14th Iberian Prion Congress in Madrid

Proceedings of the Annual Iberian Prion Conference

Edited by Juan Carlos Espinosa & Jesús Requena in Madrid, Spain.

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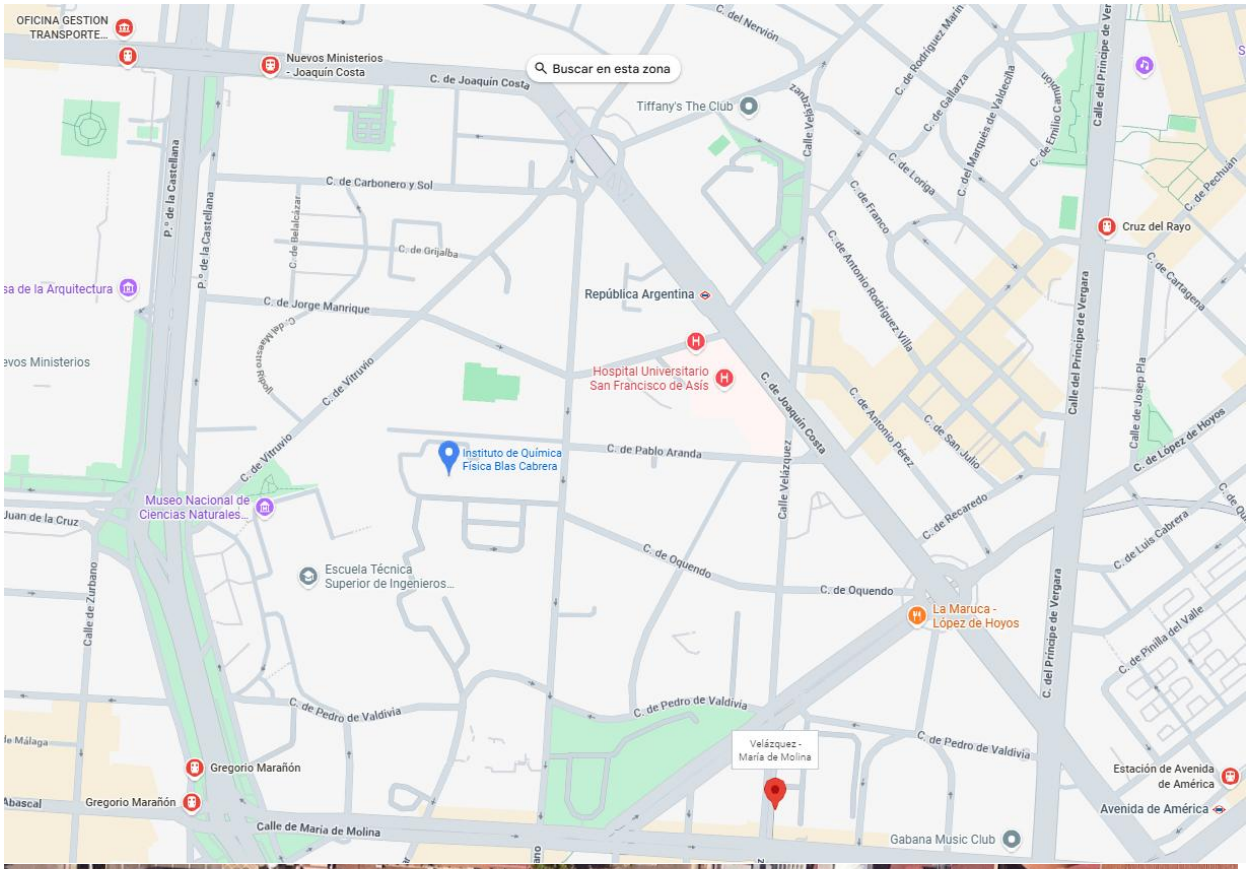
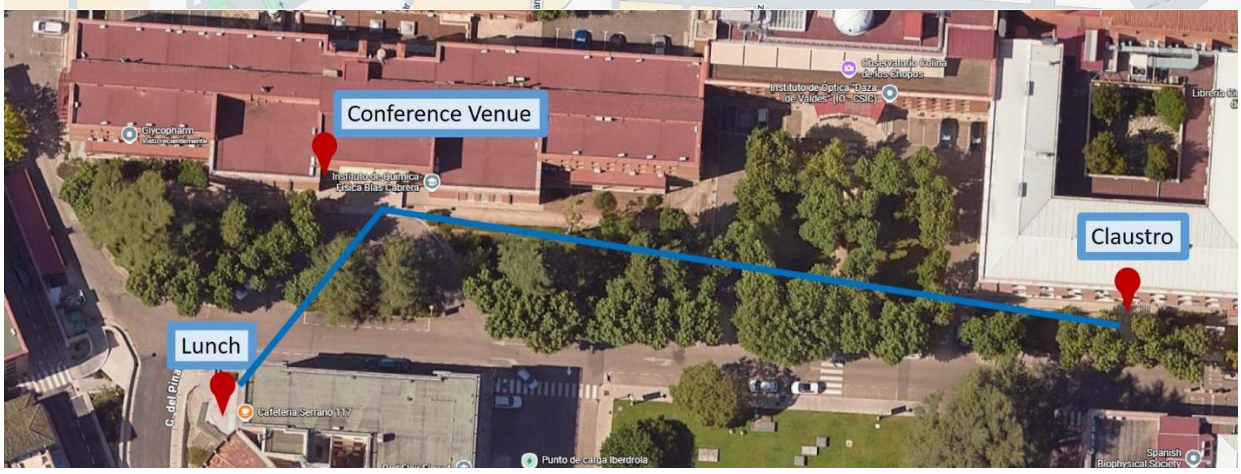
Venue

