Sub-Ångström cryo-EM structure of a prion protofibril reveals a polar clasp

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The atomic structure of the infectious, protease-resistant, β-sheet-rich and fibrillar mammalian prion remains unknown. Through the cryo-EM method MicroED, we reveal the sub-Ångström-resolution structure of a protofibril formed by a wild-type segment from the β2-α2 loop of the bank vole prion protein. The structure of this protofibril reveals a stabilizing network of hydrogen bonds that link polar zippers within a sheet, producing motifs we have named ‘polar claps’.

Micro electron diffraction (MicroED) is a cryo-EM method that facilitates the determination of atomic structures from submicrometer-thin protein nanocrystals and fragmented crystallites12–14. Several high-resolution amyloid structures have been determined by MicroED, including two structures from the toxic core of the Parkinson’s-associated protein α-synuclein1, two from the type-2-diabetes-associated protein IAPP2, and five αβP-sheet structures15. These structures add to those of the fibril cores of α-synuclein1, amyloid-β (ref. 16), tau2, and a fungal prion17 recently determined by complementary methods. However, an atomic structure of the infectious scrapie form of the mammalian prion protein (PrPSc) remains unknown11. PrPSc shares some structural hallmarks of amyloid, appearing as rope-like filaments or rods made of tightly mating β-sheets12,13. However, PrPSc also differs from other amyloids: it resists proteolysis and denaturation, is infectious, and can spread within and between species to cause disease11,14,15.

To evaluate the source of PrPSc stability, we investigated segments in mammalian prion proteins (PrP) that might form the core of PrPSc fibrils. Informed by structure-based prediction of amyloid-prone sequences16, we identified 168-QYNQNFFV-176, a segment of the β2-α2 loop of the bank vole (Myodes glareolus) PrP (Fig. 1), a universal prion acceptor17,18. This segment lies within the predicted cross-β core of PrP fibrils (Supplementary Fig. 1), shows high conservation in rodents and other mammals (Supplementary Fig. 1), and is rich in asparagines19,20, which may stabilize prion fibrils11. At sub-millimolar concentrations, this segment produces highly ordered aggregates (Supplementary Fig. 2) that, when aligned and illuminated by X-rays, produce cross-β diffraction (Supplementary Fig. 2). Like PrPSc, aggregates formed by this segment are resistant to high concentrations of urea, guanidine, and a range of pH, but are sensitive to sodium hydroxide (Supplementary Fig. 3). Given its shared biological properties with PrPSc, we labeled our segment proto-PrPSc and set out to uncover the structural basis for its stability.

From optimized microcrystals (Supplementary Fig. 4), we determined a high-completeness 0.75-Å-resolution structure of proto-PrPSc (Supplementary Table 1). The conditions in which we observed microcrystals of proto-PrPSc also produced showers of nanocrystals, evident in electron micrographs (Fig. 1). These nanocrystals diffracted to 0.72 Å by MicroED (Fig. 1). Merging diffraction from multiple crystals, we achieved a high-completeness 0.75-Å-resolution dataset (Supplementary Table 1). From these data, we obtained an ab initio solution that was similar to our microfocal X-ray diffraction structure of proto-PrPSc (Supplementary Fig. 4) and suitable for atomic refinement (Supplementary Fig. 5). This ultrahigh-resolution MicroED structure of proto-PrPSc shows features that are invisible in the X-ray structure and critical to our understanding of its stability.

The structure of proto-PrPSc reveals a prion protofibril with amyloid-like features: β-strands parallel and in register as a class 2 steric zipper21 in which sheets pair front to back (Fig. 2). Two tightly mating curved sheets make up the proto-PrPSc fibril (Fig. 2), although side chains in these sheets interdigitate less in proto-PrPSc than those observed in conventional amyloid structures12. Although sheets stack in a parallel face-to-back configuration, the convex face of one sheet nestles against the concave face of its neighbor, approximately 10.3 Å away, with a high degree of surface complementarity (S, 0.807) (Fig. 2). The interface between these sheets is large, concealing 204.5 Å² per strand, and is entirely devoid of waters at its core (Fig. 1). Atoms in the MicroED structure of proto-PrPSc are extremely well ordered, with an average B factor of 6.0 Å² overall and 2.8 Å² within its core (Supplementary Fig. 6 and Supplementary Table 1). These values are less than half of the overall B factor in our X-ray diffraction structure of proto-PrPSc (Supplementary Table 1), thus confirming a greater degree of order in our nanocrystallites compared to that of larger microcrystals of the same segment.

