A human CCT5 gene mutation causing distal neuropathy impairs hexadecamer assembly in an archaeal model

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Molecular chaperones are indispensable cellular components that assist folding and assembly of newly synthesized proteins, translocation of proteins across membranes, as well as refolding and degrading of misfolded and aggregated proteins1. A class of chaperones, the chaperonins (Cpns) are large, hollow, ATP-dependent nanomachines that promote correct folding of a wide range of proteins2–7. Cpns are divided into two groups: group I Cpns, represented by GroEL/GroES, in bacteria, mitochondria and chloroplasts, and group II Cpns, occurring in eukaryotes and archaea8–10. They share similar quaternary structures consisting of a double toroid cylinder assembled into two rings stacked back to back. This arrangement generates two dynamic internal cavities that allow unfolded proteins to fold correctly in a confined environment that opens and shuts in a cycle powered by ATP hydrolysis11,12. Group I Cpns are composed of 14 identical subunits arranged in two heptameric rings and require a co-chaperone, GroES, to facilitate protein folding. The Group II Cpns, which are very similar in Archaea and Eukarya, form double rings with an eight or nine fold rotary symmetry and have built-in lids to function without a co-chaperone13–15.

The human CCT (chaperonin containing TCP1) is a complex assembly of eight similar but nonidentical subunits which functions to fold non-native proteins through the alternative opening and closing of the two chambers. By contrast, many hyperthermophilic Archaea, including Pyrococcus furiosus and several groups such as Pyrodictium, Methanopyrus, and Pyrobaculum spp have a single group II Cpn subunit. The double ring of these archaea is therefore composed of 16 identical subunits and is minimally complex16. Hyperthermophile Cpns are exceptionally stable in vitro17, affording a suitable model for studying oligomerization of group II Cpns such as the human CCT complex.

Mutations in human Group II CHT subunits 4 and 5 cause rare debilitating diseases18–20. For example, the His147Arg mutation in human CCT5 causes mutilating peripheral sensory neuropathy20. Little is known concerning the pathogenic mechanisms of these CCT mutations at the molecular level, and on the impact of the mutations upon the structure and function of the complex. Mutations that allow survival of the carriers are likely...
to have very subtle mechanistic deficits since major functional disruption would be lethal due to the critical and multiple roles of this ubiquitous chaperone. Therefore, pathogenic mutations with living phenotypes have to be considered a priori difficult to characterize at the molecular level with the systems available today. Ways must be found to amplify a defect to make it measurable. For this purpose, we focused on modeling the CCT5 mutation, using the Cpn60 from *Pyrococcus furiosus* (PI), which has only one subunit homolog that assembles into a hexadecameric double-ring complex like the human counterpart.

**Results**

Our strategy to use the CCT homolog from a hyperthermophile was based on the conservation of the sequence and inferred structure of the human CCT5 and the archaeal subunit shown in Fig. 1 (see also Supplementary Fig. S1). The colinearity of the alignment and the high similarity of the sequences suggest that the double-ring structure would be a simplified version (homo-oligomer) with functional equivalence to the extremely complex (hetero-oligomer) human CCT hexadecamers. The archaeon *Pyrococcus furiosus* grows optimally at 100°C. In a previous study we have characterized a C-terminal structural motif that is a major determinant of extreme thermal stability. Deletion or modification of this sequence does not disrupt the hexadecameric structure, but “detunes” the activity without loss of ATPase or protein folding properties. The deletion of 22 amino acids from the C-terminus of the Pf Cpn60 removes the “modulator domain” that controls thermostability in this exceptionally thermostable protein. Consequently, the thermal stability and T_{max} for activity are downgraded to 50°C. In Fig. 1, the alignment of the archaeal Cpn60 (PfCD) and the human CCT5 shows that there is strong conservation between these polypeptides. The human CCT5 has 44% amino acid sequence identity and 65% similarity with PfCD. The amino acid His147 in CCT5 matches Ile138 in PfCD and the upstream and downstream sequences show conservation and colinearity.