Assembly Hall of the Blas Cabrera (former Rocasolano)
Institute of Physical Chemistry (119 Serrano Street. CSIC Campus)





SSID: **Priones2026**
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14th IBERIAN MADRID PRION CONGRESS 2026

Programme

Thursday May 21st 2026	
9:00 - 10:00	**Registration, poster set-up, and coffee in the Claustro (Serrano 121)**
10:00 - 10:30	Welcome session at the Assembly Hall of the **Blas Cabrera** (former Rocasolano) Institute. (Serrano 119). Natalia Fernández-Borges and Juan Carlos Espinosa (Congress Chairpersons)
10:30 - 12:30	**Prion Structure & Biology I (Chairs: Jesús Requena – Leticia Lucero)**
10:30 - 11:00	**Invited speaker: Sanaz Sabzehei** (University of Santiago de Compostela) *Unfold to refold: tracking PrP^{C} unfolding as a pre-requisite of PrP^{Sc} propagation*
11:00 - 11:15	**Chiara Zucchelli** (Università Vita-Salute San Raffaele) *Structural determinants of dual anti-prion activity: investigating Zn(II)-BnPyP binding to the PrP^{C} octapeptide repeat region*
11:15 - 11:30	**Marta Rigoli** (Italian Institute of Technology) *Exploring the toxicity of mutant prion proteins: an in silico study*
11:30 - 11:45	**Pedro Fernández-Fúnez** (University of Minnesota Medical School) *The β2α2 loop confers high conformational dynamics and toxicity to the human prion protein*
11:45 - 12:00	**Human Rezaei** (INRAe) *Non-fibrillar prion protein oligomers transmit structural information during early assembly*
12:00 - 12:15	**Emiliano Biasini (University of Trento)** *Preclinical development of folding-interfering degraders targeting the cellular prion protein*
12:15 - 12:30	**Leire Hervella-Barrio** (CIC bioGUNE) *Beyond nature's designs: rational engineering of artificial prions to better understand protein misfolding*
12:30 - 14:00	**Lunch (Cafetería Serrano 117)**
14:00 - 15:15	**Prion Structure & Biology II (Chairs: Emiliano Biasini – Josu Galarza)**
14:00 - 14:15	**Francesca Peccati** (CIC bioGUNE) *Structural basis of glycoform preferences in prion strains RML and ME7*
14:15 - 14:30	**Vincent Béringue** (INRAe) *Prion infection disrupts endosomal trafficking and induces vacuolation in epithelial cells*
14:30 - 14:45	**Giuseppe Legname** (SISSA) *The non-octa-repeat region is the main structural determinant of prion conversion*
14:45 - 15:00	**Anna Burato** (SISSA) *Prion protein deficiency results in synaptic, neural network and behavioral alterations*
15:00 - 15:15	**Elisa Nicolini** (University of Trento) *A BiFC-based CRISPR screening platform for the identification of early genetic regulators of PrP biogenesis*
15:15 - 16:45	**Prion Diseases in Animals I (Chairs: Juan María Torres – Diego Sola)**
15:15 - 15:45	**Invited speaker: Barry Bradford** (The Roslin Institute) *Microglia regulate the rate of prion neuropathogenesis*
15:45 - 16:00	**Vincent Béringue** (Veterinary Research. Editor in Chief) *Publishing prion research in Veterinary Research: from fundamental mechanisms to population-level*
16:00 - 16:15	**Glenn Telling** (Colorado State University) *Updates on the properties of emergent CWD prions*
16:15 - 16:30	**Gage Rowden** (University of Minnesota) *Growing evidence for dust-borne, environmental, Chronic Wasting Disease prions: Insights from Northern Minnesota*
16:30 - 16:45	**Lars A. Folkman** (Norwegian University of Life Sciences) *Oral inoculation of sheep with reindeer CWD results in prion amplification in the gut-associated lymphoid tissues and the central nervous system*
16:45 - 18:30	**Poster party in the Claustro (Serrano 121: coffee, soft drinks, snacks and sandwiches)**

