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Review Article

Are fibrinaloid microclots a cause of autoimmunity in Long Covid and other post-infection diseases?

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It is now well established that the blood-clotting protein fibrinogen can polymerise into an anomalous form of fibrin that is amyloid in character; the resultant clots and microclots entrap many other molecules, stain with fluorogenic amyloid stains, are rather resistant to fibrinolysis, can block up microcapillaries, are implicated in a variety of diseases including Long COVID, and have been referred to as fibrinaloids. A necessary corollary of this anomalous polymerisation is the generation of novel epitopes in proteins that would normally be seen as 'self', and otherwise immunologically silent. The precise conformation of the resulting fibrinaloid clots (that, as with prions and classical amyloid proteins, can adopt multiple, stable conformations) must depend on the existing small molecules and metal ions that the fibrinogen may (and is some cases is known to) have bound before polymerisation. Any such novel epitopes, however, are likely to lead to the generation of autoantibodies. A convergent phenomenology, including distinct conformations and seeding of the anomalous form for initiation and propagation, is emerging to link knowledge in prions, prionoids, amyloids and now fibrinaloids. We here summarise the evidence for the above reasoning, which has substantial implications for our understanding of the genesis of autoimmunity (and the possible prevention thereof) based on the primary process of fibrinaloid formation.

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Introduction

Starting with analyses using the electron microscope (e.g. [1–7]), we observed anomalous structures in the fibrin networks of blood clots from individuals exhibiting inflammation arising from a variety of causes, including in a number of chronic diseases. These were originally referred to as 'dense matted deposits'. We subsequently showed [8] that these 'anomalous' clots exhibited the properties of amyloids, in that they stained effectively, and in the same places [9], with stains such as thioflavin T [10] and the commercial oligothiophene Amytracker™ dyes known to stain amyloid forms of proteins [11,12]. Because these blood microclots based on fibrin and other entrapped molecules have this amyloid character, they are now referred to as 'fibrinaloid' microclots [13,14].

Many chronic diseases (that are usually considered noncommunicable) share a variety of observables such as inflammatory markers, iron dysregulation [15–18], hypercoagulability and hypofibrinolysis [19], and fibrinaloid microclots, along with considerable evidence that they may in fact have an infectious origin (e.g. [12,20–29]. This prevalence of fibrinaloid microclots is especially true of both acute and long COVID-19 [30–39].

A characteristic of amyloid proteins, including prion proteins (e.g. [40,41]), is that they retain the primary sequence of their normal, non-amyloid form but adopt a very different set of secondary structures (involving crossed beta-sheets [42–48]) and hence tertiary structure(s).

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An inevitable consequence of this 'amyloid' type of change in conformation, the details of which must also depend on the nature and concentrations of small molecules and metal ions present at the time of fibrinogen polymerisation, is the generation of novel epitopes of what are otherwise normal host proteins. This might then be thought to lead equally inevitably to the generation of autoantibodies, that may contribute to the symptoms associated with these diseases, and in particular to Long COVID. We here develop and assess the evidence for this idea.

Multiple protein macro-conformations and amyloidogenesis

While it is well established that proteins, including enzymes, can adopt a great many isoenergetic conformations [49,50] or microstates, it had been widely assumed — following the famous protein refolding experiments of Anfinsen [51,52] — that the main, 'ground' macrostate adopted by a typical protein following its synthesis was also that of the thermodynamically lowest free energy. While this was necessarily an assumption (the total number of possible states is uncomputably high [53–56]), it was arguably the discovery of prion proteins [57–59] that showed that the assumption could demonstrably be false; stabler conformations of the 'amyloid proteins', i.e. ones of lower free energy, were indeed possible [60,61], and the 'usual' conformation was simply one of the more kinetically accessible [62]. Figure 1A is an illustration (adapted from [62]) of a thermodynamic model for the energetics of the conversion of a PrP protein in the PrP^{C} conformation into PrP^{Sc}. Protein interactions with other ligands may result in protein misfolding and has been shown to cause amyloidogenic changes to all kinds of proteins. Figure 1B is our interpretation of the structural changes in fibrin clots (plus entrapped molecules) in diseases such as Long COVID that exhibit them. Interactions between plasma proteins (mainly fibrinogen) and inflammatory molecules in circulation can result in plasma protein misfolding and have been shown to cause amyloidogenic changes to fibrinogen. This is illustrated by electron micrographs of fibrin clots (created by adding thrombin to platelet poor plasma). Although we are not aware of any experimental observations of the conversion in process, *ab initio* computer simulations [63–66] are beginning to provide a valuable indication of the precise mechanisms by which it may take place.

Amyloidogenic proteins: prions

As is well known, the classical prion protein PrP normally exists in a relatively stable and 'benign' form known as PrP^{C}, but can adopt a substantially more stable or 'rogue' and toxic [67] form (Figure 1A) known as PrP^{Sc}, in which alpha-helices in the PrP^{C} form are converted into (crossed) beta sheets [68]. PrP^{Sc} can be ultra-stable; indeed its resistance to proteinase K digestion is often used in its assay [69–71], and essentially accounts for its heritability [72]. It is also relatively insoluble, a fact that has until recently hampered the determination of its structures [71,73–83]], much as with other amyloids [84]. The chief problems caused by PrP^{Sc} arise from the fact that it can itself catalyse ('seed' or 'template') the conversion of PrP^{C} to further molecules of the membrane-disruptive PrP^{Sc} [85], making the process of PrP^{Sc} production autocatalytic, such that absolutely miniscule amounts of PrP^{Sc} can be toxic [86–89].

Mechanisms of fibril formation in prions and classical amyloid proteins

As illustrated in Figure 2 a fibril from a typical amyloidogenic protein [90–92] is ∼7–12 nm in diameter; as templated by a single molecule of the 'rogue' form, soluble oligomer fibrils begin to form, and these assemble to form the insoluble rogue or amyloid form or aggregates that are observable e.g. by atomic force microscopy [93–97], or stainable by stains such as thioflavin T that are widely used to follow the process [98–108]. Fibres formed by most amyloidogenic proteins are in the range 10–20 nm in diameter or lower, e.g. [109–114]).

We note here that in contrast with the classical prion and amyloid fibrils, the diameter of fibres in fibrinaloid microclots is generally similar to the diameter of 'typical' healthy fibrin fibres in the range 80 to 110 nm [19,115–117], although it may be higher (e.g. in Alzheimer's type dementia [115]) or lower (as in stroke [116]). That seen in most inflammatory diseases is lower [11], but necessarily involves multiple copies of the fibrinogen protein for the same length element.

Multiple amyloid conformations and self-propagation

While it is recognised that amyloid proteins are much stabler than are their parent proteins, a very important feature of amyloid or prion proteins (especially from the present perspective) is that they can in fact adopt

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Figure 1. Illustration of the thermodynamics of prion proteins. Part 1 of 2

(**A**) Illustration of a model for the energetics of the conversion of PrP^{C} sequences into PrP^{Sc} (adapted from [62]). (**B**) Protein–protein interactions may result in protein misfolding and has been shown to cause amyloidogenic changes to fibrinogen as

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Figure 1. Illustration of the thermodynamics of prion proteins. Part 2 of 2

illustrated by electron micrographs of fibrin clots (created by adding thrombin the platelet poor plasma). Reprinted from an Open Access CC-BY 4.0 publication at [11], based on [62]. Created by BioRender.com.

multiple stable states or conformations, sometimes referred to as 'polymorphisms' [118,119], 'strains' [120–122], or 'subtypes' [123], albeit they have the same sequence [46]. In effect the first molecule to convert into the rogue form acts as a 'template' to guide further molecules into a conformation with which it can pack, and that then catalyses further formation of the ultimately insoluble fibrils and aggregates. This has led to so-called 'seed amplification' (sometimes referred to as 'quaking-induced conversion') assays in which a seed molecule in the amyloid form effectively amplifies itself (Figure 3), thereby allowing the detection of tiny amounts of initial protein in a rogue conformation, whether for prions (e.g. [86,124–129]) or other amyloidogenic proteins such as the alpha-synuclein involved in Parkinson's disease (e.g. [130–134]) or the tau aggregates that can accompany Pick disease [135] or Alzheimer's dementia [136,137]. (We also note, although we do not pursue it here, that polymorphisms including amorphous and crystalline forms are also an extremely important and likely related feature of the structure and behaviour of both small pharmaceutical drugs (e.g. [138,139]) and organic but non-biological polymers and plastics (e.g. [140–142]).

It is generally the case [143] that they catalyse the production of their own specific conformations or aggregates (e.g. [45,61,76,77,81,85,144–170]). Thus, while an individual amyloidogenic protein also has a 'set' conformation, to which the host is presumably adapted and is seen as 'self', the amyloid forms (including in tauopathies [171–173]) can adopt a variety of individual and self-propagating [174–178] conformations, and thus, presumably, display a variety of novel and different epitopes depending upon which 'polymorphism' is produced.

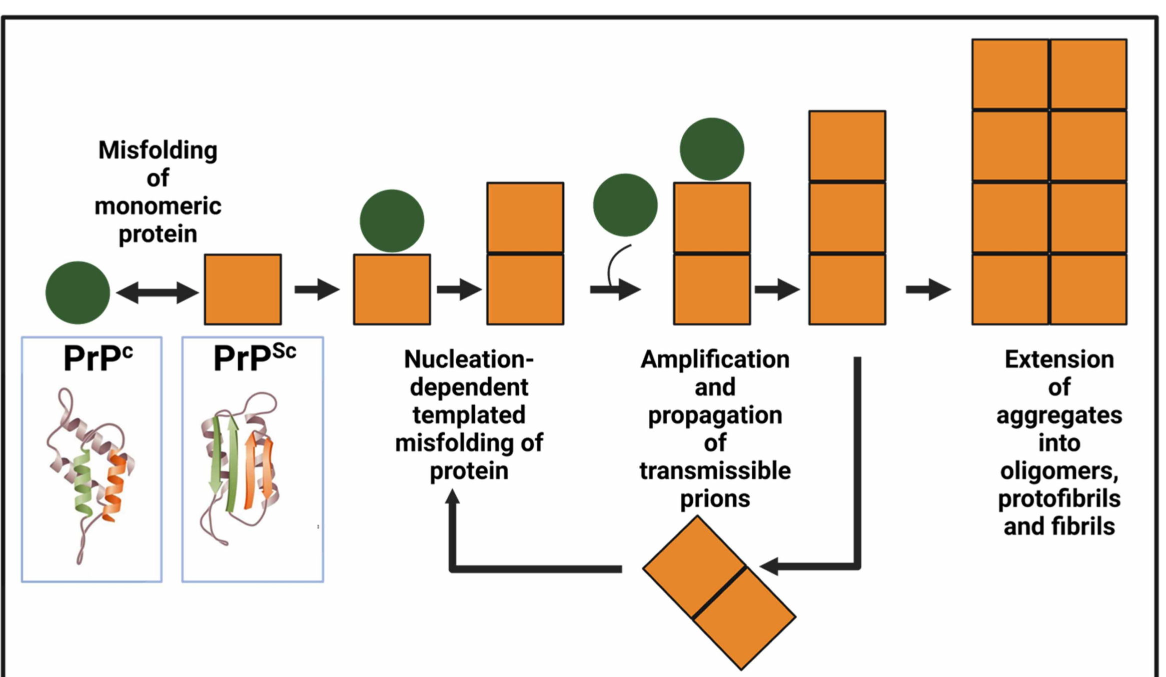

Figure 2. An illustration of a typical amyloidogenic 'rogue' protein folding illustrated by using a nucleation-dependent model of prion replication (adapted from [102]).

PrP^{c} converts into its infectious disease-associated isoform, which is PrP^{Sc}. PrP^{Sc} then induces nucleation-dependent misfolding in other PrP^{C} molecules that in turn leads to amplification and propagation of transmissible prions. Growing aggregates extend into oligomers, protofibrils, and then fibrils that form the protein aggregates characteristic of disease. Created by Biorender.com.

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Figure 3. Seeding nucleation mechanism and amplification.
Example given of of misfolded alphaSyn aggregates (Adapted from [132].) Created with BioRender.com.

Given that in many cases the starting (and of course finishing) protein sequences are the same, it is not precisely known in general what determines which polymorphisms are produced, but realistically it can only be because the starting molecules do in fact differ from each other by first having bound something else. For prions and amyloids, the most evidenced candidates for the 'something else' are small molecules [179–193], and/or metal ions [15,194–209] and/or anions [208,210,211]. In a certain sense this is little more than a recognition that allosteric interactions of small molecules with proteins (which are much more widespread than commonly recognised [212,213]) can change the conformation of the latter. The further general assumption, then, is that the closer the sequence and/or starting structure, the more likely it will self- or co-polymerise ([214], and see below).

In the case of fibrinogen, we know that amyloidogenesis into a variety of clot morphologies can be catalysed by the presence of miniscule amounts of bacterial cell wall components such as lipopolysaccharide [9,23,24,26,215,216,2724] or lipoteichoic acid [24], or the spike protein from SARS-CoV-2 [36] (which is itself amyloidogenic [217]) (Figure 4).

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Figure 4. Misfolding of fibrinogen, result in a variety of pathological clot morphologies, catalysed by the presence of miniscule amounts of bacterial cell wall components such as lipopolysaccharide, lipoteichoic acid or the spike protein from SARS-CoV-2 (which is itself amyloidogenic).
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Prionoids: a convergence of thinking on amyloidogenic proteins

Although in science a commonplace siloing means that parallel fields do not learn from each other as swiftly as they might, the fields of prions and non-prion amyloids do seem to be converging, as they come to recognise that the phenomena they study have many things in common (e.g. [102,218]). Note that as well as the classical amyloids (such as Abeta, synuclein, tau and so on involved in diseases known as amyloidoses) many other proteins can adopt amyloid forms, including lysozyme [219–225] and insulin [185,224,226–231]. These include multiple stable conformations of the proteins of interest that are insoluble and stainable by amyloid stains (and that differ from the 'benign', normal form), and the ability of a given rogue form to seed (however briefly [232], and ignoring post-translational modifications [233]), the propagation of forms similar to itself. This has led to the more general concept of prionoids [234–242] to describe this set of linked properties. As rehearsed

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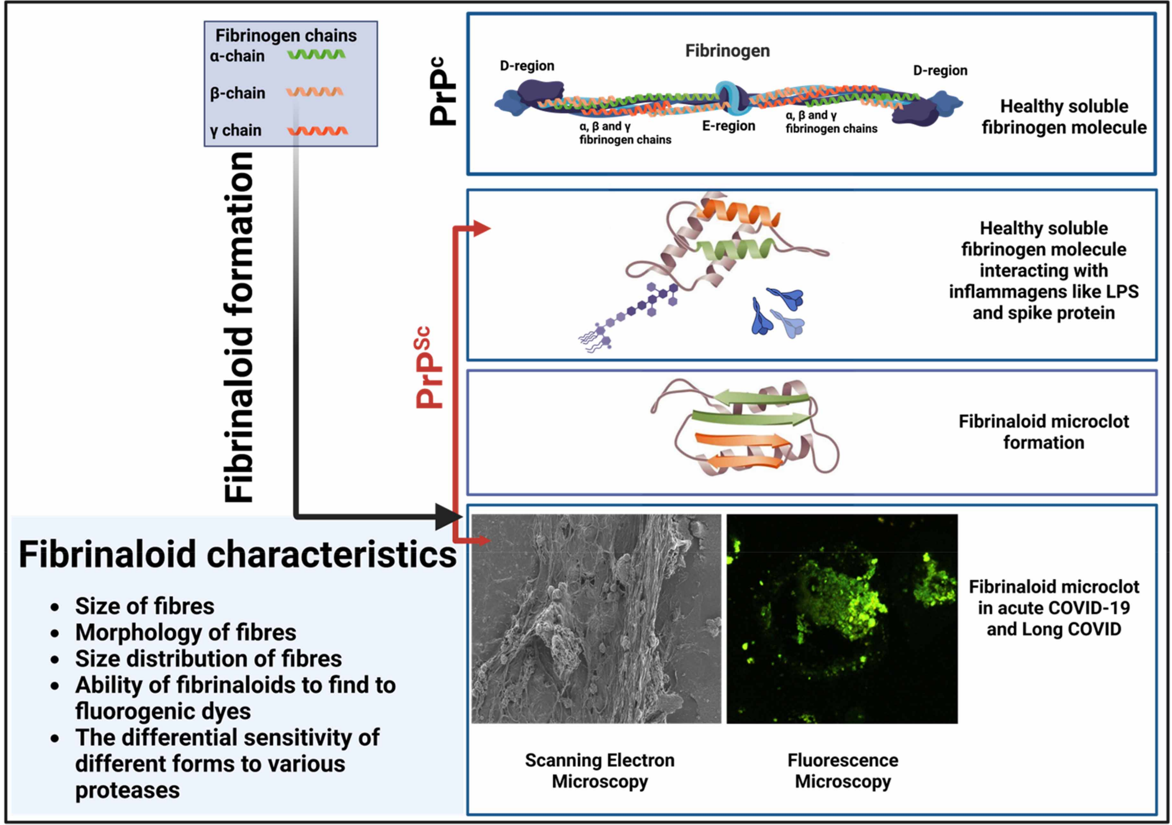

Figure 5. Features of fibrinaloid microclots (without the addition of thrombin).
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in detail elsewhere [11], these are also features common to the generation of fibrinaloid microclots (see Figure 5). We now begin to address the immunological consequences of such amyloidogenesis, starting with a brief section on autoantibodies.

Autoantibodies

The classical function of immune systems is to discriminate self from non-self, or to detect 'danger' [243–245], and to arrange to produce either innate immune responses [246] or actual antibodies [247,248] against elements (usually epitopes of protein sequence or structure) that are considered foreign. In favourable cases the offending, exogeneous invaders, usually microbes, are then suppressed or eliminated.

However, in the case of the adaptive immune response involving antibodies, usually mediated by B- and T-lymphocytes, an unwelcome phenomenon can occur: if the epitope possessed or (as we rehearse here) generated by the microbe is also shared by the host, the resultant autoantibody can also recognise this epitope and attack the host itself, causing an autoimmune disease that can share many of the characteristics of Long COVID and ME/CFS [249].

