Prion protein—Semisynthetic prion protein (PrP) variants with posttranslational modifications

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Deciphering the pathophysiologic events in prion diseases is challenging, and the role of posttranslational modifications (PTMs) such as glypidation and glycosylation remains elusive due to the lack of homogeneous protein preparations. So far, experimental studies have been limited in directly analyzing the earliest events of the conformational change of cellular prion protein (PrPC) into scrapie prion protein (PrPSc) that further propagates PrPC misfolding and aggregation at the cellular membrane, the initial site of prion infection, and PrP misfolding, by a lack of suitably modified PrP variants. PTMs of PrP, especially attachment of the glycosylphosphatidylinositol (GPI) anchor, have been shown to be crucially involved in the PrPSc formation. To this end, semisynthesis offers a unique possibility to understand PrP behavior invitro and invivo as it provides access to defined site-selectively modified PrP variants. This approach relies on the production and chemoselective linkage of peptide segments, amenable to chemical modifications, with recombinantly produced protein segments. In this article, advances in understanding PrP conversion using semisynthesis as a tool to obtain homogeneous posttranslationally modified PrP will be discussed.

KEYWORDS
glycosylphosphatidylinositol (GPI) anchor, membrane interaction, prion protein (PrP), protein semisynthesis

Abbreviations: aa, amino acid; AD, Alzheimer disease; ADAM, a disintegrin and metalloproteinase; Adgrg6, adhesion G protein–coupled receptor G6; AFM, atomic force microscopy; AL, amyloid light chain; ALS, amyotrophic lateral sclerosis; ATPase, adenosine 5′-triphosphatase; BSE, bovine spongiform encephalopathy; CBP, chitin-binding domain; CCV, clathrin-coated vesicle; Cdc42, cell division control protein 42 homolog; CDP, chronic demyelinating polyneuropathy; CFC, cell-free conversion; CJD, Creutzfeldt-Jakob disease; CNS, central nervous system; cryo-EM, cryo electron microscopy; CWD, chronic wasting disease; DOPC, 1,2-dioleoyl-sn-glycero-3-phosphocholine; DRM, Detergent-resistant membrane; E coli, Escherichia coli; eQuIC, enhanced quaking-induced conversion; EPL, expressed protein ligation; ER, endoplasmic reticulum; ESR, electron spin resonance; FFI, fatal familial insomnia; FTD, frontotemporal dementia; GPI AP, glycosylphosphatidylinositol anchored protein; GSS, Gerstmann-Sträussler-Scheinker syndrome; Hsp, heat shock protein; KD, knockdown; Kd, dissociation constant; LC, light chain; LRP1, low-density lipoprotein receptor-related protein 1; MESNA, sodium 2-mercaptoethanesulfonate; MSA, multiple system atrophy; Mxe, mycobacterium xenopi gyrA; NCL, native chemical ligation; NMR, nuclear magnetic resonance; OR, octapeptide repeats; ORF, open reading frame; PAc, phenacyl; PD, Parkinson disease; PDB, protein data bank; PEG, polyethylene glycol; PI-PLC, phosphatidylinositol-specific phospholipase C; PIRIβ5, parallel β-register intermolecular β-sheet; PK, proteinase K; PMA, protein misfolding cyclic amplification; POPG, 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1′-rac)glycerol (sodium salt); PPO, polyethylene glycol polyamide oligomer; PrP, prion protein; PrPC, cellular prion protein; PrPSc, (PK)-resistant prion protein; PrPSc, scrapie prion protein; PTM, posttranslational modification; Pts, protein trans-splicing; QuIC, quaking-induced conversion; RT-QuIC, real-time quaking-induced conversion; Scn2a, Scapie-infected mouse neuroblastoma cells; ShA, Syrian hamster; SPPS, Solid phase peptide synthesis; 5-QuIC, standard quaking-induced conversion; TEV, tobacco etch virus; THF, thioflavin T; TSEs, transmissible spongiform encephalopathies

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PRION DISEASES

Prion diseases or transmissible spongiform encephalopathies (TSEs) are incurable, neurodegenerative disorders affecting humans and animals. They include scrapie of sheep and goats, bovine spongiform encephalopathy (BSE) of cattle, chronic wasting disease (CWD) of cervids, and several human diseases such as kuru, Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome (GSS), and fatal familial insomnia (FFI). The disease progression is accompanied by the loss of cognitive skills and neuronal dysfunction, and can be of inherited sporadic or iatrogenic origin. The central pathophysiologic event is insomnia (FFI). The disease progression is accompanied by the loss of cognitive skills and neuronal dysfunction and can be of inherited sporadic or iatrogenic origin. The central pathophysiologic event is insomnia (FFI).

High expression levels of PrPC are found in the central nervous system (CNS), but it exists in other cell types and tissues, such as lymphoid organs, as well. Accessing the gene-encoding SHaPrPC, Prnp, entitled its further identification in numerous other species and illustrated a highly conserved sequence. The entire open reading frame (ORF) is contained within a single exon and primarily translates into a protein composed of 254 amino acids (aa). The first 22 aa reflect an N-terminal signal sequence for PrP entering the secretory pathway. Upon its cleavage, glycosylation at asparagine residues and formation of a disulfide bond occur in the endoplasmic reticulum (ER). Lastly, cleavage of the C-terminal signal sequence facilitates the attachment of the glycosylphosphatidylinositol (GPI) anchor, providing mature, posttranslationally modified PrP at the outer leaflet of the cell membrane, typical for glycosylphosphatidylinositol anchored proteins (GPI-APs). Interestingly, PrP can be found in three topologic forms at the ER. Apart from the fully translocated PrP, two transmembranal types occur with the N- or C-terminus facing the ER lumen, denoted as NtmPrP or CtmPrP, respectively. Normally, NtmPrP and CtmPrP only comprise a small portion of PrPC, whereas an excess of CtmPrP induces neurotoxicity. Neuronal cell death is caused in the absence of PrPSc formation, obviously by an aberrant metabolism of PrPSc. PrPSc mislocalization represents another mechanistic possibility for prion toxicity next to the alteration of PrPC-mediated signaling and PrP-derived oligomeric species. First structural studies on PrPSc isolated from brains of SHaS demonstrated a predominantly α-helical content. As these measurements agreed well with subsequent spectroscopic data of recombinant PrP, accessible in larger amounts, it was considered an appropriate surrogate in biochemical experiments, as well as in solving nuclear magnetic resonance (NMR) and crystal structures of PrP.

In more detail, the N-terminal segment consists of a nonapeptide (POQGGGWGQ) followed by four octapeptide (PHGGGWGQ) repeats (OR) with a high affinity for copper, and other diveral cations, adjacent to a charged cluster (CC) or polybasic region (Figure 2). Note-worthy, the configuration of the copper binding region in hPrP (aa 23-231) has been determined combining different experimental methods by using synthetic octapeptide and tetraoctapeptide as well as full-length hPrP. Depending on the concentration of the metal and pH, the OR region is capable to bind up to four copper ions in distinct coordination geometries. Current estimates for dissociation constant (Kd) values vary between the micromolar and femtomolar range. The central hydrophobic domain (HD), comprising of aas 113 to 135, serves as a transmembrane domain and includes a palindromic region (AAGAAAGA, aa 113-120) thought to be important in the PrPC, PrPSc conversion. Within the C-terminal region, three α-helices (aa 144-154, 175-193, and 200-219), with two of them connected by a disulfide bond, and a small antiparallel β-sheet (aa 128-131 and 161-164) are present. As posttranslational modifications (PTMs), a C-terminal GPI anchor linked to serine 231 and two N-linked glycosylation sites at asparagines 181 and 197 exist. PrPC can occur in nonglycosylated, monoglycosylated, and diglycosylated forms. Variations in glycan structures attached to PrP may be differentially distributed depending on the areas of the CNS. Molecular dynamics

PROPERTIES AND STRUCTURES OF THE PRP

2.1 Cellular prion protein

High expression levels of PrPC are found in the central nervous system (CNS), but it exists in other cell types and tissues, such as lymphoid
simulations indicate that the N-linked oligosaccharides located at two helices within the structured region of PrP contribute to its stabilization in generating a negative electrostatic field covering the helical surface,64 thus impacting strain diversity and prion infection.65-68 The C-terminal GPI anchor tethers PrP to the outer leaflet of the plasma membrane.69

It has been postulated that mutations in the Prnp gene facilitate the pathogenic process by destabilizing the tertiary structure of PrPC. More than 30 mutations in Prnp could be linked to inherited prion diseases.70 In affecting the primary sequence of PrP, concomitant changes in its 3D structure may arise, and not cause, but influence a person’s risk of developing a disease. Indeed, thermodynamic measurements of mutated PrP variants indicated destabilizing effects only for some of them.71 For example by comparing the wild-type variant to the E200K mutant almost identical structures resulted, but major perturbations of the surface electrostatic potential were found. This suggests that these defects cause abnormalities in PrP interactions and should be considered as key determinants in the misfolding process.72

Moreover, it has been speculated that methionine oxidation in PrPC plays a destabilizing role and supports spontaneous conversion into PrPSc. Wolschner et al73 found a strong proaggregation behavior for hPrPSc with oxidized methionine residues and a variant with methionine replaced by hydrophilic methoxinine as a stable substitute for oxidation-sensitive methionine. These findings suggest a pivotal role of oxidative stress in PrP conversion.

2.2  Scrapie prion protein

PrPSc is the toxic, misfolded isoform of PrP. It is, as PrPC, encoded by the Prnp gene and exhibits identical PTMs, but distinct structural, biochemical, and physiological features.13 Despite a large interest in elucidating the structure of PrPSc, there are only limited data about its molecular details available.74 To date, obtaining a high-resolution structure of PrPSc has been impeded by its insolubility, propensity to aggregate, and heterogeneity. Structural variations, such as differences in the glycosylation patterns, suggested to correlate with biochemical changes, including the extent of the proteinase K (PK) resistance, the electrophoretic mobility of the proteolytic fragments, and the conformational stability, depend on the distinct strains and complicate the determination of PrPSc structure.75 Besides, in agreement with discussions from the Prion 2018 round tables,76 the diversity of PrP assemblies implicates that there may be no single PrPSc structure. Data generated by biochemical and physical methods, such as spectroscopy analysis, electron microscopy, and limited proteolysis, have led to several 3D structural models. Govaerts and colleagues suggested that left-handed β-helices assembled into trimers, also known as the 4-rung β-solenoid model.77 Based on electron spin resonance (ESR) measurements, Cobb et al proposed a parallel in-register intermolecular β-sheet (PIRIBS) architecture where PrPSc consists of β-strands and short turns and/or loops with no residual α-helices.78 Still, so far, all models display discrepancies with experimental data.79

Notably, cryo electron microscopy (cryo-EM) is a technique providing high-resolution structures.80,81 By rapidly cooling samples, proteins can be observed in their native state. In 2016, Wille and colleagues82 employed cryo-EM to analyze GPI anchorless PrP 27-30 amyloid fibrils. PrP 27-30 was purified from brains of transgenic mice infected with prions. Further inoculation of wt mice with the purified GPI anchorless PrP 27-30 confirmed the development of typical neurological signs of prion disease. The structure of GPI anchorless PrP 27-30 amyloid fibrils was found to agree with a 4-rung β-solenoid architecture; 3D reconstruction revealed two distinct protofilaments and an average molecular height of approximately 17.7 Å. However, Collinge, Wadsworth, and coworkers83 studied the structural features distinguishing infectious
ex vivo mammalian prions from noninfectious fibrillar assemblies generated in vitro. Applying cryo-EM and atomic force microscopy (AFM) measurements noninfectious recombinant PrP fibrils were identified as 10-nm-wide single fibers with a double helical repeating substructure, agreeing with the structure described by Wille and colleagues.\textsuperscript{82} Prion-infected transgenic mice replicate prions, but they mainly develop PrP amyloid plaques, which are not seen in prion-inoculated wt mice.\textsuperscript{84,85} Caughey and coworkers\textsuperscript{86-88} have described two morphologically distinct PrP fibril assemblies in prion-infected transgenic mice. Therefore, considering the lower infectivity titer of PrP 27-30 in the studies of Wille and colleagues,\textsuperscript{82} it appears that the more abundant, single nonglycosylated PrP fibrils, corresponding essentially to recombinant PrP, has been described rather than the infectious glycosylated PrP rods. Collinge, Wadsworth, and coworkers\textsuperscript{83} characterized infectious PrP rods, 20 nm in width, that contained two fibers, each with a double helical repeating substructure separated by a central gap of 8 to 10 nm. The gap between the paired fibers consists of irregularly structured material compositionally distinct to the protein surface. Thus, it was proposed as a location of the N-linked glycans of PrP. The structure of the infectious PrP rods differentiates them from all other protein assemblies so far studied in neurodegenerative diseases. This includes characterizations by cryo-EM of tau filaments from AD\textsuperscript{89} and monoclonal immunoglobulin light chain (LC) fibrils from amyloid light-chain (AL) amyloidosis.\textsuperscript{90} To date, cryo-EM studies of tau and AL represent the only structural data of fibrils directly extracted from human tissue under pathologic conditions. For tau-paired helical and straight filaments could be identified with cores made of two identical protofilaments that adopt a combined cross-β/β-helix structure. AL fibrils were found to be helical with a single protofilament showing a cross-β architecture. It is widely accepted that during the PrP\textsuperscript{C}-PrP\textsuperscript{Sc} conversion, the β-strand content increases vastly\textsuperscript{91,92} and the PK resistance of the "folded core" (aa approximately 90-231), as well.\textsuperscript{93,14} Whereas PrP\textsuperscript{C} is dominated by α-helices, monomeric, soluble, and highly susceptible to proteolytic digestion, PrP\textsuperscript{Sc} contains predominantly β-sheets (≈43%).\textsuperscript{92} aggregates into amyloid fibrils,\textsuperscript{93} is insoluble in detergents and partially resistant to proteolysis.\textsuperscript{35,94} These biochemical differences between the PrP isoforms appear to be associated with the changes of the secondary structure in PrP\textsuperscript{Sc}.