Friday May 22nd 2026	
9:00 - 10:30	**Prion Diseases in Animals II (Chairs: Vincent Béringue - Carlos M. Domínguez)**
9:00 - 9:15	**Samia Hannaoui** (University of Calgary) *When prions travel South: gastrointestinal dysfunction as a route- and strain-dependent feature of CWD*
9:15 - 9:30	**Sabine Gilch** (University of Calgary) *Of mice and deer: understanding prion conformational variability and transmission barriers in chronic wasting disease*
9:30 - 9:45	**Oihane Alzuguren** (INRAe) *When scrapie masks BSE in co-infected sheep: limitations of standard discriminatory tests*
9:45 - 10:00	**Cristina Sampedro-Torres-Quevedo** (CIC bioGUNE) *Rapid generation of prion disease models using AAV-delivered PrP variants in knockout mice*
10:00 - 10:15	**Sonja Ernst** (Friedrich-Loeffler-Institut) *A comparative analysis of animal prion strains around the globe*
10:15 - 10:30	**Jason C. Bartz** (Creighton University) *Emergence of minor prion strains following heat treatment*
10:30 - 11:15	**Coffee Break and posters discussion in the Claustro (Serrano 121)**
11:15 - 12:30	**Prion and Prion-like Diseases in Humans I (Chairs: Ana Rita Alvaro – Valentino Panico)**
11:15 - 11:45	**Invited speaker: Giuseppe Bufano** (Istituto Neurologico Carlo Besta) *Prion detection in the urine of individuals at genetic risk for fatal familial insomnia*
11:45 - 12:00	**Ralf Ernsberger** (BMG LABTECH GmbH, Ortenberg, Germany) *The perfect match for RT-QuIC: FLUOstar Omega microplate reader from BMG LABTECH*
12:00 - 12:15	**Bárbara Ramalho** (Center for Neuroscience and Cell Biology. University of Coimbra) *Altered circadian and clock gene profiles as potential biomarkers for fatal familial insomnia*
12:15 - 12:30	**Remarh Bsoul** (Danish Reference Center for Prion Diseases, Rigshospitalet) *Prodromal detection of skin α-Synuclein and clinical relevance of Seed Amplification Assay*
12:30 - 14:00	**Lunch (Cafetería Serrano 117)**
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14:30 - 14:45	**Sara Canoyra** (CISA-INIA-CSIC) *Generation of a G114V humanized transgenic mouse model and phenotypic characterization of the spontaneous disease*
14:45 - 15:00	**Ilaria Vanni** (ISS) *Evaluation of PrP endoproteolytic processing in a humanized G114V transgenic mouse model*
15:00 - 15:15	**Ana Rita Álvaro** (Center for Neuroscience and Cell Biology. University of Coimbra) *Patient-derived iPSC-generated hypothalamic neurons as a model for fatal familial insomnia*
15:15 - 15:30	**Jorge Moreno Charco** (Atlas Molecular Pharma) *An oral small molecule inhibitor of PrP translocation extends survival by nearly 50% in a transgenic mouse model of human GSS prion disease*
15:30 - 15:45	**Sara González-Navarro**, President (Spanish Prion Disease Foundation) *The power of synergy*
15:45 - 16:00	Poster prize
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16:30 - 18:30	**Farewell party (coffee, soft drinks, snacks), poster removal and closing in the Claustro (Serrano 121)**
≈19:00	Informal walk through Madrid on the way to the symposium venue
20:00	Conference Symposium (networking about prions with food). 1881 Madrid Ventas Hotel (Calle Alcalá 269)

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• Natalia Fernández Borges (CISA INIA CSIC, Spain)

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• Tuane C. R. G. Vieira (Universidade Federal do Rio de Janeiro, Brazil)
• Vincent Beringue (INRAe, France)

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2. Structural determinants of dual anti-prion activity: investigating Zn(II)-BnPyP binding to the PrP^{C} octapeptide repeat region. **Chiara Zucchelli** (Università Vita-Salute San Raffaele).

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51. Altered circadian and clock gene profiles as potential biomarkers for fatal familial insomnia **Bárbara Ramalho** (Center for Neuroscience and Cell Biology. University of Coimbra).

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53. Anti-prion drugs reduce endoplasmic reticulum stress and protect human dopaminergic neurons from death. **Cécile Voisset** (INSERM).