A well-worked example, described in detail by Ebringer [250–253] (and reviewed by us elsewhere [21]), involves members of the genus *Proteus* (viz *mirabilis* and *vulgaris*). These organisms are a common cause of urinary tract infections, especially in women, and can evoke substantial antibodies in patients who go on to develop rheumatoid arthritis. The relevant epitopes of *Proteus* are the amino acid sequences ESRRAL and IRRET; the former (e.g. in *Proteus* haemolysin and urease) mimics a 'shared epitope' EQR(K)RAA while the latter displays molecular mimicry with LRREI found in collagen XI of hyaline cartilage. The fact that these epitopes contain arginine doublets, which can be acted upon by peptidyl arginine deiminase, also provides a straightforward explanation for the early appearance of anti-citrullinated protein antibodies in patients with RA.

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In a similar vein, SARS-CoV-2 elicits an array of autoantibodies [249,254–257], albeit the mechanisms and cross-reactivities towards the host are not yet well understood (though information is emerging in the related ME/CFS [258,259]). Of particular interest is the fact that 'anti-ACE2 IgM is found in 27% of individuals with severe COVID-19, which can initiate complement binding and alter the permeability of vascular microvessel endothelium [260]' [249]. However, our focus here is simply to suggest a particular mechanism of novel epitope creation that allowed 'self' sequences to become seen as non-self (as they are not normally exposed and thus able to elicit antibodies).

Immunological responses to prion proteins

A chief premise of this review is that the anomalous folding of proteins in amyloid forms, and in particular of fibrinaloids, leads to the display of novel epitopes (neoepitopes or neoantigens) that — unlike their parent protein, which is seen as 'self' — can induce autoantibodies that may also attack the normal form of the target. This has been anticipated for PrP^{C} ('We hypothesised that subtle conformational alterations of pathogenic PrP^{C} variants could stochastically generate immunogenic neoepitopes, which in turn might elicit a protective humoral anti-PrP^{C} immune response' [261]. Some such anti-amyloid(ogenic) antibodies can be protective [262–268]. Consistent with the view that PrP^{Sc} involves neo-epitope formation, antibodies can be found that react with PrP^{Sc} and with aggregates of PrP^{C} but not with soluble PrP^{C} [269–272]. A particularly interesting example is the discovery that certain antibodies can cross-react with beta-amyloid and the prion protein [273], implying the existence of a common element. This is certainly true for small oligomers [274,275].

Antigenic properties of amyloid forms of protein

The existence of autoantibodies following an infection is a commonplace, resulting from a molecular mimicry between the antigen and host antigens/targets (e.g. [21,250,276–279]). However, whether they are initially generated by an amyloid form of their target protein is not discussed because almost all studies fail to distinguish the conformation of the target antigen. This said, examples of autoantibodies that are selective for amyloid forms of amyloidogenic proteins include those for beta-amyloid [280,281], lysozyme [282], tau [283], transthyretin [284], and TSH receptor [285], as well as exogenous proteins such as fish beta-parvalbumin [286]. They may also be designed and selected for [287–289]. Of course there is also considerable interest in developing exogenous therapeutic antibodies against such targets (e.g. [290–295]), but these latter studies are beyond the present scope of this review.

Interactions between different amyloid proteins; cross-seeding

That amyloidogenic proteins are typically capable of catalysing the production of amyloid homopolymers is both well known and has been discussed above. In some cases (where sequences differ) there is no cross-reactivity, i.e. there exists what amounts to a species specificity among prion proteins with regard to the PrP^{Sc}-catalysed or -templated conversion of PrP^{C} to PrP^{Sc} [296–303]. The same can be true for antibodies against various prion forms [304].

More generally, though, the opposite kinds of phenomena ('promiscuity') have been found, namely within a given host or *in vitro* incubation there can be co-propagation of heterogeneous species [305,306], and such cross-reaction can depend on the anions present [210]. Thus, there is evidence for extensive amyloid-amyloid interactions [307], often referred to as a cross-seeding [308–316], in which the amyloid form of one protein induced amyloidogenesis of a different type of protein. This can often lead to a co-aggregation [317–322]. This can even occur with proteins not necessarily considered as classically amyloid, including proteins in the influenza virus [323]. Table 1 gives some other examples.

Proteomics of amyloid aggregates

Of course at a trivial level, the production of (i) insoluble protein aggregates in a variety of amyloid diseases (e.g. Lewy bodies in Parkinson's disease [333] and dementia [334]) and (ii) inclusion bodies during recombinant protein production [335]) is well known. Certainly proteomics shows that a huge variety of heterogeneous molecules can be entrapped in such aggregates (e.g. [32,336–338]). Our interests here lie in some of the consequences, since the trapping of unfolded variants of any proteins may well lead to the generation of novel epitopes. Thus in recent proteomics studies of fibrinaloids [32,338] we discovered that the clots contain various

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Table 1 Some examples of amyloidogenic biopolymers ('polymer 1') known to induce amyloid formation in others ('polymer 2') to which they are exposed ('crosstalk')

Amyloidogenic polymer 1	Amyloidogenic polymer 2	Reference
Amyloid-beta	Islet amyloid polypeptide Alpha-synuclein	[324] [325]
CsgA	Alpha-synuclein	[326,327]
Gut proteins	Alpha-synuclein	[328]
Insulin	Amylin	[329]
Islet amyloid polypeptide	Alpha-synuclein	[327,330]
Islet amyloid polypeptide	PrP	[331]
Serum amyloid A	Fibrin(ogen)	[319]
Alpha-synuclein	Tau	[332]

entrapped molecules. We found various clotting proteins, molecules involved in cellular functions and lipid metabolism that were both increased and reduced in the microclots, Interestingly, we also found numerous immunoglobulin molecules (or fragments thereof) that were found to be increased in the Long COVID sample compared with healthy samples.

Consequences of cross-seeding — even further novel epitope creation

Small molecules that bind to fibrinogen

Recognising that amyloids can adopt multiple, self-polymerising structures, and that this may depend on small molecules that were bound to fibrinogen before polymerisation, we here summarise what turns out to be fairly

Table 2 Some small molecules known to bind to fibrinogen

Small molecule	Nature of evidence	Reference (s)
Acebutolol	Biophysical binding assays; Binding and calculations	[347]; [339]
Benzothiazole	Fluorescence-based binding assays	[348]
Bilirubin	Binding assay	[349]
Clozapine	Binding assay, and structural changes in fibrin	[350]
Curcumin	UV/Vis spectroscopy; fluorescence quenching	[351]
Dihydro-alpha-lipoic acid	Binding assay and structural changes	[352]
Flavonoids	Spectroscopy, docking	[353]
Lipopolysaccharide and lipoteichoic acids	Amyloidogenesis	[8]
24dium-chain fatty acids	Calorimetric binding studies	[354]
Beta-oestradiol	Fluorescence-based binding assays	[355]
Penicillins	Calorimetric binding assays	[356]
Propranolol	Binding assay and calculations	[339,357]
Resveratrol	Binding assay	[358]
SARS-CoV-2 spike protein	Amyloidogenesis	[36]
Various, including bilirubin, resveratrol and dihydrolipoic acid	Binding assays	[340]

We do not include those fluorogenic stains such as thioflavin T [10] and oligothiophenes [359] that stain the fibrinaloid form.

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considerable evidence (Table 2) that such small molecule binding to fibrinogen does indeed occur [339,340]. This would be consistent with the fact that typical pharmaceutical drugs are known to bind at least six separate target and off-target proteins [341–343], and have significant roles in affecting protein–protein interactions (e.g. [344–346].

Pharmacological approaches to inhibiting fibrinaloid formation

Since the amyloid forms of proteins are normally more stable than are their soluble forms, it follows that they must function by raising the kinetic barrier to amyloid formation, by stabilising the native state [360] or by inhibiting folding intermediates in its formation [193]. Molecules known to inhibit various kinds of amyloid formation include L-DOPA [181], promethazine [361], cholic acid [184], and various halo-aromatic drugs [360]. Fibrinogen in its native form [362] is thus a reasonable target to avoid fibrinaloid formation. It follows that at least some of those molecules in Table 2 might serve to stabilise the native state of fibrinogen and thereby inhibit fibrinaloid formation, though as far as we know no screens for anti-fibrinaloid formation have yet been performed. This seems to be a significant opportunity.

Fibrin amyloids and how their structures may differ

As noted above, the structural biology of amyloids has lagged due to their insolubility, although it is certainly known even for homopolymers that different parallel or antiparallel cross-beta and macro-structures are formed [45,119,363]. Because fibrinogen is itself a heteropolymer, and the fibrinaloid microclots entrap a great many other proteins [32,338], the details may be difficult to establish. As also mentioned above, the fibrinaloid fibres have a substantially greater diameter than do those involved in the conventional amyloidoses. However, we recognise that at least four general properties of the fibres making up the fibrinaloid microclots might be expected to differ between the different forms (see Figure 5):

(i) the size, morphologies, and distribution in size of the fibrinaloid microclots themselves
(ii) the ability of the different fibrinaloids to bind different molecules, including the fluorogenic ones commonly used to detect them (and where spectral differences may result [364–368])
(iii) the differential sensitivity of different forms to various proteases (e.g. those caused by SARS-CoV-2 are most resilient [32,338]), as is established with the differential protease sensitivities of different prion forms [369]
(iv) spectral properties of different dyes bound to the different microclots [364–366,370–373].

Since we know that both acute [374–376] and long COVID [33,39] can be ameliorated by suitable anticoagulants, without the addition of fibrinolytics, the question arises as to whether they may be orally available fibrinolytics [377] that could serve.

Degradation of fibrinaloids *in vitro* and *in vivo*

As also reviewed elsewhere [13,14], the enzymes nattokinase [378] (also active against spike protein [379]), serrapeptase [380,381] and lumbricase [382] have been shown to degrade fibrin clots (see also [229,230,383,384]). They would seem to have potentially useful roles, not least in removing any novel antigens appearing in fibrinaloids.

Concluding remarks and agenda

Much of this review involves the recognition that many (indeed likely most) proteins typically fold into kinetically stable but thermodynamically unstable states, and that under certain circumstances they can form stabler amyloids. This is true for prions, classical amyloids, and the more recently discovered fibrinaloids in blood; it is also a hallmark of the proteins involved in energy transduction [385,386]. These conformations are radically different from the those of the 'parent' protein as synthesised; some amyloids can even then catalyse chemical reactions at significant rates [387–391]. Some of the conformations are particularly stable in the sense that they tend to catalyse homopolymerisation of the parent conformation; others are more promiscuous. This leads to a recognition that many infectious diseases involve fibrinaloid formation en route to autoantibodies (albeit some are elicited by biomimicry directly [250,251,392,393]). Acceptance of the significance of autoantibody-driven inflammation in syndromes such as ME/CFS and Long COVID also implies that there may be value in testing

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treatments (such as monoclonal antibodies [394] or small molecules) against TNF-α that are known to help in other autoimmune diseases like rheumatoid arthritis.

It is very likely that precisely which fibril conformations are formed depends on which small molecule and/or metal ligands may have pre-bound to the amyloids. Given the combinatorial explosion contingent upon making and testing multiple variants, the general sequence- and ligand-based rules are largely unknown, though the methods of synthetic biology [395] will allow us to begin to find out. A feature of note is that the modern and powerful computational folding algorithms such as Alphafold [396–402] and RosettaFold [403–405] have been trained mainly or only on the native conformations of amyloidogenic proteins so at this stage (albeit they are non-deterministic [406]) they are unable to predict the structures of amyloids. Solving this would seem to be of high importance.

Our special focus is on the fibrinaloid microclots that have been shown to entrap many other molecules, and that also contain a variety of autoantibodies. An important agenda item is thus to determine what these novel epitopes are, and to characterise the autoantibodies that they elicit. This may also hold out the hope that — whatever the autoantibody half lives — the removal of fibrinaloid formation will lead to such autoantibody elimination.

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Competing Interests

E.P. is a named inventor on a patent application covering the use of fluorescence methods for microclot detection in Long COVID.

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CRediT Author Contribution

Douglas Kell: Conceptualization, Resources, Funding acquisition, Investigation, Methodology, Writing — original draft, Project administration, Writing — review and editing. **Etheresia Pretorius:** Conceptualization, Resources, Data curation, Funding acquisition, Investigation, Visualization, Methodology, Writing — original draft, Project administration, Writing — review and editing.

References

1 Pretorius, E., Oberholzer, H.M., van der Spuy, W.J., Swanepoel, A.C. and Soma, P. (2011) Qualitative scanning electron microscopy analysis of fibrin networks and platelet abnormalities in diabetes. *Blood Coagul. Fibrinol.* **22**, 463–467 https://doi.org/10.1097/MBC.0b013e3283468a0d

2 Swanepoel, A.C., Visagie, A., de Lange, Z., Emmerson, O., Nielsen, V.G. and Pretorius, E. (2016) The clinical relevance of altered fibrinogen packaging in the presence of 17beta-estradiol and progesterone. *Thromb. Res.* **146**, 23–34 https://doi.org/10.1016/j.thromres.2016.08.022

3 Pretorius, E., Oberholzer, H.M., van der Spuy, W.J., Swanepoel, A.C. and Soma, P. (2012) Scanning electron microscopy of fibrin networks in rheumatoid arthritis: a qualitative analysis. *Rheumatol. Int.* **32**, 1611–1615 https://doi.org/10.1007/s00296-011-1805-2

4 Pretorius, E., Vermeulen, N., Bester, J., Lipinski, B. and Kell, D.B. (2013) A novel method for assessing the role of iron and its functional chelation in fibrin fibril formation: the use of scanning electron microscopy. *Toxicol. Mech. Methods* **23**, 352–359 https://doi.org/10.3109/15376516.2012.762082

5 Pretorius, E., Bester, J., Vermeulen, N., Lipinski, B., Gericke, G.S. and Kell, D.B. (2014) Profound morphological changes in the erythrocytes and fibrin networks of patients with hemochromatosis or with hyperferritinemia, and their normalization by iron chelators and other agents. *PLoS ONE* **9**, e85271 https://doi.org/10.1371/journal.pone.0085271

6 Pretorius, E. and Kell, D.B. (2014) Diagnostic morphology: biophysical indicators for iron-driven inflammatory diseases. *Integr. Biol.* **6**, 486–510 https://doi.org/10.1039/C4IB00025K

7 Pretorius, E., Bester, J., Vermeulen, N., Alummoottil, S., Soma, P., Buys, A.V. et al. (2015) Poorly controlled type 2 diabetes is accompanied by significant morphological and ultrastructural changes in both erythrocytes and in thrombin-generated fibrin: implications for diagnostics. *Cardiovasc. Diabetol.* **13**, 30 https://doi.org/10.1186/s12933-015-0192-5

8 Pretorius, E., Mbotwe, S., Bester, J., Robinson, C.J. and Kell, D.B. (2016) Acute induction of anomalous and amyloidogenic blood clotting by molecular amplification of highly substoichiometric levels of bacterial lipopolysaccharide. *J. R. Soc. Interface* **123**, 20160539 https://doi.org/10.1098/rsif.2016.0539

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9 de Waal, G.M., Engelbrecht, L., Davis, T., de Villiers, W.J.S., Kell, D.B. and Pretorius, E. (2018) Correlative light-electron microscopy detects lipopolysaccharide and its association with fibrin fibres in Parkinson's disease, Alzheimer's disease and type 2 diabetes mellitus. *Sci. Rep.* **8**, 16798 https://doi.org/10.1038/s41598-018-35009-y

10 Biancalana, M. and Koide, S. (2010) Molecular mechanism of thioflavin-T binding to amyloid fibrils. *Biochim. Biophys. Acta* **1804**, 1405–1412 https://doi.org/10.1016/j.bbapap.2010.04.001

11 Kell, D.B. and Pretorius, E. (2017) Proteins behaving badly. Substoichiometric molecular control and amplification of the initiation and nature of amyloid fibril formation: lessons from and for blood clotting. *Progr. Biophys. Mol. Biol.* **123**, 16–41 https://doi.org/10.1016/j.pbiomolbio.2016.08.006

12 Pretorius, E., Page, M.J., Engelbrecht, L., Ellis, G.C. and Kell, D.B. (2017) Substantial fibrin amyloidogenesis in type 2 diabetes assessed using amyloid-selective fluorescent stains. *Cardiovasc. Diabetol.* **16**, 141 https://doi.org/10.1186/s12933-017-0624-5

13 Kell, D.B., Laubscher, G.J. and Pretorius, E. (2022) A central role for amyloid fibrin microclots in long COVID/PASC: origins and therapeutic implications. *Biochem. J.* **479**, 537–559 https://doi.org/10.1042/BCJ20220016

14 Kell, D.B. and Pretorius, E. (2022) The potential role of ischaemia-reperfusion injury in chronic, relapsing diseases such as rheumatoid arthritis, long COVID and ME/CFS: evidence, mechanisms, and therapeutic implications. *Biochem. J.* **479**, 1653–1708 https://doi.org/10.1042/BCJ20220154

15 Kell, D.B. (2009) Iron behaving badly: inappropriate iron chelation as a major contributor to the aetiology of vascular and other progressive inflammatory and degenerative diseases. *BMC Med. Genom.* **2**, 2 https://doi.org/10.1186/1755-8794-2-2

16 Kell, D.B. and Pretorius, E. (2018) No effects without causes. The iron dysregulation and dormant microbes hypothesis for chronic, inflammatory diseases. *Biol. Rev.* **93**, 1518–1557 https://doi.org/10.1111/brv.12407

17 Kell, D.B. and Pretorius, E. (2014) Serum ferritin is an important disease marker, and is mainly a leakage product from damaged cells. *Metallomics* **6**, 748–773 https://doi.org/10.1039/C3MT00347G

18 Venter, C., Bezuidenhout, J.A., Laubscher, G.J., Lourens, P.J., Steenkamp, J., Kell, D.B. et al. (2020) Erythrocyte, platelet, serum ferritin and P-selectin pathophysiology implicated in severe hypercoagulation and vascular complications in COVID-19. *Int. J. Mol. Sci.* **21**, 8234 https://doi.org/10.3390/ijms21218234

19 Kell, D.B. and Pretorius, E. (2015) The simultaneous occurrence of both hypercoagulability and hypofibrinolysis in blood and serum during systemic inflammation, and the roles of iron and fibrin(ogen). *Integr. Biol.* **7**, 24–52 https://doi.org/10.1039/c4ib00173g

20 Itzhaki, R.F., Lathe, R., Balin, B.J., Ball, M.J., Braak, H., Bearer, E.L. et al. (2016) Microbes and Alzheimer's disease. *J. Alzheimers Dis.* **51**, 979–984 https://doi.org/10.3233/JAD-160152