Structural representations of the human CCT5 and PfCD were generated in the Swiss-Model server, using their primary amino-acid sequences and as template the crystal structure of the Cpn60 α subunit from *Thermococcus* KS-1 (KS-1α; PDB ID: 1Q3Q), which was crystallized with an ATP analogue, AMP-PNP, and fluoride ion in the active site. Overall structural similarities and characteristic features of group II chaperonins are evident around the equatorial domain (Fig. 2A). This domain comprises the ATP/ADP binding site, is the region where the mutation H147R occurs in the case of the human CCT5-associated neuropathy, and is essentially superimposed. Noteworthy, the α-helices in which are located His147 in CCT5, or Ile138 in PfCD overlap, and the side chain of His147 of human CCT5 and Ile138 of PfCD showed the same configuration as Ile138 of KS-1α. To analyze the structural consequences of mutation I138R in PfCD, we superimposed the PfCD structure onto the crystal structure of the KS-1α (1Q3Q) (Fig. 2B). The mutation I138R is located in the equatorial domain, in the N-terminus of Cpn, and this position is close to the intermediate domain, which serves as a hinge to allow binding of ATP/ADP when the apical alpha-helical protrusion domain of Cpn recognizes client proteins. Arg138 is contiguous to the conserved motif Gly94-Thr99 constituting the ATP binding site in group II chaperonins, suggesting that steric hindrance might impede the conformational change necessary for ATP binding. A similar situation is apparent in the case of mutant human CCT5 with Arg147, instead of His147 (Fig. 2C).

In Fig. 3, the optimal temperatures for ATPase activity of the Pf Cpn wild type as well as I138H and I138R were shown to be 50°C. The PfCD and I138H Cpons showed much higher ATPase activities than I138R Cpn over the whole temperature range investigated. Fig. 3 also shows that the residual activity of I138R Cpn was ~12% of that of PfCD, whereas I138H retained ~88% of ATPase activity compared with PfCD. These data indicate that Arg located at position 138 caused the loss of ~88% of the total ATPase activity displayed by PfCD.

Fig. 4 shows the wild type, I138H and I138R complexes separated on a 4–9% gradient native-PAGE gel. Group II Cpons typically form double-ring hexadecamers, the quaternary structures of group II Cpn, consisting of a double toroid cylinder assembled into two rings of subunits stacked back-to-back. This conformation is optimal for

![Figure 1](image-url)
Figure 2 | (A). Human CCT5 and *P. furiosus* PfCD superposed onto the crystal structure of *Thermococcus* strain KS-1 α. **LEFT**: The structure (Swiss-Model [http://swissmodel.expasy.org/]) of PfCD (gold) superposed onto the crystal structure of KS1α (monomers, displayed in marine blue, violet, and deep teal as surface, and in cyan color as ribbon). The hexadecamer, double-ring structure, is depicted by a dotted line, as is on the right of the subsequent panels B and C. **RIGHT**: Magnified image of the superposed structures of the PfCD (gold) and Human CCT5 (orange) onto KS1α; cyan ribbon). Side chains of isoleucine at 138 of PfCD (blue) and isoleucine at 138 of KS1α (deep teal), and side chain of histidine at 147 of human CCT5 (red) are represented as ball and stick. AMP-PNP (green stick; also shown on the left in panels B and C) and magnesium ion (yellow ball). (B). PfCD around the Arg138 mutation and its predicted matching areas in the crystal structure of KS1α. LEFT: Mutant Arg138 (orange) and its adjacent Arg421 (red) in the opposite α helix are drawn with stick in the PfCD structure (marine blue). **RIGHT**: Arg138 mutation area was superposed onto the crystal structure of KS1α (monomers are displayed in various shades of blue). (C). Human CCT5 structure around the Arg147 mutation viewed from different angles and predicted matching areas in the crystal structure of KS1α. LEFT: Wild-type His147 (blue), mutant Arg147 (red) and their adjacent amino acids, Ser428 and Cys429 (magenta) in the opposite α helix are drawn with ball and stick and transparent surface in the human CCT5 structure (orange ribbon). **RIGHT**: The predicted Arg147 mutation area in the monomers is shown as a red surface on the crystal structure of KS1α (monomers are shown as surface in marine blue, violet, deep teal, and cyan colors).
promoting correct folding of non-native cellular proteins. Whereas there was no clear difference in molar concentration ratios of hexadecamer between PfCD and I138H, a relatively lower concentration of double-ringed Cpn was observed in the case of I138R (Fig. 4, top panel). Densitometric analysis revealed that I138 formed double ring approximately 4–6 fold less than PfCD or I138H, indicating a functional deficit in maintaining the double ring structure (results not shown).