\section{Physiology of the PrP}

\subsection{Function of PrP\textsuperscript{C}}

Although the relevance of PrP\textsuperscript{C} in TSEs is widely accepted, its physiological function remains enigmatic. Studies with PrP knockout mice have failed on this regard. Transgenic mice lacking PrP were found to develop normally.\textsuperscript{95,96} A multitude of functions has been ascribed to PrP in different tissues, cells, and experimental settings, although not always without controversy or questionable reproducibility. Among others, PrP\textsuperscript{C} has been connected to developmental processes,\textsuperscript{97} cell adhesion,\textsuperscript{98,99} neurite outgrowth, synapse formation,\textsuperscript{100-104} neuroprotection,\textsuperscript{105-107} and regulation of the circadian rhythm.\textsuperscript{108} Moreover, there is evidence for PrP contributing to myelin maintenance,\textsuperscript{109-112} cellular homeostasis of divalent cations,\textsuperscript{113-115} and signaling events.\textsuperscript{116-118} A more detailed discussion can be found in reviews by the group of Aguzzi.\textsuperscript{109,119} Recently, other functions have been attributed to PrP\textsuperscript{C}, that is acting as a receptor for the aggre gated proteins Aβ oligomers\textsuperscript{120-122} and α-synuclein.\textsuperscript{123} By mediating the uptake of Aβ and α-synuclein, PrP\textsuperscript{Sc} is unable to replicate in their presence.

Already 10 years ago, it was suspected that reports on the function of PrP represent just specific aspects of a more complex physiological role of PrP\textsuperscript{C}.\textsuperscript{23} Causes for the functional diversity of PrP\textsuperscript{C} might not only be its alternating transient binding partners in different cellular locations but also its proteolytic processing.\textsuperscript{124,125} For once, PrP fragmentation may inhibit association with some binding partners while possibly allowing new interactions with others. Then again, the cleaved products may act as soluble ligands facilitating protein interactions over large distances. These findings contribute to the biological complexity of the physiological function of PrP. In fact, four different but highly conserved cleavage events have been significantly characterized (Figure 2).\textsuperscript{126-128} During transport to the cell surface, α-cleavage results in a soluble flexible N1- and a globular membrane-bound C1 part. Myelin maintenance has been initially linked to this C1 part derived from α-cleavage. Mice expressing PrP mutants not able to undergo α-cleavage suffered from chronic demyelinating polyneuropathy (CDP).\textsuperscript{110,111,119} Interestingly, recent work found a specific ligand role of the flexible N1 part towards the G-protein coupled receptor Adgrg6, promoting myelin homeostasis.\textsuperscript{112} During shedding, PrP\textsuperscript{C} is released from the plasma membrane by a disintegrin and metalloproteinase (ADAM) enzyme, namely, ADAM10, in a glycosylated form without the GPI anchor and designated as "shed PrP"\textsuperscript{120} Although definite functions of "shed PrP" are not known to date, the shedding process regulates the membrane levels of PrP\textsuperscript{C} and thus its functions at the cell surface. Similar to α-synuclein,\textsuperscript{123} recent work by Jarosz-Griffiths et al.\textsuperscript{131} found that the toxicity and cellular binding of Aβ oligomers can be reduced by shedding of PrP\textsuperscript{C}, thereby pointing towards a contribution as a receptor in AD. Moreover, PrP\textsuperscript{C} is expressed in immune cells as well, particularly on mast cells.\textsuperscript{132} Upon activation of these cells, the PrP\textsuperscript{C} shedding process is enhanced, proposing PrP involvement in the inflammatory mast cell response. Under pathological conditions and in response to oxidative stress, incidences of β-cleavage occurring around aa position 90 are increased.\textsuperscript{133,134} Lastly, γ-cleavages restricted to unglycosylated PrP generate a large soluble N3 and a short C3 part by taking place in a region between aas 170 and 200. While prevalence and relevance of this cleavage requires further investigation, increased amounts of C3 in CJD brain samples suggest a pathophysiological role.\textsuperscript{135}

Despite multiple evidence of PrP in physiological processes, the functional diversity based on its manifold binding partners and proteolytic fragments complicate an exact definition of its physiological function. Yet successful elucidation of pathways and roles of PrP could help to understand its linkage to toxicity in prion diseases and to other neurodegenerative diseases.\textsuperscript{136}
3.2 Trafficking of PrP
c
As the PrP function is closely intertwined with the cellular compart-
ments where the protein is located, having a closer look at trafficking
may assist in elucidating its involvement in pathological and physiolog-
ic processes. PrP
c is tethered via its GPI anchor to the outer leaflet
of the plasma membrane.69 In 1993, data by Shyng et al137 revealed
constitutive cycling of PrP
c between the cell surface and endocytic
compartments on varying times scales dependent on the cell line, as
demonstrated in later work.138 From the cellular membrane, PrP
c can
enter the cell via multiple pathways, mediated mainly by the unstruc-
tured N-terminal domain.139,140 Evidence for a cooperation between
clathrin138,141,142 and rafts143,145 in the internalization of PrP
c was
found.146 Clathrin is a large, oligomeric protein assembling into lattice
structures on the inner surface of the plasma membrane. Thereby, it
causes the membrane to invaginate and pinch off to form clathrin-
coated vesicles (CCVs), which can then fuse with other intracellular
organelles.147 Although a clathrin-dependent internalization might
appear unusual since PrP lacks a cytoplasmic domain necessary for the
direct interaction with clathrin and the adaptor protein, GPI APs can
indeed enter the clathrin-dependent pathway upon interaction with
transmembrane proteins possessing a clathrin-coated pit internalization
signal.144 Moreover, the endocytosis of PrP
c was found to be associ-
ated with the low-density lipoprotein receptor-related protein 1
(LRP1)142,148 that belongs to a receptor family of cell-surface
transmembrane proteins capable of binding a variety of ligands and
internalizing via clathrin-coated pits.149,150 As a nonclassical clathrin-
independent pathway, the raft-dependent internalization route
distinguishes caveolae-dependent and caveolae-independent endocy-
tosis.151 Caveolae are membrane invaginations, originating from the
oligomerization of caveolins, their integral coat proteins, and are consid-
ered to be specialized raft domains.152,153 Due to the presence of PrP
c in caveolae-like domains145,155 and its colocalization with caveolin-1
(cav-1),143,156 the involvement of caveolin in PrP
c endocytosis had
been suggested earlier. To this end, Sarnataro et al157 could provide evi-
dence that the raft-mediated pathway is not affected by caveolin
expression. Still, PrP
c internalization was found to be impacted by cho-
lesterol depletion and activation of the cell division control protein 42
homolog (Cdc-42), a member of the Rho family of GTPases being specif-
ically involved in clathrin-independent endocytosis of GPI APs.158 Addi-
tionally Sarnataro et al157 reported that in coimmunoprecipitation
studies of clathrin and PrP
c, the latter remained associated with
detergent-insoluble microdomains. This fact supports a cooperation
between rafts and clathrin in the internalization process. PrP
c suscepti-
bility to various endocytic pathways could also be the basis for its neu-
roprotective and neurodegenerative functions.

4 MECHANISM OF PrP\textsubscript{C}-PrP\textsubscript{Sc} CONVERSION

To date, despite considerable knowledge about the characteristics of
the infective prion pathogen, its mechanism of replication and the
molecular pathways leading to neurodegeneration are largely
unknown. There is evidence from invitro and transgenic mouse studies
that the conversion to PrP\textsubscript{Sc} implicates PrP\textsubscript{C}-PrP\textsubscript{Sc} interactions.84,159-
163 The rate of PrP\textsubscript{Sc} formation and disease progression appears to be
directly proportional to the level of PrP\textsubscript{C} expression, indicated by
PrP knockout mice not propagating scrapie infectivity and transgenic
mice heterozygous for a disrupted PrP gene requiring prolonged incu-
bation times upon prion inoculation.164-166 in agreement with the
“protein-only hypothesis,” these findings have raised two models
explaining prion replication (Figure 3). The template-directed refolding
model by Prusiner167 proposes that a high-energy barrier prevents the
spontaneous PrP\textsubscript{C}–PrP\textsubscript{Sc} conversion. Upon interaction, monomeric
PrP\textsubscript{Sc} induces PrP\textsubscript{C} to convert into PrP\textsubscript{Sc}. However, until now, there
is no experimental evidence for the existence of a stable PrP\textsubscript{Sc} mono-
mer.168 PrP\textsubscript{Sc} seeds in this prion propagation process are not consid-
ered essential. Alternatively, in the more accredited seeded
nucleation model by Jarrett and Lansbury,169 a reversible thermody-
namic equilibrium between PrP\textsubscript{C} and PrP\textsubscript{Sc} is postulated. In the pre-
scible oligomeric PrP\textsubscript{Sc} aggregates, the conversion from PrP\textsubscript{C}
to PrP\textsubscript{Sc} is favored, thus making PrP\textsubscript{Sc} aggregates (seeds) inevitable
for prion spread. Fragmentation of PrP\textsubscript{Sc} aggregates increases the
number of nuclei capable of recruiting further PrP\textsubscript{Sc}. In fact, these sol-
uble oligomers produced during the PrP amyloid aggregation have
emerged as the primary neurotoxic species, supporting the seeded
nucleation model.170-172

Ultimately, evidence for a direct PrP\textsubscript{C}–PrP\textsubscript{Sc} interaction in the con-
version to PrP\textsubscript{Sc} came from invitro systems. Pioneering studies from
Caughey and colleagues173 succeeded within a cell-free conversion
(CFC) assay in the generation of protease-resistant (res), radioactive
PrP\textsubscript{res} from mixed PrP\textsubscript{C} substrate and an excess of unlabeled PrP\textsubscript{Sc}.
This in vitro PrP\textsubscript{res} propagation recapitulates the species and strain
specificity of prion transmission invitro.173,174 Mechanistically, it has
identified structural factors underlying the species barrier and optimal
conditions for the PrP\textsubscript{res} formation.168,175,176 The ability to generate
PrP\textsubscript{res} not only from purified but also recombinant protein177 provides

\textbf{FIGURE 3} Models for the conversion of cellular prion protein (PrP\textsubscript{C})
into scrapie prion protein (PrP\textsubscript{Sc}). The model for template-directed
refolding (top) and seeded nucleation (bottom) are depicted. The
figure was modified from Aguzzi and Calella23
a unique opportunity to study prion propagation. CFC assays can be used as screening experiments as they have the potential to identify compounds directly inhibiting the PrP<sup>C</sup>-PrP<sup>Sc</sup> interaction or its subsequent conversion. Still, the proportionally large amount of PrP<sup>Sc</sup> seeds required to drive the CFC assay (PrP<sup>Sc</sup>-PrP<sup>C</sup> = 50:1) has prevented it from generating de novo infectivity. 