A 3D network of hydrogen bonds stabilizes proto-PrPSc (Supplementary Table 2); hydrogen at its core participate in intra-residue C5 bonds22 (Fig. 3), and asparagine and glutamine residues stack along its fibril axis (Fig. 2). Glutamines and asparagines...
in proto-PrPSc form networks of hydrogen bonds reminiscent of proton wires\(^\text{13}\), polar ladders\(^\text{14}\), and the polar zippers first proposed by Max Perutz\(^\text{25,26}\) (Supplementary Fig. 7). Neighboring polar ladders in proto-PrPSc are additionally linked by hydrogen bonds within a strand (Fig. 3), a motif we refer to as a ‘polar clasp’ (Supplementary Fig. 7). Stacks of phenylalanine and tyrosine residues shield clasps at the core of proto-PrPSc (Figs. 1 and 2). The importance of this aromatic embrace is underscored by a lack of clasps in structures of shorter segments from this region of PrP that lack Tyr\(^169\) (refs. \(^\text{12,27}\)). On this evidence, we hypothesize that polar clasps and stacked aromatic residues act in concert to stabilize proto-PrPSc, as they might for PrPSc.

Our hypothesis of proto-PrPSc stability relies on the locations of hydrogen atoms throughout the structure. Hydrogens in the MicroED structure of proto-PrPSc are unambiguously assigned, informed by pronounced difference density in ultrahigh-resolution maps (Supplementary Figs. 5, 8, and 9 and Supplementary Table 4). Hydrogens at the core of proto-PrPSc are as evident as those seen in structures of small organic compounds determined by electron diffraction\(^\text{28}\), including our own structure of carbamazepine (Supplementary Fig. 10 and Supplementary Table 3). Density in ultrahigh-resolution maps of proto-PrPSc suggests that hydrogen may occupy positions that deviate from idealized geometry (Supplementary Fig. 5). Improved hydrogen positions indicated by ultrahigh-resolution maps in MicroED could bolster the accuracy of calculations based on observed hydrogen bond networks.

Ultrahigh-resolution maps of proto-PrPSc mirror features that appear in electron density maps of the highest resolution X-ray
**Methods**

Methods, including statements of data availability and any associated accession codes and references, are available at https://doi.org/10.1038/s41594-017-0018-0.
12. Sawaya, M. R. et al. Atomic structures of amyloid cross-beta spines reveal varied steric zippers. *Nature* **447**, 453–457 (2007).

13. Eisenberg, D. & Jucker, M. The amyloid state of proteins in human diseases. *Cell* **148**, 1188–1203 (2012).

14. McKinley, M. P., Bolton, D. C. & Prusiner, S. B. A protease-resistant protein is a structural component of the scrapie prion. *Cell* **35**, 57–62 (1983).

15. Kurt, T. D. & Sigurdson, C. J. Cross-species transmission of CWD prions. *Prion* **10**, 83–91 (2016).

16. Goldschmidt, L., Teng, P. K., Riek, R. & Eisenberg, D. Identifying the amyloidome, proteins capable of forming amyloid-like fibrils. *Proc. Natl. Acad. Sci. USA* **107**, 3487–3492 (2010).

17. Watts, J. C. et al. Evidence that bank vole PrP is a universal acceptor for prions. *PLoS. Pathog.* **10**, e1003990 (2014).

18. Kurt, T. et al. The molecular basis for cross-species prion transmission. *FASEB J.* **30**, 814.7 (2016).

19. Halfmann, R. et al. Opposing effects of glutamine and asparagine govern prion formation by intrinsically disordered proteins. *Mol. Cell.* **43**, 72–84 (2011).

20. Zambrano, R. et al. PrionW: a server to identify proteins containing glutamate/asparagine rich prion-like domains and their amyloid cores. *Nucleic Acids Res.* **43**, W331–W337 (2015).