Fig. 5 shows that protection against heat denaturation of two enzymes by PfCD and its mutants is lower for I138R as compared with I138H and PfCD when hetero-oligomeric preparations are tested. However, hexadecamers of all three variants of the chaperonin, freshly purified by gel permeation chromatography, all showed intact heat protection activity. The enzymes tested at various temperatures were porcine mitochondrial malate dehydrogenase (MDH) and shrimp alkaline phosphatase (SAP). The results with hetero-oligomeric preparations, incubated with MDH at 37 or 42 °C are shown in panels A and B, respectively, while the results with freshly purified hexadecamers are shown in panels C (MDH, at 37 °C) and D (SAP, at 50 °C).

In Fig. 6, the ability of the archaeal Cpn60 to deconstruct and disperse amyloid fibrils under mild conditions, a system that has been described in detail elsewhere, is confirmed. The top panel shows the activity of the PfCD Cpn60 to disrupt fibrils completely in 30 min. The activity of the I138H mutant is similar to PfCD (middle panel), whereas the I138R mutant is defective, and the fibrils are intact at 60 min (bottom panel).

Since the study of the activity of pure monomers was not feasible because of their strong tendency to oligomerize, their response to heat stress was examined in silico, via simulations. The results are shown in Fig. 7 and the overall conclusions from these analyses are displayed in Table 1. It can be seen that the archaeal chaperonin with optimal temperature at 42 °C, or higher, is rigid (it loses flexibility) at 37 °C, a stressful temperature for this molecule. Similarly, CCT5 is flexible at its physiological temperature of 37 °C but is rigid, i.e., it loses flexibility, at the stressful temperature of 42 °C. It can also be seen that the Arg mutations in both the archaeal and the human chaperonins, result in a loss of flexibility in the two molecules, even at their respective optimal temperatures.

Discussion

Human TRiC/CCT is a double-ring complex that interacts with ~10% of the human cytosolic proteins and is essential to achieve folding and stabilization of many essential proteins in human and yeast. In addition, TRiC modulates and suppresses the aggregation of neurologically toxic proteins with polyglutamine motifs. We have set out to model the function of mutant forms of the TRiC hexadecamer using an archaeal Cpn60 homolog. The results show overall structural similarities and characteristic features of group II chaperonins around the equatorial domain (Fig. 2A), including the ATP/ADP binding site, which is the region where the mutation His147Arg occurs in the case of the human CCT5-associated neuropathy. Remarkably, the α-helices that include His147 in CCT5, and Ile138 in PfCD overlap, and the side chain of His147 of human CCT5 and Ile138 of the archaeal model colocalized with Ile138. Assuming that His 147 was the wild type residue at the position of the mutation causing the peripheral sensory neuropathy, we mutated Ile138 to histidine to recreate the human structural element and tested the effect on chaperone stability and function in vitro. H138R is located in the equatorial domain, near the N-terminus, close to the intermediate domain, which serves as a hinge with a large articulation angle, to allow binding of ATP/ADP when the apical alpha-helical protrusion domain of Cpn recognizes client proteins. Interestingly, our I138R structure representation revealed...
that the Arg138 mutation formed a close contact with a neighboring Arg421 in the adjacent α-helix. It seems that the side chain of Arg could cause a steric hindrance through the repulsive effects of closing the distance between two internal positive charges, thus affecting the helix-helix interface (Fig. 2C). Arg138 is contiguous to the highly conserved motif Gly94-Thr99 for ATP binding in group II chaperonins25, suggesting steric hindrance might impede the necessary conformational change for ATP binding.

Figure 2 also shows that the “humanized” version had little effect on the structure and function of the complex. In contrast, the mutation I138R (Fig. 2C), sharply reduced the catalytic capability resulting in low ATPase activity, and poor heat protection of mesophilic vertebrate enzymes. The quaternary structures of group II chaperonins, consisting of two rings of subunits stacked back to back, were determined on a gradient native-PAGE gel shown in Fig. 4, similar to analysis of assembly deficient N terminal mutants 22. Interestingly, whereas there was no difference in molar concentration ratios of hexadecamer in preparations of PfCD, or I138H, double-ringed Cpn at ~1 mDa was sharply reduced in the case of I138R (Fig. 4, top panel). Densitometric analysis revealed that I138R formed less than 20% of double rings than PfCD or I138H indicating a functional deficit in maintaining the double ring structure (Supplementary material).