A more efficient method for mimicking the autocatalytic replication of PrP<sup>Sc</sup> was provided by Soto and colleagues in affording a larger than 10-fold increase in PrP<sup>res</sup> with the usage of a 1:100 ratio of PrP<sup>Sc</sup> to PrP<sup>C</sup>. By subjecting scrapie-infected and normal brain homogenate to the so-called protein misfolding cyclic amplification (PMCA) procedure, PrP<sup>res</sup> is amplified in cycles of sonication and incubation. Successive rounds of PMCAs and fragmentation of PrP<sup>Sc</sup> rise the available amounts of replication-competent species. Thus, with automation, this assay offers a promising diagnostic tool in presymptomatic blood screening and eventually, it has facilitated the detection of de novo infectivity in hamsters. However, the levels of infectivity still remain lower than with a similar quantity of brain-derived PrP<sup>Sc</sup>, and the usage of complex brain homogenate itself represents an obstacle in thoroughly elucidating the conversion and association of infectivity with PrP<sup>res</sup>. Besides, the distinct efficiency differences between the CFC and PMCA assays applying purified and crude brain-derived PrP<sup>Sc</sup> proposed that cellular accessory factors are involved in the generation of PrP<sup>res</sup>. In fact, polyanionic molecules were identified as factors present in the brain homogenate that contribute to the conversion efficiency. Ongoing development of PMCA assays aiming to detect and early diagnose TSEs has led to the quaking-induced conversion (QuIC) method. Sonication is replaced by reproducible and easier controllable shaking during the prion amplification process, which enables the application of standardized protocols. This accomplishment is reflected by the multiple variations currently available, such as standard (S-QuIC), real-time (RT-QuIC), and enhanced QuIC (eQuIC).

Apart from the autocatalytic propagation of PrP<sup>Sc</sup>, another crucial hallmark of the PrP<sup>C</sup>-PrP<sup>Sc</sup> conversion is the de novo generation of infectivity. However, when inoculated into animals, PrP fibrillar assemblies can range from being biologically inert to fully infectious, pathogenic, and transmissible in subsequent passages. Legname and coworkers inoculated transgenic mice expressing truncated PrP<sup>C</sup> (aa 89-231) with amyloid fibrils formed from recombinant PrP (aa 89-230). The outcomes were low infectious titers and the affection of only that single line of transgenic mice Tg9949, probably due to the high expression and truncation of the transgene sequence enhancing the susceptibility to prion infection within the mice. Hence, according to Supattapone, these highly concentrated samples of PrP amyloid fibrils are not suitable in mimicking the infectious properties of PrP<sup>Sc</sup>. In contrast, Wang et al succeeded in the formation of infectious de novo recombinant PrP amyloid fibrils associated with neurological signs in wild-type mice after approximately 130 days. Here, PrP<sup>res</sup> was formed in PMCA assays in the presence of negatively charged lipids, namely, 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1′-rac-glycerol) (sodium salt) (POPG). In their earlier work, they had shown that POPG promotes the conversion to PrP<sup>res</sup> under physiological conditions. In further studies, Wang et al. could attribute a crucial role in the PrP-lipid interaction to the highly conserved middle region of PrP that induced conformational change.

### 4.1 PrP<sup>C</sup>-PrP<sup>Sc</sup> conversion in cells

The findings regarding PrP<sup>res</sup> formation in the presence of POPG support the possibility of the plasma membrane being the cellular localization of PrP<sup>Sc</sup> formation as a posttranslational event. At this position, contact between endogenous PrP<sup>C</sup> and exogenous PrP<sup>Sc</sup> can easily occur. This is supported by the finding that by releasing PrP<sup>C</sup> from the cell surface or interrupting its transport to the plasma membrane prevents the formation of PrP<sup>Sc</sup>. More precisely, both PrP isoforms were found to be associated with rafts. These are defined as highly dynamic microdomains wherein specific lipids stabilize larger lipid platforms and compartmentalize cellular processes at the membrane. Impairing the integrity of the cholesterol-enriched rafts associated with PrP by lowering the intracellular levels of cholesterol reduced the formation of PrP<sup>Sc</sup> in infected cells. Moreover, PrP<sup>C</sup>- and PrP<sup>Sc</sup>-associated rafts were found to have distinct characteristics, as they can be separated from each other by solubilization and flotation on density gradients. According to Campana et al., this proposes that either the types of raft or the membrane association of each isoform has different characteristics. However, Baron et al. illustrated that the PrP<sup>Sc</sup>-PrP<sup>C</sup> conversion only takes place in the presence of fused PrP<sup>Sc</sup>- and PrP<sup>C</sup>-containing membranes, suggesting that the two PrP isoforms need to be inserted into contiguous membranes. Alternatively, rafts were proposed to stabilize PrP in its conformation via a direct interaction with cholesterol. Thus, changes in the local lipid environment can mediate PrP conformation. Studies on model lipid bilayers regarding the impact of the PrP-lipid interaction on structure and affinity of PrP support the idea that predominantly α-helical PrP<sup>C</sup> is stabilized upon binding to raft membranes, whereas binding to negatively charged lipid (nonraft) membranes leads to an increased β-sheet content. Interestingly, the PrP-raft association is mediated by the GPI anchor and the N-terminal region of PrP. Unlike for a typical GPI-anchored protein, for PrP, this raft association occurs already earlier in the secretory pathway and appears to be involved in the maturation and folding process of PrP<sup>C</sup>. Alternatively to the plasma membrane, the formation of PrP<sup>Sc</sup> was suggested to involve additional cellular places. Immediately after PrP internalization, the PrP<sup>C</sup>-PrP<sup>Sc</sup> conversion may occur in the endolysosomal compartment, in the Golgi apparatus and/or the ER following retrograde transport. In infected cells, stimulation of retrograde transport towards the ER leads to an increase in PrP<sup>Sc</sup> formation from PrP<sup>C</sup> precursor, suggesting that the ER may represent an amplification compartment for PrP<sup>Sc</sup>. Participation of the endocytic pathway is indicated by PrP<sup>Sc</sup> accumulation in the late endosomes. Still, as demonstrated by Goold et al., the plasma membrane is the initial site of prion conversion.
and consequently of most interest in studying the earliest events in prion infection and PrP misfolding.

4.2 Impact of GPI anchor on PrP<sub>C</sub>-PrP<sub>Sc</sub> conversion

Typically, PrP is attached to membranes by its GPI anchor (Figure 6A). A better understanding of the interplay between membranes, GPI-anchored PrP, and PrP<sub>C</sub>-PrP<sub>Sc</sub> conversion is provided by work from Baron and Caughey. First, they studied the conditions necessary for PrP<sub>Sc</sub> formation of PrP associated with detergent-resistant membranes (DRMs). Based on that, in CFC assays, Baron and Caughey investigated the impact of GPI-anchoring of PrP associated with model membranes on PrP<sub>Sc</sub> formation. PrP was isolated by immunoprecipitation from mammalian cell lines expressing GPI-anchored and anchorless PrP, respectively. GPI-anchored PrP bound to liposomes could not be converted to PrP<sub>Sc</sub> upon exposure to exogenous PrP<sub>Sc</sub> in microsomes until phosphatidylinositol-specific phospholipase C (PI-PLC) was added or the combined membrane fractions were treated with a membrane-fusing agent. These findings indicate for the initiation and propagation of PrP<sub>Sc</sub> that at the membrane surface, an insertion of PrP<sub>Sc</sub> into the host cell membrane is necessary for the conversion. Whereas if the conversion occurred extracellularly, PrP<sub>C</sub> needed to be released from the cell membrane. In contrast, anchorless PrP bound to liposomes was converted to PrP<sub>Sc</sub> without any treatments necessary. Hence, contradictory to PrP conversion occurring at the cellular membrane, only the membrane-associated form containing PrP attached to a GPI anchor could resist the conversion induced by exogenous PrP<sub>Sc</sub>. Moreover, Chesebro et al. found that anchorless PrP results in infectious amyloid disease but without typical clinical TSE. Scrapie infection of transgenic mice lacking GPI-anchored PrP<sub>C</sub> leads to a formation of amyloid plaques in contrast to nonamyloid deposits, typically observed in wild-type mice. Although neuropathological lesions were induced, clinical manifestations were minimal. Surprisingly, the combined expression of anchorless and wild-type PrP accelerated the onset of clinical disease. This suggests that GPI-anchored PrP may be critically involved in the pathogenesis of prion diseases. Overall, the findings mentioned above indicate a major contribution of the GPI anchor in the toxicity of the PrP<sub>C</sub>-PrP<sub>Sc</sub> conversion.

5 TOWARDS THE ELUCIDATION OF PrP CONVERSION

Recombinant PrP is an appropriate surrogate for PrP<sub>C</sub>, as determined by spectroscopic measurements, including circular dichroism (CD), that eventually facilitated solving the NMR and crystal structures of PrP. However, it can be a suitable representative only under certain conditions, including thioflavin T (ThT) fluorescence-based following of the aggregation process. Even though with this method, insights into the characteristics and kinetics of in vitro fibril formation have been gained, just recently, the molecular basis of PrP replication was established in detail by applying a single-molecule fluorescence methodology to characterize individual aggregates. With total internal reflection fluorescence (TIRF) microscopy, Klenerman and colleagues studied fibril fragmentation and elongation of individual murine PrP aggregates from seeded aggregation invitro. PK-resistant PrP fibrils elongated until length-dependent fragmentation resulted in PK-sensitive fragments. This method allowed direct observation of heterogeneous, transient, metastable oligomers during aggregation, found to be the most infectious PrP particles. Additionally, a spreading model for aggregate propagation through the brain could be predicted, and a framework was established to start determining the main factors that control the rate of prion spreading in animals. In 2011, Goold et al. analyzed a PrP knockdown (KD) neuroblastoma cell line expressing epitope-tagged PrP<sub>C</sub> upon infection with exogenous PrP<sub>Sc</sub>. After facing the limitation of immunological differentiation between PrP<sub>Sc</sub> and PrP<sub>C</sub> expressed on the recipient cell from cell lines susceptible to prion infection, epitope-tagged PrP<sub>C</sub> appeared an elegant solution. However, several previous attempts had failed in generating PrP molecules capable of prion conversion, probably due to the sequence sensitivity in this process, particularly in certain key regions of the PrP molecule. Eventually, out of eight different constructs, Goold et al. succeeded with a PrP-224AlaMYC construct, in which the tag is inserted within the C-terminal domain. A detailed analysis of the cells shortly after prion exposure demonstrated that PrP<sub>Sc</sub> is formed on the plasma membrane. Furthermore, PI-PLC treatment effectively removed PrP<sub>C</sub> from the plasma membrane of PrP-224AlaMYC cells and reduced the generation of PrP<sub>Sc</sub>. However, immunostaining is only feasible on fixed cells and impedes dynamic studies revealing molecular details involved in the PrP conversion and propagation processes. ThT-based detection of preformed PrP amyloid fibrils applied in a cellular environment cannot exclude ThT binding to other structures unrelated to amyloid and guarantees binding to all aggregates, as the binding mechanism is not fully understood yet.

To this end, labeling of PrP with an organic fluorophore is required for dynamic studies in cells. Recombinant PrP differs compared with PrP<sub>C</sub> in a complete lack of PTMs causing distinct infectivity and membrane interaction characteristics. These properties can be mainly ascribed to the GPI anchor, tethering PrP to the cellular membrane, as demonstrated in various studies. Advances in semisynthetic strategies based on solid-phase peptide synthesis (SPPS), protein engineering, native chemical ligation (NCL), and expressed protein ligation (EPL) have facilitated access to homogeneous membrane-anchored labeled PrP variants that allow to directly observe the biophysical properties of PrP upon interaction with the cellular membrane.

6 SEMISYNTHESIS STRATEGIES FOR PrP

To date, the majority of studies on the function and structure of PrP have been carried out with recombinant protein lacking all PTMs, including the GPI anchor, or with heterogeneous protein preparations isolated from mammalian cell lines. Still, there have been attempts towards generating defined membrane-anchored PrP.
of PrP, as the lipids were attached via disulfide bonds. Different strategies were utilized by Baskakov, Baldwin, and Moroder with coworkers: Baskakov and colleagues applied maleimide chemistry to introduce a myristoyl chain at the C-terminus of genetically engineered PrP(S230C). This modification did not alter the structure of the protein. Interestingly, an increasing affinity of PrP for the cell membrane and a decreased extent of fibrillation was found. Baldwin and coworkers chemically synthesized several PrP segments, including a 106 residue “mini-prion” (PrP106) by connecting PrP (aa 90-141) to PrP (aa 178-231) via a native peptide bond using NCL, a selective reaction that links an unprotected peptide containing a C-terminal α-thioester to another peptide with an N-terminal cysteine. A membrane anchor made of a lipophilic myristoyl chain was introduced at the C-terminus of shorter PrP peptides via an orthogonally side-chain-protected lysine. Immunofluorescence analysis indicated that only myristylated PrP peptides could be targeted to the cell surface. The group of Moroder applied click and ligation chemistry to obtain lipidated peptides corresponding to the C-terminal PrP segment (aa 214-231). Confocal images of HeLa cells revealed a direct transfer of fluorescently labeled lipopeptides to the cellular membrane. Thus, lipopeptides can be used as mimics of the GPI anchor’s ability to attach PrP to the cell membrane. A similar strategy with regard to using a lipidated peptide as a GPI-mimicking membrane anchor was pursued by Becker et al., when starting to develop semisynthetic strategies, based on EPL and protein trans-splicing (PTS) (Figure 4), to access to different posttranslationally modified PrP variants (Figure 5).