21. Kurt, T. D. et al. Asparagine and glutamine ladders promote cross-species prion conversion. *J. Biol. Chem.* https://doi.org/10.1074/jbc.M117.794107 (2017).

22. Newberry, R. W. & Raines, R. T. A prevalent intraresidue hydrogen bond stabilizes proteins. *Nat. Chem. Biol.* **12**, 1084–1088 (2016).

23. Nagle, J. F. & Morowitz, H. J. Molecular mechanisms for proton transport in membranes. *Proc. Natl. Acad. Sci. USA* **75**, 298–302 (1978).

24. Yoder, M. D., Lietzke, S. E. & Jurnak, F. Unusual structural features in the parallel β-helix in pectate lyases. *Structure* **1**, 241–251 (1993).

25. Perutz, M. F., Staden, R., Moens, L. & De Baere, I. Polar zippers. *Nature* **355**, 241–251 (1993).

26. Goldschmidt, L., Teng, P. K., Riek, R. & Eisenberg, D. Identifying the amyloidome, proteins capable of forming amyloid-like fibrils. *Proc. Natl. Acad. Sci. USA* **107**, 3487–3492 (2010).

27. Wiltzius, J. J. W. et al. Molecular mechanisms for protein-encoded parallel β-helix stabilization. *Acta Crystallogr. A* **58**, 162–170 (2002).

28. Jelsch, C. et al. Accurate protein crystallography at ultra-high resolution: valence electron distribution in crambin. *Proc. Natl. Acad. Sci. USA* **97**, 3171–3176 (2000).

29. Zhong, S., Dadarlat, V. M., Glaser, R. M., Head-Gordon, T. & Downing, K. H. Modeling chemical bonding effects for protein electron crystallography: the transferable fragmental electrostatic potential (TFESP) method. *Acta Crystallogr. A* **58**, 162–170 (2002).

30. Glaeser, R. M. & Downing, K. H. High-resolution electron crystallography of protein molecules. *Ultramicroscopy* **52**, 478–486 (1993).

31. Zhang, S., Dadarlat, V. M., Glaser, R. M., Head-Gordon, T. & Downing, K. H. Modeling chemical bonding effects for protein electron crystallography: the transferable fragmental electrostatic potential (TFESP) method. *Acta Crystallogr. A* **58**, 162–170 (2002).

32. Jelsch, C. et al. Accurate protein crystallography at ultra-high resolution: valence electron distribution in crambin. *Proc. Natl. Acad. Sci. USA* **97**, 3171–3176 (2000).

33. Zuo, J. M., Kim, M., O’Keeffe, M. & Spence, J. C. H. Direct observation of d-orbital holes and Cu–Cu bonding in Cu2O. *Nature* **401**, 49–52 (1999).

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Author contributions

J.A.R. directed the work. J.A.R., J.M., E.H., M.W.M., and C.G. grew, evaluated, and directed the work. J.A.R., J.M., E.H., M.W.M., and C.G. grew, evaluated, and directed the work. J.A.R., D.C. collected data. J.A.R., L.G., D.S.E., and T.G. analyzed the data. C.G., M.G.-J., M.R.S., D.C., M.W.M., G.F.H., E.H., L.G., D.S.E., and T.G. analyzed the data. C.G., M.G.-J., and J.A.R. wrote the article, with input from all authors.

Competing interests

The authors declare no competing interests.

Additional information

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Methods

Phylogenetic and sequence analysis of bank vole PrP 27–30. The top 130 most similar wild-type sequences to bank vole PrP 90–231 were generated using NCBI BLAST. Of these 130 sequences, all sequences containing the proto-PrPSc peptide were selected and aligned.

Characterization of proto-PrPSc peptide. The synthetic peptide, QYNQQNQNFV, corresponding to residues 168–176 of the bank vole prion protein was purchased from GenScript. The peptide used for our experiments was received at >98% purity achieved by reverse-phase HPLC. The peptide was qualified on a Bruker (ultralifeXtreme) MALDI-TOF/TOF mass spectrometer, reported as m/z (intensity, arbitrary units). The spectrum has a mass list that includes a [M+H]+ peak at 1,141.3 Da (expected, 1,140.5 Da), a [M+Na]+ peak at 1,163.4 Da (expected, 1,162.49 Da), and a [M+K]+ peak at 1,179.3 Da (1,178.46 Da).