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Prion Structure & Biology

1. Unfold to refold: Tracking PrP^{C} unfolding as a pre-requisite of PrP^{Sc} propagation

Sanaz Sabzehei[1], Marta Rigoli[2,a], Raúl Cacheiro[1], Iria Díaz-Arias[1], Hasier Eraña[3,4,5], Rubén P. Lago[1], Arcadio Guerra[6], Human Rezaei[7], Joaquín Castilla[3,5,8], Emiliano Biasini[2], Víctor M. Sánchez-Pedregal[9,b], Manuel Martín-Pastor[10,b], Jesús R. Requena[1,b]

[1]CIMUS Biomedical Research Institute and Department of Medical Sciences, University of Santiago de Compostela-IDIS, Spain; [2]Department of Cellular, Computational and Integrative Biology, University of Trento, Povo, TN, Italy;[3]CIC BioGUNE, Basque Research and Technology Alliance (BRTA), Prion Research Lab, Derio, Spain; [4]ATLAS Molecular Pharma S. L. Bizkaia Technology Park, Derio, Spain; [5]Centro de Investigación Biomédica en Red de Enfermedades Infecciosas (CIBERINFEC), Carlos III National Health Institute, Madrid, Spain; [6]CIQUS Research Institute, University of Santiago de Compostela, Spain; [7]Université Paris-Saclay, INRAE, UVSQ, VIM, Jouy-en-Josas, France; [8]IKERBASQUE, Basque Foundation for Science, Bilbao, Spain; [9]Department of Organic Chemistry, University of Santiago de Compostela, Spain; [10]Unidade de Resonancia Magnética, CACTUS, University of Santiago de Compostela, Spain.
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[b]Equal contributors.

Keywords: Prion propagation, PrP^{C} unfolding, PrP^{Sc}, thermal unfolding, NMR, Procrustean bed.

It might have been believed that elucidation of the atomistic structure of PrP^{Sc} would lead to an immediate understanding of the mechanism of prion propagation. However, PrP^{Sc}, now known to be a "simple" amyloid, can only template a previously unfolded polypeptide chain. Thus, PrP^{Sc} can easily template the disordered ~90-120 domain of an incoming PrP^{C} molecule, but not its ~121-231 folded domain (FD). The FD needs to accommodate into the ~121-230 PrP^{Sc} surface, an essentially inert "Procrustean bed". Thus, a mechanism for concerted unfolding/refolding of the FD must exist, with FD unfolding as a key element. To explore how this might happen, we performed thermal unfolding of recombinant bank vole (BV) PrP^{C}(90-231), a universal PrP^{Sc} propagator, tracking changes at the residue level with solution NMR to pinpoint early unfolding propensity. Our data suggest that a key early event is the destabilization of the short β1-β2 assembly, and that the segment contiguous to the disordered tail, ~121-140, encompassing β1 and its adjacent coils, is the most likely region to unfold first [1]. As this region unfolds, it can be easily trapped by its isosequential stretch on the nearby PrP^{Sc} surface. This would leave a diminished ~140-231 FD tethered to the templating surface. Our ongoing experimental work with recombinant BV(137-231) and published data relative to HuPrP137-230 show this sequence to display substantially diminished stability, suggestive of an unfolding cascade effect. Spectroscopic data obtained at higher temperatures suggest that portions of alpha helix α2 are likely the last elements of the FD to unfold and refold. This agrees with published data showing that α2-α3 constitute a stable, yet flexible, standalone structure. Molecular Dynamics simulations assisted the interpretation of these changes and suggest eventual separation of α1 and the β2-α2-α3 ensemble [1], suggesting a late motion of α2-α3 to their anchoring area in the C-terminal lobe of PrP^{Sc}. Our data provide a conceivable timeline of events in PrP^{Sc}-assisted conversion of PrP^{C}, pointing to unfolding of segments of the FD in a "block zipper-like" N to C fashion. Our ultimate goal is to develop a computational atomistic model of PrP^{Sc} propagation informed by such timeline.

[1] S. Sabzehei *et al*. Exploring PrP^{C} unfolding as a critical step preceding its refolding in the context of PrP^{Sc} propagation. Proc. Natl. Acad. Sci. USA. In the press.

We dedicate this communication to the memory of Byron Caughey, who contributed to the studies described with critical discussion.

2. Structural determinants of dual anti-prion activity: investigating Zn(II)-BnPyP binding to the PrP^C octapetide repeat region

Zucchelli Chiara,[1] Albanesi Riccardo,[1] Ghitti Michela,[2] Anna Grasso,[4] Antonio Masone,[4] Enrico Caruso,[3] Roberto Chiesa,[4] Musco Giovanna[2]

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2 Biomolecular NMR Unit, Division of Genetics and Cell Biology, IRCCS Ospedale San Raffaele, Milan, Italy
3 Department of Biotechnology and Life Sciences, Università degli Studi dell'Insubria, Varese, Italy
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Keywords: zn-BnPyP, prion protein, anti-prion activity, NMR spectroscopy, structure-activity relationship (SAR) study