In the EPL reaction, a protein thioester, obtained by cleaving a fusion protein consisting of the protein of interest (POI) and an intein, is linked to a chemically synthesized peptide containing an N-terminal cysteine in a reaction similar to NCL. An initial transesterification leads to formation of a thioester linking the recombinant and synthetic PrP segments, and a subsequent irreversible S → N acyl shift establishes the amide bond at the ligation site (Figure 4A). The recombinant protein α-thioesters can be accessed using engineered inteins. Inteins are self-processing protein segments, which mediate protein splicing. In the course of this intramolecular process, the intein excises itself and joins the C- and N-terminal flanking protein segments (C- and N-extein). In more detail, a nucleophilic side chain, namely, a hydroxy or thiol group for serine and threonine or cysteine residues, accomplishes an N → O or N → S acyl shift. Then, in a trans-(thio)esterification, the N-extein gets attached to a conserved N-terminal serine or cysteine of the C-extein. The instable branched intermediate is resolved via an intramolecular rearrangement involving a conserved asparagine residue of the intein producing an intein with a C-terminal succinimide, and an O → N or S → N acyl shift resulting in ligated exteins with a native bond at the ligation site. Mutations of the C-terminal asparagine of the intein and the N-terminal cysteine, threonine, or serine residue of the C-extein to alanine block the splicing process and only allow the initial N → S acyl shift, which enables the generation of a protein α-thioester by addition of an excess of a thiol, such as sodium 2-mercaptoethanesulfonate (MESNA), to trap the protein thioester. PTS is a process that relies on the assembly of two divided segments of inteins, so-called split inteins, to form a functional intein. Upon assembly of the split inteins, PTS occurs and links the N- and C-exteins in a similar sequence of events as described above (Figure 4B).

The generation and biophysical characterization of PrP constructs containing a GPI anchor mimic started more than 10 years ago in the Becker laboratory with work described in Olschewski et al. Two strategies based on the EPL approach provided PrP\textsubscript{Palm}, an N-terminally truncated PrP variant (T_\textsubscript{PrP} [aa 90-231]) that is missing the unfolded N-terminal domain (aa 23-89) and modified at the C-terminus with chemically synthesized membrane anchor peptides (Figure 5). At that time, the protease-resistant PrP fragment comprising residues aa 90-231 had been considered as the structure crucially involved in TSEs. The GPI anchor mimics (Figure 6C) feature two palmitoyl modifications (Palm) that induce a high affinity towards DOPC liposomes and locate PrP in its native conformation to the detergent-resistant domains (DRMs) of cell membranes. A tobacco etch virus (TEV) protease recognition site (ENLYFQ) facilitates controlled release of PrP from the membrane, a polyethylene glycol (PEG) polyamide oligomer (PPO) functions as solubilization tag to handle the palmitoylated peptides in aqueous buffers, and a fluorescent dye can be incorporated for tracking of the semisynthetic PrP\textit{invivo} and invivo (Figure 6C).

One of the initial EPL-based strategies relies on the expression of PrP in fusion with the Mxe GyRa intein and a combination of two affinity tags, namely, a His tag and a chitin-binding domain (CBD) in Escherichia coli (E coli). Cleavage of this construct is achieved with an excess of thiol to generate PrP with a C-terminal thioester. This PrP-thioester is incubated with the GPI anchor-mimicking peptides and gives the C-terminally modified PrP (denoted as PrP\textsubscript{Palm}, Figure 5A). A second strategy is based on PTS by expressing PrP fused to the N-terminal segment of the DnaE split intein (DnaE\textsubscript{C}, 36 aa) linked to the GPI anchor-mimicking peptides by a prior NCL reaction. Both DnaE segments spontaneously associate when folded and form a functional intein, which excises itself to give the desired modified PrP with its C-terminal membrane anchor (Figure 5B). Aggregation assays based on PK resistance and ThT binding revealed an extended lag time for vesicle-attached PrP\textsubscript{Palm} with respect to conversion into PrP\textsubscript{res} and fibril formation than for PrP in control experiments. Further, binding to zwitterionic DOPC liposomes indicated a very strong membrane interaction for PrP\textsubscript{Palm} in contrast to PrP Transfert of PrP\textsubscript{Palm} onto neuronal cells gave rise to similar patterns observed for native PrP\textsubscript{C} by immunostaining. Together with extraction experiments of the cell membrane, this provided proof that soluble PrP\textsubscript{Palm} is attached to detergent-resistant domains (DRMs) similar to wild-type PrP\textsubscript{C} with a native GPI anchor.
Next, Becker et al. developed a synthetic strategy for the preparation of PrP with a native, homogeneous GPI anchor that can also be applied for other GPI-anchored proteins. A challenge lies here in the chemical diversity of GPI anchors on the same protein. Different glycoforms of native PrP GPI anchors have been reported with the exact linkage positions and anomeric configuration of the oligosaccharide branches not defined. At the same time, details about the lipids attached to these GPI anchors are not fully clear (Figure 6A).

In view of this structural uncertainty, a core GPI pseudopentasaccharide, containing three mannose (Man), a glucoseamine (GlcN), and an inositol (Ino) glycan connected in an $\alpha$-Man$(1 \rightarrow 2)\alpha$-Man$(1 \rightarrow 6)\alpha$-Man$(1 \rightarrow 4)\alpha$-GlcN$(1 \rightarrow 6)$-myo-Ino way, was selected. The incorporation of a cysteine residue on the 2-aminoethyl phosphate moiety of the GPI backbone prior to global deprotection provided a synthetic, cysteine-tagged GPI anchor suitable for NCL reactions (Figure 6B). In a following EPL reaction, PrP with a C-terminal thioester was linked to this synthetic GPI anchor. Analysis of the secondary structure of PrP attached to the synthetic GPI revealed that the CD curves are indistinguishable from the spectra of PrP and comparable with the spectra of PrP$_{\text{Palm}}$. Moreover, the CD spectra were found to agree with the spectra of PrP$_{\text{Palm}}$. This observation confirms the successful application of the GPI anchor-mimicking peptides (Figure 6C) as an alternative to circumvent the elaborate synthesis of a GPI anchor (Figure 6B). Even though the synthesis of the GPI anchor succeeded, it remains a challenge to provide sufficient amounts for subsequent experiments and extension to other proteins. Isolating mostly homogeneous, cysteine-carrying GPI anchors from natural sources could help to avoid this problem, and first steps have been made towards this goal by using yeast as an expression system for GPI-anchored proteins, from which the GPI anchor is proteolytically released and purified. GPI-anchored PrP was also found to quantitatively bind to DOPC vesicles. This emphasizes the contribution of GPI anchors in the membrane association of PrP. Noteworthy, the group of Silva et al. is also working on intein-based semisynthesis schemes to obtain homogeneous GPI-anchored proteins, including PrP, using synthetic GPI anchors.

A major limitation of obtaining semisynthetic PrP variants by EPL lies in the series of denaturation and renaturation steps required to obtain functional PrP-intein fusion constructs due to expression into inclusion bodies in E. coli. The subsequent folding steps required for PrP$_{\text{Palm}}$ and GPI-anchored PrP also limits the overall yield of EPL reactions. Deposition in inclusion bodies in E. coli is probably due to misprocessing of newly generated PrP and the overproduction that impedes proper folding, including the formation of the structurally important disulfide bridge. Hence, to improve the semisynthetic access to posttranslationally modified PrP Chu and Becker developed a strategy for soluble expression of PrP-intein constructs in E. coli. Ultimately, the overexpression of a PrP-intein construct N-terminally fused to the ATPase domain of heat shock protein (Hsp) 70 DnaK chaperone gave high quantities of soluble PrP. This approach offers an alternative way to produce PrP-thioester for subsequent EPL reactions but also requires an additional step for removing the N-terminal ATPase domain by using sortase A.
With robust semisynthetic strategies established, the critical membrane attachment of PrP was studied by Chu et al. using three PrP variants, including full-length FL_PrP (aa 23-231), central hydrophobic region deleted ΔCR_PrP (aa 23-231 with Δ105-125) and N-terminally truncated T_PrP (aa 90-231), all equipped with a C-terminal membrane anchor. Interactions of the lipidated PrP constructs with phospholipid membranes demonstrated binding modes distinct from the nonmodified PrPs and impacts on the biochemical and conformational properties of PrP. Whereas nonmodified PrPs showed a conversion into β-sheet–enriched structures upon interaction with anionic POPG vesicles, lipidated ΔCR_PrP and T_PrP retained their α-helical structure and lipidated FL_PrP partially converted into random coil. Evidence indicating pore formation of lipidated ΔCR_PrP was found in fluorescence-based assays and supported by patch clamp electrophysiological measurements of cells transfected with lipidated ΔCR_PrP. ΔCR_PrP was previously found to be neurotoxic in vivo. Yet, expressed in cultured cells, it is identically localized as wild-type PrP. Thus, altered binding interactions had been suggested to cause the deleterious signaling pathways. Based on these results, critical roles for both C-terminal

**FIGURE 5** Semisynthetic strategies for prion protein (PrP) variants, developed in the Becker laboratory. Via an expressed protein ligation (EPL)- (A) and a protein trans-splicing (PTS)-based (B) approach, PrP variants equipped with a glycosylphosphatidylinositol (GPI) anchor mimic can be obtained. With the strategy displayed in C, PrP variants modified with monodisperse PEG chains as mimics of N-glycans can be accessed. Selective desulfurization of the introduced cysteine following EPL (A) is depicted in D.
membrane attachment and the N-terminal domain of PrP have been suggested.

PTMs in PrP comprise not only a C-terminal GPI anchor but also N-glycosylation of two asparagine residues at positions 181 and 197. Different prion strains and prion-related diseases (TSEs) possess distinct glycosylation patterns of PrP. Studying the influence of these PTMs in prion pathogenesis has not been forthcoming mainly due to the confusing complexity and heterogeneity of these glycans. Shi et al reported a strategy based on linking three segments of murine PrP, in which a recombinant PrP segment (aa 90-177S) was ligated with two synthetic peptide segments (aa 178-212 and aa 213-230). This strategy was aimed at introduction sugars into PrP but did not fully succeed. Shortly after that, Araman et al demonstrated a semisynthetic approach to generate PrP variants modified with monodisperse PEG chains as mimics of N-glycans that are similar in size and molar mass (Figure 5C). A new EPL strategy was established to achieve this, in which expressed PrP (aa 23-177) with a C-terminal thioester is used in EPL reactions with PEGylated, synthetic peptides (aa 179-231). Selective desulfurization of the β-mercapto-aspartate at the ligation site gives homogeneous PEGylated full-length PrP constructs. Interestingly, in vitro aggregation was completely abrogated for all PEGylated PrP constructs under conditions at which wild-type PrP aggregated. Furthermore, the addition of only 10% of PEGylated PrP completely blocked aggregation of wild-type PrP. This has raised the question if large N-glycans interfere with aggregation in vivo. Recently, Mishra et al introduced lactosyl and mannobiosyl glycans in huPrP (aa 90-231) at positions 181 and 197 via Asn to Cys mutations. In agreement with our results, they found that glycosylated PrPs are less prone to spontaneous fibril nucleation. Such a strategy raises the question if added cysteine residues influence PrP structure by disulfide shuffling and if this affects the modification reaction used by Mishra et al. A similar question can arise from the cysteine residues introduced during the EPL reactions described above as in our previous approach depicted in Figure 5A. The introduced ligation site cysteine at the C-terminus of T_PrP (aa 90-231) was left undesulfurized, which could potentially be problematic for the folding of PrP. To finally prove that such an additional C-terminal cysteine residue does not influence PrP folding, we employed a strategy recently introduced by Matveenko et al, in which the two native cysteines in recombinant PrP (aa 23-231) are protected by a phenacyl (PAC) protecting group. This protection allowed selective desulfurization of the introduced cysteine following EPL (Figure 5D). Comparing PrP variants containing a
cysteine at the ligation site and an alanine (by CD) proved that the introduced cysteine did not disturb the folding to native PrP.

7 | CONCLUSION AND OUTLOOK

Based on the continuous progress in protein (semi)synthesis, access to homogeneous, posttranslationally modified PrP variants was facilitated over the past decade and a set of differently modified variants could be characterized with respect to their biophysical and conformational properties, including their interaction with membranes. Semisynthetic PrP variants have the potential to shed light on the crucial steps in PrP conversion, transmission, and pathogenicity, eg., by allowing for direct observation of the protein at the cellular membrane. Understanding these key features in prion diseases can further serve as paradigm for other neurodegenerative diseases.