Characterization of carbamazepine. Lyophilized powder of carbamazepine (3H-dibenzo[1,2,3]azepine-5-carboxamide, CH3N2H2O) with purity >99% was purchased from Sigma-Aldrich and crystallized without further purification.

Aggregation of proto-PrPSc. Proto-PrPSc peptide was solubilized in ultrapure water at 0.2–1.75 mM. 50 µl of each sample was added to a 96-well clear flat bottom plate in triplicate and evaluated for aggregate formation by reading absorbance at 350 nm on an Infinite M1000 Pro plate reader (Tecan). Readings were measured immediately after solubilization after 3 h and after 6 h of shaking at 900 r.p.m. at 37 °C. Wells were imaged after 6 h using a Leica M205 C light microscope (Leica Microsystems) and after 3 and 6 h by electron microscopy as described below. Electron microscope images are representative of more than five images taken at each concentration and time point.

Fibril diffraction from proto-PrPSc aggregates. Solutions containing aggregates of proto-PrPSc were clarified by centrifugation. Pelletted aggregates were resuspended in a concentrated volume in water, applied between two pulled capillaries and left to dry overnight. Oriented aggregates formed between the capillary ends were resupplied with additional solution containing aggregates and left to dry again. This process was repeated several times to grow the bulk of aligned aggregates on a capillary. Aligned aggregates were then diffused using 5-min exposures to a FIRE+ rotating anode generator with V ARIMAX HR confocal optics producing Cu Kα radiation (Rigaku, Tokyo, Japan) and detected using a RIGAKU R-AXIS HTC imaging plate detector at a distance of 156 mm from the source.

Chemical denaturation of proto-PrPSc aggregates. Proto-PrPSc was solubilized at approximately 3.5 mM and allowed to form aggregates. This solution was diluted to approximately 1:4 before treatment with either 0.5–6.0 M urea, 0.5–4.5 M guanidinium-HCl, 0.75 M HCl, 0.75 M of either MES, pH 2, acetate, pH 4, MES, pH 6, Tris-HCl, pH 8 or 10, or 0.75 M NaOH. Aggregate content was measured by absorbance compared to a control solution consisting of 0.01% (w/v) 1-µm latex spheres in water. Spectra across the visible range (250–700 nm) were collected using a Nanodrop One (Thermo).

Transmission electron microscopy. Approximately 2 µl of aggregated proto-PrPSc were applied to 300-mesh Formvar-carbon coated grids (Ted Pella Inc.) for 2 min before excess liquid was removed and grids left to dry. Grids were imaged either on a Tecnai T12 or F20 electron microscope (Thermo Fisher, formerly FEI). Samples were imaged at a magnification of 2,100 e–/Å2.

MicroED sample preparation. Nanoscale needle crystals of proto-PrPSc were grown in batch in 0.1 M MES, pH 6.0, and 10% ethanol. Crystals were diluted in mother liquor and fragmented by force of pipetting to create an approximately monodisperse solution of crystals. Carbamazepine was crystallized in batches by dilution into neat isopropanol at 100 µg/mL. 2 µl of either solution were placed on a holey carbon grid (1/4, 2/2, 2/4, #300 copper; Ted Pella Inc.) before plunge freezing into liquid ethane and transferring into liquid nitrogen for storage. Grids were held by a liquid-nitrogen-cooled Gatan 626 cryo-holder for transfer into and manipulation within the electron microscope.

