Newly deciphered structure suggests how infectious prions replicate

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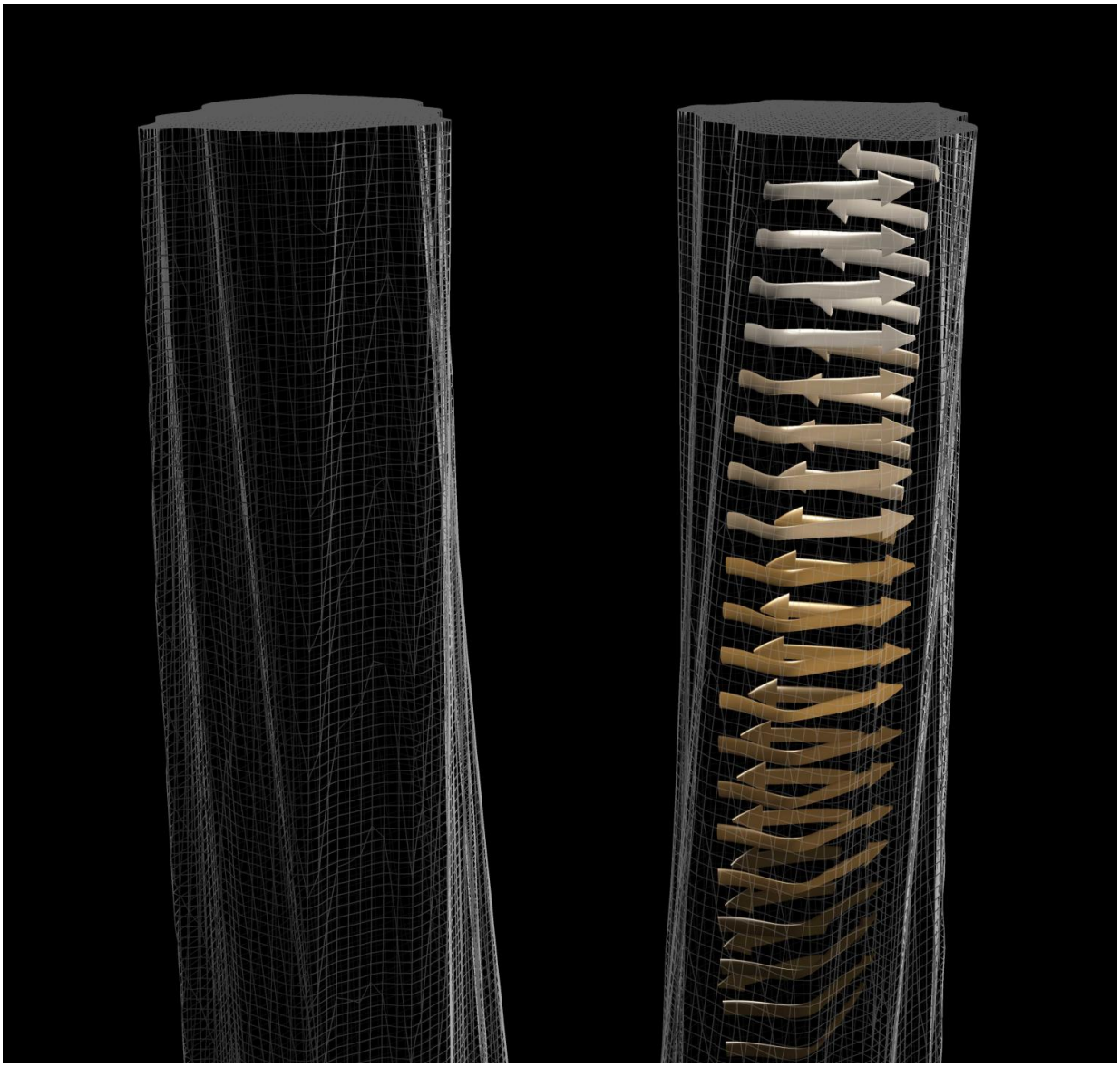

Molecular architecture of an infectious mammalian prion is shown. Credit: Ester

Vázquez-Fernández, Howard Young, Holger Wille, Jesús Requena.

