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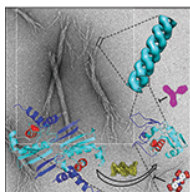
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## *EXTRA VIEWS*

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### Getting to the core of prion superstructural variability

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**ABSTRACT.** The phenomenon of protein superstructural polymorphism has become the subject of increased research activity. Besides the relevance to explain the existence of multiple prion strains, such activity is partly driven by the recent finding that in many age-related neurodegenerative diseases highly ordered self-associated forms of peptides and proteins might be the structural basis of prion-like processes and strains giving rise to different disease phenotypes.

Biophysical studies of prion strains have been hindered by a lack of tools to characterize inherently noncrystalline, heterogeneous and insoluble proteins. A description of the pressure response of prion quaternary structures might change this picture. This is because applying pressure induces quaternary structural changes of PrP, such as misfolding and self-assembly. From the thermodynamics of these processes, structural features in terms of associated volume changes can then be deduced. We suggest that conformation-enciphered prion strains can be distinguished in terms of voids in the interfaces of the constituting PrP protomers and thus in their volumetric properties.

**KEYWORDS.** prion, amyloid, oligomer, pressure, strain, protein misfolding

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The assembly of complex protein superstructures, such as oligomers and amyloid fibrils, from simpler misfolded proteins or peptides is a hallmark of various, seemingly unrelated disorders.<sup>1</sup> These include prion diseases, Alzheimer's, synucleinopathies, and type II diabetes, all of which are progressive disorders with associated high morbidity and mortality.

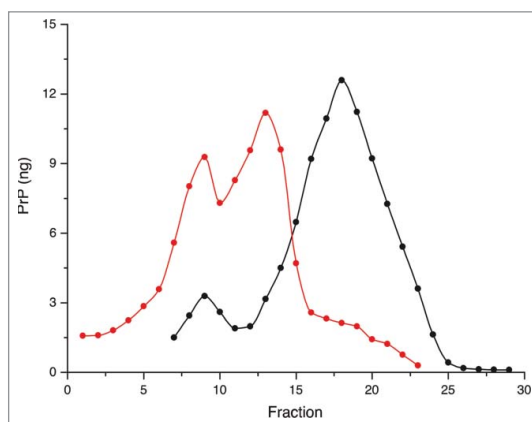
In prion diseases, the prion protein (PrP<sup>C</sup>) misfolds and assembles into various self-propagating quaternary structures with predominantly  $\beta$  sheet secondary structure, designated PrP<sup>Sc</sup> (Sc for scrapie, a prion disease of small ruminants).<sup>2</sup> PrP assemblies formed in vitro can show significant variability in their structures and biological effects,<sup>3-8</sup> yet the structural basis of PrP<sup>Sc</sup> in vivo has remained elusive so far. It is generally accepted that the extreme structural plasticity of brain-derived PrP<sup>Sc</sup> occurs through conformational changes within protomers or through rearrangements of the subunits relative to each other. Besides underlying the generally observed amyloid polymorphism, these changes appear sufficiently different and stable to allow the existence of biologically distinct prion strains within the same host-species.<sup>9-16</sup> Within the context of the protein-only hypothesis, a strain is therefore viewed as a protein superstructural identity that confers distinctive symptomology, as well as a precise pathobiology and infectious character to PrP<sup>Sc</sup>. Moreover, such PrP superstructures can be maintained indefinitely, under defined conditions, by repeated passaging in laboratory animals.