We have previously characterized the C-terminus as a primary thermostability determinant, which can be tuned to achieve lower temperatures for optimal activity of the archaeal Cpn60 22. Thus, the Cpn60 complex from the hyperthermophile Pyrococcus furiosus (Topt for growth 100°C), which was originally active and stable at 95°C, was lowered to 50–55°C by the deletion of the C-terminus. Thus detuned, the Cpn60 can be used for refolding and salvage of mesophilic enzymes such as porcine mitochondrial MDH as shown in Fig. 5. Porcine MDH is known to lose activity rapidly at 51°C 31. Incubation at 50°C in the absence of Cpn resulted in rapid first-order loss of MDH activity \( t_{1/2} \approx 13 \) min. Both the PfCD and I138H Cpons were effective in protecting MDH against heat-denaturation at both 42 and 50°C compared to unprotected MDH (Figs 5A, and B, respectively). By contrast, I138R showed drastically diminished inhibition of heat-denaturation of MDH \( t_{1/2} \approx 36 \) min, as indicated
Figure 6 | Dispersion of amyloid fibrils by archaeal PCD (top strip of panels), partial dispersion by I138H (middle strip of panels) and lack of dispersion by I138R (bottom strip of panels).

Homology modelling. The 3D structures of the wild type molecules (PCD, and CCT5-His67) and their mutants (PCD Ile138His, and His138Arg; and CCT5 Arg147) were constructed by homology modeling. This was performed using PRIME software (PRIME, 2012) and FASTA sequences. The sequences of CCT5 and PCD were retrieved from the UniProt KB/TreMBL database. Basic Local Alignment Search Tool (BLAST) searches predicted that the crystal structure of the chaperonin from Thermococcus strain KS-1, PDB code: I0Q3; resolution 3.0 Å, was most suitable average structure. Alignments between the template and the target sequences were performed using ClustalW 2.0 with default parameters. The structures of all proteins were generated using the PRIME module (PRIME, version 3.1, Schrödinger, LLC, New York, NY, 2012). The generated models were relaxed by molecular dynamic simulations and were further validated.

Chlorins, enzymes and reagents. Restriction endonucleases, Tag polymerase and DNA ligase were purchased from New England Biolabs (Beverly, MA). Malate, ATP, EDTA, DTT, β-NADH and NAD were purchased from Sigma–Aldrich (St. Louis, MO). Porcine heart malate dehydrogenase (MDH) was purchased from Amresco (Solon, OH). PageRuler Broad Range unstained protein ladder in SDS-PAGE and NativeMark+ unstained molecular weight protein standard were from Thermo scientific (Rockford, IL) and Invitrogen (Carlsbad, CA), respectively.

Methods

Sequence comparison. The following molecules were studied and compared: Human CCT5 (CCT5) wild type (His147) and its mutant CCT5 Arg147 (His147Arg); Pyrococcus furiosus Chaperonin 60 with Ile138, with the last 22 amino acids deleted (PCD)17,34; wild type for this work); PCD mutant Ile138His, and PCD Arg138 (His138Arg, equivalent to the human His147Arg mutant).

Gradient native-PAGE. For oligomeric pattern analysis of recombinant PCD, I138H and I138R, the GFC purified samples were analysed in 4–9% gradient native-PAGE. Stacking and two separating gel buffers with 4% or 9% acrylamide were prepared without SDS. In preparation of separating gel buffers with 9% acrylamide, glycerol plus Bromophenol Blue (0.001%) was added to a final concentration of 15% and vertical acrylamide gradient formed via specific gravity difference between 4% and 9% separating gel during gel solidification. Bromophenol Blue in separating gel buffers with 9% acrylamide served to visualize the gradient after gel formation. Purified chaperonins were preheated under ATPase activity assay conditions at 50°C for 30 min to oligomerize complexes, prior to mixing with PAGE gel sample buffer without β-mercaptoethanol and SDS. Gradient native gels were run at constant voltage (20 mV) for 3 h at 23°C and PCD, I138H and I138R protein bands on native SDS-PAGE gel were visualized by staining with Coomassie Brilliant Blue R-250. See Supplementary Figs. S4 and S5.