Infectious prions or PrPSc—misfolded versions of the normal cellular prion protein PrPC—convert their normal counterparts into copies of themselves and thereby cause fatal disease. How this conversion works at the molecular level has remained largely a mystery. A study published on September 8th in *PLOS Pathogens* reports the three-dimensional structure of a large part of PrPSc. The structure argues against existing theories of conversion and suggests how the process might actually work.

The discovery of the structure of DNA in 1953 made it immediately obvious how DNA could be copied, or replicated. The three-dimensional structure of PrPSc has remained elusive, but the hope is that its discovery would likewise promote the understanding of prion replication, as well as lead to the development of structure-based therapeutic interventions. Convinced that the structure of what they call 'infectious conformers'—PrPSc from the brain of diseased animals—will be most informative, a team led by Holger Wille and Howard Young from the University of Alberta in Edmonton, Canada, and Jesús Requena from the University of Santiago de Compostela, Spain, is applying electron cryomicroscopy (cryo-EM) to the problem.

In this study, they used cryo-EM to record and analyze the structure of PrPSc isolated from the brain of infected mice. Prion-infected mouse and human brains contain a mix of different versions of PrPSc because different types of molecules such as lipids and sugars have been attached to the core protein. The heterogeneity of these modified brain-derived PrPSc makes it difficult to analyze their structure. To avoid this difficulty, the researchers started with PrPSc molecules that were truncated to delete the attachment of one type of modification, the so-called GPI lipid anchor. By using as a source the brains of transgenic

mice expressing a GPI-anchorless form of the prion protein, they were able to analyze a more homogeneous version of PrPSc that nonetheless retained its ability to cause disease and convert normal cellular prion proteins.

In the diseased brain, PrPSc molecules are often arranged in fibrils. The cryo-EM images of the mouse GPI-anchorless PrPSc fibrils, and their subsequent analysis, showed that they consist of two intertwined protofilaments of defined volume. As cryo-EM preserves the native structure of specimens, this information sets a structural restraint for the conformation of GPI-anchorless PrPSc, with the implication that PrPSc molecules can form protofilaments with the observed dimensions only if they are folded up onto themselves.

Based on their own analyses (and consistent with data from related studies), the researchers conclude that the cryo-EM data reveal a four-rung ß-solenoid architecture as the basic element for the structure of the mammalian prion GPI-anchorless PrPSc. ß-solenoids are protein structures that consist of an array of repetitive elements with secondary structures that are predominantly beta sheets. These PrPSc beta-sheet rungs, the researchers propose, serve as templates for new unfolded PrPSc molecules.

What they have learned about the structure of GPI-anchorless PrPSc and its four-rung ß solenoid architecture, the researchers say, allows them to rule out all previously proposed templating mechanisms for the replication of infectious prions in vivo. Discussing their ideas for the conversion of PrPC to PrPSc, the researchers note that the molecular forces responsible for the templating are fundamentally similar to those operating during the replication of DNA. "Because the exquisite specificity of the A:T and G:C pairings is lacking", they conclude that "a much more complex array of forces controls the pairing of the pre-existing and nascent ß-rungs".

"Templating based on a four-rung ß-solenoid architecture", they say, "must involve the upper- and lowermost ß-solenoid rungs [which] are inherently aggregation-prone". "Once an additional ß-rung has formed", they propose, "it creates a fresh "sticky" edge ready to continue templating until the incoming unfolded PrP molecule has been converted into another copy of the infectious conformer".

The researchers acknowledge that higher resolution structures and resolution of structures of other PrPSc molecules will be needed. Nonetheless, they conclude, "we present data based on cryo-EM analysis that strongly support the notion that GPI-anchorless PrPSc fibrils consist of stacks of four-rung ß-solenoids. Two of such protofilaments intertwine to form double fibrils [...]. The four-rung ß-solenoid architecture of GPI-anchorless PrPSc provides unique and novel insights into the molecular mechanism by which mammalian prions replicate".

More information: Vázquez-Fernández E, Vos MR, Afanasyev P, Cebey L, Sevillano AM, Vidal E, et al. (2016) The Structural Architecture of an Infectious Mammalian Prion Using Electron Cryomicroscopy. *PLoS Pathog* 12(9): e1005835. DOI: 10.1371/journal.ppat.1005835