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REFERENCES

1. Weissmann C. Molecular genetics of transmissible spongiform encephalopathies. J. Biol. Chem. 1999;274(1):3-6.
2. Kovačs GG, Trabattoni G, Hainfellner JA, Ironside JW, Knight RSG, Budka H. Mutations of the Prion Protein Gene. J. Neurov. 2002;249 (11):1567-1582. https://doi.org/10.1007/s00415-002-0896-9
3. Wadsworth JD, Collinge J. Molecular pathology of human prion disease. Acta Neuropathol. 2011;121(1):69-77. https://doi.org/10.1007/s00401-010-0735-5
4. Aguzzi A, Weissmann C. Prion diseases. Haemophilia. 1998;4 (4):619-627.
5. Gajdusek DC, Gibbs CJ, Alpers M. Experimental transmission of a kuru-like syndrome to chimpanzees. Nature. 1966;209(5025):794-796.
6. Alper T, Cramp WA, Haig DA, Clarke MC. Does the agent of scrapie replicate without nucleic acid? Nature. 1967;214(5090):764-766.
7. Griffith JS. Self-replication and scrapie. Nature. 1967;215 (5105):1043-1044.
8. Prusiner SB. Novel proteinaceous infectious particles cause scrapie. Science. 1982;216(4542):136-144.
9. Prusiner SB. Nobel Lecture: Prions. Proc. Natl. Acad. Sci. U. S. A. 1998;95 (23):13363-13383. https://doi.org/10.1073/pnas.95.23.13363
10. Bolton DC, McKinley MP, Prusiner SB. Identification of a protein that purifies with the scrapie prion. Science. 1982;218(4579):1309-1311.
11. Prusiner SB, Bolton DC, Groth DF, Bowman KA, Cochran SP, McKinley MP. Further purification and characterization of scrapie prions. Biochemistry. 1982;21(26):6942-6950.
12. Prusiner SB, Groth DF, Bolton DC, Kent SB, Hood LE. Purification and structural studies of a major scrapie prion protein. Cell. 1984;38 (1):127-134.
13. Basler K, Oesch B, Scott M, et al. Scrapie and cellular PrP isoforms are encoded by the same chromosomal gene. Cell. 1986;46(3):417-428. https://doi.org/10.1016/0092-8674(86)90662-8
14. Oesch B, Westaway D, Walchli M, et al. A cellular gene encodes scrapie PrP 27-30 protein. Cell. 1985;40(4):735-746.
15. Meyer RK, McKinley MP, Bowman KA, et al. Separation and properties of cellular and scrapie prion proteins. Proc. Natl. Acad. Sci. U. S. A. 1986;83(8):2310-2314. https://doi.org/10.1073/pnas.83.8.2310
16. Locht C, Chesebro B, Race R, Keith JM. Molecular cloning and complete sequence of prion protein cDNA from mouse brain infected with the scrapie agent. Proc. Natl. Acad. Sci. U. S. A. 1986;83(17):6372-6376.
17. Prusiner SB. Prions and neurodegenerative diseases. N. Engl. J. Med. 1987;317(25):1571-1581. https://doi.org/10.1056/NEJM198712173172505
18. Leighton PL, Allison WT. Protein misfolding in prion and prion-like diseases: reconsidering a required role for protein loss-of-function. J. Alzheimers Dis. 2016;54(1):3-29. https://doi.org/10.3233/jad-160361
19. Verma A. Prions, prion-like prionoids, and neurodegenerative disorders. Ann. Indian Acad. Neurol. 2016;19(2):169-174. https://doi.org/10.4103/0972-2327.179979
20. Aguzzi A, Lakkaraju AK. Cell biology of prions and prionoids: a status report. Trends Cell Biol. 2016;26(1):40-51. https://doi.org/10.1016/j.tcb.2015.08.007
21. Prusiner SB, Woerman AL, Mordes DA, et al. Evidence for a α-synuclein prions causing multiple system atrophy in humans with parkinsonism. Proc. Natl. Acad. Sci. U. S. A. 2015;112(38):E5308-E5317. https://doi.org/10.1073/pnas.1514475112
22. Jaunmuktane Z, Mead S, Ellis M, et al. Evidence for human transmission of amyloid-β pathology and cerebral amyloid angiopathy. Nature. 2015;525(7568):247-250. https://doi.org/10.1038/nature15369
23. Aguzzi A, Calella AM. Prions: protein aggregation and infectious diseases. Physiol. Rev. 2009;89(4):1105-1152. https://doi.org/10.1152/physrev.00006.2009
24. Kretzschmar HA, Prusiner SB, Stowing LE, DeArmond SJ. Scraie prion proteins are synthesized in neurons. Am. J. Pathol. 1986;122 (1):1-5.
25. Linden R, Martins VR, Prado MA, et al. Physiology of the prion protein. Physiol. Rev. 2008;88(2):673-728. https://doi.org/10.1152/physrev.00007.2007
26. Hermes J, Tings T, Gall S, et al. Evidence of presynaptic location and function of the prion protein. J. Neurosci. 1999;19(20):8866-8875.
27. Chesebro B, Race R, Wehrly K, et al. Identification of scrapie prion protein-specific mRNA in scrapie-infected and uninfected brain. Nature. 1985;315(6017):331-333.
28. Schatzl HM, Da Costa M, Taylor L, Cohen FE, Prusiner SB. Prion protein gene variation among primates. J. Mol. Biol. 1995;245(4):362-374.
29. Wopfner F, Weidenhofer G, Schneider R, et al. Analysis of 27 mammalian and 9 avian PrPs reveals high conservation of flexible regions of the prion protein. J. Mol. Biol. 1999;289(5):1163-1178. https://doi.org/10.1006/jmbi.1999.2831
30. Puckett C, Concannon P, Casey C, Hood L. Genomic structure of the human prion protein gene. Am. J. Hum. Genet. 1991;49(2):320-329.
31. Kretzschmar HA, Stowing LE, Westaway D, et al. Molecular cloning of a human prion protein cDNA. DNA. 1986;5(4):315-324.
32. Tatzelt J, Winklhofer KF. Folding and misfolding of the prion protein in neurodegenerative disease. Trends Cell Biol. 2009;19(4):133-144.
33. Hegde RS, Mastrianni JA, Scott MR, et al. A transmembrane form of the prion protein in neurodegenerative disease. Science. 1998;279 (5352):827-834.
34. Hegde RS, Tremblay P, Groth D, DeArmond SJ, Prusiner SB, Lingappa VR. Transmissible and genetic prion diseases share a common
pathway of neurodegeneration. Nature. 1999;402(6763):822-826. https://doi.org/10.1038/45574

35. Pan KM, Baldwin M, Nguyen J, et al. Conversion of alpha-helices into beta-sheets features in the formation of the scrapie prion proteins. Proc. Natl. Acad. Sci. U. S. A. 1993;90(23):10962-10966. https://doi.org/10.1073/pnas.90.23.10962

36. Timmes AG, Moore RA, Fischer ER, Priola SA. Recombinant prion protein refolded with lipid and RNA has the biochemical hallmarks of a prion but lacks in vivo infectivity. PLoS One. 2013;8(7):e71081. https://doi.org/10.1371/journal.pone.0071081

37. Wang F, Wang X, Orrú CD, et al. Self-propagating, protease-resistant, recombinant prion protein conformers with or without in vivo pathogenicity. PLoS Pathog. 2017;13(7):e1006491. https://doi.org/10.1371/journal.ppat.1006491

38. Castilla J, Saa P, Hetz C, Soto C. In vitro generation of infectious scrapie prions. Cell. 2005;121(2):195-206. https://doi.org/10.1016/j.cell.2005.02.011

39. Wang F, Wang X, Yuan CG, Ma J. Generating a prion with bacterially expressed recombinant prion protein. Science. 2010;327(5969):1132-1135. https://doi.org/10.1126/science.1183748

40. Homemann S, Korth C, Oesch B, et al. Recombinant full-length murine prion protein, mPrP(23-231): purification and spectroscopic characterization. FEBS Lett. 1997;413(2):277-281.

41. Riek R, Homemann S, Wider G, Billiter M, Glogshuber R, Wüthrich K. NMR structure of the mouse prion protein domain PrP(121-231). Nature. 1996;382(6587):180-182. https://doi.org/10.1038/382180a0

42. Calzolai L, Lysek DA, Perez DR, Guntert P, Wuthrich K. Prion protein NMR structures of chickens, turtles, and frogs. Proc. Natl. Acad. Sci. U. S. A. 2005;102(3):651-655. https://doi.org/10.1073/pnas.0408939102

43. Donne DG, Viles JH, Groth D, et al. Structure of the recombinant full-length hamster prion protein PrP(29-231): the N terminus is highly flexible. Proc. Natl. Acad. Sci. U. S. A. 1997;94(25):13452-13457. https://doi.org/10.1073/pnas.94.25.13452

44. James TL, Liu H, Ulyanov NB, et al. Solution structure of a 142-residue recombinant prion protein corresponding to the infectious fragment of the scrapie isoform. Proc. Natl. Acad. Sci. U. S. A. 1997;94(19):10086-10091.

45. Lysek DA, Schorn C, Nivon LG, et al. Prion protein NMR structures of cats, dogs, pigs, and sheep. Proc. Natl. Acad. Sci. U. S. A. 2005;102(3):640-645. https://doi.org/10.1073/pnas.0408937102

46. Lopez Garcia F, Zahn R, Riek R, Wuthrich K. NMR structure of the bovine prion protein. Proc. Natl. Acad. Sci. U. S. A. 2000;97(15):8334-8339. https://doi.org/10.1073/pnas.97.15.8334

47. Zahn R, Liu A, Luhrs T, et al. NMR solution structure of the human prion protein. Proc. Natl. Acad. Sci. U. S. A. 2000;97(1):145-150.

48. Stöckel J, Safar J, Wallace AC, Cohen FE, Prusiner SB. Prion protein selectively binds copper (II) ions. Biochemistry. 1998;37(20):7185-7193. https://doi.org/10.1021/bi972827k

49. Villes JH, Cohen FE, Prusiner SB, Goodin DB, Wright PE, Dyson HJ. Copper binding to the prion protein: structural implications of four identical cooperative binding sites. Proc. Natl. Acad. Sci. U. S. A. 1999;96(5):2042-2047.

50. Walter ED, Chattopadhyay M, Millhauser GL. The affinity of copper binding to the prion protein octarepeat domain: evidence for negative cooperativity. Biochemistry. 2006;45(43):13083-13092. https://doi.org/10.1021/bi060948r

51. Miura T, Sasaki S, Toyama A, Takeuchi H. Copper reduction by the octapeptide repeat region of prion protein: pH dependence and implications in cellular copper uptake. Biochemistry. 2005;44(24):8712-8720. https://doi.org/10.1021/bi0501784

52. Walter ED, Stevens DJ, Visconte MP, Millhauser GL. The prion protein is a combined zinc and copper binding protein: Zn²⁺ alters the distribution of Cu²⁺ coordination modes. J. Am. Chem. Soc. 2007;129(50):15440-15441. https://doi.org/10.1021/ja071146j

53. Renner C, Fiori S, Fiorino F, et al. Micellar environments induce structuring of the N-terminal tail of the prion protein. Biopolymers. 2004;73(4):421-433. https://doi.org/10.1002/bip.20015

54. Mentler M, Weiss A, Granter K, et al. A new method to determine the structure of the metal environment in metalloproteins: investigation of the prion protein octapeptide repeat Cu²⁺ complex. European biophysics journal: EBj. 2005;34(2):97-112. https://doi.org/10.1007/s00249-004-0434-z

55. del Pino P, Weiss A, Bertsch U, et al. The configuration of the Cu²⁺ binding region in full-length human prion protein. European biophysics journal: EBj. 2007;36(3):239-252. https://doi.org/10.1007/s00249-006-0124-0

56. Weiss A, Del Pino P, Bertsch U, et al. The configuration of the Cu²⁺ binding region in full-length human prion protein compared with the isolated octapeptide. Veterinary microbiology. 2007;123(4):358-366. https://doi.org/10.1016/j.vetmic.2007.04.008

57. Burns CS, Aronoff-Spencer E, Legname G, et al. Copper coordination in the full-length, recombinant prion protein. Biochemistry. 2003;42(22):6794-6803. https://doi.org/10.1021/bi207139+.