ATPase activity and heat protection assays using MDH or SAP. The standard ATPase activity assay reaction buffer composed of 25 mM Hepes–KOH (pH 7.2), 300 mM KCl, 1 mM MgCl2 in the presence or absence of 0.05 mg/ml tin peroxide or absence of 0.05 mg/ml ascorbate. The reaction proceeded for 15 min at each temperature in a PCR thermocycler (Bio-Rad), followed by perchloric acid addition to a final concentration of 2% v/v to quench the reaction. The liberated P1 was determined by the Malachite Green assay at 630 nm. All reactions and controls were performed under liquid mineral oil (Sigma–Aldrich) for avoiding evaporation during reaction. Standard deviation was calculated from assays repeated three times.

Porcine malate dehydrogenase (MDH) heat protection by PCD, I138H and I138R was analyzed by comparing protection activities at 37 or 42°C. MDH samples (1 mg/ml) were incubated in reaction buffer (25 mM Hepes–KOH, pH 8.0, 300 mM KCl, 1 mM MgCl2, and 0.5 mM ammonium sulfate) plus 4 mM ATP at each temperature in the presence or absence of Cpn (0.2 mg/ml). Residual MDH enzyme activities after different incubation time intervals (0, 3, 6, 9, 12 and 15 min at 37°C and 0, 1, 2, 3, 4, and 5 min at 42°C) were measured immediately as described at 25°C in modified assay mixture (90 mM Hepes–KOH, pH 8.0, 0.22 mM α-NADH, 0.55 mM oxaloacetate (Sigma–Aldrich)). The time dependent oxidation of β-NADH was measured by recording the decreasing OD 340 nm. To measure residual shrimp alkaline phosphatase activity (enzyme activity of phosphatase (nNPP) supported by nNPP Phosphatase Assay Kit (BioAssay Systems, Hayward, CA). The nNPP is a chromogenic substrate which was converted via SAP to β-nitrophenol was measured at 405 nm under alkaline conditions.

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Figure 7 | Root mean square deviation (RMSD) plot of Cz atoms at 310 and 315 K. Top panel: PfCD at 310 K (blue line) and at 315 K (red line). Middle panel, I138H at 310 K (blue line) and at 315 K (red line); I138R at 310 K (green line) and at 315 K (violet line). Bottom panel: Human CCT5 with H at position 147 at 310 and 315 K (blue and red lines, respectively); Human CCT5 with R at position 147 at 310 and 315 K (green and violet lines, respectively).
Fibril dispersion assays and Atomic Force Microscopy. Amyloid fibrils prepared from bovine insulin (I5500, Sigma-Aldrich) were subjected to dispersion assays by the chaperonins from bovine insulin (I5500, Sigma-Aldrich) were subjected to dispersion assays by the chaperonins from bovine insulin (I5500, Sigma-Aldrich) were subjected to dispersion assays by the chaperonins.