21 Pretorius, E., Akeredolu, O.-O., Soma, P. and Kell, D.B. (2017) Major involvement of bacterial components in rheumatoid arthritis and its accompanying oxidative stress, systemic inflammation and hypercoagulability. *Exp. Biol. Med.* **242**, 355–373 https://doi.org/10.1177/1535370216681549

22 Pretorius, E., Bester, J. and Kell, D.B. (2016) A bacterial component to Alzheimer-type dementia seen via a systems biology approach that links iron dysregulation and inflammagen shedding to disease. *J. Alzheimers Dis.* **53**, 1237–1256 https://doi.org/10.3233/JAD-160318

23 Pretorius, E., Mbotwe, S. and Kell, D.B. (2017) Lipopolysaccharide-binding protein (LBP) reverses the amyloid state of fibrin seen in plasma of type 2 diabetics with cardiovascular comorbidities. *Sci. Rep.* **7**, 9680 https://doi.org/10.1038/s41598-017-09860-4

24 Pretorius, E., Page, M.J., Hendricks, L., Nkosi, N.B., Benson, S.R. and Kell, D.B. (2017) Both lipopolysaccharide and lipoteichoic acids potently induce anomalous fibrin amyloid formation: assessment with novel Amytracker™ stains. *J. R. Soc. Interface* **15**, 20170941 https://doi.org/10.1098/rsif.2017.0941

25 Pretorius, E., Swanepoel, A.C., DeVilliers, S. and Bester, J. (2017) Blood clot parameters: thromboelastography and scanning electron microscopy in research and clinical practice. *Thromb. Res.* **154**, 59–63 https://doi.org/10.1016/j.thromres.2017.04.005

26 Pretorius, E., Page, M.J., Mbotwe, S. and Kell, D.B. (2018) Lipopolysaccharide-binding protein (LBP) can reverse the amyloid state of fibrin seen or induced in Parkinson's disease. *PLoS ONE* **13**, e0192121 https://doi.org/10.1371/journal.pone.0192121

27 Pretorius, E., Bester, J., Page, M.J. and Kell, D.B. (2018) The potential of LPS-binding protein to reverse amyloid formation in plasma fibrin of individuals with Alzheimer-type dementia. *Front. Aging Neurosci.* **10**, 257 https://doi.org/10.3389/fnagi.2018.00257

28 Nunes, J.M., Kruger, A., Proal, A., Kell, D.B. and Pretorius, E. (2022) The occurrence of hyperactivated platelets and fibrinaloid microclots in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS). *Pharmaceuticals (Basel)* **15**, 931 https://doi.org/10.3390/ph15080931

29 Nunes, J.M., Kell, D.B. and Pretorius, E. (2023) Cardiovascular and haematological pathology in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS): a role for viruses. *Blood Rev.* **60**, 101075 https://doi.org/10.1016/j.blre.2023.101075

30 Pretorius, E., Venter, C., Laubscher, G.J., Lourens, P.J., Steenkamp, J. and Kell, D.B. (2020) Prevalence of amyloid blood clots in COVID-19 plasma. *medRxiv* https://doi.org/10.1186/s12933-020-01165-7

31 Pretorius, E., Venter, C., Laubscher, G.J., Lourens, P.J., Steenkamp, J. and Kell, D.B. (2020) Prevalence of readily detected amyloid blood clots in 'unclotted' type 2 diabetes mellitus and COVID-19 plasma: a preliminary report. *Cardiovasc. Diabetol.* **19**, 193 https://doi.org/10.1101/2020.07.28.20163543

32 Pretorius, E., Vlok, M., Venter, C., Bezuidenhout, J.A., Laubscher, G.J., Steenkamp, J. et al. (2021) Persistent clotting protein pathology in long COVID/ post-Acute sequelae of COVID-19 (PASC) is accompanied by increased levels of antiplasmin. *Cardiovasc. Diabetol.* **20**, 172 https://doi.org/10.1186/s12933-021-01359-7

33 Pretorius, E., Venter, C., Laubsher, G.J., Kotze, M.J., Moremi, K., Oladejo, S. et al. (2021) Combined triple treatment of fibrin amyloid microclots and platelet pathology in individuals with long COVID/ post-acute sequelae of COVID-19 (PASC) can resolve their persistent symptoms. *Res. Square* https://doi.org/10.21203/rs.3.rs-1205453/v1

34 Pretorius, E., Venter, C., Laubscher, G.J., Kotze, M.J., Oladejo, S., Watson, L.R. et al. (2022) Prevalence of symptoms, comorbidities, fibrin amyloid microclots and platelet pathology in individuals with long COVID/ post-acute sequelae of COVID-19 (PASC). *Cardiovasc. Diabetol.* **21**, 148 https://doi.org/10.1186/s12933-022-01579-5

35 Bunch, C.M., Moore, E.E., Moore, H.B., Neal, M.D., Thomas, A.V., Zackariya, N. et al. (2022) Immuno-thrombotic complications of COVID-19: implications for timing of surgery and anticoagulation. *Front. Surg.* **9**, 889999 https://doi.org/10.3389/fsurg.2022.889999

36 Grobbelaar, L.M., Venter, C., Vlok, M., Ngoepe, M., Laubscher, G.J., Lourens, P.J. et al. (2021) SARS-CoV-2 spike protein S1 induces fibrin(ogen) resistant to fibrinolysis: implications for microclot formation in COVID-19. *Biosci. Rep.* **41**, BSR20210611 https://doi.org/10.1042/BSR20210611

37 Grobler, C., Maphumulo, S.C., Grobbelaar, L.M., Bredenkamp, J.C., Laubscher, J., Lourens, P.J. et al. (2020) COVID-19: the rollercoaster of fibrin (ogen), D-dimer, von Willebrand factor, P-selectin and their interactions with endothelial cells, platelets and erythrocytes. *Int. J. Mol. Sci.* **21**, 5168 https://doi.org/10.3390/ijms21145168

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38 Laubscher, G.J., Lourens, P.J., Venter, C., Kell, D.B. and Pretorius, E. (2021) TEG®, microclot and platelet mapping for guiding early management of severe COVID-19 coagulopathy. *J. Clin. Med.* **10**, 5381 https://doi.org/10.3390/jcm10225381

39 Laubscher, G.J., Khan, M.A., Venter, C., Pretorius, J.H., Kell, D.B. and Pretorius, E. (2023) Treatment of long COVID symptoms with triple anticoagulant therapy. *Res. Square* https://doi.org/10.21203/rs.3.rs-2697680/v1

40 Aguzzi, A. and O'Connor, T. (2010) Protein aggregation diseases: pathogenicity and therapeutic perspectives. *Nat. Rev. Drug Discov.* **9**, 237–248 https://doi.org/10.1038/nrd3050

41 Prusiner, S.B. (2013) Biology and genetics of prions causing neurodegeneration. *Annu. Rev. Genet.* **47**, 601–623 https://doi.org/10.1146/annurev-genet-110711-155524

42 Jahn, T.R., Tennent, G.A. and Radford, S.E. (2008) A common beta-sheet architecture underlies *in vitro* and *in vivo* beta2-microglobulin amyloid fibrils. *J. Biol. Chem.* **283**, 17279–17286 https://doi.org/10.1074/jbc.M710351200

43 Baldwin, M.A., Pan, K.M., Nguyen, J., Huang, Z., Groth, D., Serban, A. et al. (1994) Spectroscopic characterization of conformational differences between PrP^{C} and PrP^{Sc}: an alpha-helix to beta-sheet transition. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **343**, 435–441 https://doi.org/10.1098/rstb.1994.0041

44 Groveman, B.R., Dolan, M.A., Taubner, L.M., Kraus, A., Wickner, R.B. and Caughey, B. (2014) Parallel in-register intermolecular beta-sheet architectures for prion-seeded prion protein (PrP) amyloids. *J. Biol. Chem.* **289**, 24129–24142 https://doi.org/10.1074/jbc.M114.578344

45 Toyama, B.H. and Weissman, J.S. (2011) Amyloid structure: conformational diversity and consequences. *Annu. Rev. Biochem.* **80**, 557–585 https://doi.org/10.1146/annurev-biochem-090908-120656

46 Tycko, R. and Wickner, R.B. (2013) Molecular structures of amyloid and prion fibrils: consensus versus controversy. *Acc. Chem. Res.* **46**, 1487–1496 https://doi.org/10.1021/ar300282r

47 Morris, K.L. and Serpell, L.C. (2013) From Molecular to Supramolecular Amyloid Structures: Contributions from Fiber Diffraction and Electron Microscopy. In *Amyloid Fibrils and Prefibrillar Aggregates: Molecular and Biological Properties* (Otzen, D.E., ed.), pp. 63–84, Wiley-VCH, Weinheim

48 Terry, C. and Wadsworth, J.D.F. (2019) Recent advances in understanding mammalian prion structure: a mini review. *Front. Mol. Neurosci.* **12**, 169 https://doi.org/10.3389/fnmol.2019.00169

49 Beece, D., Eisenstein, L., Frauenfelder, H., Good, D., Marden, M.C., Reinisch, L. et al. (1980) Solvent viscosity and protein dynamics. *Biochemistry* **19**, 5147–5157 https://doi.org/10.1021/bi00564a001

50 Frauenfelder, H., Fenimore, P.W. and Young, R.D. (2007) Protein dynamics and function: insights from the energy landscape and solvent slaving. *IUBMB Life* **59**, 506–512 https://doi.org/10.1080/15216540701194113

51 Anfinsen, C.B., Haber, E., Sela, M. and White, F.H. (1961) The kinetics of formation of native ribonuclease during oxidation of the reduced polypeptide chain. *Proc. Natl Acad. Sci. U.S.A.* **47**, 1309–1314 https://doi.org/10.1073/pnas.47.9.1309

52 Anfinsen, C.B. (1973) Principles that govern the folding of protein chains. *Science* **181**, 223–230 https://doi.org/10.1126/science.181.4096.223

53 Levinthal, C. (1969) How to Fold Graciously. In *Mossbauer Spectroscopy in Biological Systems* (Debrunner, P., Tsibris, J.C.M. and Münck, E., eds), pp. 22–24, University of Illinois Press, Monticello, Illinois

54 Zwanzig, R., Szabo, A. and Bagchi, B. (1992) Levinthal's paradox. *Proc. Natl Acad. Sci. U.S.A.* **89**, 20–22 https://doi.org/10.1073/pnas.89.1.20

55 Karplus, M. (1997) The levinthal paradox: yesterday and today. *Fold Des.* **2**, S69–S75 https://doi.org/10.1016/s1359-0278(97)00067-9

56 Martínez, L. (2014) Introducing the levinthal's protein folding paradox and Its solution. *J. Chem. Educ.* **91**, 1918–1923 https://doi.org/10.1021/ed300302h

57 Prusiner, S.B. (1982) Novel proteinaceous infectious particles cause scrapie. *Science* **216**, 136–144 https://doi.org/10.1126/science.6801762

58 Prusiner, S.B. (1998) Prions. *Proc. Natl Acad. Sci. U.S.A.* **95**, 13363–13383 https://doi.org/10.1073/pnas.95.23.13363

59 Zabel, M.D. and Reid, C. (2015) A brief history of prions. *Pathog. Dis.* **73**, ftv087 https://doi.org/10.1093/femspd/ftv087

60 Chiti, F. and Dobson, C.M. (2006) Protein misfolding, functional amyloid, and human disease. *Annu. Rev. Biochem.* **75**, 333–366 https://doi.org/10.1146/annurev.biochem.75.101304.123901

61 Eisenberg, D. and Jucker, M. (2012) The amyloid state of proteins in human diseases. *Cell* **148**, 1188–1203 https://doi.org/10.1016/j.cell.2012.02.022

62 Cohen, F.E. and Prusiner, S.B. (1998) Pathologic conformations of prion proteins. *Annu. Rev. Biochem.* **67**, 793–819 https://doi.org/10.1146/annurev.biochem.67.1.793

63 Spagnolli, G., Rigoli, M., Orioli, S., Sevillano, A.M., Faccioli, P., Wille, H. et al. (2019) Full atomistic model of prion structure and conversion. *PLoS Pathog.* **15**, e1007864 https://doi.org/10.1371/journal.ppat.1007864

64 Spagnolli, G., Requena, J.R. and Biasini, E. (2020) Understanding prion structure and conversion. *Prog. Mol. Biol. Transl. Sci.* **175**, 19–30 https://doi.org/10.1016/bs.pmbts.2020.07.005

65 Terruzzi, L., Spagnolli, G., Boldrini, A., Requena, J.R., Biasini, E. and Faccioli, P. (2020) All-atom simulation of the HET-s prion replication. *PLoS Comput. Biol.* **16**, e1007922 https://doi.org/10.1371/journal.pcbi.1007922

66 Mullapudi, V., Vaquer-Alicea, J., Bommareddy, V., Vega, A.R., Ryder, B.D., White, C.L. et al. (2023) Network of hotspot interactions cluster tau amyloid folds. *Nat. Commun.* **14**, 895 https://doi.org/10.1038/s41467-023-36572-3

67 Hughes, D. and Halliday, M. (2017) What Is Our current understanding of PrP(Sc)-associated neurotoxicity and Its molecular underpinnings? *Pathogens* **6**, 63 https://doi.org/10.3390/pathogens6040063

68 Pan, K.M., Baldwin, M., Nguyen, J., Gasset, M., Serban, A., Groth, D. et al. (1993) Conversion of alpha-helices into beta-sheets features in the formation of the scrapie prion proteins. *Proc. Natl Acad. Sci. U.S.A.* **90**, 10962–10966 https://doi.org/10.1073/pnas.90.23.10962

69 Aguzzi, A. and Calella, A.M. (2009) Prions: protein aggregation and infectious diseases. *Physiol. Rev.* **89**, 1105–1152 https://doi.org/10.1152/physrev.00006.2009

70 Silva, C.J., Vazquez-Fernández, E., Onisko, B. and Requena, J.R. (2015) Proteinase K and the structure of PrP^{Sc}: the good, the bad and the ugly. *Virus Res.* **207**, 120–126 https://doi.org/10.1016/j.virusres.2015.03.008

71 Wang, F., Wang, X., Abskharon, R. and Ma, J. (2018) Prion infectivity is encoded exclusively within the structure of proteinase K-resistant fragments of synthetically generated recombinant PrP(Sc). *Acta Neuropathol. Commun.* **6**, 30 https://doi.org/10.1186/s40478-018-0534-0

72 Telling, G.C. (2022) The shape of things to come: structural insights into how prion proteins encipher heritable information. *Nat. Commun.* **13**, 4003 https://doi.org/10.1038/s41467-022-31460-8