Prion diseases are rare, rapidly progressing and fatal neurodegenerative disorders caused by conversion of the cellular prion protein (PrP^C) into a pathogenic form (PrP^{Sc}). No effective therapies are currently available. We previously identified Zn(II)-BnPyP porphyrin (ZnB) as the first molecule with potent, strain-independent, dual anti-prion activity: ZnB binds both the globular domain and N-terminal tail of PrP^C, thereby inhibiting its conformational conversion to PrP^{Sc} and reducing PrP^C levels [1,2]. Although ZnB shows poor blood-brain barrier permeability, limiting its *in vivo* efficacy, it provides a powerful tool to dissect the structural determinants of dual anti-prion activity. Because both the metal ion and meso substituents of ZnB influence its anti-prion effects [1], our goals are to (i) generate experimentally-driven structural models of ZnB in complex with both PrP^C domains, and (ii) define the optimal metal and meso substituents required for effective PrP^C engagement and dual anti-prion activity. Here, we present our work in progress on ZnB binding to the PrP^C N-terminal tail.
Using NMR spectroscopy restraints and computational methods we determined an ensemble of structures of the octapeptide repeat (OR) motif (PHGGGWGQ) bound to ZnB. Selected OR conformers are being used to build docking models of the OR-ZnB complex and to perform molecular dynamics simulations. Analysis of these simulations aims to identify a representative ensemble of ZnB-OR complex structures that is consistent with the NMR-derived atomic distances observed between ZnB and OR protons. In parallel, we are experimentally assessing how different metals in the BnPyP porphyrin scaffold affect binding to the PrP^C N-terminal tail and reduction of PrP^C levels in HEK293T cells, the cellular phenotype mediated by OR-dependent, ZnB engagement of PrP^C[1].
Dual anti-prion activity represents a major conceptual advance for the development of effective anti-prion compounds. Robust structural models of ligand-PrP^C complexes, together with structure-activity relationship (SAR) analysis, are the essential prerequisite for rational therapeutic design. Insights from our structural and SAR studies on ZnB binding to PrP^Cdomains are expected to guide the design of next-generation molecules with dual anti-prion activity and improved drug-like properties beyond the porphyrin scaffold.

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3. Exploring the Toxicity of Mutant Prion Proteins: An *in Silico* Study

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Keywords: PrPC, Molecular Dynamics, ΔCR mutant, Molecular Modeling, Neurotoxicity.

Prion diseases are fatal neurodegenerative disorders caused by the conversion of the cellular prion protein (PrPC) into the misfolded, pathogenic conformer [1]. In addition to acting as a substrate for prion propagation, PrPC is considered to promote neurotoxic signaling [2]. In this context, PrPC central region has emerged as key element. Indeed, the deletion of central region's residues 105-125 (ΔCR) causes spontaneous neurodegeneration in vivo [3], and induces abnormal ionic currents in cultured cells and primary neurons [4,5], suggesting that central region is essential for controlling the toxicity of the N-terminal domain. A current molecular model suggests that the N-terminus acts as toxic effector whose activity is regulated by the C-terminal domain [6]. This interplay may be crucial for PrP physiological role, and the alterations of this regulatory mechanism could contribute to neurodegeneration. Here, we investigated how the deletion of the central region alters the structure and the dynamics of full-length PrPC. To address this question, we generated membrane-bound models of full-length, diglycosylated wild-type PrPC and of neurotoxic ΔCR PrPC mutant, and compared their conformational dynamics through Molecular Dynamics simulations. The two proteins clearly displayed distinct behaviors. The wild-type PrPC molecule adopted a more compact conformational ensemble of the N-terminal domain, consistent with stabilizing interactions between the flexible N-terminal and the C-terminal domains. In contrast, the ΔCR PrPC molecule showed more extended conformations and significant redistribution of intramolecular contacts with respect to wild-type PrPC, including the loss of specific molecular interactions between the disordered tail and the globular domain. This altered organization was associated with an increased tendency of the N-terminal to approach the membrane surface in the mutant form. These findings support a model in which the central region contributes to an intramolecular interaction network that restrains the neurotoxic activity of the N-terminus. Its deletion appears to weaken this control, promoting N-terminal domain and membrane association, and potentially favoring toxic processes. This framework can be extended to further PrP variants, sharing ΔCR related pathophysiology, like G114V mutant, associated with genetic Creutzfeldt-Jakob disease [7]. Additionally, it may guide the rational design of molecules that restore interdomain contacts in mutant PrP.