Another layer of complexity in describing PrP structural features comes from recent findings suggesting that beyond the inter-strain variability, a prion strain itself may be composed of an ensemble of superstructures. Indeed, PrP<sup>Sc</sup> might exist as an ensemble of nonidentical but related molecular forms in a dynamic equilibrium (i.e. that can interconvert).<sup>17</sup> Such a structural network or quasispecies,<sup>18,19</sup> might be maintained in vivo under the selection pressure imposed by complex interactions with other macromolecular partners. In this regard, a number of molecules have been proposed to be involved in the in vivo PrP misfolding.<sup>20-22</sup> We have recently demonstrated that both

endogenous Shadoo, a protein that resembles the flexibly disordered N-terminal domain of PrP<sup>C</sup>, and acetylcholinesterase can affect PrP structural dynamics and therefore modify the physicochemical properties of PrP<sup>Sc</sup>.<sup>23,24</sup> From a thermodynamic point of view, a particular environment leads predictably to a dominant PrP<sup>Sc</sup> morphotype preferentially stabilized/propagated, together with less populated superstructures. The "strain shift" or "strain mutation" phenomenon that is observed in prion diseases upon passage to another host,<sup>25</sup> could therefore be readily explained by a preferential selection of alternative dominant superstructures, probably due to changes in the PrP primary sequence (between species) or to a fine-tuned, tissue-specific interactome (within and between species).

While a close correlation between diverse prion strain phenotypes and PrP<sup>Sc</sup> conformational stability (against thermal or chaotropic denaturation), as well as relative susceptibility to proteolytic cleavage is widely recognized,<sup>5,26-32</sup> less is known about the underlying physical and structural basis characterizing such assemblies. There is limited evidence showing the diversity and complexity of PrP assemblies within an isolated strain. Our recent results obtained applying sedimentation fractionation techniques to a panel of prion strains are consistent with the essential role of the hydrodynamic properties (hydrodynamic size) of PrP<sup>Sc</sup> in prion conversion efficiency and duration of disease.<sup>33</sup> Another key parameter distinguishing various PrP<sup>Sc</sup> superstructures, and differing between PrP<sup>Sc</sup> and PrP<sup>C</sup> is the volume (i.e density).<sup>34</sup> Of particular interest is that PrP<sup>Sc</sup> is found in fractions of lower density than PrP<sup>C</sup> after isopycnic density gradient centrifugation (equilibrium separation) (Fig. 1 and ref. 33). This observation is consistent with the presence of packing defects that might arise during the assembly of the PrP protomers to form the PrP<sup>Sc</sup> assemblies. Remarkably, the sedimentation profile of several distinct prion strains from different species reveals at least 2 PrP<sup>Sc</sup> populations with different density properties (Fig. 1 and ref. 33), supporting the existence of prion superstructural heterogeneity.

FIGURE 1. Solubilized brain material from uninfected (black) or 263K (red) infected hamsters was fractionated by sedimentation at the equilibrium. The collected fractions (numbered from top to bottom of the gradient) were analyzed for PrP content by immunoblot. The amount (in ng) of PrP per fraction was reported on the graph by using a standard curve of recombinant PrP. For methodological details see ref. 34.



