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# Proteins behaving badly. Substoichiometric molecular control and amplification of the initiation and nature of amyloid fibril formation: lessons from and for blood clotting☆

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ABSTRACT

The chief and largely terminal element of normal blood clotting is considered to involve the polymerisation of the mainly α-helical fibrinogen to fibrin, with a binding mechanism involving 'knobs and holes' but with otherwise little change in protein secondary structure. We recognise, however, that extremely unusual mutations or mechanical stressing can cause fibrinogen to adopt a conformation containing extensive β-sheets. Similarly, prions can change morphology from a largely α-helical to largely β-sheet conformation, and the latter catalyses both the transition and the self-organising polymerisation of the β-sheet structures. Many other proteins can also do this, where it is known as amyloidogenesis. When fibrin is formed in samples from patients harbouring different diseases it can have widely varying diameters and morphologies. We here develop the idea, and summarise the evidence, that in many cases the anomalous fibrin fibre formation seen in such diseases actually amounts to amyloidogenesis. In particular, fibrin can interact with the amyloid-β (Aβ) protein that is misfolded in Alzheimer's disease. Seeing these unusual fibrin morphologies as true amyloids explains a great deal about fibrin(ogen) biology that was previously opaque, and provides novel strategies for treating such coagulopathies. The literature on blood clotting can usefully both inform and be informed by that on prions and on the many other widely recognised (β-)amyloid proteins. A preprint has been lodged in bioRxiv (Kell and Pretorius, 2016).



**Contents**



☆ Paper number 9 in the series "a dormant blood microbiome in chronic, inflammatory disease".

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"Novel but physiologically important factors that affect fibrinolysis have seldom been discovered and characterized in recent years" (Weisel, 2011)

## 1. Introduction: the thermodynamics of protein folding and prion proteins, and the existence of multiple macrostates

Starting with Anfinsen's famous protein re-folding experiments (Anfinsen, 1973; Anfinsen et al., 1961), showing that an unfolded protein would refold reliably to its commonest (and original) state as found in the cell, it was widely assumed that the normal macrostate of a folded protein was that of its lowest free energy.

If one allows each amino acid to have $n$ distinct conformational substates, the total number $n^m$ scales exponentially with the number m of amino acids (Kell, 2012), and until recently exhaustive calculations to determine whether the 'preferred' conformation was of lowest free energy were prohibitively expensive (Piana et al., 2014, 2012, 2013; Verma and Wenzel, 2009); indeed, they still are save for small proteins, so this question of whether the 'normal' conformation is that of lowest free energy (±kT) is certainly not settled in general terms, and (as we shall see in many cases) forms of lower free energy than the 'normal' one are in fact both common and of high biological significance.

In particular, as is again well known (Aguzzi and Calella, 2009; Caughey et al., 2009; Colby and Prusiner, 2011; Prusiner, 1998), and starting with Virchow's observations in 1854 (Sipe and Cohen, 2000), a number of proteins of a given sequence can exist in at least two (or more) highly distinct conformations (e.g. (Chiti and Dobson, 2006; Eisenberg and Jucker, 2012)). Typically the normal ('benign') form, as produced initially within the cell, will have a significant α-helical content and a very low amount of β-sheet, but the abnormal ('rogue') form, especially when in the form of an insoluble amyloid (Dobson, 2013), will have a massively increased amount of β-sheet (Baldwin et al., 1994; Groveman et al., 2014; Harrison et al., 1997; Jack et al., 2006; Jahn et al., 2008; Pan et al., 1993) (but cf (Ow and Dunstan, 2014)), whether parallel or antiparallel (Tycko and Wickner, 2013). The canonical example is the prion protein $PrP^c$, whose abnormal form is known as $PrP^{Sc}$, and whose $PrP^c$ structure is shown in Fig 1. As is also well known, the monomers of the abnormal form may catalyse their own formation from the normal form, and will typically go on to self-assemble to form oligomers, protofibrils and finally insoluble fibrils (Colby and Prusiner, 2011). (A particular hallmark of $PrP^{Sc}$, and indeed a common basis for its assay, is its very great resistance to proteolysis relative to $PrP^c$, typically assessed using proteinase K (Basu et al., 2007; Grassi et al., 2000; Mishra et al., 2004; Saá and Cervenakova, 2015; Saleem et al., 2014; Saverioni et al., 2013; Silva et al., 2015)).

**Fig. 1.** $PrP^c$ conformation of human prion protein (1HJM at PDB).

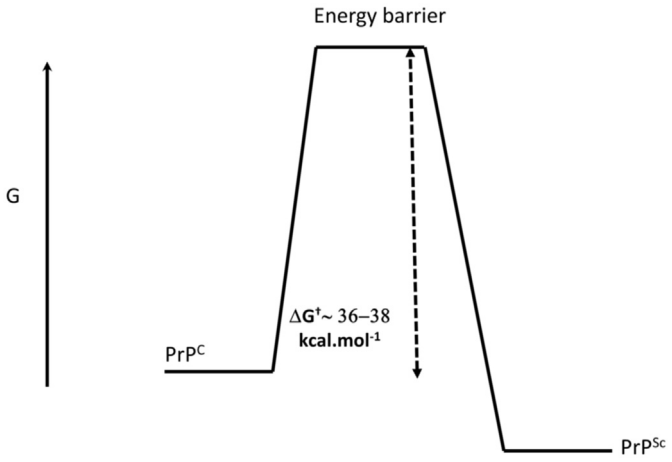


**Fig. 2.** Kinetic isolation of $PrP^{Sc}$ from $PrP^C$ (based on (Cohen and Prusiner, 1998).