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4. The β2α2 loop confers high conformational dynamics and toxicity to the human prion protein

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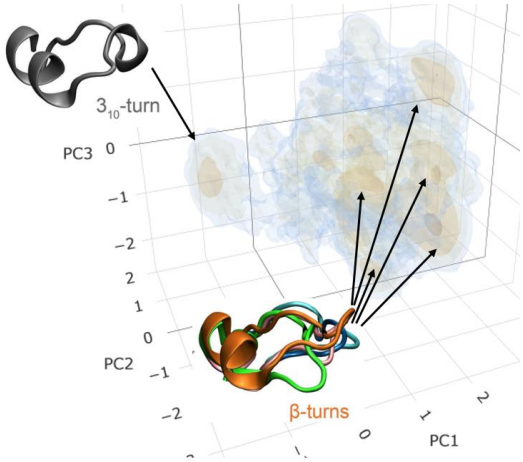
Keywords: human PrP, conformation, molecular dynamics, Drosophila

A key goal in the study of prion diseases and other proteinopathies is uncovering the molecular mechanisms governing protein misfolding and neurotoxicity. Here, we sought to identify the intrinsic (sequence / conformation / dynamics) determinants of human PrP neurotoxicity combining molecular dynamics (MD) simulations with in vivo Drosophila assays. Our **HYPOTHESIS** is that human-specific residues in a discontinuous C-terminal 3D domain (**CT3DD**) comprising the β2-α2 loop and the C-terminus of helix 3 encode a heightened propensity to misfold into neurotoxic conformations. Multiple studies have proposed that the CT3DD, which contains high sequence variability, govern PrP conformational dynamics and initiates PrP misfolding (1-3). Structural changes in the β2-α2 loop (rigid vs disordered) critically modulate PrP conversion and disease susceptibility in ungulates (4-6). Still, no precise molecular mechanism explains how this domain modulates disease susceptibility since either rigid or disordered loops can cause disease. We used transgenic flies to compare the toxicity of PrP variants (different animals, mutations) and found that human PrP exhibits higher toxicity than other PrPs (7-8). We showed that human PrP carrying the Y225A substitution from rabbit PrP robustly suppresses neurotoxicity and aggregation in flies (9). MD simulations revealed showed that in human PrP-WT the β2-α2 loop equally populates five distinct β-turn and one 3_{10}-turn conformations, whereas PrP-**Y225A** favors the 3_{10}-turn. The 3_{10}-turn conformation lowers solvent exposure of Y169, increasing overall loop stability. Since Y225A impacts the dynamics of the β2-α2 loop at a distance, we examined how residues in the loop itself impact its dynamics. Human / ape PrP are the only mammals carrying M166 in the loop, suggesting a key contribution to the dynamics of the CT3DD. In MD simulations human PrP-**M166V** strongly favors the 3_{10}-turn; flies expressing human PrP-M166V show suppressed toxicity. Notably, changes to D167 or E168 had minimal effects on their own or combined with M166V, thus, M166 is the main driver of the high conformational dynamics of human PrP. We propose that the humans / ape loop sequence 166-MDE causes high conformational dynamics and predispose to misfolding, corresponding to sporadic human disease.



Structural diversity of the β2-α2 loop in human PrP. The 3D space for the different conformations of the β2-α2 loop obtained from MD simulations (Myers et al., 2023) was created from Gibbs free energy isocontours of the first three principal components from the φ/ψ dihedrals. Darker indicates more stable regions. We identified six conformations, including five β-turns and the 3_{10}-turn, and their relative locations are indicated by arrows.

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5. Non-fibrillar prion protein oligomers transmit structural information during early assembly

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Keywords: Replication, Oligomer, templating, structural complementation

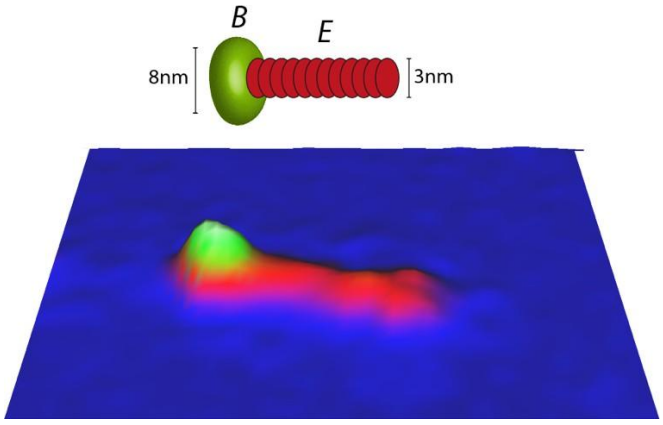
The prion paradigm is defined by the transmission of structural information from an auto-replicative aggregated protein assembly to a monomeric substrate, a process commonly referred to as templating (1). In prevailing models of amyloid and prion propagation, this transfer of folding information is largely attributed to fibrillar assemblies and is thought to occur predominantly at their extremities through an end-elongation mechanism (2, 3). Whether non-fibrillar oligomeric assemblies can also support such information transfer remains poorly understood.