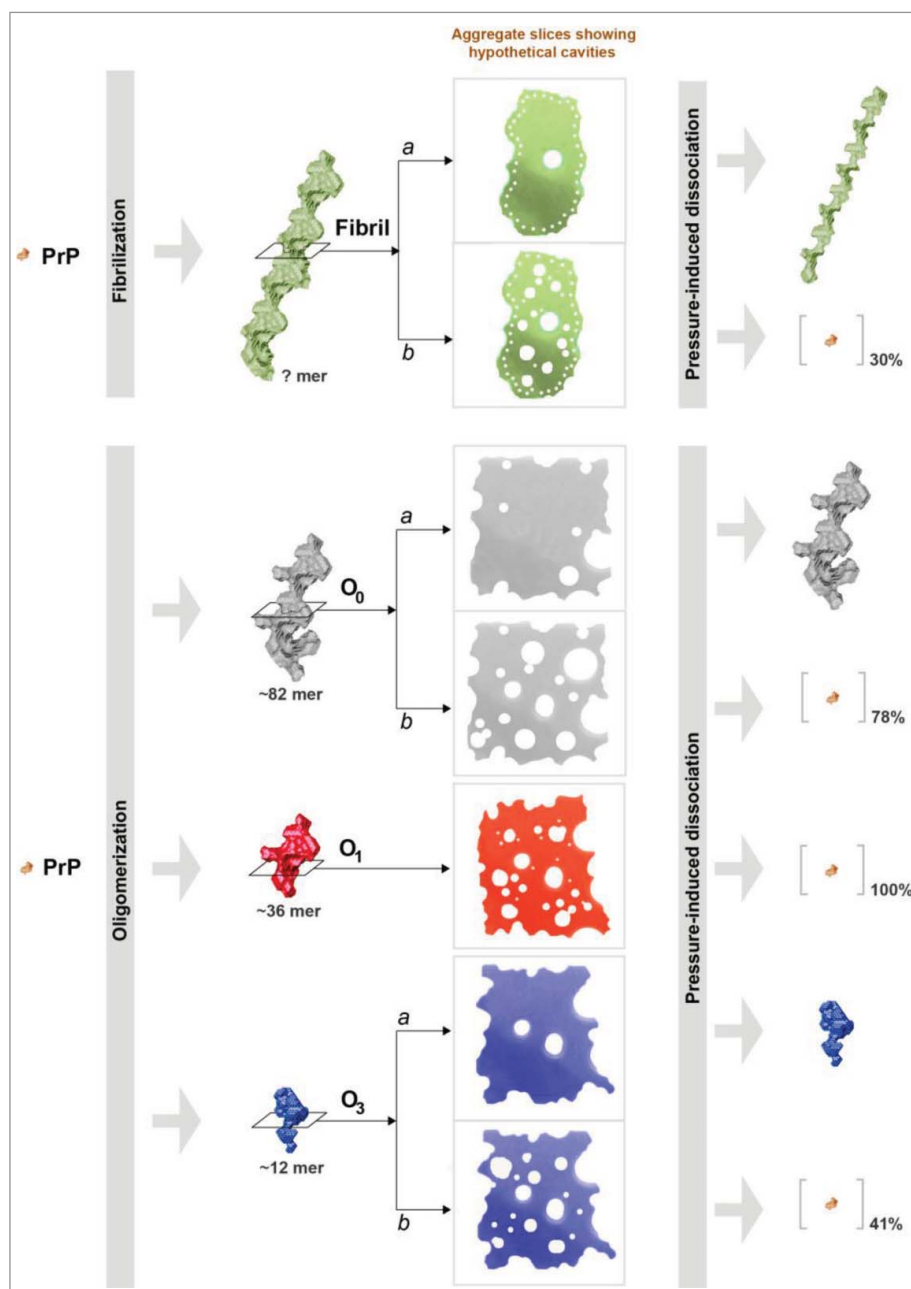
It is therefore likely that thermodynamically and conformationally unique PrP superstructures differ in their hydration and packing, 2 properties that modify directly the volume of the system. In line with this view, a recent study using 2 PrP amyloid fibrils formed from the recombinant protein demonstrated that stability differences might be governed by distinct packing arrangements.<sup>35</sup> Hence, we believe that the bedrock principle of PrP<sup>Sc</sup> polymorphism must be largely determined by the interfaces of the constituting PrP protomers. These interfaces should exhibit significantly different packing defects between distinct superstructures, yet conserving their basic structural arrangement of cross  $\beta$ -sheet. Nevertheless, the physical nature of such protein boundaries, the influence of inter-subunit cavities in PrP conformational changes, and its functional link to prion replication and pathogenesis remain to be determined.

It was recently demonstrated that eliminating the cavity volume in a protein acts as the principal determinant for its destabilization by pressure.<sup>36</sup> In contrast to temperature, or the use of exogenous chemical agents, pressure is a

fundamental thermodynamic parameter that affects only the volume of the system under study. Pressure changes the Gibbs free energy of a reversible process in proportion to its volume variation, favoring processes accompanied by a volume decrease. Because of its unique potential, the influence of pressure on the energy landscapes for protein folding and aggregation has not escaped the consideration of the scientific community.<sup>37,38</sup> The pressure response of a protein mainly consists in the unfolding of its 3 dimensional structure and, in the case of a protein assembly, in its dissociation.