MicroED data collection. MicroED data collection from nine sub-micron-thick needle crystals, and a single sub-micron carbamazepine crystal was performed as previously described. Briefly, crystals of either proto-PrPSc or carbamazepine lying in a frozen-hydrated state on holey carbon grids were inspected visually in overfocus diffraction mode to either a cryo-cooled FEI Tecnai F20 microscope operated at 200 kV (Janelia Research Campus) or a Titan environmental TEM operated at 300 kV (Environmental Molecular Sciences Lab, PNNL). Diffraction patterns used for structure determination were collected on a TVIPS TemCam-F416 CMOS detector in rolling-shutter mode with 3-s exposures while proto-PrPSc crystals were unidirectionally rotated at a constant rate of 0.27° s−1 over angular wedges ranging from −55° to +72°. A single carbamazepine crystal was rotated at a speed of 0.2° s−1 over an angular wedge ranging between −45° to +45° with 5-s exposures. Beam intensity was held constant, with an average dose rate of 0.003–0.005 e− Å−2 sec−1, corresponding to a total dose of −1–3 e− Å−2 per dataset. We used a camera length of 520 mm, the equivalent of a sample-to-detector distance of 950 mm in a corresponding lensless system. All diffraction was performed using a circular selected area aperture of −1 µm in projection.

MicroED data processing. Diffraction movies were converted to the SMV file format using TVIPS tools as previously described. Indexing and integration were performed using XDS. Integrated intensity data was obtained from partial datasets of nine different proto-PrPSc crystals were sorted and merged in XSCEATE. Merged intensities were converted to amplitudes at various resolution cutoffs to produce separate 1.0–Å, 3.0–Å, 5.0–Å, 7.0–Å, and 7.5–Å datasets. Ab initio structure determination was performed on each of these datasets using SHELDX. Phases obtained from the atomic assembly generated by direct methods were used to produce maps of sufficient quality for subsequent model building in Coot and refinement in PHENIX using electron scattering form factors to produce a final structure in space group P1 with a final R(F) = Rw = 24/25. Refinement in REFMAC was carried out in parallel to a final R(F) = Rw = 23/25. The structure refined in PHENIX (PDBeX) was used in all subsequent analyses and is shown in figures. Ab initio structure determination for carbamazepine was performed in SHEXL in which a solution was found in space group P2121, with no errors in chemical assignment or atom positions for all carbon, nitrogen, and oxygen atoms. This solution was refined in SHEXL using electron form factors to an R value of 21.8%. A structure with hydrogen positions refined to best match difference density in the map lowered the R value to 19.8%.

Analysis of buried surface area (Sb) and surface complementarity (Ss) for proto-PrPSc.

Growth of proto-PrPSc crystals. Peptide powder was weighed and dissolved in ultrapure water at near maximum solubility, 3.5 mM. Crystals were grown at room temperature by the hanging-drop method in a 96-well Wizard screen. Crystals appeared as rod-like crystals and were further optimized in 24-well hanging drop vapor diffusion experiments. The best crystals of proto-PrPSc grew within 24 h at a peptide concentration of 1.75 mM in the presence of 0.1 M 2-(N-morpholino)ethanesulfonic acid (MES), pH 6.0, and either 10% ethanol or 10% 2-Methyl-2,4-pentanediol (MPD).

Transmission electron microscopy. Approximately 2 µl of aggregated proto-PrPSc were applied to 300-mesh Formvar-carbon coated grids (Ted Pella Inc.) for 2 min before excess liquid was removed and grids left to dry. Grids were imaged either on a Tecnai T12 or F20 electron microscope (Thermo Fisher, formerly FEI). Samples were imaged at a magnification of 2,100 e–/Å2 with a dose rate of <30 e−/Å2.

Microfocus X-ray data collection. Crystals grown in 0.1 M MES, pH 6.0, and 10% ethanol and mixed with 100% glycerol as cryoprotectant were harvested from 24-well hanging drop using MiTeGen loops and flash frozen in liquid nitrogen. 72 diffraction images were collected, each spanning a 3° wedge, from a single crystal at a temperature of 100K at the advanced photon source (APS) beamline 24-ID-E equipped with an ADSC Q315 CCD detector, using a 10-µm beam with a 0.98-Å wavelength.

Microfocus X-ray data processing and structure determination. Diffraction images collected from a single crystal of proto-PrPSc were indexed and integrated in Denzo, yielding a dataset with 80.75% overall completeness at 1.1-Å resolution in space group P1. A suitable molecular replacement solution was obtained from this data using the PHASER program and an idealized β-strand nonapeptide alanine model as a probe. The model was refined using REFMAC against the measured data to a final Rfree / Rwork of 0.14 / 0.16.