58. Acevedo-Morantes CY, Wille H. The Structure of Human Prions: From Biology to Structural Models—Considerations and Pitfalls. Viruses. 2014;6(10):3875-3892. https://doi.org/10.3390/v6103875

59. Norstrom EM, Mastriani JA. The AGAAAAGA palindrome in PrP is required to generate a productive PrPSc-PrP complex that leads to prion propagation. J. Biol. Chem. 2005;280(29):27236-27243. https://doi.org/10.1074/jbc.M413441200

60. Maiti NR, Surewicz WK. The role of disulfide bridge in the folding and stability of the recombinant human prion protein. Biochemistry. 2000;39(22):6794-6803. https://doi.org/10.1021/bi9901784

61. 10.10.959

62. 10.10.959

63. 10.10.959

64. 10.10.959

65. 10.10.959

66. 10.10.959

67. Tuzi NL, Cancellotti E, Baybutt H, et al. Host PrP glycosylation: a pathway of neurodegeneration. Nature. 2003;421(6902):672-677. https://doi.org/10.1038/nature01441

68. 10.10.959

69. 10.10.959

70. 10.10.959

71. 10.10.959

72. 10.10.959

73. 10.10.959

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81. 10.10.959

82. 10.10.959

83. 10.10.959

84. 10.10.959

85. 10.10.959

86. 10.10.959

87. 10.10.959
68. Baskakov IV, Katorcha E, Makarava N. Prion strain-specific structure and pathology: a view from the perspective of glycobiology. Viruses. 2018;10(12). https://doi.org/10.3390/v10120723

69. Stahl N, Borchelt DR, Hsiao K, Prusiner SB. Scrapie prion protein contains a phosphatidylinositol glycolipid. Cell. 1987;51(2):229-240. https://doi.org/10.1016/0092-8674(87)90150-4

70. Collinge J. Prion diseases of humans and animals: their causes and molecular basis. Annu. Rev. Neurosci. 2001;24(1):519-550. https://doi.org/10.1146/annurev.neuro.24.1.519

71. Swietnicki W, Petersen RB, Gambetti P, Surewicz WK. Familial mutations and the thermodynamic stability of the recombinant human prion protein. J. Biol. Chem. 1998;273(47):31048-31052.

72. Zhang Y, Swietnicki W, Zagorski MG, Surewicz WK, Sonnichsen FD. Solution structure of the E200K variant of human prion protein. Implications for the mechanism of pathogenesis in familial prion diseases. J. Biol. Chem. 2000;275(43):33650-33654. https://doi.org/10.1074/jbc.C000483200

73. Wolschner C, Giese A, Kretzschmar HA, Huber R, Moroder L, Budisa N. Design of anti- and pro-aggregation variants to assess the effects of methionine oxidation in human prion protein. Proc. Natl. Acad. Sci. U. S. A. 2009;106(19):7756-7761. https://doi.org/10.1073/pnas.0902688106

74. Díaz-Espinoza R, Soto C. High-resolution structure of infectious prion protein: the final frontier. Nat. Struct. Mol. Biol. 2012;19(4):370-377.

75. Colby DW, Prusiner SB. De novo generation of prion strains. Nat. Rev. Microbiol. 2011;9(11):771. https://doi.org/10.1038/nrmicro2650

76. Baskakov IV, Caughey B, Requena JR, Sevillano AM, Surewicz WK, Wille H. The prion 2018 round tables (I): the architecture of human prion protein amyloid: a parallel, in-register β-sheet architecture of the scrapie prion protein (PrP) amyloids. J. Biol. Chem. 2014;289(35):24129-24142. https://doi.org/10.1074/jbc.M114.578344

77. Groveman BR, Dolan MA, Taubner LM, Kraus A, Wickner RB, Caughey B. Parallel in-register intermolecular β-sheet architectures for prion-seeded prion protein (PrP) amyloids. J. Biol. Chem. 2009;284(35):36700-36709. https://doi.org/10.1074/jbc.M109.073619

78. Vázquez Fernández E, Vos MR, Afanasyev P, et al. The structural architecture of an infectious mammalian prion using electron cryomicroscopy. J. Mol. Biol. 2016;12(9):e1005835. https://doi.org/10.1038/s41467-019-09133-w

79. Halliez S, Passet B, Martin-Lannereau S, et al. To develop with or without the prion protein. Front. Cell Dev. Biol. 2014;12(5):e1005835. https://doi.org/10.1038/s41598-018-36700-w

80. Chesebro B, Traylro M, Race R, et al. Anchorless prion protein results in infectious amyloid disease without clinical scrapie. Science. 2005;308(5727):1435-1439. https://doi.org/10.1126/science.1110837

81. Amin L, Nguyen XTA, Rolle IG, et al. Characterization of prion protein function by focal neurite stimulation. J. Cell Sci. 2016;129(20):3878-3891. https://doi.org/10.1242/jcs.183137

82. Santuccione A, Sytnyk V, Leshchyns’ka I, Schachner M. Prion protein recruits its neuronal receptor NCAM to lipid rafts to activate p59fyn lack of prion protein membrane anchoring. PLoS Pathog. 2010;6(3):e1000800. https://doi.org/10.1371/journal.ppat.1000800
and to enhance neurite outgrowth. J. Cell Biol. 2005;169(2):341-354. https://doi.org/10.1083/jcb.200409127

103. Kanaani J, Prusiner SB, Diacono J, Baekkeskov S, Legname G. Recombinant prion protein induces rapid polarization and development of synapses in embryonic rat hippocampal neurons in vitro. J. Neurochem. 2005;95(5):1373-1386. https://doi.org/10.1111/j.1471-4159.2005.03469.x

104. Martins VR, Reraldo FH, Hajj GN, et al. Prion protein: orchestrating cellular prion protein mediates impairment of synaptic plasticity by amyloid-β oligomers. Nature. 2009;457(7233):1128-1132. https://doi.org/10.1038/nature07761

105. Martins VR, Beraldo FH, Hajj GN, et al. Prion protein—does proteolytic processing hold the key? Biochim. Biophys. Acta Mol. Cell. Res. 2017;1864(11, Part B):2128-2137. https://doi.org/10.1016/j.bbamcr.2017.06.022

106. Bounhar Y, Zhang Y, Goodyer CG, LeBlanc A. Prion protein protects human neurons against Bax-mediated apoptosis. J. Biol. Chem. 2001;276(42):39145-39149. https://doi.org/10.1074/jbc.C100432000

107. Rooucou X, Gains M, LeBlanc AC. Neuroprotective functions of prion protein. J. Neurosci. Res. 2004;75(2):153-161. https://doi.org/10.1002/jnr.10864

108. Tobler I, Gaus SE, Deboer T, et al. Altered circadian activity rhythms and sleep in mice devoid of prion protein. Nature. 1996;380 (6575):639-642. https://doi.org/10.1038/380639a0

109. Nuvolone M, Hermann M, Sorce S, et al. Strictly co-isogenic C57BL/6J–PrpC–/– mice: a rigorous resource for prion science. J. Exp. Med. 2016;213(3):313-327. https://doi.org/10.1084/jem.20151610

110. Baumann F, Tlonay M, Brabec K, et al. Lethal recessive myelin toxicity of prion protein lacking its central domain. EMBO J. 2007;26 (2):538-547. https://doi.org/10.1038/sj.emboj.7601510

111. Bremer J, Baumann F, Tiberi C, et al. Axonal prion protein is required for peripheral myelin maintenance. Nat. Neurosci. 2010;13 (3):310-318. https://doi.org/10.1038/nn.2483

112. Küffer A, Lakkaraju AKK, Mogha A, et al. The prion protein is an agonistic ligand of the G protein-coupled receptor AdgrG6. Nature. 2016;536(7617):464-468. https://doi.org/10.1038/nature19312

113. Brown DR, Qin K, Herms JW, et al. The cellular prion protein binds copper in vivo. Nature. 1997;390(6661):684-687. https://doi.org/10.1038/37783

114. Watt NT, Taylor DR, Kerrigan TL, et al. Prion protein facilitates uptake of zinc into neuronal cells. Nat. Commun. 2012;3(1):1134. https://doi.org/10.1038/ncomms2135

115. Haigh CL, Marom SY, Collins SJ. Copper, endoproteolytic processing of the prion protein and cell signalling. Front. Biosci. Landmark Ed. 2010;15(1):1086-1104.

116. Mouillet-Richard S, Emronval M, Chebassier C, et al. Signal transduction through prion protein. Science. 2000;289(5486):1925-1928. https://doi.org/10.1126/science.289.5486.1925

117. Chiarini LB, Freitas ARO, Zanata SM, Brentani RR, Martins VR, Linden R. Cellular prion protein transduces neuroprotective signals. EMBO J. 2002;21(13):3317-3326. https://doi.org/10.1093/emboj/cdf324

118. Spielmüller C, Schatzl HM. PrPC directly interacts with proteins involved in signaling pathways. J. Biol. Chem. 2001;276 (48):44604-44612. https://doi.org/10.1074/jbc.M103289200

119. Wulf MA, Senatore A, Aguzzi A. The biological function of the cellular prion protein: an update. BMC Biol. 2017;15(1):34. https://doi.org/10.1186/s12915-017-0375-5

120. Lauren J, Gimbel DA, Nygaard HB, Gilbert JW, Strittmatter SM. Cellular prion protein mediates impairment of synaptic plasticity by endoproteolytic processing event. Cell. Mol. Life Sci. 2016;73 (3):667-683. https://doi.org/10.1007/s00018-015-2022-z
136. Linden R. The Biological Function of the Prion Protein: A Cell Surface Scaffold of Sorting Modules. Front. Mol. Neurosci. 2017;10:77. https://doi.org/10.3389/fnmol.2017.00077

137. Shyng SL, Huber MT, Harris DA. A prion protein cycles between the cell surface and an endocytic compartment in cultured neuroblastoma cells. J. Biol. Chem. 1993;268(21):15922-15928.

138. Sunyach C, Jen A, Deng J, et al. The mechanism of internalization of glycosphosphatidylinositol-anchored prion protein. EMBO J. 2003;22(14):3591-3601. https://doi.org/10.1093/embojd/cdg344

139. Shyng SL, Moulder KL, Lesko A, Harris DA. The N-terminal domain of a glycolipid-anchored prion protein is essential for its endocytosis via clathrin-coated pits. J. Biol. Chem. 1995;270(24):14793-14800.

140. Nunziante M, Gilch S, Schatzl HM. Essential role of the prion protein N terminus in subcellular trafficking and half-life of cellular prion protein. J. Biol. Chem. 2003;278(6):3726-3734. https://doi.org/10.1074/jbc.M206313200

141. Shyng SL, Heuser JE, Harris DA. A glycolipid-anchored prion protein is endocytosed via clathrin-coated pits. J. Cell Biol. 1994;125(6):1239-1250.

142. Taylor DR, Hooper NM. The low-density lipoprotein receptor-related protein 1 (LRP1) mediates the endocytosis of the cellular prion protein. Biochim. J. 2007;402(1):17-23. https://doi.org/10.1042/bj20061736

143. Peters PJ, Mironov A, Perez D, et al. Trafficking of prion proteins through a caveola-mediated endosomal pathway. J. Cell Biol. 2003;162(4):703-717. https://doi.org/10.1083/jcb.200304140

144. Mayor S, Riezman H. Sorting GPI-anchored proteins. Nat. Rev. Mol. Cell Biol. 2004;5(2):110-120. https://doi.org/10.1038/nrm1309

145. Galvan C, Camoletto PG, Dotti CG, Aguzzi A, Ledesma MD. Proper axonal distribution of PrPC depends on cholesterol-spHINGomyelin-enriched membrane domains and is developmentally regulated in hippocampal neurons. Mol. Cell. Neurosci. 2005;30(3):304-315. https://doi.org/10.1016/j.mcn.2005.07.003

146. Samataro D, Caputo A, Casanova P, et al. Lipid rafts and clathrin cooperate in the internalization of PrP in epithelial FRT cells. PLoS One. 2009;4(6):e5829. https://doi.org/10.1371/journal.pone.0005829

147. Schmid SL. Clathrin-coated vesicle formation and protein sorting: an integrated process. Annu. Rev. Biochem. 1997;66(1):511-548. https://doi.org/10.1146/annurev.biochem.66.1.511

148. Parkyn CJ, Vermeulen EGM, Moootoonsy RC, et al. LRP1 controls biosynthetic and endocytic trafficking of neuronal prion protein. J. Cell Sci. 2008;121(6):773-783. https://doi.org/10.1242/jcs.021816

149. Willnow TE. The low-density lipoprotein receptor gene family: multiple roles in lipid metabolism. J. Mol. Med. 1999;77(3):306-315.

150. Strickland DK, Gonias SL, Argraves WS. Diverse roles for the LDL receptor family. Trends Endocrinol. Metab. 2002;13(2):66-74.