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73 Diaz-Espinoza, R. and Soto, C. (2012) High-resolution structure of infectious prion protein: the final frontier. *Nat. Struct. Mol. Biol.* **19**, 370–377 https://doi.org/10.1038/nsmb.2266
74 Tycko, R. (2016) Structure of aggregates revealed. *Nature* **537**, 492–493 https://doi.org/10.1038/nature19470
75 Hoyt, F., Standke, H.G., Artikis, E., Schwartz, C.L., Hansen, B., Li, K. et al. (2022) Cryo-EM structure of anchorless RML prion reveals variations in shared motifs between distinct strains. *Nat. Commun.* **13**, 4005 https://doi.org/10.1038/s41467-022-30458-6
76 Hoyt, F., Alam, P., Artikis, E., Schwartz, C.L., Hughson, A.G., Race, B. et al. (2022) Cryo-EM of prion strains from the same genotype of host identifies conformational determinants. *PLoS Pathog.* **18**, e1010947 https://doi.org/10.1371/journal.ppat.1010947
77 Kraus, A., Hoyt, F., Schwartz, C.L., Hansen, B., Artikis, E., Hughson, A.G. et al. (2021) High-resolution structure and strain comparison of infectious mammalian prions. *Mol. Cell* **81**, 4540–4551.e4546 https://doi.org/10.1016/j.molcel.2021.08.011
78 Artikis, E., Kraus, A. and Caughey, B. (2022) Structural biology of ex vivo mammalian prions. *J. Biol. Chem.* **298**, 102181 https://doi.org/10.1016/j.jbc.2022.102181
79 Frontzek, K., Bardelli, M., Senatore, A., Henzi, A., Reimann, R.R., Bedir, S. et al. (2022) A conformational switch controlling the toxicity of the prion protein. *Nat. Struct. Mol. Biol.* **29**, 831–840 https://doi.org/10.1038/s41594-022-00814-7
80 Manka, S.W., Zhang, W., Wenborn, A., Betts, J., Joiner, S., Saibil, H.R. et al. (2022) 2.7 å cryo-EM structure of ex vivo RML prion fibrils. *Nat. Commun.* **13**, 4004 https://doi.org/10.1038/s41467-022-30457-7
81 Manka, S.W., Wenborn, A., Collinge, J. and Wadsworth, J.D.F. (2022) Prion strains viewed through the lens of cryo-EM. *Cell Tissue Res.* **392**, 167–178 https://doi.org/10.1007/s00441-022-03676-z
82 Requena, J.R. (2022) Unlatching a window into the molecular landscape of prion toxicity. *Nat. Struct. Mol. Biol.* **29**, 733–735 https://doi.org/10.1038/s41594-022-00817-4
83 Shoup, D. and Priola, S.A. (2022) Cell biology of prion strains *in vivo* and *in vitro*. *Cell Tissue Res.* **392**, 269–283 https://doi.org/10.1007/s00441-021-03572-y
84 Rodriguez, J.A., Ivanova, M.I., Sawaya, M.R., Cascio, D., Reyes, F.E., Shi, D. et al. (2015) Structure of the toxic core of alpha-synuclein from invisible crystals. *Nature* **525**, 486–490 https://doi.org/10.1038/nature15368
85 Colby, D.W. and Prusiner, S.B. (2011) Prions. *Cold Spring Harb. Perspect. Biol.* **3**, a006833 https://doi.org/10.1101/cshperspect.a006833
86 Saborio, G.P., Permanne, B. and Soto, C. (2001) Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding. *Nature* **411**, 810–813 https://doi.org/10.1038/35081095
87 Colby, D.W., Zhang, Q., Wang, S., Groth, D., Legname, G., Riesner, D. et al. (2007) Prion detection by an amyloid seeding assay. *Proc. Natl Acad. Sci. U.S.A.* **104**, 20914–20919 https://doi.org/10.1073/pnas.0710152105
88 Atarashi, R., Satoh, K., Sano, K., Fuse, T., Yamaguchi, N., Ishibashi, D. et al. (2011) Ultrasensitive human prion detection in cerebrospinal fluid by real-time quaking-induced conversion. *Nat. Med.* **17**, 175–178 https://doi.org/10.1038/nm.2294
89 Takatsuki, H., Satoh, K., Sano, K., Fuse, T., Nakagaki, T., Mori, T. et al. (2015) Rapid and quantitative assay of amyloid-Seeding activity in human brains affected with prion diseases. *PLoS ONE* **10**, e0126930 https://doi.org/10.1371/journal.pone.0126930
90 Serpell, L.C. (2000) Alzheimer's amyloid fibrils: structure and assembly. *Biochim. Biophys. Acta* **1502**, 16–30 https://doi.org/10.1016/S0925-4439(00)00029-6
91 Varadi, M., De Baets, G., Vranken, W.F., Tompa, P. and Pancsa, R. (2018) Amypro: a database of proteins with validated amyloidogenic regions. *Nucleic Acids Res.* **46**, D387–D392 https://doi.org/10.1093/nar/gkx950
92 Louros, N., Konstantoulea, K., De Vleeschouwer, M., Ramakers, M., Schymkowitz, J. and Rousseau, F. (2020) WALTZ-DB 2.0: an updated database containing structural information of experimentally determined amyloid-forming peptides. *Nucleic Acids Res.* **48**, D389–D393 https://doi.org/10.1093/nar/gkz758
93 Gosal, W.S., Myers, S.L., Radford, S.E. and Thomson, N.H. (2006) Amyloid under the atomic force microscope. *Protein Pept. Lett.* **13**, 261–270 https://doi.org/10.2174/092986606775338498
94 Visser, M.J.E. and Pretorius, E. (2019) Atomic force microscopy: the characterisation of amyloid protein structure in pathology. *Curr. Top. Med. Chem.* **19**, 2958–2973 https://doi.org/10.2174/1568026619666191121143240
95 Serem, W.K., Bett, C.K., Ngunjiri, J.N. and Garno, J.C. (2011) Studies of the growth, evolution, and self-aggregation of beta-amyloid fibrils using tapping-mode atomic force microscopy. *Microsc. Res. Tech.* **74**, 699–708 https://doi.org/10.1002/jemt.20940
96 Drolle, E., Hane, F., Lee, B. and Leonenko, Z. (2014) Atomic force microscopy to study molecular mechanisms of amyloid fibril formation and toxicity in Alzheimer's disease. *Drug Metab. Rev.* **46**, 207–223 https://doi.org/10.3109/03602532.2014.882354
97 Watanabe-Nakayama, T., Sahoo, B.R., Ramamoorthy, A. and Ono, K. (2020) High-speed atomic force microscopy reveals the structural dynamics of the amyloid-beta and amylin aggregation pathways. *Int. J. Mol. Sci.* **21**, 4287 https://doi.org/10.3390/ijms21124287
98 Almstedt, K., Nyström, S., Nilsson, K.P. and Hammarström, P. (2009) Amyloid fibrils of human prion protein are spun and woven from morphologically disordered aggregates. *Prion* **3**, 224–235 https://doi.org/10.4161/pri.3.4.10112
99 Sandberg, A. and Nyström, S. (2018) Purification and fibrillation of recombinant human amyloid-beta, prion protein, and tau under native conditions. *Methods Mol. Biol.* **1779**, 147–166 https://doi.org/10.1007/978-1-4939-7816-8_10
100 Ostapchenko, V., Gasset, M. and Baskakov, I.V. (2012) Atomic force fluorescence microscopy in the characterization of amyloid fibril assembly and oligomeric intermediates. *Methods Mol. Biol.* **849**, 157–167 https://doi.org/10.1007/978-1-61779-551-0_11
101 Sengupta, U., Nilson, A.N. and Kayed, R. (2016) The role of amyloid-beta oligomers in toxicity, propagation, and immunotherapy. *EBioMedicine* **6**, 42–49 https://doi.org/10.1016/j.ebiom.2016.03.035
102 Ugalde, C.L., Finkelstein, D.I., Lawson, V.A. and Hill, A.F. (2016) Pathogenic mechanisms of prion protein, amyloid-beta and alpha-synuclein misfolding: the prion concept and neurotoxicity of protein oligomers. *J. Neurochem.* **139**, 162–180 https://doi.org/10.1111/jnc.13772
103 Gade Malmos, K., Blancas-Mejia, L.M., Weber, B., Buchner, J., Ramirez-Alvarado, M., Naiki, H. et al. (2017) Tht 101: a primer on the use of thioflavin T to investigate amyloid formation. *Amyloid* **24**, 1–16 https://doi.org/10.1080/13506129.2017.1304905
104 Meisl, G., Knowles, T.P. and Klenerman, D. (2020) The molecular processes underpinning prion-like spreading and seed amplification in protein aggregation. *Curr. Opin. Neurobiol.* **61**, 58–64 https://doi.org/10.1016/j.conb.2020.01.010

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105 Cawood, E.E., Karamanos, T.K., Wilson, A.J. and Radford, S.E. (2021) Visualizing and trapping transient oligomers in amyloid assembly pathways. *Biophys. Chem.* **268**, 106505 https://doi.org/10.1016/j.bpc.2020.106505

106 Ziaunys, M., Sakalauskas, A., Mikalauskaite, K. and Smirnovas, V. (2021) Exploring the occurrence of thioflavin-T-positive insulin amyloid aggregation intermediates. *PeerJ* **9**, e10918 https://doi.org/10.7717/peerj.10918

107 Zhang, P. and Tan, C. (2022) Cross-reactive fluorescent sensor array for discrimination of amyloid beta aggregates. *Anal. Chem.* **94**, 5469–5473 https://doi.org/10.1021/acs.analchem.2c00579

108 Cristóvão, J.S., Henriques, B.J. and Gomes, C.M. (2023) Biophysical and Spectroscopic Methods for Monitoring Protein Misfolding and Amyloid Aggregation. In *Protein Misfolding Diseases: Methods and Protocols* (Gomes, C.M., ed.), pp. 3–18, Springer, Berlin

109 Uversky, V.N., Li, J. and Fink, A.L. (2001) Evidence for a partially folded intermediate in alpha-synuclein fibril formation. *J. Biol. Chem.* **276**, 10737–10744 https://doi.org/10.1074/jbc.M010907200

110 Foguel, D., Suarez, M.C., Ferrao-Gonzales, A.D., Porto, T.C., Palmieri, L., Einsiedler, C.M. et al. (2003) Dissociation of amyloid fibrils of alpha-synuclein and transthyretin by pressure reveals their reversible nature and the formation of water-excluded cavities. *Proc. Natl Acad. Sci. U.S.A.* **100**, 9831–9836 https://doi.org/10.1073/pnas.1734009100

111 Ivanova, M.I., Sawaya, M.R., Gingery, M., Attinger, A. and Eisenberg, D. (2004) An amyloid-forming segment of beta 2-microglobulin suggests a molecular model for the fibril. *Proc. Natl Acad. Sci. U.S.A.* **101**, 10584–10589 https://doi.org/10.1073/pnas.0403756101

112 Klement, K., Wieligmann, K., Meinhardt, J., Hortschansky, P., Richter, W. and Fändrich, M. (2007) Effect of different salt ions on the propensity of aggregation and on the structure of Alzheimer's abeta(1-40) amyloid fibrils. *J. Mol. Biol.* **373**, 1321–1333 https://doi.org/10.1016/j.jmb.2007.08.068

113 Engel, M.F.M., Khemtémourian, L., Kleijer, C.C., Meeldijk, H.J.D., Jacobs, J., Verkleij, A.J. et al. (2008) Membrane damage by human islet amyloid polypeptide through fibril growth at the membrane. *Proc. Natl Acad. Sci. U.S.A.* **105**, 6033–6038 https://doi.org/10.1073/pnas.0708354105

114 Kollmer, M., Meinhardt, K., Haupt, C., Liberta, F., Wulff, M., Linder, J. et al. (2016) Electron tomography reveals the fibril structure and lipid interactions in amyloid deposits. *Proc. Natl Acad. Sci. U.S.A.* **113**, 5604–5609 https://doi.org/10.1073/pnas.1523496113

115 Bester, J., Soma, P., Kell, D.B. and Pretorius, E. (2015) Viscoelastic and ultrastructural characteristics of whole blood and plasma in Alzheimer-type dementia, and the possible role of bacterial lipopolysaccharides (LPS). *Oncotarget* **6**, 35284–35303 https://doi.org/10.18632/oncotarget.6074

116 Pretorius, E., Steyn, H., Engelbrecht, M., Swanepoel, A.C. and Oberholzer, H.M. (2011) Differences in fibrin fiber diameters in healthy individuals and thromboembolic ischemic stroke patients. *Blood Coagul. Fibrinolysis* **22**, 696–700 https://doi.org/10.1097/MBC.0b013e32834bdb32

117 Weigandt, K.M., White, N., Chung, D., Ellingson, E., Wang, Y., Fu, X.Y. et al. (2012) Fibrin clot structure and mechanics associated with specific oxidation of methionine residues in fibrinogen. *Biophys. J.* **103**, 2399–2407 https://doi.org/10.1016/j.bpj.2012.10.036

118 Tycko, R. (2014) Physical and structural basis for polymorphism in amyloid fibrils. *Protein Sci.* **23**, 1528–1539 https://doi.org/10.1002/pro.2544

119 Riek, R. (2017) The three-dimensional structures of amyloids. *Cold Spring Harb. Perspect. Biol.* **9**, a023572 https://doi.org/10.1101/cshperspect.a023572

120 Prusiner, S.B. (1991) Molecular biology of prion diseases. *Science* **252**, 1515–1522 https://doi.org/10.1126/science.1675487

121 Colby, D.W. and Prusiner, S.B. (2011) *De novo* generation of prion strains. *Nat. Rev. Microbiol.* **9**, 771–777 https://doi.org/10.1038/nrmicro2650

122 Zink, R.M. (2021) Considering the use of the terms strain and adaptation in prion research. *Heliyon* **7**, e06801 https://doi.org/10.1016/j.heliyon.2021.e06801

123 Qiang, W., Yau, W.M., Lu, J.X., Collinge, J. and Tycko, R. (2017) Structural variation in amyloid-beta fibrils from Alzheimer's disease clinical subtypes. *Nature* **541**, 217–221 https://doi.org/10.1038/nature20814

124 Saá, P. and Cervenakova, L. (2015) Protein misfolding cyclic amplification (PMCA): current status and future directions. *Virus Res.* **207**, 47–61 https://doi.org/10.1016/j.virusres.2014.11.007

125 Shi, S., Wagner, J., Mitteregger-Kretzschmar, G., Ryazanov, S., Leonov, A., Griesinger, C. et al. (2015) Quantitative real-Time quaking-Induced conversion allows monitoring of disease-modifying therapy in the urine of prion-infected mice. *J. Neuropathol. Exp. Neurol.* **74**, 924–933 https://doi.org/10.1097/NEN.0000000000000233

126 Kang, H.E., Mo, Y., Abd Rahim, R., Lee, H.M. and Ryou, C. (2017) Prion diagnosis: application of real-Time quaking-Induced conversion. *Biomed. Res. Int.* **2017**, 5413936 https://doi.org/10.1155/2017/5413936

127 Coysh, T. and Mead, S. (2022) The future of seed amplification assays and clinical trials. *Front. Aging Neurosci.* **14**, 872629 https://doi.org/10.3389/fnagi.2022.872629

128 Poleggi, A., Baiardi, S., Ladogana, A. and Parchi, P. (2022) The Use of real-Time quaking-induced conversion for the diagnosis of human prion diseases. *Front. Aging Neurosci.* **14**, 874734 https://doi.org/10.3389/fnagi.2022.874734

129 Vascellari, S., Orru, C.D. and Caughey, B. (2022) Real-Time quaking- induced conversion assays for prion diseases, synucleinopathies, and tauopathies. *Front. Aging Neurosci.* **14**, 853050 https://doi.org/10.3389/fnagi.2022.853050

130 Russo, M.J., Orru, C.D., Concha-Marambio, L., Giaisi, S., Groveman, B.R., Farris, C.M. et al. (2021) High diagnostic performance of independent alpha-synuclein seed amplification assays for detection of early Parkinson's disease. *Acta Neuropathol. Commun.* **9**, 179 https://doi.org/10.1186/s40478-021-01282-8

131 Bellomo, G., De Luca, C.M.G., Paoletti, F.P., Gaetani, L., Moda, F. and Parnetti, L. (2022) alpha-Synuclein seed amplification assays for diagnosing synucleinopathies: the way forward. *Neurology* **99**, 195–205 https://doi.org/10.1212/WNL.0000000000200878

132 Concha-Marambio, L., Pritzkow, S., Shahnawaz, M., Farris, C.M. and Soto, C. (2023) Seed amplification assay for the detection of pathologic alpha-synuclein aggregates in cerebrospinal fluid. *Nat. Protoc.* **18**, 1179–1196 https://doi.org/10.1038/s41596-022-00787-3

133 Siderowf, A., Concha-Marambio, L., Lafontant, D.-E., Farris, C.M., Ma, Y., Urenia, P.A. et al. (2023) Assessment of heterogeneity among participants in the Parkinson's progression markers initiative cohort using α-synuclein seed amplification: a cross-sectional study. *Lancet Neurol.* **22**, 407–417 https://doi.org/10.1016/S1474-4422(23)00109-6

134 Vaneyck, J., Yousif, T.A., Segers-Nolten, I., Blum, C. and Claessens, M. (2023) Quantitative seed amplification assay: a proof-of-Principle study. *J. Phys. Chem. B* **127**, 1735–1743 https://doi.org/10.1021/acs.jpcb.2c08326

135 Saijo, E., Groveman, B.R., Kraus, A., Metrick, M., Orru, C.D., Hughson, A.G. et al. (2019) Ultrasensitive RT-QuIC seed amplification assays for disease-associated tau, alpha-synuclein, and prion aggregates. *Methods Mol. Biol.* **1873**, 19–37 https://doi.org/10.1007/978-1-4939-8820-4_2

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136 Manca, M. and Kraus, A. (2020) Defining the protein seeds of neurodegeneration using real-time quaking-Induced conversion assays. *Biomolecules* **10**, 1233 https://doi.org/10.3390/biom10091233
137 Standke, H.G. and Kraus, A. (2022) Seed amplification and RT-QuIC assays to investigate protein seed structures and strains. *Cell Tissue Res.* **392**, 323–335 https://doi.org/10.1007/s00441-022-03595-z
138 Yu, L. (2001) Amorphous pharmaceutical solids: preparation, characterization and stabilization. *Adv. Drug Deliv. Rev.* **48**, 27–42 https://doi.org/10.1016/s0169-409x(01)00098-9
139 Shi, Q., Chen, H., Wang, Y., Xu, J., Liu, Z. and Zhang, C. (2022) Recent advances in drug polymorphs: aspects of pharmaceutical properties and selective crystallization. *Int. J. Pharm.* **611**, 121320 https://doi.org/10.1016/j.ijpharm.2021.121320
140 Strobl, G. (2003) *The Physics of Polymers*, 3rd edn, Spriger, Berlin
141 Stempfle, F., Ortmann, P. and Mecking, S. (2016) Long-chain aliphatic polymers to bridge the gap between semicrystalline polyolefins and traditional polycondensates. *Chem. Rev.* **116**, 4597–4641 https://doi.org/10.1021/acs.chemrev.5b00705
142 DelRe, C., Jiang, Y., Kang, P., Kwon, J., Hall, A., Jayapurna, I. et al. (2021) Near-complete depolymerization of polyesters with nano-dispersed enzymes. *Nature* **592**, 558–563 https://doi.org/10.1038/s41586-021-03408-3
143 Maji, S.K., Wang, L., Greenwald, J. and Riek, R. (2009) Structure-activity relationship of amyloid fibrils. *FEBS Lett.* **583**, 2610–2617 https://doi.org/10.1016/j.febslet.2009.07.003
144 Chien, P., DePace, A.H., Collins, S.R. and Weissman, J.S. (2003) Generation of prion transmission barriers by mutational control of amyloid conformations. *Nature* **424**, 948–951 https://doi.org/10.1038/nature01894
145 Chien, P., Weissman, J.S. and DePace, A.H. (2004) Emerging principles of conformation-based prion inheritance. *Annu. Rev. Biochem.* **73**, 617–656 https://doi.org/10.1146/annurev.biochem.72.121801.161837
146 Petkova, A.T., Leapman, R.D., Guo, Z., Yau, W.M., Mattson, M.P. and Tycko, R. (2005) Self-propagating, molecular-level polymorphism in Alzheimer's beta-amyloid fibrils. *Science* **307**, 262–265 https://doi.org/10.1126/science.1105850
147 Weissmann, C. (2005) Birth of a prion: spontaneous generation revisited. *Cell* **122**, 165–168 https://doi.org/10.1016/j.cell.2005.07.001
148 Collinge, J. and Clarke, A.R. (2007) A general model of prion strains and their pathogenicity. *Science* **318**, 930–936 https://doi.org/10.1126/science.1138718
149 Toyama, B.H., Kelly, M.J.S., Gross, J.D. and Weissman, J.S. (2007) The structural basis of yeast prion strain variants. *Nature* **449**, 233–237 https://doi.org/10.1038/nature06108
150 Makarava, N. and Baskakov, I.V. (2008) The same primary structure of the prion protein yields two distinct self-propagating states. *J. Biol. Chem.* **283**, 15988–15996 https://doi.org/10.1074/jbc.M800562200
151 Wiltzius, J.J.W., Landau, M., Nelson, R., Sawaya, M.R., Apostol, M.I., Goldschmidt, L. et al. (2009) Molecular mechanisms for protein-encoded inheritance. *Nat. Struct. Mol. Biol.* **16**, 973–978 https://doi.org/10.1038/nsmb.1643
152 Collinge, J. (2010) Prion strain mutation and selection. *Science* **328**, 1111–1112 https://doi.org/10.1126/science.1190815
153 Greenwald, J. and Riek, R. (2010) Biology of amyloid: structure, function, and regulation. *Structure* **18**, 1244–1260 https://doi.org/10.1016/j.str.2010.08.009
154 Cushman, M., Johnson, B.S., King, O.D., Gitler, A.D. and Shorter, J. (2010) Prion-like disorders: blurring the divide between transmissibility and infectivity. *J. Cell Sci.* **123**, 1191–1201 https://doi.org/10.1242/jcs.051672
155 Cortez, L.M. and Sim, V.L. (2013) Implications of prion polymorphisms. *Prion* **7**, 276–279 https://doi.org/10.4161/pri.25566
156 Poggiolini, I., Saverioni, D. and Parchi, P. (2013) Prion protein misfolding, strains, and neurotoxicity: an update from studies on mammalian prions. *Int. J. Cell Biol.* **2013**, 910314 https://doi.org/10.1155/2013/910314
157 Gill, A.C. (2014) beta-hairpin-mediated formation of structurally distinct multimers of neurotoxic prion peptides. *PLoS ONE* **9**, e87354 https://doi.org/10.1371/journal.pone.0087354
158 Sano, K., Atarashi, R., Ishibashi, D., Nakagaki, T., Satoh, K. and Nishida, N. (2014) Conformational properties of prion strains can be transmitted to recombinant prion protein fibrils in real-time quaking-induced conversion. *J. Virol.* **88**, 11791–11801 https://doi.org/10.1128/JVI.00585-14
159 Wickner, R.B., Edskes, H.K., Bateman, D.A., Kelly, A.C., Gorkovskiy, A., Dayani, Y. et al. (2014) Amyloid diseases of yeast: prions are proteins acting as genes. *Essays Biochem.* **56**, 193–205 https://doi.org/10.1042/bse0560193
160 Kobayashi, A., Teruya, K., Matsuura, Y., Shirai, T., Nakamura, Y., Yamada, M. et al. (2015) The influence of PRNP polymorphisms on human prion disease susceptibility: an update. *Acta Neuropathol.* **130**, 159–170 https://doi.org/10.1007/s00401-015-1447-7
161 Le, N.T., Narkiewicz, J., Aulic, S., Salzano, G., Tran, H.T., Scaini, D. et al. (2015) Synthetic prions and other human neurodegenerative proteinopathies. *Virus Res.* **207**, 25–37 https://doi.org/10.1016/j.virusres.2014.10.020
162 Moda, F., Le, T.N., Aulic, S., Bistaffa, E., Campagnani, I., Virgilio, T. et al. (2015) Synthetic prions with novel strain-specified properties. *PLoS Pathog.* **11**, e1005354 https://doi.org/10.1371/journal.ppat.1005354
163 Tycko, R. (2015) Amyloid polymorphism: structural basis and neurobiological relevance. *Neuron* **86**, 632–645 https://doi.org/10.1016/j.neuron.2015.03.017
164 Bartz, J.C. (2016) Prion strain diversity. *Cold Spring Harb. Perspect. Med.* **6**, a024349 https://doi.org/10.1101/cshperspect.a024349
165 Curcio, L., Sebastiani, C., Di Lorenzo, P., Lasagna, E. and Biagetti, M. (2016) Review: a review on classical and atypical scrapie in caprine: prion protein gene polymorphisms and their role in the disease. *Animal* **10**, 1585–1593 https://doi.org/10.1017/S1751731116000653
166 Morales, R. (2017) Prion strains in mammals: different conformations leading to disease. *PLoS Pathog.* **13**, e1006323 https://doi.org/10.1371/journal.ppat.1006323
167 Igel-Egalon, A., Béringue, V., Rezaei, H. and Sibille, P. (2018) Prion strains and transmission barrier phenomena. *Pathogens* **7**, 5 https://doi.org/10.3390/pathogens7010005
168 Killian, A.N., Miller, S.C. and Hines, J.K. (2019) Impact of amyloid polymorphism on prion-chaperone interactions in yeast. *Viruses* **11**, 349 https://doi.org/10.3390/v11040349
169 Scialò, C., De Cecco, E., Manganotti, P. and Legname, G. (2019) Prion and prion-like protein strains: deciphering the molecular basis of heterogeneity in neurodegeneration. *Viruses* **11**, 261 https://doi.org/10.3390/v11030261