3. Goñi-Puertas, P., Martín-Benito, J., Carrascosa, J. L., Willison, K. R. & Valpuesta, J. M. The substrate recognition mechanisms in chaperonins. J. Mol. Biol. 323–355 (2013).
4. Macario, A. J. L., Conway de Macario, E. & Cappello, F. Conformational cycling by catalyzing nucleotide exchange. Proc. Natl. Acad. Sci. USA 1993 Apr 5; 90, 3775. PMID, 8096339 (1993). Erratum in: Proc Natl Acad Sci USA 2011 Jan 21; 108, 9022. DOI: 10.7554/eLife.00710 (2013).
5. Zhang, J. et al. Cryo-EM structure of a group II chaperonin in the prehydrolysate ATP-bound state leading to lid closure. Structure. 2011 May 11; 19, 633–639. DOI: 10.1016/j.str.2011.03.005 (2011).
6. Laksanalamai, P., Whitehead, T. A. & Robb, F. T. Minimal protein-folding systems in hyperthermophilic archaea. Nature Rev. Microbiol. 2, 315–324 (2004).
7. Le9071u, H., Laksanalamai, P. & Robb, F. T. An exceptionally stable Group II chaperonin from the hyperthermophile Pyrococcus furiosus. Arch. Biochem. Biophys. 486, 12–18 (2009).
8. Macario, A. J. L. & Conway de Macario, E. Sick chaperones, cellular stress and disease. New Eng. J. Med. 353, 1489–1501 (2005).
9. Macario, A. J. L., Conway de Macario, E. & Cappello, F. Chaperonopathies. Diseases with Defective Molecular Chaperones. (Ebook) DOI: 10.1007/978-94-007-4667-1 (2013).
10. Springer Dordrecht-Heidelberg-New York-London. (2013).
11. Bouhouche, A., Benomar, A., Bouslam, N., Chikil, T. & Yahyaoui, M. Mutation in the epsilon subset of the cytosolic chaperonin-containing t-complex peptide-1 (Cct5) gene causes autosomal recessive mutilating sensory neuropathy with spastic paraplegia. J. Med. Genet. 44, 441–443 (2007).
12. Luo, H. & Robb, F. T. Protein folding systems in thermophiles. [Chapter 4.9 pp. 584–597] in The Extremophile Handbook (Springer, Tokyo). Horikoshi, K., Grant, W.R., Antranikian, G. & Robb F.T. (eds.) (2010).
13. Luo, H. & Robb, F. T. A modulator domain controlling thermal stability in the Group II chaperonins of Archaea. Arch. Biochem. Biophys. 512, 111–118 (2011).
14. Czakó, E. et al. Characterization of archaean group II chaperonin-ADP-metal fluoride complexes: implications that group II chaperonins operate as a “two-stroke engine” J. Biol.Chem. 280, 40375–40383. Epub 2005 Sep 23. PMID, 16183634 (2005).
15. Booth, C. R. et al. Mechanism of lid closure in the eukaryotic chaperonin TRiC/CCT. Nat. Struct. Mol. Biol. 15, 746–753. DOI: 10.1038/nmsb.1436 (2008).
16. Ditzl, L. et al. Crystal structure of the thermosome, the archaean chaperonin and homolog of CCT. Cell 93, 125–138 (1998).
17. Kurokusi, D., Luo, H., Sereva, V., Robb, F. T. & Lednev, I. K. Deconstruction of stable cross-Beta fibrillar structures into toxic and nontoxic products using a mutated archaean chaperonin. ACS Chem. Biol. 8, 2095–2101 (2013).
18. Yarn, A. V. et al. Defining the TRiC/CCT interactome links chaperonin function to stabilization of newly made proteins with complex topologies. Nat. Structural Mol. Biol. 15, 1255–62. DOI: 10.1038/nsmb.1515 (2008).
19. Amit, M. et al. Equivalent mutations in the eight subunits of the chaperonin CCT produce dramatically different cellular and gene expression phenotypes. J. Mol. Biol. 401, 532–543. DOI: 10.1016/j.jmb.2010.06.037 (2010).
20. Kitamura, A. et al. Cytosolic chaperonin prevents polyglutamate toxicity with altering the aggregation state. Nat. Cell Biol. 10, 1163–1170, Epub 2006 Sep 17. PMID, 16909585 (2006).
21. Shahmoradian, S. H. et al. TRiC’s tricks inhibit huntingtin aggregation. Elife 2, e00710, DOI: 10.7554/eLife.00710 (2013).
22. Hartman, D. J., Surin, B. P., Dixon, N. E., Hoogenraad, N. J. & Heij, P. B. Substoichiometric amounts of the molecular chaperones GroEL and GroES prevent thermal denaturation and aggregation of mammalian mitochondrial malate dehydrogenase in vitro. Proc. Natl. Acad. Sci. USA. 2001 Apr 3; 98, 5977–5982. DOI: 10.1073/pnas.980449798 (2001).
23. Kurokusi, D., Luo, H., Sereva, V., Robb, F. T. & Lednev, I. K. Rapid degradation of amyloid fibrils controls their smallest possible fragment size. J. Mol. Biol. 376, 1155–1167 (2008).
24. Luo, H., Zhang, P. & Robb, F. T. Oligomerization of an archaean group II chaperonin is mediated by N-terminal salt bridges. Biochem. Biophys. Res. Commun. 2011 Sep 23; 413, 389–394, PMID, 21893040 (2011).
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Author contributions

F.T.R., E. C.de M., A.J.L.M. and F. C. conceived and designed the experiments. A.L. and A.A.M. carried out molecular modeling and the simulation experiments. H.L., F.A. and F.T.R. designed mutant gene construction. W.K.M. and F.A. conducted recombinant protein expression and purification. W.K.M., F.A. and M.S. carried out protein characterization and enzyme stability-protection studies. M.S. and I.L. carried out fibril deconstruction experiments. F.T.R., E. C.de M., A.J.L.M. and F.C. wrote the paper.

Additional information

Supplementary information accompanies this paper at http://www.nature.com/scientificreports

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