151. Parton RG, Richards AA. Lipid rafts and caveolae as portals for endocytosis: new insights and common mechanisms. Traffic. 2003;4(11):724-738.

152. Parton RG, Simons K. The multiple faces of caveolae. Nat. Rev. Mol. Cell Biol. 2007;8(3):185-194. https://doi.org/10.1038/nrm2122

153. Parton RG, Joggerst B, Simons K. Regulated internalization of caveolae. J. Cell Biol. 1994;127(5):1199-1215.

154. Vey M, Pilkuhn S, Wille H, et al. Subcellular colocalization of the cellular and scarpie prion proteins in caveola-like membranous domains. Proc. Natl. Acad. Sci. U. S. A. 1996;93(25):14945-14949.

155. Kaneko K, Vey M, Scott M, Pilkuhn S, Cohen FE, Prusiner SB. COOH-terminal sequence of the cellular prion protein directs subcellular trafficking and controls conversion into the scrapie isoform. Proc. Natl. Acad. Sci. U. S. A. 1997;94(6):2333-2338. https://doi.org/10.1073/pnas.94.6.2333

156. Marella M, Lehmann S, Grassi J, Chabry J. Filipin prevents pathological prion protein accumulation by reducing endocytosis and inducing cellular PrP release. J. Biol. Chem. 2002;277(28):25457-25464. https://doi.org/10.1074/jbc.M203248200

157. Samataro D, Caputo A, Casanova P, et al. Lipid rafts and clathrin cooperate in the internalization of PrP in epithelial FRT cells. PLoS One. 2009;4(6):e5829. https://doi.org/10.1371/journal.pone.0005829

158. Sabharanjak S, Sharma P, Parton RG, Mayor S. GPI-anchored proteins are delivered to recycling endosomes via a distinct cdc42-regulated, clathrin-independent pinocytic pathway. Dev. Cell. 2002;2(4):411-423.

159. Kim JL, Surewicz K, Gambetti P, Surewicz WK. The role of glycosphosphatidylinositol anchor in the amplification of the scrapie isoform of prion protein in vitro. FEBS Lett. 2009;583(22):3671-3675. https://doi.org/10.1016/j.febslet.2009.10.049

160. Priola SA, McNally KL. The role of the prion protein membrane anchor in prion infection. Prion. 2009;3(3):134-138.

161. Saborio GP, Permanne B, Soto C. Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding. Nature. 2001;411(6839):810-813.

162. Prusiner SB, Scott M, Foster D, et al. Transgenetic studies implicate interactions between homologous PrP isoforms in scrapie prion replication. Cell. 1990;63(4):673-686.

163. Meier P, Genoud N, Prinz M, et al. Soluble dimeric prion protein binds PrPSc in vivo and antagonizes prion disease. Cell. 2003;113(1):49-60.

164. Sailer A, Bueler H, Fischer M, Aguzzi A, Weissmann C. No propagation of prions in mice devoid of PrP. Cell. 1994;77(7):967-968.

165. Bueler H, Raebel A, Sailer A, et al. High prion and PrPSc levels but delayed onset of disease in scrapie-inoculated mice heterozygous for a disrupted PrP gene. Mol. Med. 1994;11(1):19-30.

166. Manson JC, Clarke AR, McBride PA, McConnell I, Hope J. PrP gene dosage determines the timing but not the final intensity or distribution of lesions in scrapie pathology. Neurodegeneration. 1994;3(4):331-340.

167. Prusiner SB. Molecular biology of prion diseases. Science. 1991;252(5012):1515-1522.

168. Zhou Z, Xiao G. Conformational conversion of prion protein in prion diseases. Acta Biochim. Biophys. Sin. 2013;45(6):465-476. https://doi.org/10.1093/abbs/gmt027

169. Jarrett JT, Lansbury PT Jr. Seeding one-dimensional crystallization” of amyloid: A pathogenic mechanism in Alzheimer’s disease and scrapie? Cell. 1993;73(6):1055-1058.

170. Combet S, Cousin F, Rezaei H, Noinville S. Membrane interaction of off-pathway prion oligomers and lipid-induced on-pathway intermediates during prion conversion: A clue for neurotoxicity. Biochem. Biophys. Acta Biomembr. 2018;1861(2):514-523. https://doi.org/10.1016/j.bbamed.2018.12.001

171. Sandberg MK, Al-Doujaily H, Sharps B, Clarke AR, Collinge J. Prion propagation and toxicity in vivo occur in two distinct mechanistic phases. Nature. 2011;470(7335):540-542. https://doi.org/10.1038/nature09768

172. Sandberg MK, Al-Doujaily H, Sharps B, et al. Prion neuropathology follows the accumulation of alternate prion protein isoforms after infective titre has peaked. Nat. Commun. 2014;5(1):4347. https://doi.org/10.1038/ncomms5347

173. Kocisko DA, Come JH, Priola SA, et al. Cell-free formation of protease-resistant prion protein. Nature. 1994;370(6489):471-474. https://doi.org/10.1038/370471a0
174. Bessen RA, Kocisko DA, Raymond GJ, Nandan S, Lansbury PT, Caughey B. Non-genetic propagation of strain-specific properties of scrapie prion protein. Nature. 1995;375(6533):698-700. https://doi.org/10.1038/375698a0

175. Priola SA, Chabry J, Chan K. Efficient conversion of normal prion protein (PrP) by abnormal hamster PrP is determined by homology at amino acid residue 155. J. Virol. 2001;75(10):4673-4680. https://doi.org/10.1128/jvi.75.10.4673-4680.2001

176. Horiiuchi M, Caughey B. Specific binding of normal prion protein to the scrapie form via a localized domain initiates its conversion to the protease-resistant state. EMBO J. 1999;18(12):3193-3203. https://doi.org/10.1093/emboj/18.12.3193

177. Kirby L, Birkett CR, Rudyk H, Gilbert IH, Hope J. In vitro cell-free conversion of bacterial recombinant PrP to PrPRes as a model for conversion. J. Gen. Virol. 2003;84(PT 4):1013-1020. https://doi.org/10.1099/vir.0.18903-0

178. Caughey WS, Raymond LD, Horiiuchi M, Caughey B. Inhibition of protease-resistant prion protein formation by porphyrins and phthalocyanines. Proc. Natl. Acad. Sci. U. S. A. 1998;95(21):12117-12122.

179. Maxson L, Wong C, Herrmann LM, Caughey B, Baron GS. A solid-phase assay for identification of modulators of prion protein interactions. Anal. Biochem. 2003;323(1):54-64.

180. Hill AF, Antoniou M, Collinge J. Protease-resistant prion protein produced in vitro lacks detectable infectivity. J. Gen. Virol. 1999;80 (1):11-14.

181. Castilla J, Gonzalez-Romero D, Saa P, et al. Crossing the Species Barrier by PrPSc Replication In Vivo Generates Unique Infectious Prions. Cell. 2008;134(5):757-768. https://doi.org/10.1016/j.cell.2008.07.030

182. Castilla J, Morales A, Saa P, et al. Cell-free propagation of prion strains. EMBO J. 2008;27(19):2557-2566. https://doi.org/10.1038/emboj.2008.181

183. Green KM, Castilla J, Seward TS, et al. Accelerated high fidelity prion amplification within and across prion species barriers. PLoS Pathog. 2008;4(8):e1000139. https://doi.org/10.1371/journal.ppat.1000139

184. Caughey WS, Raymond LD, Horiiuchi M, Caughey B. Inhibiting the formation of protease-resistant prion protein by porphyrins and phthalocyanines. Proc. Natl. Acad. Sci. U. S. A. 1998;95(21):12117-12122.

185. Maxson L, Wong C, Herrmann LM, Caughey B, Baron GS. A solid-phase assay for identification of modulators of prion protein interactions. Anal. Biochem. 2003;323(1):54-64.

186. Hill AF, Antoniou M, Collinge J. Protease-resistant prion protein produced in vitro lacks detectable infectivity. J. Gen. Virol. 1999;80 (1):11-14.

187. Castilla J, Gonzalez-Romero D, Saa P, et al. Crossing the Species Barrier by PrPSc Replication In Vivo Generates Unique Infectious Prions. Cell. 2008;134(5):757-768. https://doi.org/10.1016/j.cell.2008.07.030

188. Castilla J, Morales A, Saa P, et al. Cell-free propagation of prion strains. EMBO J. 2008;27(19):2557-2566. https://doi.org/10.1038/emboj.2008.181

189. Atarashi R, Sano K, Satoh K, Nishida N. Real-time quaking-induced conversion: a highly sensitive assay for prion detection. Prion. 2011;5(3):150-153. https://doi.org/10.1016/j.prion.2011.05.001

190. Wilham JM, Orru CD, Bessen RA, et al. Rapid end-point quantitation of prion seeding activity with sensitivity comparable to bioassays. PLoS Pathog. 2010;6(12):e1001217. https://doi.org/10.1371/journal.ppat.1001217
prion protein and its scrapie isoform. J. Biol. Chem. 1997;272(10):6324-6331.

209. Baron GS, Wehrly K, Dorward DW, Chesebro B, Caughey B. Conversion of raft associated prion protein to the protease-resistant state requires insertion of PrP-res (PrP Sc) into contiguous membranes. EMBO J. 2002;21(5):1031-1040. https://doi.org/10.1093/emboj/21.5.1031

210. Baron GS, Caughey B. Effect of glycosylphosphatidylinositol anchor-dependent and -independent prion protein association with model raft membranes on conversion to the protease-resistant isoform. J. Biol. Chem. 2003;278(17):14883-14892. https://doi.org/10.1074/jbc.M210840200

211. Taraboulos A, Scott M, Semenov A, et al. Cholesterol depletion and modification of COOH-terminal targeting sequence of the prion protein inhibit formation of the scrapie isoform. J. Cell Biol. 1995;129(1):121-132.

212. Critchley P, Kazlauskaite J, Eason R, Pinheiro TJ. Binding of prion proteins to lipid membranes. Biochem. Biophys. Res. Commun. 2004;313(3):559-567.

213. Sanghera N, Pinheiro TJ. Binding of prion protein to lipid membranes and implications for prion conversion. J. Mol. Biol. 2002;315(5):1241-1256. https://doi.org/10.1006/jmbi.2001.5322

214. Kaneo K, Vey M, Scott M, Pilkuhn S, Cohen FE, Prusiner SB. COOH-terminal sequence of the cellular prion protein directs subcellular trafficking and controls conversion into the scrapie isoform. Proc. Natl. Acad. Sci. U. S. A. 1997;94(6):2333-2338.

215. Walmsley AR, Zeng F, Hooper NM. The N-terminal region of the prion protein ectodomain contains a lipid raft targeting determinant. J. Biol. Chem. 2003;278(39):37241-37248. https://doi.org/10.1074/jbc.M302036200

216. Samataro D, Campana V, Paladino S, Stornaiuolo M, Nitsch L, Zurzolo C. PrP-res association with lipid rafts in the early secretory pathway stabilizes its cellular differentiation. Mol. Biol. Cell. 2004;15(9):4031-4042. https://doi.org/10.1091/mbc.e03-05-0271

217. Samataro D, Paladino S, Campana V, Grassi J, Nitsch L, Zurzolo C. PrP-Sc association is sorted to the basolateral membrane of epithelial cells independently of its association with rafts. Traffic. 2002;3(11):810-821.

218. Harris DA. Trafficking, turnover and membrane topology of PrP: protein function in prion disease. Br. Med. Bull. 2003;66(1):71-85. https://doi.org/10.1093/bmb/66.1.71

219. Zavodszky E, Hegde RS. Misfolded GPI-anchored proteins are escorted through the secretory pathway by ER-derived factors. eLife. 2019;8. https://doi.org/10.7554/eLife.46740

220. Beranger F, Mange A, Goud B, Lehmann S. Stimulation of PrP(2-50) Translocation to the Endoplasmic Reticulum Increases Accumulation of PrP(Sc) In Prion-infected Cells. J. Biol. Chem. 2002;277(41):38972-38977. https://doi.org/10.1074/jbc.M205110200

221. Hegde RS, Rane NS. Prion protein trafficking and the development of neurodegeneration. Trends Neurosci. 2003;26(7):337-339. https://doi.org/10.1016/S0166-2236(03)00143-7

222. Arnold JE, Tipler C, Laszló L, Hope J, Landon M, Mayer RJ. The abnormal isoform of the prion protein accumulates in late-endosom-like organelles in scrapie-infected mouse brain. J. Pathol. 1995;176(4):403-411. https://doi.org/10.1002/path.1711760412

223. McKinley MP, Taraboulos A, Kenaga L, et al. Ultrastructural localization of scrapie prion proteins in cytoplasmic vesicles of infected cultured cells. Lab. Invest. 1991;65(6):622-630.