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170 Arifin, M.I., Hannaoui, S., Chang, S.C., Thapa, S., Schatzl, H.M. and Gilch, S. (2021) Cervid prion protein polymorphisms: role in chronic wasting disease pathogenesis. *Int. J. Mol. Sci.* **22**, 2271 https://doi.org/10.3390/ijms22052271
171 Vaquer-Alicea, J., Diamond, M.I. and Joachimiak, L.A. (2021) Tau strains shape disease. *Acta Neuropathol.* **142**, 57–71 https://doi.org/10.1007/s00401-021-02301-7
172 Han, Z.Z., Kang, S.G., Arce, L. and Westaway, D. (2022) Prion-like strain effects in tauopathies. *Cell Tissue Res.* **392**, 179–199 https://doi.org/10.1007/s00441-022-03620-1
173 Hromadkova, L., Siddiqi, M.K., Liu, H. and Safar, J.G. (2022) Populations of tau conformers drive prion-like strain effects in Alzheimer's disease and related dementias. *Cells* **11**, 2997 https://doi.org/10.3390/cells11192997
174 Kaufman, S.K., Sanders, D.W., Thomas, T.L., Ruchinskas, A.J., Vaquer-Alicea, J., Sharma, A.M. et al. (2016) Tau prion strains dictate patterns of cell pathology, progression rate, and regional vulnerability In vivo. *Neuron* **92**, 796–812 https://doi.org/10.1016/j.neuron.2016.09.055
175 Kaufman, S.K., Thomas, T.L., Del Tredici, K., Braak, H. and Diamond, M.I. (2017) Characterization of tau prion seeding activity and strains from formaldehyde-fixed tissue. *Acta Neuropathol. Commun.* **5**, 41 https://doi.org/10.1186/s40478-017-0442-8
176 Mudher, A., Colin, M., Dujardin, S., Medina, M., Dewachter, I., Alavi Naini, S.M. et al. (2017) What is the evidence that tau pathology spreads through prion-like propagation? *Acta Neuropathol. Commun.* **5**, 99 https://doi.org/10.1186/s40478-017-0488-7
177 Vaquer-Alicea, J. and Diamond, M.I. (2019) Propagation of protein aggregation in neurodegenerative diseases. *Annu. Rev. Biochem.* **88**, 785–810 https://doi.org/10.1146/annurev-biochem-061516-045049
178 Ziaunys, M., Sakalauskas, A., Mikalauskaite, K., Snieckute, R. and Smirnovas, V. (2021) Temperature-dependent structural variability of prion protein amyloid fibrils. *Int. J. Mol. Sci.* **22**, 5075 https://doi.org/10.3390/ijms22105075
179 Cawood, E.E., Guthertz, N., Ebo, J.S., Karamanos, T.K., Radford, S.E. and Wilson, A.J. (2020) Modulation of amyloidogenic protein self-assembly using tethered small molecules. *J. Am. Chem. Soc.* **142**, 20845–20854 https://doi.org/10.1021/jacs.0c10629
180 Bett, C.K., Ngunjiri, J.N., Serem, W.K., Fontenot, K.R., Hammer, R.P., McCarley, R.L. et al. (2010) Structure-activity relationships in peptide modulators of beta-amyloid protein aggregation: variation in alpha,alpha-disubstitution results in altered aggregate size and morphology. *ACS Chem. Neurosci.* **1**, 608–626 https://doi.org/10.1021/cn100045q
181 Nusrat, S., Zaidi, N., Siddiqi, M.K., Zaman, M., Siddique, I.A., Ajmal, M.R. et al. (2017) Anti-Parkinsonian L-Dopa can also act as anti-systemic amyloidosis-A mechanistic exploration. *Int. J. Biol. Macromol.* **99**, 630–640 https://doi.org/10.1016/j.ijbiomac.2017.03.028
182 Barreca, M.L., Iraci, N., Biggi, S., Cecchetti, V. and Biasini, E. (2018) Pharmacological agents targeting the cellular prion protein. *Pathogens* **7**, 27 https://doi.org/10.3390/pathogens7010027
183 Lee, S.M., Kim, S.S., Kim, H. and Kim, S.Y. (2019) THERPA v2: an update of a small molecule database related to prion protein regulation and prion disease progression. *Prion* **13**, 197–198 https://doi.org/10.1080/19336896.2019.1689789
184 Majid, N., Siddiqi, M.K., Khan, A.N., Shabnam, S., Malik, S., Alam, A. et al. (2019) Biophysical elucidation of amyloid fibrillation inhibition and prevention of secondary nucleation by cholic acid: an unexplored function of cholic acid. *ACS Chem. Neurosci.* **10**, 4704–4715 https://doi.org/10.1021/acschemneuro.9b00482
185 Gancar, M., Kurin, E., Bednarikova, Z., Marek, J., Mucaji, P., Nagy, M. et al. (2020) Amyloid aggregation of insulin: an interaction study of green tea constituents. *Sci. Rep.* **10**, 9115 https://doi.org/10.1038/s41598-020-66033-6
186 Ishibashi, D., Nakagaki, T., Ishikawa, T., Atarashi, R., Watanabe, K., Cruz, F.A. et al. (2016) Structure-based drug discovery for prion disease using a novel binding simulation. *EBioMedicine* **9**, 238–249 https://doi.org/10.1016/j.ebiom.2016.06.010
187 Li, L., Wei, W., Jia, W.J., Zhu, Y., Zhang, Y., Chen, J.H. et al. (2017) Discovery of small molecules binding to the normal conformation of prion by combining virtual screening and multiple biological activity evaluation methods. *J. Comput. Aided Mol. Des.* **31**, 1053–1062 https://doi.org/10.1007/s10822-017-0086-6
188 Li, L., Zhu, Y., Zhou, S., An, X., Zhang, Y., Bai, Q. et al. (2017) Experimental and theoretical insights into the inhibition mechanism of prion fibrillation by resveratrol and its derivatives. *ACS Chem. Neurosci.* **8**, 2698–2707 https://doi.org/10.1021/acschemneuro.7b00240
189 Massignan, T., Sangiovanni, V., Biggi, S., Stincardini, C., Elezgarai, S.R., Maietta, G. et al. (2017) A small-molecule inhibitor of prion replication and mutant prion protein toxicity. *ChemMedChem* **12**, 1286–1292 https://doi.org/10.1002/cmdc.201700302
190 Ishibashi, D., Ishikawa, T., Mizuta, S., Tange, H., Nakagaki, T., Hamada, T. et al. (2020) Novel compounds identified by structure-based prion disease drug discovery using In silico screening delay the progression of an illness in prion-Infected mice. *Neurotherapeutics* **17**, 1836–1849 https://doi.org/10.1007/s13311-020-00903-9
191 Reidenbach, A.G., Mesleh, M.F., Casalena, D., Vallabh, S.M., Dahlin, J.L., Leed, A.J. et al. (2020) Multimodal small-molecule screening for human prion protein binders. *J. Biol. Chem.* **295**, 13516–13531 https://doi.org/10.1074/jbc.RA120.014905
192 Linsenmeier, L., Mohammadi, B., Shafiq, M., Frontzek, K., Bar, J., Shrivastava, A.N. et al. (2021) Ligands binding to the prion protein induce its proteolytic release with therapeutic potential in neurodegenerative proteinopathies. *Sci. Adv.* **7**, eabj1826 https://doi.org/10.1126/sciadv.abj1826
193 Spagnolli, G., Massignan, T., Astolfi, A., Biggi, S., Rigoli, M., Brunelli, P. et al. (2021) Pharmacological inactivation of the prion protein by targeting a folding intermediate. *Commun. Biol.* **4**, 62 https://doi.org/10.1038/s42003-020-01585-x
194 Kawahara, M., Kato-Negishi, M. and Tanaka, K. (2017) Cross talk between neurometals and amyloidogenic proteins at the synapse and the pathogenesis of neurodegenerative diseases. *Metallomics* **9**, 619–633 https://doi.org/10.1039/c7mt00046d
195 Petersingham, G., Zaman, M.S., Johnson, A.J., Reddy, N., Torres, A.M. and Wu, M.J. (2022) Molecular details of aluminium-amyloid beta peptide interaction by nuclear magnetic resonance. *Biometals* **35**, 759–769 https://doi.org/10.1007/s10534-022-00399-0
196 Brown, D.R., Qin, K., Herms, J.W., Madlung, A., Manson, J., Strome, R. et al. (1997) The cellular prion protein binds copper *in vivo*. *Nature* **390**, 684–687 https://doi.org/10.1038/37783
197 Stöckel, J., Safar, J., Wallace, A.C., Cohen, F.E. and Prusiner, S.B. (1998) Prion protein selectively binds copper(II) ions. *Biochemistry* **37**, 7185–7193 https://doi.org/10.1021/bi972827k
198 Wadsworth, J.D., Hill, A.F., Joiner, S., Jackson, G.S., Clarke, A.R. and Collinge, J. (1999) Strain-specific prion-protein conformation determined by metal ions. *Nat. Cell Biol.* **1**, 55–59 https://doi.org/10.1038/9030
199 Whittal, R.M., Ball, H.L., Cohen, F.E., Burlingame, A.L., Prusiner, S.B. and Baldwin, M.A. (2000) Copper binding to octarepeat peptides of the prion protein monitored by mass spectrometry. *Protein Sci.* **9**, 332–343 https://doi.org/10.1110/ps.9.2.332

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200 Nadal, R.C., Davies, P., Brown, D.R. and Viles, J.H. (2009) Evaluation of copper2+ affinities for the prion protein. *Biochemistry* **48**, 8929–8931 https://doi.org/10.1021/bi9011397
201 Wärmländer, S., Tiiman, A., Abelein, A., Luo, J., Jarvet, J., Söderberg, K.L. et al. (2013) Biophysical studies of the amyloid beta-peptide: interactions with metal ions and small molecules. *ChemBioChem* **14**, 1692–1704 https://doi.org/10.1002/cbic.201300262
202 Stanyon, H.F., Patel, K., Begum, N. and Viles, J.H. (2014) Copper(II) sequentially loads onto the N-terminal amino group of the cellular prion protein before the individual octarepeats. *Biochemistry* **53**, 3934–3999 https://doi.org/10.1021/bi500643b
203 Pan, K., Yi, C.W., Chen, J. and Liang, Y. (2015) Zinc significantly changes the aggregation pathway and the conformation of aggregates of human prion protein. *Biochim. Biophys. Acta* **1854**, 907–918 https://doi.org/10.1016/j.bbapap.2015.04.020
204 McDonald, A.J., Leon, D.R., Markham, K.A., Wu, B., Heckendorf, C.F., Schilling, K. et al. (2019) Altered domain structure of the prion protein caused by Cu^{2+} binding and functionally relevant mutations: analysis by cross-linking, MS/MS, and NMR. *Structure* **27**, 907–922.e905 https://doi.org/10.1016/j.str.2019.03.008
205 Salzano, G., Giachin, G. and Legname, G. (2019) Structural consequences of copper binding to the prion protein. *Cells* **8**, 770 https://doi.org/10.3390/cells8080770
206 Salzano, G., Brennich, M., Mancini, G., Tran, T.H., Legname, G., D'Angelo, P. et al. (2020) Deciphering copper coordination in the mammalian prion protein amyloidogenic domain. *Biophys. J.* **118**, 676–687 https://doi.org/10.1016/j.bpj.2019.12.025
207 Singh, O., Kumar Das, B. and Chakraborty, D. (2022) Influence of Ion specificity and concentration on the conformational transition of intrinsically disordered sheep prion peptide. *Chemphyschem* **23**, e202200211 https://doi.org/10.1002/cphc.202200211
208 Lorentzon, E., Horvath, I., Kumar, R., Rodrigues, J.I., Tamas, M.J. and Wittung-Stafshede, P. (2021) Effects of the toxic metals arsenite and cadmium on alpha-synuclein aggregation *in vitro* and in cells. *Int. J. Mol. Sci.* **22**, 11455 https://doi.org/10.3390/ijms222111455
209 Wittung-Stafshede, P. (2022) Crossroads between copper ions and amyloid formation in Parkinson's disease. *Essays Biochem.* **66**, 977–986 https://doi.org/10.1042/EBC20220043
210 Sharma, A., Bruce, K.L., Chen, B., Gyoneva, S., Behrens, S.H., Bommarius, A.S. et al. (2016) Contributions of the prion protein sequence, strain, and environment to the species barrier. *J. Biol. Chem.* **291**, 1277–1288 https://doi.org/10.1074/jbc.M115.684100
211 Sasanian, N., Bernson, D., Horvath, I., Wittung-Stafshede, P. and Esbjorner, E.K. (2020) Redox-dependent copper ion modulation of amyloid-beta (1-42) aggregation *in vitro*. *Biomolecules* **10**, 924 https://doi.org/10.3390/biom10060924
212 Li, X., Gianoulis, T.A., Yip, K.Y., Gerstein, M. and Snyder, M. (2010) Extensive *in vivo* metabolite-protein interactions revealed by large-scale systematic analyses. *Cell* **143**, 639–650 https://doi.org/10.1016/j.cell.2010.09.048
213 Kell, D.B. (2011) Metabolites do social networking. *Nat. Chem. Biol.* **7**, 7–8 https://doi.org/10.1038/nchembio.505
214 Tessier, P.M. and Lindquist, S. (2007) Prion recognition elements govern nucleation, strain specificity and species barriers. *Nature* **447**, 556–561 https://doi.org/10.1038/nature05848
215 Nunes, J.M., Fillis, T., Page, M.J., Venter, C., Lancry, O., Kell, D.B. et al. (2020) Gingipain R1 and lipopolysaccharide from *Porphyromonas gingivalis* have major effects on blood clot morphology and mechanics. *Front. Immunol.* **11**, 1551 https://doi.org/10.3389/fimmu.2020.01551
216 Pretorius, E., Mbotwe, S., Bester, J., Robinson, C. and Kell, D.B. (2016) Acute induction of anomalous blood clotting by highly substoichiometric levels of bacterial lipopolysaccharide (LPS). *bioRxiv* https://doi.org/10.1101/053538
217 Nyström, S. and Hammarström, P. (2022) Amyloidogenesis of SARS-CoV-2 spike protein. *J. Am. Chem. Soc.* **144**, 8945–8950 https://doi.org/10.1021/jacs.2c03925
218 Hammarström, P. and Nyström, S. (2023) Viruses and amyloids - a vicious liaison. *Prion* **17**, 82–104 https://doi.org/10.1080/19336896.2023.2194212
219 Sunde, M. and Blake, C.C.F. (1998) From the globular to the fibrous state: protein structure and structural conversion in amyloid formation. *Q. Rev. Biophys.* **31**, 1–39 https://doi.org/10.1017/S0033583598003400
220 Dumoulin, M., Canet, D., Last, A.M., Pardon, E., Archer, D.B., Muyldermans, S. et al. (2005) Reduced global cooperativity is a common feature underlying the amyloidogenicity of pathogenic lysozyme mutations. *J. Mol. Biol.* **346**, 773–788 https://doi.org/10.1016/j.jmb.2004.11.020
221 Mossuto, M.F., Dhulesia, A., Devlin, G., Frare, E., Kumita, J.R., de Laureto, P.P. et al. (2010) The non-core regions of human lysozyme amyloid fibrils influence cytotoxicity. *J. Mol. Biol.* **402**, 783–796 https://doi.org/10.1016/j.jmb.2010.07.005
222 Chaturvedi, S.K., Khan, J.M., Siddiqi, M.K., Alam, P. and Khan, R.H. (2016) Comparative insight into surfactants mediated amyloidogenesis of lysozyme. *Int. J. Biol. Macromol.* **83**, 315–325 https://doi.org/10.1016/j.ijbiomac.2015.11.053
223 Roode, L.W.Y., Shimanovich, U., Wu, S., Perrett, S. and Knowles, T.P.J. (2019) Protein microgels from amyloid fibril networks. *Adv. Exp. Med. Biol.* **1174**, 223–263 https://doi.org/10.1007/978-981-13-9791-2_7
224 Ke, P.C., Zhou, R., Serpell, L.C., Riek, R., Knowles, T.P.J., Lashuel, H.A. et al. (2020) Half a century of amyloids: past, present and future. *Chem. Soc. Rev.* **49**, 5473–5509 https://doi.org/10.1039/c9cs00199a
225 Nagata, K., Ashikaga, R., Mori, W., Zako, T. and Shimazaki, Y. (2023) Analysis of the enzymatic degradation of lysozyme fibrils using a combination method of non-denaturing gel electrophoresis and double staining with Coomassie Brilliant Blue G-250 and R-250 dyes. *Anal. Sci.* **39**, 267–274 https://doi.org/10.1007/s44211-022-00229-w
226 Nielsen, L., Khurana, R., Coats, A., Frokjaer, S., Brange, J., Vyas, S. et al. (2001) Effect of environmental factors on the kinetics of insulin fibril formation: elucidation of the molecular mechanism. *Biochemistry* **40**, 6036–6046 https://doi.org/10.1021/bi002555c
227 Mishra, R., Sjolander, D. and Hammarström, P. (2011) Spectroscopic characterization of diverse amyloid fibrils *in vitro* by the fluorescent dye Nile red. *Mol. Biosyst.* **7**, 1232–1240 https://doi.org/10.1039/c0mb00236d
228 Frankær, C.G., Sonderby, P., Bang, M.B., Mateiu, R.V., Groenning, M., Bukrinski, J. et al. (2017) Insulin fibrillation: the influence and coordination of Zn^{2+}. *J. Struct. Biol.* **199**, 27–38 https://doi.org/10.1016/j.jsb.2017.05.006
229 Metkar, S.K., Girigoswami, A., Murugesan, R. and Girigoswami, K. (2017) In vitro and in vivo insulin amyloid degradation mediated by Serratiopeptidase. *Mater. Sci. Eng. C Mater. Biol. Appl.* **70**, 728–735 https://doi.org/10.1016/j.msec.2016.09.049
230 Metkar, S.K., Girigoswami, A., Murugesan, R. and Girigoswami, K. (2017) Lumbrokinase for degradation and reduction of amyloid fibrils associated with amyloidosis. *J. Appl. Biomed.* **15**, 96–104 https://doi.org/10.1016/j.jab.2017.01.003