We and other groups have used pressure as an external perturbation for shifting the dynamic balance between native and unfolded/misfolded PrP states.<sup>39-44</sup> These studies evidenced that the conformational changes mimicking the PrP structural conversion have important impacts in the PrP volumetric properties. Therefore, PrP structural variations are characteristically associated with changes in hydration and cavities. We previously found that the application of pressure to the native PrP state effectively populates aggregation-prone intermediates and, depending on the interplay of different parameters, such as temperature, pH, incubation time, distinct thermodynamically stable assemblies were obtained.<sup>42,43</sup> The combined effects of pressure and temperature also lead to a decrease in the infectivity titer in processed meat contaminated by prions.<sup>45-47</sup> Although not proven, these irreversible effects are certainly due to a pressure-induced destabilization of PrP<sup>Sc</sup>. This would mean that PrP assemblies (i.e., amyloid fibrils or oligomers) dissociate under pressure—at least to some extent. The impact of pressure on various PrP quaternary structures was recently studied in our group. We demonstrated that a pressure treatment leads to increasing destabilization of the assembled state, relative to the monomeric form. We therefore characterized the kinetic and energetic behavior of the pressure-induced dissociation of mature amyloid PrP fibrils<sup>48</sup> and of 3 distinct misfolded PrP oligomers of different sizes<sup>49</sup> that were generated in vitro from recombinant PrP. Figure 2 shows a schematic representation of the effects

FIGURE 2. Schematic representation of the effects of high pressure on mature PrP amyloid fibrils and 3 distinct  $\beta$ -sheet-rich PrP oligomers. This diagram illustrates the proposed model of pressure-induced structural changes, which depends on the strongly varying cavities present in their quaternary structure. The amount (% on the entire protein sample) of resolubilized PrP rescued from the superstructure after the pressure treatment is also shown.



of pressure on these various quaternary structures. The pressure-induced dissociation of fibrils was not complete, indicating structural heterogeneity of the starting amyloid fibrils.

We considered a population of initial PrP fibrils with few packing defects (denoted as “*a*” in the scheme). These fibrils underwent a conformational rearrangement under pressure that led to

a physicochemically different fibril, characterized by an irreversible loss of several amyloid specific features, including loss of thioflavin T binding. In contrast, a population of fibrils characterized by a low degree of packing (denoted as “*b*”), disaggregates into native monomers. In agreement with these results, different PrP oligomers exhibit different pressure-sensitivities. This can be explained by strongly varying void volumes in their quaternary structure. As for amyloid fibrils, we also brought evidence of 2 different degrees of barostability within a particular oligomer type (specifically for O0 and O3 oligomers), reflecting that 2 populations of PrP superstructures coexist in these samples of initial PrP fibrils.

These results open the opportunity to detect small differences in the conformational stability of quaternary structures associated with a particular strain, and between strains. Many important questions and extra-views lay ahead. Expanding this study to a large collection of brain samples from scrapie-infected animals might therefore be useful, not only to unravel the predicted wealth of PrP<sup>Sc</sup> superstructures, but also to induce a shift of the dominant superstructures. If minor PrP<sup>Sc</sup> structures that coexist during disease propagation have less solvent excluded volumes, these otherwise dismissed forms will be selected and enriched under high pressure, and could be subsequently amplified by standard techniques, such as including protein misfolding cyclic amplification (PMCA),<sup>50,51</sup> the amyloid seeding assay (ASA),<sup>52</sup> or quaking-induced conversion (QUIC).<sup>53,54</sup>

The latest results reviewed here show that defects in atomic packing may deserve consideration as a new factor that influences the PrP<sup>Sc</sup> assembly puzzle. We conclude that pressure provides an efficient tool to ascertain the highly diversified spatial arrangement between PrP protomers. Its use will provide information not only on mammalian prion self-perpetuating structural states, but also on other complex protein superstructures associated with a range of debilitating medical disorders, as well as on many newly discovered beneficial prions and prion-like proteins that are turning out to be versatile components in various physiological processes.

## DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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