224. Paulick MG, Bertozzi CR. The glycosylphosphatidylinositol anchor: a complex membrane-anchoring structure for proteins. Biochemistry. 2008;47(27):6991-7000. https://doi.org/10.1021/bi8006324

225. Tsai Y-H, Gotze S, Vilotijevic I, et al. A general and convergent synthesis of diverse glycosylphosphatidylinositol glycolipids. Chem. Sci. 2013;4(1):468-481. https://doi.org/10.1039/C2SC21515B

226. Olschewski D, Seidel R, Miesbauer M, et al. Semisynthetic murine prion protein equipped with a GPI anchor mimic incorporates into cellular membranes. Chem. Biol. 2007;14(9):994-1006. https://doi.org/10.1016/j.chembiol.2007.08.007

227. Chesebro B, Wehrly K, Caughey B, Nishio J, Ernst D, Race R. Foreign PrP expression and scrapie infection in tissue culture cell lines. Dev. Biol. Stand. 1993;80:131-140.

228. Lawson VA, Priola SA, Wehrly K, Chesebro B. N-terminal truncation of prion protein affects both formation and conformation of abnormal protease-resistant prion protein generated in vitro. J. Biol. Chem. 2001;276(38):35265-35271. https://doi.org/10.1074/jbc.M103799200

229. Caughey B, Baron GS. Prions and their partners in crime. Nature. 2006;443(7113):803-810. https://doi.org/10.1038/nature05294

230. Kundel F, Tosatto L, Whiten DR, Wirthssohn DC, Horrocks MH, Klennerman D. Shedding light on aberrant interactions - a review of modern tools for studying protein aggregates. FEBS J. 2018;285(19):3604-3630. https://doi.org/10.1111/febs.14409

231. Sang JC, Meid G, Thackray AM, et al. Direct observation of murine prion protein replication in vitro. J. Am. Chem. Soc. 2018;140(44):14789-14798. https://doi.org/10.1021/jacs.8b08311

232. Sang JC, Lee J-E, Dear AJ, et al. Direct observation of prion protein oligomer formation reveals an aggregation mechanism with multiple conformationally distinct species. Chem. Sci. 2019;10(17):4588-4597. https://doi.org/10.1039/C8SC05627G

233. Silveira JR, Raymond GJ, Hughson AG, et al. The most infectious prion protein particles. Nature. 2005;437(7056):257-261. https://doi.org/10.1038/nature03989

234. Rutschauser D, Metz KD, Moos R, et al. The comprehensive native interactome of a fully functional tagged prion protein. PLoS One. 2009;4(2):e4446. https://doi.org/10.1371/journal.pone.0004446

235. Barnada SJ, Harris DA. Visualization of prion infection in transgenic mice expressing green fluorescent protein-tagged prion protein. J. Neurolsci. 2005;25(24):5824-5832. https://doi.org/10.1523/jneurosci.1192-05.2005

236. Moore RA, Vorberg I, Priola SA. Species barriers in prion diseases—brief review. Arch. Virol. Suppl. 2005;19:187-202.

237. Caughey B. Interactions between prion protein isoforms: the kiss of death? Trends Biochem. Sci. 2001;26(4):235-242.

238. Nally KL, Ward AE, Priola SA. Cells expressing anchorageless prion protein are resistant to scrapie infection. J. Virol. 2009;83(9):4469-4475. https://doi.org/10.1128/jvi.02412-08

239. Chu NK, Shabbir W, Bove-Fenderson E, et al. A C-terminal membrane anchor affects the interactions of prion proteins with lipid membranes. J. Biol. Chem. 2014;289(43):30144-30160. https://doi.org/10.1074/jbc.M114.587345

240. Charco JM, Erana H, Venegas V, et al. Recombinant PrP and its contribution to research on transmissible spongiform encephalopathies. Pathogens. 2017;6(4). https://doi.org/10.3390/pathogens6040067

241. Merrifield RB. Solid Phase Peptide Synthesis. I. The synthesis of a tetrapeptide. J. Am. Chem. Soc. 1963;85(14):2149-2154.
244. Dawson PE, Muir TW, Clark-Lewis I, Kent SB. Synthesis of proteins by native chemical ligation. Science. 1994;266(5186):776-779.

245. Muir TW, Sondhi D, Cole PA. Expressed protein ligation: a general method for protein engineering. Proc. Natl. Acad. Sci. U. S. A. 1998;95(12):6705-6710.

246. Evans TC Jr, Benner J, Xu MQ. The in vitro ligation of bacterially expressed proteins using an intein from Methanobacterium thermoautotrophicum. J. Biol. Chem. 1999;274(7):3923-3926.

247. Gorodinsky A, Harris DA. Glycolipid-anchored proteins in neuroblastoma cells form detergent-resistant complexes without caveolin. J. Cell Biol. 1995;129(3):619-627.

248. Aguzzi A, Heppner FL. Pathogenesis of prion diseases: a progress report. Cell Death Differ. 2000;7(10):889-902. https://doi.org/10.1038/sj.jcd.4400737

249. Weissmann C. Molecular biology of prion diseases. Trends Cell Biol. 1994;4(1):10-14.

250. Eberl H, Tittmann P, Glockshuber R. Characterization of Recombinant. Membrane-attached Full-length Prion Protein. J. Biol. Chem. 2004;279(24):25056-25065. https://doi.org/10.1074/jbc.M400952200

251. Hicks MR, Gill AC, Bath IK, et al. Synthesis and structural characterization of a mimetic membrane-anchored prion protein. FEBS J. 2006;273(6):1285-1299. https://doi.org/10.1111/j.1742-4658.2006.05152.x

252. Breydo L, Sun Y, Makarava N, et al. Nonpolar substitution at the C-terminus of the prion protein, a mimic of the glycosylphosphatidylinositol anchor, impair amyloid fibril formation. Biochemistry. 2007;46(3):852-861. https://doi.org/10.1021/bi061923v

253. Ball HL, King DS, Cohen FE, Prusiner SB, Baldwin MA. Engineering the prion protein using chemical synthesis. J. Pept. Res. 2001;58(5):357-374.

254. Musiol H-J, Dong S, Kaiser M, et al. Toward semisynthetic lipoproteins by convergent strategies based on click and ligation chemistry. ChemBioChem. 2005;6(4):625-628. https://doi.org/10.1002/cbic.200400351

255. Araman C, Thompson RE, Wang S, Hackl S, Payne RJ, Becker CFW. Semisynthetic prion protein (PrP) variants carrying glycan mimics at the C-terminus of the prion protein, a mimic of the glycosylphosphatidylinositol anchor. J. Pept. Sci. 2008;14(9):6626-6632. https://doi.org/10.1002/jps.21719

256. Matveenko M, Hackl S, Becker CFW. Utility of the phenacyl protecting group in traceless protein semisynthesis via ligation-desulfurization chemistry. ChemistryOpen. 2018;7(1):106-110. https://doi.org/10.1002/open.201700180

257. Becker CF, Liu X, Olschewski D, et al. Semisynthesis of a glycosylphosphatidylinositol anchored prion protein. Angew. Chem. Int. Ed. Engl. 2008;47(43):8215-8219.

258. Olschewski D, Becker CF. Chemical synthesis and semisynthesis of membrane proteins. Mol. Biosyst. 2008;4(7):733-740. https://doi.org/10.1039/b803248c

259. Chu NK, Becker CF. Semisynthesis of membrane-attached prion proteins. Methods Enzymol. 2009;462:177-193. https://doi.org/10.1016/s0076-6879(07)62009-7

260. Chu NK, Olschewski D, Seidel R, et al. Protein immobilization on liposomes and lipid-coated nanoparticles by protein trans-splicing. J. Pept. Sci. 2010;16(10):582-588. https://doi.org/10.1002/jps.1227

261. Chu NK, Becker CF. Recombinant expression of soluble murine prion protein for C-terminal modification. FEBS Lett. 2013;587(5):430-435. https://doi.org/10.1016/j.febslet.2012.12.026

262. Volkman G, Mootz H. Recent progress in in situ research: from mechanism to directed evolution and applications. Cell. Mol. Life Sci. 2013;70(7):1185-1206. https://doi.org/10.1007/s00018-012-1120-4

263. Shah NH, Muir TW. Inteins: nature’s gift to protein chemists. Chem. Sci. 2014;5(2):446-461. https://doi.org/10.1039/C3SC52951G

264. Xu MQ, Perler FB. The mechanism of protein splicing and its modulation by mutation. EMBO J. 1996;15(19):5146-5153.

265. Wu H, Hu Z, Liu XQ. Protein trans-splicing by a split intein encoded in a split DnaE gene of Synechocystis sp. PCC6803. Proc. Natl. Acad. Sci. U. S. A. 1998;95(16):9226-9231.

266. Zacharias DA, Violin JD, Newton AC, Tsien RK. Partitioning of lipid-modified monomeric GFPs into membrane microdomains of live cells. Science. 2002;296(5569):913-916. https://doi.org/10.1126.science.1068539

267. Rose K, Vizzavona J. Stepwise solid-phase synthesis of polypeptides as linkers. J. Am. Chem. Soc. 1999;121(30):7034-7038. https://doi.org/10.1021/ja9909879

268. Martin DD, Xu MQ, Evans TC Jr. Characterization of a naturally occurring trans-splicing intein from Synechocystis sp. PCC6803. Biochemistry. 2001;40(5):1439-1402.

269. Kaneko K, Wille H, Mehlhorn J, et al. Molecular properties of complexes formed between the prion protein and synthetic peptides. J. Mol. Biol. 1997;270(4):574-586. https://doi.org/10.1006/jmbi.1997.1135

270. Baldwin MA. Analysis of glycosylphosphatidylinositol protein anchors: the prion protein. Methods Enzymol.. (Academic Press. 2005;405:172-187. https://doi.org/10.1016/S0076-6879(05)50008-1

271. Schumacher MC, Renesberger U, Seidel RP, et al. Synthesis of a GPI anchor module suitable for protein post-translational modification. Biopolymers. 2010;94(4):457-464. https://doi.org/10.1002/bip.21380

272. Vilotijevic I, Götze S, Seeberger PH, & Silva DV (2013) Chemical synthesis of GPI anchors and GPI-anchored molecules. Modern Synthetic Methods in Carbohydrate Chemistry, eds D. B. Werz & S. Vidal (Wiley-VCH), pp 335-372. https://doi.org/10.1002/9783527658947.ch12

273. Roller R, Vilotijevic I, Michel D, Seeberger HP, Silva DV. Semi-synthesis of glycosylphosphatidylinositol anchored glycopeptides and glycoproteins. J. Pept. Sci. 2014;20:547-548.

274. Grube M, Lee B-Y, Garg M, et al. Synthesis of galactosylated glycosylphosphatidylinositol derivatives from trypanosoma brucei. Chem. Eur. J. 2018;24(13):3271-3282. https://doi.org/10.1002/chem.201705511

275. Makarava N, Baskakov IV. Expression and purification of full-length recombinant PrP of high purity. Methods Mol. Biol. 2008;459:131-143. https://doi.org/10.1007/978-1-59745-234-210

276. Li A, Christensen HM, Stewart LR, Roth KA, Chiesa R, Harris DA. Neonatal lethality in transgenic mice expressing prion protein with a deletion of residues 105-125. EMBO J. 2007;26(2):548-558. https://doi.org/10.1038/sj.emboj.7601507

277. Christensen HM, Harris DA. A deleted prion protein that is neurotoxic in vivo is localized normally in cultured cells. J. Neurochem. 2009;108(1):44-56. https://doi.org/10.1111/j.1471-4159.2008.05719.x

278. Otvos L Jr, Cudic M. Post-translational modifications in prion proteins. Curr. Protein Pept. Sci. 2002;3(6):643-652.

279. Stimson E, Hope J, Chong A, Burlingame AL. Site-specific characterization of the N-linked glycans of murine prion protein by high-performance liquid chromatography/electrospray mass
spectrometry and exoglycosidase digestions. Biochemistry. 1999;38 (15):4885-4895. https://doi.org/10.1021/bi982330q

280. Shi L, Chen H, Zhang SY, et al. Semi-synthesis of murine prion protein by native chemical ligation and chemical activation for preparation of polypeptide-α-thioester. J. Pept. Sci. 2017;23(6):438-444. https://doi.org/10.1002/psc.3008

281. Mishra R, Elglad M, Begum A, et al. Impact of N-glycosylation site variants during human PrP aggregation and fibril nucleation. Biochim. Biophys. Acta Proteins Proteom. 2019;1867(10):909-921. https://doi.org/10.1016/j.bbapap.2019.03.010

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