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231 Fagihi, M.H.A. and Bhattacharjee, S. (2022) Amyloid fibrillation of insulin: amelioration strategies and implications for translation. *ACS Pharmacol. Transl. Sci.* **5**, 1050–1061 https://doi.org/10.1021/acsptsci.2c00174
232 Piccardo, P., King, D., Telling, G., Manson, J.C. and Barron, R.M. (2013) Dissociation of prion protein amyloid seeding from transmission of a spongiform encephalopathy. *J. Virol.* **87**, 12349–12356 https://doi.org/10.1128/JVI.00673-13
233 Piccardo, P., King, D., Brown, D. and Barron, R.M. (2017) Variable tau accumulation in murine models with abnormal prion protein deposits. *J. Neurol. Sci.* **383**, 142–150 https://doi.org/10.1016/j.jns.2017.10.040
234 Aguzzi, A. and Rajendran, L. (2009) The transcellular spread of cytosolic amyloids, prions, and prionoids. *Neuron* **64**, 783–790 https://doi.org/10.1016/j.neuron.2009.12.016
235 Aguzzi, A. and Lakkaraju, A.K.K. (2016) Cell biology of prions and prionoids: a status report. *Trends Cell Biol.* **26**, 40–51 https://doi.org/10.1016/j.tcb.2015.08.007
236 Ashe, K.H. and Aguzzi, A. (2013) Prions, prionoids and pathogenic proteins in Alzheimer disease. *Prion* **7**, 55–59 https://doi.org/10.4161/pri.23061
237 Liberski, P.P. (2014) Prion, prionoids and infectious amyloid. *Parkinsonism Relat. Disord.* **20**, S80–S84 https://doi.org/10.1016/S1353-8020(13)70021-X
238 Verma, A. (2016) Prions, prion-like prionoids, and neurodegenerative disorders. *Ann. Indian Acad. Neurol.* **19**, 169–174 https://doi.org/10.4103/0972-2327.179979
239 Hafner Bratkovič, I. (2017) Prions, prionoid complexes and amyloids: the bad, the good and something in between. *Swiss Med. Wkly* **147**, w14424 https://doi.org/10.4414/smw.2017.14424
240 Scheckel, C. and Aguzzi, A. (2018) Prions, prionoids and protein misfolding disorders. *Nat. Rev. Genet.* **19**, 405–418 https://doi.org/10.1038/s41576-018-0011-4
241 Wells, C., Brennan, S.E., Keon, M. and Saksena, N.K. (2019) Prionoid proteins in the pathogenesis of neurodegenerative diseases. *Front. Mol. Neurosci.* **12**, 271 https://doi.org/10.3389/fnmol.2019.00271
242 Gosset, P., Camu, W., Raoul, C. and Mezghrani, A. (2022) Prionoids in amyotrophic lateral sclerosis. *Brain Commun.* **4**, fcac145 https://doi.org/10.1093/braincomms/fcac145
243 Matzinger, P. (1994) Tolerance, danger, and the extended family. *Annu. Rev. Immunol.* **12**, 991–1045 https://doi.org/10.1146/annurev.iy.12.040194.005015
244 Matzinger, P. (2007) Friendly and dangerous signals: is the tissue in control? *Nat. Immunol.* **8**, 11–13 https://doi.org/10.1038/ni0107-11
245 Pradeu, T. and Cooper, E.L. (2012) The danger theory: 20 years later. *Front. Immunol.* **3**, 287 https://doi.org/10.3389/fimmu.2012.00287
246 Tang, D.L., Kang, R., Coyne, C.B., Zeh, H.J. and Lotze, M.T. (2012) PAMPs and DAMPs: signal 0s that spur autophagy and immunity. *Immunol. Rev.* **249**, 158–175 https://doi.org/10.1111/j.1600-065X.2012.01146.x
247 Chaplin, D.D. (2010) Overview of the immune response. *J. Allergy Clin. Immunol.* **125**, S3–23 https://doi.org/10.1016/j.jaci.2009.12.980
248 Marshall, J.S., Warrington, R., Watson, W. and Kim, H.L. (2018) An introduction to immunology and immunopathology. *Allergy Asthma Clin. Immunol.* **14**, 49 https://doi.org/10.1186/s13223-018-0278-1
249 Altmann, D.M., Whettlock, E.M., Liu, S., Arachchillage, D.J. and Boyton, R.J. (2023) The immunology of long COVID. *Nat. Rev. Immunol.* https://doi.org/10.1038/s41577-023-00904-7
250 Ebringer, A. and Rashid, T. (2009) Rheumatoid arthritis is caused by *Proteus*: the molecular mimicry theory and Karl Popper. *Front. Biosci. (Elite Ed)* **1**, 577–586 https://doi.org/10.2741/e56
251 Ebringer, A., Rashid, T. and Wilson, C. (2010) Rheumatoid arthritis, *Proteus*, anti-CCP antibodies and Karl Popper. *Autoimmun. Rev.* **9**, 216–223 https://doi.org/10.1016/j.autrev.2009.10.006
252 Ebringer, A. (2012) *Rheumatoid Arthritis and Proteus*, Springer, London
253 Ebringer, A. and Rashid, T. (2014) Rheumatoid arthritis is caused by a *Proteus* urinary tract infection. *APMIS* **122**, 363–368 https://doi.org/10.1111/apm.12154
254 Wallukat, G., Hohberger, B., Wenzel, K., Furst, J., Schulze-Rothe, S., Wallukat, A. et al. (2021) Functional autoantibodies against G-protein coupled receptors in patients with persistent long-COVID-19 symptoms. *J. Transl. Autoimmun.* **4**, 100100 https://doi.org/10.1016/j.jtauto.2021.100100
255 Wang, E.Y., Mao, T., Klein, J., Dai, Y., Huck, J.D., Jaycox, J.R. et al. (2021) Diverse functional autoantibodies in patients with COVID-19. *Nature* **595**, 283–288 https://doi.org/10.1038/s41586-021-03631-y
256 Su, Y., Yuan, D., Chen, D.G., Ng, R.H., Wang, K., Choi, J. et al. (2022) Multiple early factors anticipate post-acute COVID-19 sequelae. *Cell* **185**, 881–895.e820 https://doi.org/10.1016/j.cell.2022.01.014
257 Davis, H.E., McCorkell, L., Vogel, J.M. and Topol, E.J. (2023) Long COVID: major findings, mechanisms and recommendations. *Nat. Rev. Microbiol.* **21**, 133–146 https://doi.org/10.1038/s41579-022-00846-2
258 Phelan, J., Grabowska, A.D. and Sepulveda, N. (2020) A potential antigenic mimicry between viral and human proteins linking myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) with autoimmunity: the case of HPV immunization. *Autoimmun. Rev.* **19**, 102487 https://doi.org/10.1016/j.autrev.2020.102487
259 Renz-Polster, H. and Scheibenbogen, C. (2022) [Post-COVID syndrome with fatigue and exercise intolerance: myalgic encephalomyelitis/chronic fatigue syndrome]. *Inn. Med. (Heidelb)* **63**, 830–839 https://doi.org/10.1007/s00108-022-01369-x
260 Casciola-Rosen, L., Thiemann, D.R., Andrade, F., Trejo-Zambrano, M.I., Leonard, E.K., Spangler, J.B. et al. (2022) Igm anti-ACE2 autoantibodies in severe COVID-19 activate complement and perturb vascular endothelial function. *JCI Insight* **7**, e158362 https://doi.org/10.1172/jci.insight.158362
261 Frontzek, K., Carta, M., Losa, M., Epskamp, M., Meisl, G., Anane, A. et al. (2020) Autoantibodies against the prion protein in individuals with PRNP mutations. *Neurology* **95**, e2028–e2037 https://doi.org/10.1212/WNL.0000000000009183
262 Handisurya, A., Gilch, S., Winter, D., Shafti-Keramat, S., Maurer, D., Schatzl, H.M. et al. (2007) Vaccination with prion peptide-displaying papillomavirus-like particles induces autoantibodies to normal prion protein that interfere with pathologic prion protein production in infected cells. *FEBS J.* **274**, 1747–1758 https://doi.org/10.1111/j.1742-4658.2007.05721.x
263 Britschgi, M., Olin, C.E., Johns, H.T., Takeda-Uchimura, Y., LeMieux, M.C., Rufibach, K. et al. (2009) Neuroprotective natural antibodies to assemblies of amyloidogenic peptides decrease with normal aging and advancing Alzheimer's disease. *Proc. Natl Acad. Sci. U.S.A.* **106**, 12145–12150 https://doi.org/10.1073/pnas.0904866106

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264 Horiuchi, M., Karino, A., Furuoka, H., Ishiguro, N., Kimura, K. and Shinagawa, M. (2009) Generation of monoclonal antibody that distinguishes PrPSc from PrPC and neutralizes prion infectivity. *Virology* **394**, 200–207 https://doi.org/10.1016/j.virol.2009.08.025
265 O'Nuallain, B., Williams, A.D., McWilliams-Koeppen, H.P., Acero, L., Weber, A., Ehrlich, H. et al. (2010) Anti-amyloidogenic activity of igGs contained in normal plasma. *J. Clin. Immunol.* **30**, S37–S42 https://doi.org/10.1007/s10875-010-9413-6
266 Roettger, Y., Zerr, I., Dodel, R. and Bach, J.P. (2013) Prion peptide uptake in microglial cells–the effect of naturally occurring autoantibodies against prion protein. *PLoS ONE* **8**, e67743 https://doi.org/10.1371/journal.pone.0067743
267 Braczynski, A.K., Sevenich, M., Gering, I., Kupreichyk, T., Agerschou, E.D., Kronimus, Y. et al. (2022) Alpha-synuclein-specific naturally occurring antibodies inhibit aggregation *in vitro* and *in vivo*. *Biomolecules* **12**, 469 https://doi.org/10.3390/biom12030469
268 Senatore, A., Frontzek, K., Emmenegger, M., Chincisan, A., Losa, M., Reimann, R. et al. (2020) Protective anti-prion antibodies in human immunoglobulin repertoires. *EMBO Mol. Med.* **12**, e12739 https://doi.org/10.15252/emmm.202012739
269 Biasini, E., Seegulam, M.E., Patti, B.N., Solforosi, L., Medrano, A.Z., Christensen, H.M. et al. (2008) Non-infectious aggregates of the prion protein react with several PrP^{Sc}-directed antibodies. *J. Neurochem.* **105**, 2190–2204 https://doi.org/10.1111/j.1471-4159.2008.05306.x
270 Petsch, B., Muller-Schiffmann, A., Lehle, A., Zirdum, E., Prikulis, I., Kuhn, F. et al. (2011) Biological effects and use of PrPSc- and PrP-specific antibodies generated by immunization with purified full-length native mouse prions. *J. Virol.* **85**, 4538–4546 https://doi.org/10.1128/JVI.02467-10
271 Kang, H.E., Weng, C.C., Saijo, E., Saylor, V., Bian, J., Kim, S. et al. (2012) Characterization of conformation-dependent prion protein epitopes. *J. Biol. Chem.* **287**, 37219–37232 https://doi.org/10.1074/jbc.M112.395921
272 Tapella, L., Stravalaci, M., Bastone, A., Biasini, E., Gobbi, M. and Chiesa, R. (2013) Epitope scanning indicates structural differences in brain-derived monomeric and aggregated mutant prion proteins related to genetic prion diseases. *Biochem. J.* **454**, 417–425 https://doi.org/10.1042/BJ20130563
273 Albus, A., Gold, M., Bach, J.P., Burg-Roderfeld, M., Jördens, M., Kirchhein, Y. et al. (2018) Extending the functional characteristics of naturally occurring autoantibodies against beta-amyloid, prion protein and alpha-Synuclein. *PLoS ONE* **13**, e0202954 https://doi.org/10.1371/journal.pone.0202954
274 Kayed, R., Head, E., Thompson, J.L., McIntire, T.M., Milton, S.C., Cotman, C.W. et al. (2003) Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. *Science* **300**, 486–489 https://doi.org/10.1126/science.1079469
275 Viola, K.L., Bicca, M.A., Bebenek, A.M., Kranz, D.L., Nandwana, V., Waters, E.A. et al. (2022) The therapeutic and diagnostic potential of amyloid beta oligomers selective antibodies to treat Alzheimer's disease. *Front. Neurosci.* **15**, 768646 https://doi.org/10.3389/fnins.2021.768646
276 Vojdani, A., Vojdani, E. and Kharrazian, D. (2020) Reaction of human monoclonal antibodies to SARS-CoV-2 proteins With tissue antigens: implications for autoimmune diseases. *Front. Immunol.* **11**, 617089 https://doi.org/10.3389/fimmu.2020.617089
277 Vojdani, A., Vojdani, E., Melgar, A.L. and Redd, J. (2022) Reaction of SARS-CoV-2 antibodies with other pathogens, vaccines, and food antigens. *Front. Immunol.* **13**, 1003094 https://doi.org/10.3389/fimmu.2022.1003094
278 Root-Bernstein, R., Churchill, E. and Oliverio, S. (2023) T cell receptor sequences amplified during severe COVID-19 and multisystem inflammatory syndrome in children mimic SARS-CoV-2, Its bacterial co-infections and host autoantigens. *Int. J. Mol. Sci.* **24**, 1335 https://doi.org/10.3390/ijms24021335
279 Root-Bernstein, R. (2023) From co-infections to autoimmune disease via hyperactivated innate immunity: COVID-19 autoimmune coagulopathies, autoimmune myocarditis and multisystem inflammatory syndrome in children. *Int. J. Mol. Sci.* **24**, 3001 https://doi.org/10.3390/ijms24033001
280 Stravalaci, M., Tapella, L., Beeg, M., Rossi, A., Joshi, P., Pizzi, E. et al. (2016) The anti-Prion antibody 15B3 detects toxic amyloid-beta oligomers. *J. Alzheimers Dis.* **53**, 1485–1497 https://doi.org/10.3233/JAD-150882
281 Qu, B.X., Gong, Y., Moore, C., Fu, M., German, D.C., Chang, L.Y. et al. (2014) Beta-amyloid auto-antibodies are reduced in Alzheimer's disease. *J. Neuroimmunol.* **274**, 168–173 https://doi.org/10.1016/j.jneuroim.2014.06.017
282 Gustot, A., Raussens, V., Dehousse, M., Dumoulin, M., Bryant, C.E., Ruysschaert, J.M. et al. (2013) Activation of innate immunity by lysozyme fibrils is critically dependent on cross-beta sheet structure. *Cell. Mol. Life Sci.* **70**, 2999–3012 https://doi.org/10.1007/s00018-012-1245-5
283 Hromadkova, L. and Ovsepian, S.V. (2019) Tau-Reactive endogenous antibodies: origin, functionality, and implications for the pathophysiology of Alzheimer's disease. *J. Immunol. Res.* **2019**, 7406810 https://doi.org/10.1155/2019/7406810
284 Phay, M., Blinder, V., Macy, S., Greene, M.J., Wooliver, D.C., Liu, W. et al. (2014) Transthyretin aggregate-specific antibodies recognize cryptic epitopes on patient-derived amyloid fibrils. *Rejuvenation. Res.* **17**, 97–104 https://doi.org/10.1089/rej.2013.1524
285 Chazenbalk, G.D., McLachlan, S.M., Pichurin, P., Yan, X.M. and Rapoport, B. (2001) A prion-like shift between two conformational forms of a recombinant thyrotropin receptor A-subunit module: purification and stabilization using chemical chaperones of the form reactive with Graves' autoantibodies. *J. Clin. Endocrinol. Metab.* **86**, 1287–1293 https://doi.org/10.1210/jcem.86.3.7363
286 Martínez, J., Sánchez, R., Castellanos, M., Fernández-Escamilla, A.M., Vázquez-Cortés, S., Fernández-Rivas, M. et al. (2015) Fish beta-parvalbumin acquires allergenic properties by amyloid assembly. *Swiss Med. Wkly* **145**, w14128 https://doi.org/10.4414/smw.2015.14128
287 Lee, C.C., Julian, M.C., Tiller, K.E., Meng, F., DuConge, S.E., Akter, R. et al. (2016) Design and optimization of anti-amyloid domain antibodies specific for beta-amyloid and islet amyloid polypeptide. *J. Biol. Chem.* **291**, 2858–2873 https://doi.org/10.1074/jbc.M115.682336
288 Julian, M.C., Rabia, L.A., Desai, A.A., Arsiwala, A., Gerson, J.E., Paulson, H.L. et al. (2019) Nature-inspired design and evolution of anti-amyloid antibodies. *J. Biol. Chem.* **294**, 8438–8451 https://doi.org/10.1074/jbc.RA118.004731
289 Syvänen, S., Fang, X.T., Faresjö, R., Rokka, J., Lannfelt, L., Olberg, D.E. et al. (2020) Fluorine-18-labeled antibody ligands for PET imaging of amyloid-beta in brain. *ACS Chem. Neurosci.* **11**, 4460–4468 https://doi.org/10.1021/acschemneuro.0c00652
290 Bard, F., Barbour, R., Cannon, C., Carretto, R., Fox, M., Games, D. et al. (2003) Epitope and isotype specificities of antibodies to beta -amyloid peptide for protection against Alzheimer's disease-like neuropathology. *Proc. Natl Acad. Sci. U.S.A.* **100**, 2023–2028 https://doi.org/10.1073/pnas.0436286100
291 Westwood, M. and Lawson, A.D.G. (2015) Opportunities for conformation-selective antibodies in amyloid-related diseases. *Antibodies (Basel)* **4**, 170–196 https://doi.org/10.3390/antib4030170
292 Albus, A., Jordens, M., Moller, M. and Dodel, R. (2019) Encoding the sequence of specific autoantibodies against beta-amyloid and alpha-synuclein in neurodegenerative diseases. *Front. Immunol.* **10**, 2033 https://doi.org/10.3389/fimmu.2019.02033
293 Rofo, F., Buijs, J., Falk, R., Honek, K., Lannfelt, L., Lilja, A.M. et al. (2021) Novel multivalent design of a monoclonal antibody improves binding strength to soluble aggregates of amyloid beta. *Transl. Neurodegener.* **10**, 38 https://doi.org/10.1186/s40035-021-00258-x