Here, we investigate the early stages of recombinant prion protein oligomeric assembly to determine whether non-fibrillar oligomers (4) can transmit folding information, a defining hallmark of templating processes. Using rational mutagenesis to generate non-oligomerizing variants, hetero-oligomerization assays, arrested reaction conditions, and single molecule structural analyses, we examine how polymerization-defective variants can be recruited into assembling oligomeric structures.

We show that incorporation of such variants is not a passive process but requires structural complementation, whereby folding information supplied by wild-type PrP induces their conformational conversion into a β-enriched, assembly-competent state. This demonstrates that folding information can be transmitted during oligomerization itself, independently of fibrillar structures.

Structural analyses reveal that oligomeric assemblies adopt a modular architecture composed of a β-sheet–rich globular B domain connected to an elongated E domain. Folding information transfer is initiated at the level of the B domain, which functions as a primary structural scaffold, and is followed by domain-biased organization within the oligomer. Preformed oligomers further act as conformational templates, promoting incorporation of otherwise inactive variants and supporting a hierarchical assembly mechanism.

Together, these findings demonstrate that non-fibrillar oligomeric assemblies can transmit folding information independently of fibril ends. This work expands the conceptual framework of prion assembly by identifying oligomeric states as active contributors to structural propagation and potential alternative nucleation platforms in protein misfolding processes.



Structural model of the ovine PrP oligomer O1 ((4)), derived from high-resolution liquid atomic force microscopy (AFM), revealing a two-domain architecture. O1 is composed of a globular basement domain (B), approximately 8 nm in diameter, extended by an elongated domain (E) of approximately 3 nm in diameter.

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6. Preclinical Development of Folding-Interfering Degraders Targeting the Cellular Prion Protein

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Keywords: Targeted Protein Degradation; Prion Protein; Folding Intermediate Degraders; PPI-FIT; Prion Diseases

Targeted protein degradation is an emerging drug discovery strategy with broad therapeutic potential. We have developed a novel approach, called Pharmacological Protein Inactivation by Folding Intermediate Targeting (PPI-FIT), which promotes selective protein degradation by stabilizing folding intermediates that are recognized as misfolded by the cellular quality control machinery [1-2]. We applied this strategy to the cellular prion protein (PrP), a central player in prion diseases and most likely the best pharmacological target to treat these disorders [3-4]. The effort led to the identification of SM875, a small molecule that dose-dependently reduces PrP levels and inhibits prion propagation across multiple cell models. To advance SM875 along the drug development pipeline, we recently pursued biophysical validation of its mechanism of action, chemical optimization, and in vivo evaluation of its therapeutic potential. Native mass spectrometry showed direct binding of SM875 to a non-native PrP conformer under near-physiological conditions, supporting the proposed mechanism. SM875 displays stereoselective activity, with the (R)-enantiomer exclusively responsible for PrP reduction, while the (S)-enantiomer is inactive, highlighting key aspects of its mechanism of action and structure–activity relationships [4]. A focused library of several dozen SM875 analogues, comprising both newly synthesized and commercially available compounds, was evaluated using a novel high-content cell-based assay (called Light-Identification of Protein Suppressors, LIPS), specifically designed to identify PrP suppressors and improve their potency, selectivity, and pharmacokinetic properties [5]. Finally, in vivo administration of SM875 to wild-type mice over several weeks resulted in a robust reduction of endogenous PrP levels in peripheral organs, including the spleen and liver, as confirmed by western blot analysis. Collectively, these findings establish PPI-FIT as a novel targeted protein degradation platform and position SM875 as a first-in-class small-molecule degrader of PrP. The integration of computational modelling, chemical synthesis, biophysical characterization, and in vivo validation provides a strong foundation for the continued development of PrP-directed folding-interfering degraders as therapeutic agents for prion diseases.

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7. Beyond nature's designs: Rational engineering of artificial prions to better understand protein misfolding

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Keywords: Prion protein engineering, artificial prions, protein misfolding, PMSA (Protein Misfolding Shaking Amplification)

Evolution has constrained prion protein sequences within narrow functional boundaries across millions of years, with both sequence and native fold being highly conserved across mammalian species, leaving unexplored vast regions of alternative sequences potentially compatible with prion misfolding and propagation. We leveraged our extensive collection of approximately 1000 natural PrP variants and their PMSA-determined misfolding propensities to develop a weighted amino acid permissiveness matrix that guided the rational design of artificial prion variants challenging the evolutionary constraints of mammalian PrPs. Using bank vole PrP as scaffold, we designed 16 artificial variants by systematically incorporating amino acids with high permissiveness scores at 19 key positions (residues 99-230), achieving ≥17-19 mutations relative to any known natural PrP—a level of divergence unprecedented in this highly conserved protein family. Additionally, we engineered 9 negative control variants using amino acids with significantly lower permissiveness scores, all achieving high AlphaFold confidence scores (pLDDT 87.2-90.4) for proper structural prediction.