MicroED data analysis. The structure of carbamazepine was refined using REFMAC against the measured data to a final Rfree / Rwork of 0.162.49 / 0.14.
associated with intrastrand hydrogen bonds formed by three pairs of residues: Q168–N170, N171–N173 and Q172–N174. Polar contacts were also measured between Q168–N170, with a 3.1-Å donor-acceptor (D–A) distance and a 2.3-Å H–O distance, Q172–N174, with a 3.0-Å D–A distance and a 2.1-Å H–O distance and N171–N173, with a 3.1-Å D–A distance and a 2.3-Å H–O distance. In each case, the linked residues faced the same side of the β-strand and bridged residues at positions (N/Q)i and (N)i+2 within the strand. While the HBplus program does not identify C5 hydrogen bonds in our structure, we measured these bonds in residues N171, Q172, and N173, on the basis of the criteria that carbonyl oxygens bond with intraresidue amide protons if their geometry permits, with H–O distances shorter than 2.5 Å (ref. 22).

Amylome profiling. A subset of the predicted amylome was analyzed16, consisting of six-residue segments found to score favorably when threaded onto a template based on the structure of the yeast prion NNQQNY. We chose all segments that scored two s.d. better than the mean score for all peptides (Z score > 2). This subset of six-residue segments represents 95,381 out of 7,900,599 total segments, or 1.2% of all possible segments of this size in the human proteome. For each segment, we searched for a (Y/F) X (N/Q) X (N/Q) X (Y/F) motif across the region of the protein to which the segment belonged; a ten-residue window including two residues upstream and downstream of a profiled segment. QYNNQNNFV satisfies these metrics.

Calculation of contour maps. $2F_o - F_c$ and $F_o - F_c$ density maps were calculated from the final refined MTZ file using the FFT tool in CCP4. Maps were converted to MRC format in Chimera and imported into MATLAB. Contour plots were calculated such that the number of contours spanned the minimum to the maximum values of the maps with intervals of one s.d. (σ) between contour levels.

Life Sciences Reporting Summary. Further information on experimental design is available in the Life Sciences Reporting Summary.

Data availability. Atomic coordinates and structure factors for proto-PrPSc have been deposited in the EMDB and wwPDB; the structure obtained using MicroED is under accession codes EMD-7017 and PDB 6AXZ; the X-ray crystallography structure is under accession code PDB 6BTK. The structure of carbamazepine using microED has been deposited in the EMDB with accession code EMD-7287. Source data for all figures and files is available from the authors upon reasonable request, please see author contributions for specific datasets.

References
34. Shi, D. et al. The collection of MicroED data for macromolecular crystallography. Nat. Protoc. 11, 895–904 (2016).
35. Hattne, J., Shi, D., de la Cruz, M. J., Reyes, F. E. & Gonen, T. Modeling truncated pixel values of faint reflections in MicroED images. J. Appl. Crystallogr. 49, 1029–1034 (2016).
36. Kabsch, W. XDS. Acta Crystallogr. D. Biol. Crystallogr. 66, 125–132 (2010).
37. Sheldrick, G. M. A short history of SHELX. Acta Crystallogr. A 64, 112–122 (2008).
38. Emsley, P., Lohkamp, B., Scott, W. G. & Cowtan, K. Features and development of Coot. Acta Crystallogr. D. Biol. Crystallogr. 66, 486–501 (2010).
39. Adams, P. D. et al. PHENIX: a comprehensive Python-based system for macromolecular structure solution. Acta Crystallogr. D. Biol. Crystallogr. 66, 213–221 (2010).
40. Delano, W.L. The PyMOL Molecular Graphics System (DeLano Scientific, 2002).
41. McGaughey, G. B., Gagné, M. & Rappé, A. K. π-Stacking interactions. Alive and well in proteins. J. Biol. Chem. 273, 15458–15463 (1998).
42. McDonald, I. K. & Thornton, J. M. Satisfying hydrogen bonding potential in proteins. J. Mol. Biol. 238, 777–793 (1994).
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Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

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10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

N/A.

b. Describe the method of cell line authentication used.

N/A.

c. Report whether the cell lines were tested for mycoplasma contamination.

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Animals and human research participants

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

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