 37°C. SH-SY5Y or HEK-293T cells were cultured in 6-well plates in the minimum essential medium for 1 day and then cultured with 0 or 10 µM SOD1 fibril seeds for 3 days, and cells cultured with 20 mM Tris-HCl buffer (pH 7.4) containing 5 mM TCEP as a negative control. After prefixation with 3% paraformaldehyde and 1.5% glutaraldehyde in 1× PBS (pH 7.4), the cells were harvested and postfixed in 1% osmium tetroxide for 1 h using an ice bath; the samples were then dehydrated in graded acetone and embedded in 812 resins. SH-SY5Y sections of the cells were prepared using a Leica Ultracut S Microtome and negatively stained using 2% uranyl acetate and lead citrate. The doubly stained ultrathin sections of cells were examined using a JEM-1400 Plus transmission electron microscope (JEOL) operating at 100 kV. The TEM images were analyzed by using Origin Pro software version 8.0274 (Origin Laboratory), and p values were determined using two-sided Student’s t test. All experiments were further confirmed by biological repeats.

**Data availability** The cryo-EM density maps have been deposited in the Electron Microscopy Data Bank (EMDB) under accession code EMDB-32227 (human SOD1 fibril). The coordinates generated in this study are deposited in the Protein Data Bank (PDB) under accession code PDB 7VZF (human SOD1 fibril). Previously published structure 1HL4 is available from PDB. Biological materials are available on request. Source data are provided with this paper.

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and the SOD1 fibrils. L.-Q.W., H.-Y.Y., and M.-Y.Z. performed Congo red binding assays and cell viability assays of SOD1 fibrils and TEM of ultrathin sections of cells. L.-Q.W., H.-Y.Y., B.D., and Z.W. performed APM experiments. L.-Q.W., Y.M., H.-Y.Y., K.Z., Q.W., D.Z., and D.L. collected, processed, and/or analyzed cryo-EM data. L.-Q.W., Y.M., C.L., and Y.L. wrote the manuscript. All authors proofread and approved the manuscript.

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