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294 Bateman, R.J., Cummings, J., Schobel, S., Salloway, S., Vellas, B., Boada, M. et al. (2022) Gantenerumab: an anti-amyloid monoclonal antibody with potential disease-modifying effects in early Alzheimer's disease. *Alzheimers Res. Ther.* **14**, 178 https://doi.org/10.1186/s13195-022-01110-8
295 Wechalekar, A., Antoni, G., Al Azzam, W., Bergstrom, M., Biswas, S., Chen, C. et al. (2022) Pharmacodynamic evaluation and safety assessment of treatment with antibodies to serum amyloid P component in patients with cardiac amyloidosis: an open-label Phase 2 study and an adjunctive immuno-PET imaging study. *BMC Cardiovasc. Disord.* **22**, 49 https://doi.org/10.1186/s12872-021-02407-6
296 Scott, M., Foster, D., Mirenda, C., Serban, D., Coufal, F., Walchli, M. et al. (1989) Transgenic mice expressing hamster prion protein produce species-specific scrapie infectivity and amyloid plaques. *Cell* **59**, 847–857 https://doi.org/10.1016/0092-8674(89)90608-9
297 Kocisko, D.A., Priola, S.A., Raymond, G.J., Chesebro, B., Lansbury, Jr, P.T. and Caughey, B. (1995) Species specificity in the cell-free conversion of prion protein to protease-resistant forms: a model for the scrapie species barrier. *Proc. Natl Acad. Sci. U.S.A.* **92**, 3923–3927 https://doi.org/10.1073/pnas.92.9.3923
298 Kocisko, D.A., Engel, A.L., Harbuck, K., Arnold, K.M., Olsen, E.A., Raymond, L.D. et al. (2005) Comparison of protease-resistant prion protein inhibitors in cell cultures infected with two strains of mouse and sheep scrapie. *Neurosci. Lett.* **388**, 106–111 https://doi.org/10.1016/j.neulet.2005.06.053
299 Sweeting, B., Khan, M.Q., Chakrabartty, A. and Pai, E.F. (2010) Structural factors underlying the species barrier and susceptibility to infection in prion disease. *Biochem. Cell Biol.* **88**, 195–202 https://doi.org/10.1139/o09-172
300 Hagiwara, K., Hara, H. and Hanada, K. (2013) Species-barrier phenomenon in prion transmissibility from a viewpoint of protein science. *J. Biochem.* **153**, 139–145 https://doi.org/10.1093/jb/mvs148
301 Luers, L., Bannach, O., Stöhr, J., Wördehoff, M.M., Wolff, M., Nagel-Steger, L. et al. (2013) Seeded fibrillation as molecular basis of the species barrier in human prion diseases. *PLoS ONE* **8**, e72623 https://doi.org/10.1371/journal.pone.0072623
302 Torres, J.M., Espinosa, J.C., Aguilar-Calvo, P., Herva, M.E., Relaño-Ginés, A., Villa-Diaz, A. et al. (2014) Elements modulating the prion species barrier and its passage consequences. *PLoS ONE* **9**, e89722 https://doi.org/10.1371/journal.pone.0089722
303 Peden, A.H., Suleiman, S. and Barria, M.A. (2021) Understanding intra-species and inter-Species prion conversion and zoonotic potential using protein misfolding cyclic amplification. *Front. Aging Neurosci.* **13**, 716452 https://doi.org/10.3389/fnagi.2021.716452
304 Furuoka, H., Yabuzoe, A., Horiuchi, M., Tagawa, Y., Yokoyama, T., Yamakawa, Y. et al. (2007) Species-specificity of a panel of prion protein antibodies for the immunohistochemical study of animal and human prion diseases. *J. Comp. Pathol.* **136**, 9–17 https://doi.org/10.1016/j.jcpa.2006.09.002
305 Castilla, J., Gonzalez-Romero, D., Saa, P., Morales, R., De Castro, J. and Soto, C. (2008) Crossing the species barrier by PrP(Sc) replication in vitro generates unique infectious prions. *Cell* **134**, 757–768 https://doi.org/10.1016/j.cell.2008.07.030
306 Igel, A., Fornara, B., Rezaei, H. and Béringue, V. (2023) Prion assemblies: structural heterogeneity, mechanisms of formation, and role in species barrier. *Cell Tissue Res.* **392**, 149–166 https://doi.org/10.1007/s00441-022-03700-2
307 Burdukiewicz, M., Rafacz, D., Barbach, A., Hubicka, K., Bakąla, L., Lassota, A. et al. (2023) Amylograph: a comprehensive database of amyloid-amyloid interactions. *Nucleic Acids Res.* **51**, D352–D357 https://doi.org/10.1093/nar/gkac882
308 Hammarström, P. and Nyström, S. (2015) Porcine prion protein amyloid. *Prion* **9**, 266–277 https://doi.org/10.1080/19336896.2015.1065373
309 Morales, R., Moreno-Gonzalez, I. and Soto, C. (2013) Cross-seeding of misfolded proteins: implications for etiology and pathogenesis of protein misfolding diseases. *PLoS Pathog.* **9**, e1003537 https://doi.org/10.1371/journal.ppat.1003537
310 Sarell, C.J., Stockley, P.G. and Radford, S.E. (2013) Assessing the causes and consequences of co-polymerization in amyloid formation. *Prion* **7**, 359–368 https://doi.org/10.4161/pri.26415
311 Sarell, C.J., Woods, L.A., Su, Y., Debelouchina, G.T., Ashcroft, A.E., Griffin, R.G. et al. (2013) Expanding the repertoire of amyloid polymorphs by co-polymerization of related protein precursors. *J. Biol. Chem.* **288**, 7327–7337 https://doi.org/10.1074/jbc.M112.447524
312 Hu, R., Zhang, M., Chen, H., Jiang, B. and Zheng, J. (2015) Cross-seeding interaction between beta-amyloid and human islet amyloid polypeptide. *ACS Chem. Neurosci.* **6**, 1759–1768 https://doi.org/10.1021/acschemneuro.5b00192
313 Zhang, M., Hu, R., Chen, H., Chang, Y., Ma, J., Liang, G. et al. (2015) Polymorphic cross-seeding amyloid assemblies of amyloid-beta and human islet amyloid polypeptide. *Phys. Chem. Chem. Phys.* **17**, 23245–23256 https://doi.org/10.1039/c5cp03329b
314 Thacker, D., Sanagavarapu, K., Frohm, B., Meisl, G., Knowles, T.P.J. and Linse, S. (2020) The role of fibril structure and surface hydrophobicity in secondary nucleation of amyloid fibrils. *Proc. Natl Acad. Sci. U.S.A.* **117**, 25272–25283 https://doi.org/10.1073/pnas.2002956117
315 Ivanova, M.I., Lin, Y., Lee, Y.H., Zheng, J. and Ramamoorthy, A. (2021) Biophysical processes underlying cross-seeding in amyloid aggregation and implications in amyloid pathology. *Biophys. Chem.* **269**, 106507 https://doi.org/10.1016/j.bpc.2020.106507
316 Subedi, S., Sasidharan, S., Nag, N., Saudagar, P. and Tripathi, T. (2022) Amyloid cross-seeding: mechanism, implication, and inhibition. *Molecules* **27**, 1776 https://doi.org/10.3390/molecules27061776
317 Thangakani, A.M., Nagarajan, R., Kumar, S., Sakthivel, R., Velmurugan, D. and Gromiha, M.M. (2016) CPAD, curated protein aggregation database: a repository of manually curated experimental data on protein and peptide aggregation. *PLoS ONE* **11**, e0152949 https://doi.org/10.1371/journal.pone.0152949
318 Bondarev, S.A., Antonets, K.S., Kajava, A.V., Nizhnikov, A.A. and Zhouravleva, G.A. (2018) Protein co-aggregation related to amyloids: methods of investigation, diversity, and classification. *Int. J. Mol. Sci.* **19**, 2292 https://doi.org/10.3390/ijms19082292
319 Page, M.J., Thomson, G.J.A., Nunes, J.M., Engelbrecht, A.M., Nell, T.A., de Villiers, W.J.S. et al. (2019) Serum amyloid A binds to fibrin(ogen), promoting fibrin amyloid formation. *Sci. Rep.* **9**, 3102 https://doi.org/10.1038/s41598-019-39056-x
320 Rawat, P., Prabakaran, R., Sakthivel, R., Mary Thangakani, A., Kumar, S. and Gromiha, M.M. (2020) CPAD 2.0: a repository of curated experimental data on aggregating proteins and peptides. *Amyloid* **27**, 128–133 https://doi.org/10.1080/13506129.2020.1715363
321 Zaman, M. and Andreasen, M. (2020) Cross-talk between individual phenol-soluble modulins in *Staphylococcus aureus* biofilm enables rapid and efficient amyloid formation. *eLife* **9**, e59776 https://doi.org/10.7554/eLife.59776
322 Chatterjee, D., Jacob, R.S., Ray, S., Navalkar, A., Singh, N., Sengupta, S. et al. (2022) Co-aggregation and secondary nucleation in the life cycle of human prolactin/galanin functional amyloids. *eLife* **11**, e73835 https://doi.org/10.7554/eLife.73835
323 Hara, H. and Sakaguchi, S. (2021) Virus infection, genetic mutations, and prion infection in prion protein conversion. *Int. J. Mol. Sci.* **22**, 12439 https://doi.org/10.3390/ijms222212439

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Biochemical Journal (2023) 480 1217–1240
https://doi.org/10.1042/BCJ20230241

324 Oskarsson, M.E., Paulsson, J.F., Schultz, S.W., Ingelsson, M., Westermark, P. and Westermark, G.T. (2015) *In vivo* seeding and cross-seeding of localized amyloidosis: a molecular link between type 2 diabetes and Alzheimer disease. *Am. J. Pathol.* **185**, 834–846 https://doi.org/10.1016/j.ajpath.2014.11.016
325 Ono, K., Takahashi, R., Ikeda, T. and Yamada, M. (2012) Cross-seeding effects of amyloid beta-protein and alpha-synuclein. *J. Neurochem.* **122**, 883–890 https://doi.org/10.1111/j.1471-4159.2012.07847.x
326 Sampson, T.R., Challis, C., Jain, N., Moiseyenko, A., Ladinsky, M.S., Shastri, G.G. et al. (2020) A gut bacterial amyloid promotes alpha-synuclein aggregation and motor impairment in mice. *eLife* **9**, e53111 https://doi.org/10.7554/eLife.53111
327 Werner, T., Horvath, I. and Wittung-Stafshede, P. (2020) Crosstalk between alpha-synuclein and other human and non-human amyloidogenic proteins: consequences for amyloid formation in Parkinson's disease. *J. Parkinsons Dis.* **10**, 819–830 https://doi.org/10.3233/JPD-202085
328 Wittung-Stafshede, P. (2022) Gut power: modulation of human amyloid formation by amyloidogenic proteins in the gastrointestinal tract. *Curr. Opin. Struct. Biol.* **72**, 33–38 https://doi.org/10.1016/j.sbi.2021.07.009
329 Baram, M., Gilead, S., Gazit, E. and Miller, Y. (2018) Mechanistic perspective and functional activity of insulin in amylin aggregation. *Chem. Sci.* **9**, 4244–4252 https://doi.org/10.1039/c8sc00481a
330 Horvath, I. and Wittung-Stafshede, P. (2016) Cross-talk between amyloidogenic proteins in type-2 diabetes and Parkinson's disease. *Proc. Natl Acad. Sci. U.S.A.* **113**, 12473–12477 https://doi.org/10.1073/pnas.1610371113
331 Gal, N., Morag, A., Kolusheva, S., Winter, R., Landau, M. and Jelinek, R. (2013) Lipid bilayers significantly modulate cross-fibrillation of two distinct amyloidogenic peptides. *J. Am. Chem. Soc.* **135**, 13582–13589 https://doi.org/10.1021/ja4070427
332 Lu, J., Zhang, S., Ma, X., Jia, C., Liu, Z., Huang, C. et al. (2020) Structural basis of the interplay between alpha-synuclein and Tau in regulating pathological amyloid aggregation. *J. Biol. Chem.* **295**, 7470–7480 https://doi.org/10.1074/jbc.RA119.012284
333 Hall, H., Reyes, S., Landeck, N., Bye, C., Leanza, G., Double, K. et al. (2014) Hippocampal Lewy pathology and cholinergic dysfunction are associated with dementia in Parkinson's disease. *Brain* **137**, 2493–2508 https://doi.org/10.1093/brain/awu193
334 Chiu, S.Y., Wyman-Chick, K.A., Ferman, T.J., Bayram, E., Holden, S.K., Choudhury, P. et al. (2023) Sex differences in dementia with Lewy bodies: focused review of available evidence and future directions. *Parkinsonism Relat. Disord.* **107**, 105285 https://doi.org/10.1016/j.parkreldis.2023.105285
335 Kopp, J. and Spadiut, O. (2023) Inclusion bodies: status quo and perspectives. *Methods Mol. Biol.* **2617**, 1–13 https://doi.org/10.1007/978-1-0716-2930-7_1
336 Gottwald, J. and Röcken, C. (2021) The amyloid proteome: a systematic review and proposal of a protein classification system. *Crit. Rev. Biochem. Mol. Biol.* **56**, 526–542 https://doi.org/10.1080/10409238.2021.1937926
337 Drummond, E., Kavanagh, T., Pires, G., Marta-Ariza, M., Kanshin, E., Nayak, S. et al. (2022) The amyloid plaque proteome in early onset Alzheimer's disease and down syndrome. *Acta Neuropathol. Commun.* **10**, 53 https://doi.org/10.1186/s40478-022-01356-1
338 Kruger, A., Vlok, M., Turner, S., Venter, C., Laubscher, G.J., Kell, D.B. et al. (2022) Proteomics of fibrin amyloid microclots in long COVID/ post-Acute sequelae of COVID-19 (PASC) shows many entrapped pro-inflammatory molecules that may also contribute to a failed fibrinolytic system. *Cardiovasc. Diabetol.* **21**, 190 https://doi.org/10.1186/s12933-022-01623-4
339 González-Durruthy, M., Concu, R., Vendrame, L.F.O., Zanella, I., Ruso, J.M. and Cordeiro, M. (2020) Targeting beta-blocker drug-drug interactions with fibrinogen blood plasma protein: a computational and experimental study. *Molecules* **25**, 5425 https://doi.org/10.3390/molecules25225425
340 Gligorijević, N., Simeon, M., Mirjana, R., Steva, L., Milan, N., Tanja, Ć.V et al. (2021) Ligand binding to fibrinogen influences its structure and function. *Biol. Serb.* **43**, 24–31 https://doi.org/10.5281/zenodo.5512285
341 Mestres, J., Gregori-Puigjané, E., Valverde, S. and Solé, R.V. (2009) The topology of drug-target interaction networks: implicit dependence on drug properties and target families. *Mol. Biosyst.* **5**, 1051–1057 https://doi.org/10.1039/b905821b
342 Mestres, J. and Gregori-Puigjané, E. (2009) Conciliating binding efficiency and polypharmacology. *Trends Pharmacol. Sci.* **30**, 470–474 https://doi.org/10.1016/j.tips.2009.07.004
343 Kell, D.B., Dobson, P.D., Bilsland, E. and Oliver, S.G. (2013) The promiscuous binding of pharmaceutical drugs and their transporter-mediated uptake into cells: what we (need to) know and how we can do so. *Drug Disc. Today* **18**, 218–239 https://doi.org/10.1016/j.drudis.2012.11.008
344 Winter, A., Higueruelo, A.P., Marsh, M., Sigurdardottir, A., Pitt, W.R. and Blundell, T.L. (2012) Biophysical and computational fragment-based approaches to targeting protein-protein interactions: applications in structure-guided drug discovery. *Q. Rev. Biophys.* **45**, 383–426 https://doi.org/10.1017/S0033583512000108
345 Nero, T.L., Morton, C.J., Holien, J.K., Wielens, J. and Parker, M.W. (2014) Oncogenic protein interfaces: small molecules, big challenges. *Nat. Rev. Cancer* **14**, 248–262 https://doi.org/10.1038/nrc3690
346 Skolnick, J. and Zhou, H. (2022) Implications of the essential role of small molecule ligand binding pockets in protein-protein interactions. *J. Phys. Chem. B* **126**, 6853–6867 https://doi.org/10.1021/acs.jpcb.2c04525
347 Hassan, N., Ruso, J.M. and Somasundaran, P. (2011) Mechanisms of fibrinogen-acebutolol interactions: insights from DSC, CD and LS. *Colloids Surf. B Biointerfaces* **82**, 581–587 https://doi.org/10.1016/j.colsurfb.2010.10.020
348 González-Durruthy, M., Scanavachi, G., Rial, R., Liu, Z., Cordeiro, M., Itri, R. et al. (2020) Mapping the underlying mechanisms of fibrinogen benzothiazole drug interactions using computational and experimental approaches. *Int. J. Biol. Macromol.* **163**, 730–744 https://doi.org/10.1016/j.ijbiomac.2020.07.044
349 Gligorijević, N., Miniić, S., Robajac, D., Nikolić, M., Ćirković Veličković, T. and Nedić, O. (2019) Characterisation and the effects of bilirubin binding to human fibrinogen. *Int. J. Biol. Macromol.* **128**, 74–79 https://doi.org/10.1016/j.ijbiomac.2019.01.124
350 Gligorijević, N., Vasović, T., Lević, S., Miljević, Č., Nedić, O. and Nikolić, M. (2020) Atypical antipsychotic clozapine binds fibrinogen and affects fibrin formation. *Int. J. Biol. Macromol.* **154**, 142–149 https://doi.org/10.1016/j.ijbiomac.2020.03.119
351 Leung, M.H.M. and Kee, T.W. (2009) Effective stabilization of curcumin by association to plasma proteins: human serum albumin and fibrinogen. *Langmuir* **25**, 5773–5777 https://doi.org/10.1021/la804215v
352 Gligorijević, N., Šukalović, V., Penezić, A. and Nedić, O. (2020) Characterisation of the binding of dihydro-alpha-lipoic acid to fibrinogen and the effects on fibrinogen oxidation and fibrin formation. *Int. J. Biol. Macromol.* **147**, 319–325 https://doi.org/10.1016/j.ijbiomac.2020.01.098
353 Li, X., Duan, H., Song, Z. and Xu, R. (2022) Comparative study on the interaction between fibrinogen and flavonoids. *J. Mol. Struct.* **1262**, 132963 https://doi.org/10.1016/j.molstruc.2022.132963

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354 Hassan, N., Barbosa, L.R., Itri, R. and Ruso, J.M. (2011) Fibrinogen stability under surfactant interaction. *J. Colloid Interface Sci.* **362**, 118–126 https://doi.org/10.1016/j.jcis.2011.06.010

355 Gonçalves, S., Santos, N.C., Martins-Silva, J. and Saldanha, C. (2007) Fluorescence spectroscopy evaluation of fibrinogen-beta-estradiol binding. *J. Photochem. Photobiol. B* **86**, 170–176 https://doi.org/10.1016/j.jphotobiol.2006.09.001

356 González-Durruthy, M., Rial, R., Cordeiro, M.N.D.S., Liu, Z. and Ruso, J.M. (2021) Exploring the conformational binding mechanism of fibrinogen induced by interactions with penicillin β-lactam antibiotic drugs. *J. Mol. Liq.* **324**, 114667 https://doi.org/10.1016/j.molliq.2020.114667

357 González-Durruthy, M., Scanavachi, G., Rial, R., Liu, Z., Cordeiro, M.N.D.S., Itri, R. et al. (2019) Structural and energetic evolution of fibrinogen toward to the betablocker interactions. *Int. J. Biol. Macromol.* **137**, 405–419 https://doi.org/10.1016/j.ijbiomac.2019.06.229

358 Gligorijević, N., Radomirović, M., Rajković, A., Nedić, O. and Ćirković Veličković, T. (2020) Fibrinogen increases resveratrol solubility and prevents it from oxidation. *Foods* **9**, 780 https://doi.org/10.3390/foods9060780

359 Schütz, A.K., Hornemann, S., Walti, M.A., Greuter, L., Tiberi, C., Cadalbert, R. et al. (2017) Binding of polythiophenes to amyloids: structural mapping of the pharmacophore. *ACS Chem. Neurosci.* **9**, 475–481 https://doi.org/10.1021/acschemneuro.7b00397

360 Hammarström, P., Wiseman, R.L., Powers, E.T. and Kelly, J.W. (2003) Prevention of transthyretin amyloid disease by changing protein misfolding energetics. *Science* **299**, 713–716 https://doi.org/10.1126/science.1079589

361 Nusrat, S., Zaman, M., Masroor, A., Siddqi, M.K., Zaidi, N., Neelofar, K. et al. (2018) Deciphering the enhanced inhibitory, disaggregating and cytoprotective potential of promethazine towards amyloid fibrillation. *Int. J. Biol. Macromol.* **106**, 851–863 https://doi.org/10.1016/j.ijbiomac.2017.08.081

362 Gaule, T.G. and Ajjan, R.A. (2021) Fibrin(ogen) as a therapeutic target: opportunities and challenges. *Int. J. Mol. Sci.* **22**, 6916 https://doi.org/10.3390/ijms22136916

363 Iadanza, M.G., Jackson, M.P., Hewitt, E.W., Ranson, N.A. and Radford, S.E. (2018) A new era for understanding amyloid structures and disease. *Nat. Rev. Mol. Cell Biol.* **19**, 755–773 https://doi.org/10.1038/s41580-018-0060-8

364 Nilsson, K.P.R., Ikenberg, K., Åslund, A., Fransson, S., Konradsson, P., Röcken, C. et al. (2010) Structural typing of systemic amyloidoses by luminescent-conjugated polymer spectroscopy. *Am. J. Pathol.* **176**, 563–574 https://doi.org/10.2353/ajpath.2010.080797

365 Klingstedt, T., Åslund, A., Simon, R.A., Johansson, L.B.G., Mason, J.J., Nyström, S. et al. (2011) Synthesis of a library of oligothiophenes and their utilization as fluorescent ligands for spectral assignment of protein aggregates. *Org. Biomol. Chem.* **9**, 8356–8370 https://doi.org/10.1039/c1ob05637a

366 Wegenast-Braun, B.M., Skodras, A., Bayraktar, G., Mahler, J., Fritschi, S.K., Klingstedt, T. et al. (2012) Spectral discrimination of cerebral amyloid lesions after peripheral application of luminescent conjugated oligothiophenes. *Am. J. Pathol.* **181**, 1953–1960 https://doi.org/10.1016/j.ajpath.2012.08.031

367 Rasmussen, J., Mahler, J., Beschorner, N., Kaeser, S.A., Häsler, L.M., Baumann, F. et al. (2017) Amyloid polymorphisms constitute distinct clouds of conformational variants in different etiological subtypes of Alzheimer's disease. *Proc. Natl Acad. Sci. U.S.A.* **114**, 13018–13023 https://doi.org/10.1073/pnas.1713215114

368 Stepanchuk, A.A., Morgan, M.L., Joseph, J.T. and Stys, P.K. (2022) Dual-probe fluorescence spectroscopy for sensitive quantitation of Alzheimer's amyloid pathology. *Acta Neuropathol. Commun.* **10**, 153 https://doi.org/10.1186/s40478-022-01456-y

369 Diack, A.B., Ritchie, D.L., Peden, A.H., Brown, D., Boyle, A., Morabito, L. et al. (2014) Variably protease-sensitive prionopathy, a unique prion variant with inefficient transmission properties. *Emerg. Infect. Dis.* **20**, 1969–1979 https://doi.org/10.3201/eid2012.140214

370 Åslund, A., Herland, A., Hammarström, P., Nilsson, K.P.R., Jonsson, B.H., Inganäs, O. et al. (2007) Studies of luminescent conjugated polythiophene derivatives: enhanced spectral discrimination of protein conformational states. *Bioconjug. Chem.* **18**, 1860–1868 https://doi.org/10.1021/bc700180g

371 Klingstedt, T. and Nilsson, K.P.R. (2012) Luminescent conjugated poly- and oligo-thiophenes: optical ligands for spectral assignment of a plethora of protein aggregates. *Biochem. Soc. Trans.* **40**, 704–710 https://doi.org/10.1042/BST20120009

372 Klingstedt, T., Shirani, H., Åslund, K.O.A., Cairns, N.J., Sigurdson, C.J., Goedert, M. et al. (2013) The structural basis for optimal performance of oligothiophene-based fluorescent amyloid ligands: conformational flexibility is essential for spectral assignment of a diversity of protein aggregates. *Chemistry* **19**, 10179–10192 https://doi.org/10.1002/chem.201301463

373 Stepanchuk, A., Tahir, W., Nilsson, K.P.R., Schatzl, H.M. and Stys, P.K. (2021) Early detection of prion protein aggregation with a fluorescent pentameric oligothiophene probe using spectral confocal microscopy. *J. Neurochem.* **156**, 1033–1048 https://doi.org/10.1111/jnc.15148

374 ATTACC Investigators, ACTIV-4a Investigators, REMAP-CAP Investigators, Lawler, P.R., Goligher, E.C., Berger, J.S. et al. (2021) Therapeutic anticoagulation with heparin in noncritically Ill patients with COVID-19. *N. Engl. J. Med.* **385**, 790–802. https://doi.org/10.1056/NEJMoa2105911

375 REMAP-CAP Investigators, A. CTIV-4a Investigators, ATTACC Investigators, Goligher, E.C., Bradbury, C.A., McVerry, B.J. et al. (2021) Therapeutic anticoagulation with heparin in critically Ill patients with COVID-19. *N. Engl. J. Med.* **385**, 777–789. https://doi.org/10.1056/NEJMoa2103417

376 Cuker, A., Tseng, E.K., Schünemann, H.J., Angchaisuksiri, P., Blair, C., Dane, K. et al. (2022) American Society of Hematology living guidelines on the use of anticoagulation for thromboprophylaxis for patients with COVID-19: March 2022 update on the use of anticoagulation in critically ill patients. *Blood Adv.* **6**, 4975–4982 https://doi.org/10.1182/bloodadvances.2022007940

377 Kumar, S.S. and Sabu, A. (2019) Fibrinolytic enzymes for thrombolytic therapy. *Adv. Exp. Med. Biol.* **1148**, 345–381 https://doi.org/10.1007/978-981-13-7709-9_15

378 Yuan, L., Liangqi, C., Xiyu, T. and Jinyao, L. (2022) Biotechnology, bioengineering and applications of *Bacillus* nattokinase. *Biomolecules* **12**, 980 https://doi.org/10.3390/biom12070980

379 Tanikawa, T., Kiba, Y., Yu, J., Hsu, K., Chen, S., Ishii, A. et al. (2022) Degradative effect of nattokinase on spike protein of SARS-CoV-2. *Molecules* **27**, 5405 https://doi.org/10.3390/molecules27175405

380 Jadhav, S.B., Shah, N., Rathi, A., Rathi, V. and Rathi, A. (2020) Serratiopeptidase: insights into the therapeutic applications. *Biotechnol. Rep. (Amst)* **28**, e00544 https://doi.org/10.1016/j.btre.2020.e00544

381 Sharma, C., Jha, N.K., Meeran, M.F.N., Patil, C.R., Goyal, S.N. and Ojha, S. (2021) Serratiopeptidase, A serine protease anti-inflammatory, fibrinolytic, and mucolytic drug, can be a useful adjuvant for management in COVID-19. *Front. Pharmacol.* **12**, 603997 https://doi.org/10.3389/fphar.2021.603997

382 Altaf, F., Wu, S.R. and Kasim, V. (2021) Role of fibrinolytic enzymes in anti-thrombosis therapy. *Front. Mol. Biosci.* **8**, 680397 https://doi.org/10.3389/fmolb.2021.680397

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383 Metkar, S.K., Ghosh, S., Girigoswami, A. and Girigoswami, K. (2019) The potential of serratiopetidase and lumbrokinase for the degradation of prion peptide 106-126: an *in vitro* and *in silico* perspective. *CNS Neurol. Disord. Drug Targets* **18**, 723–731 https://doi.org/10.2174/1871527318666191021150002
384 Metkar, S.K., Girigoswami, A., Bondage, D.D., Shinde, U.G. and Girigoswami, K. (2022) The potential of lumbrokinase and serratiopeptidase for the degradation of Aβ 1–42 peptide: an in vitro and in silico approach. *Int. J. Neurosci.*, 1–12 https://doi.org/10.1080/00207454.2022.2089137
385 Welch, G.R. and Kell, D.B. (1986) Not just catalysts; the bioenergetics of molecular machines. In *The Fluctuating Enzyme* (Welch, G., ed.), pp. 451–492, John Wiley, New York
386 Kell, D.B. (2021) A protet-based, protonic charge transfer model of energy coupling in oxidative and photosynthetic phosphorylation. *Adv. Micr. Physiol.* **78**, 1–177 https://doi.org/10.1016/bs.ampbs.2021.01.001
387 Castellanos, M., Torres-Pardo, A., Rodriguez-Perez, R. and Gasset, M. (2018) Amyloid assembly endows Gad m 1 with biomineralization properties. *Biomolecules* **8**, 13 https://doi.org/10.3390/biom8010013
388 Arad, E., Leshem, A.B., Rapaport, H. and Jelinek, R. (2021) Beta-amyloid fibrils catalyze neurotransmitter degradation. *Chem. Catal.* **1**, 908–922 https://doi.org/10.1016/j.checat.2021.07.005
389 Arad, E., Yosefi, G., Kolusheva, S., Bitton, R., Rapaport, H. and Jelinek, R. (2022) Native glucagon amyloids catalyze key metabolic reactions. *ACS Nano* **16**, 12889–12899 https://doi.org/10.1021/acsnano.2c05166
390 Arad, E. and Jelinek, R. (2022) Catalytic amyloids. *Trends Chem.* **4**, 907–917 https://doi.org/10.1016/j.trechm.2022.07.001
391 Horvath, I. and Wittung-Stafshede, P. (2023) Amyloid fibers of alpha-synuclein catalyze chemical reactions. *ACS Chem. Neurosci.* **14**, 603–608 https://doi.org/10.1021/acschemneuro.2c00799
392 Lanz, T.V., Brewer, R.C., Ho, P.P., Moon, J.S., Jude, K.M., Fernandez, D. et al. (2022) Clonally expanded B cells in multiple sclerosis bind EBV EBNA1 and GlialCAM. *Nature* **603**, 321–327 https://doi.org/10.1038/s41586-022-04432-7
393 Wekerle, H. (2022) Epstein-Barr virus sparks brain autoimmunity in multiple sclerosis. *Nature* **603**, 230–232 https://doi.org/10.1038/d41586-022-00382-2
394 Scallon, B., Cai, A., Solowski, N., Rosenberg, A., Song, X.Y., Shealy, D. et al. (2002) Binding and functional comparisons of two types of tumor necrosis factor antagonists. *J. Pharmacol. Exp. Ther.* **301**, 418–426 https://doi.org/10.1124/jpet.301.2.418
395 Currin, A., Swainston, N., Day, P.J. and Kell, D.B. (2015) Synthetic biology for the directed evolution of protein biocatalysts: navigating sequence space intelligently. *Chem. Soc. Rev.* **44**, 1172–1239 https://doi.org/10.1039/c1034cs00351a
396 Ren, F., Ding, X., Zheng, M., Korzinkin, M., Cai, X., Zhu, W. et al. (2023) Alphafold accelerates artificial intelligence powered drug discovery: efficient discovery of a novel CDK20 small molecule inhibitor. *Chem. Sci.* **14**, 1443–1452 https://doi.org/10.1039/d2sc05709c
397 Yuan, Z., Shen, T., Xu, S., Yu, L., Ren, R. and Sun, S. (2023) AF2-mutation: adversarial sequence mutations against AlphaFold2 on protein tertiary structure prediction. *arXiv* 2305.08929 https://doi.org/10.48550/arXiv.2305.08929
398 Alkhouri, I., Jha, S., Beckus, A., Atia, G., Velasquez, A., Ewetz, R. et al. (2023) On the robustness of alphaFold: a COVID-19 case study. *arXiv* https://doi.org/10.48550/arXiv.2301.04093
399 Abdel-Rehim, A., Orhobor, O., Lou, H., Ni, H. and King, R.D. (2023) Beating the best: improving on AlphaFold2 at protein structure prediction. *arXiv* https://doi.org/10.48550/arXiv.2301.07568
400 Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O. et al. (2021) Highly accurate protein structure prediction with alphaFold. *Nature* **596**, 583–589 https://doi.org/10.1038/s41586-021-03819-2
401 Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O. et al. (2021) Applying and improving AlphaFold at CASP14. *Proteins* **89**, 1711–1721 https://doi.org/10.1002/prot.26257
402 Liu, S., Wu, K. and Chen, C. (2022) The computational models of AlphaFold2 and RoseTTAfold carry protein foldability information. *bioRxiv* https://doi.org/10.1101/2022.01.27.477978
403 Liang, T., Jiang, C., Yuan, J., Othman, Y., Xie, X.Q. and Feng, Z. (2022) Differential performance of RoseTTAFold in antibody modeling. *Brief Bioinform.* **23**, bbac152 https://doi.org/10.1093/bib/bbac152
404 Wang, J., Lisanza, S., Juergens, D., Tischer, D., Watson, J.L., Castro, K.M. et al. (2022) Scaffolding protein functional sites using deep learning. *Science* **377**, 387–394 https://doi.org/10.1126/science.abn2100
405 Watson, J.L., Juergens, D., Bennett, N.R., Trippe, B.L., Yim, J., Eisenach, H.E. et al. (2022) Broadly applicable and accurate protein design by integrating structure prediction networks and diffusion generative models. *bioRxiv* https://doi.org/10.1101/2022.12.09.519842
406 Casadevall, G., Duran, C., Estévez-Gay, M. and Osuna, S. (2022) Estimating conformational heterogeneity of tryptophan synthase with a template-based Alphafold2 approach. *Protein Sci.* **31**, e4426 https://doi.org/10.1002/pro.4426

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