All 16 rationally designed variants successfully underwent spontaneous misfolding via PMSA despite unprecedented sequence divergence from natural variants, while the 9 negative controls failed to undergo efficient misfolding as predicted, validating our design approach. These synthetic prions exhibited characteristic protease resistance and efficient *in vitro* propagation in homologous substrates. Strikingly, 15/16 variants successfully propagated in TgVole(I109)1x brain homogenate, demonstrating preserved infectivity. Using AAV-mediated neuronal expression, we confirmed that artificial prions maintain propagation capacity in authentic brain environments with native post-translational processing, remaining stable through serial passages at 1:100 dilutions. *In vivo* infectivity studies in transgenic models are currently ongoing.

We have successfully created functional prions existing far beyond natural evolutionary boundaries, demonstrating that the sequence diversity compatible with prion propagation extends dramatically beyond what evolution has determined. This establishes proof-of-concept for rational prion design and provides unprecedented opportunities for dissecting structure-function relationships, identifying potential therapeutic targets, and understanding fundamental principles governing transmissible protein misfolding.

8. Structural Basis of Glycoform Preferences in Prion Strains RML and ME7

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Keywords: prion strains, molecular dynamics, sialic acid

Prion diseases originate from the pathological misfolding of the cellular sialoglycoprotein prion protein (PrP^{C}), universally found across mammalian species, into an aberrant conformation termed PrP^{Sc}, which exhibits high aggregation propensity and neurotoxicity. Distinct conformations of the misfolded and aggregated PrP^{Sc}, termed prion strains, can cause different disease phenotypes and transmission characteristics. Different prion strains exhibit well-defined and distinct glycoform preferences arising from two sialylated, N-linked glycans. Glycosylation, and in particular sialylation, have been demonstrated to modulate the replication rate of PrP^{Sc}, with profound implications for the propagation of prion diseases.[1,2] In this contribution, we leverage high-resolution cryo-EM structural data[3,4] and all-atom molecular dynamics simulations to elucidate the molecular basis of the glycoform preferences in mouse strains RML and ME7. We show that these preferences are likely determined by differential engagement of the major basic patch and palindromic region of PrP, shedding light on a long elusive, fundamental aspect of prion biology.[5]

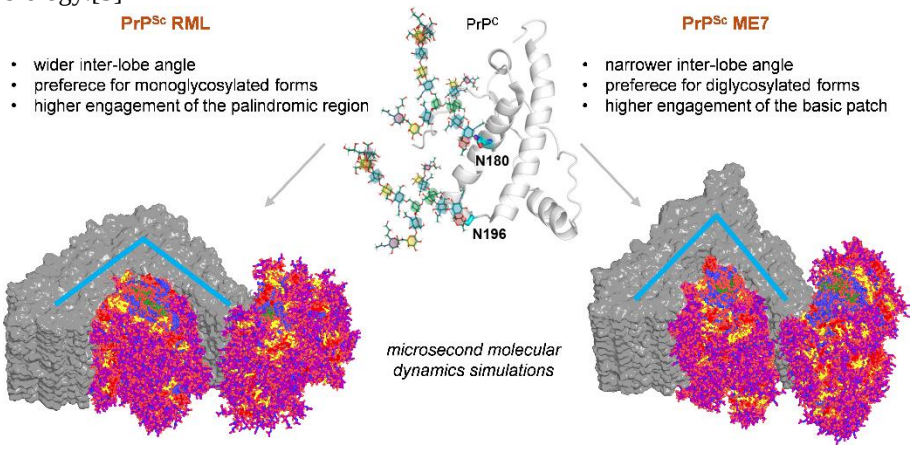

Figure All-atom models of PrP^{C} and PrP^{Sc} (RML and ME7 strains) glycosylated with sialylated glycans, highlighting the determinants underlying strain-specific structural differences and glycoform preferences.

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9. Prion infection disrupts endosomal trafficking and induces vacuolation in epithelial cells

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Keywords: prion disease; endosomal trafficking; vacuolation; PrP; epithelial cell model

Mammalian prion diseases are fatal infectious neurodegenerative disorders characterized by accumulation of misfolded prion protein (PrP^{Sc}) and spongiform vacuolation in the brain. The cellular origin of these vacuoles remains unclear.

Here, we describe RovP2FJ6, a novel epithelial cell model constitutively expressing ovine PrP^{C}, which is highly permissive to multiple prion strains and uniquely develops cytoplasmic vacuoles upon infection.
Using electron microscopy, vital dye staining and pharmacological inhibition, we show that these vacuoles derive from weakly acidic, pre-lysosomal compartments exhibiting late endosomal markers, including Rab GTPases, but failing to acquire key components of the lysosomal fusion machinery, indicating a stall at the late endosome-lysosome interface.
Vacuolation was reduced by the V-ATPase inhibitors bafilomycin-A1 and concanamycin A, suggesting V-ATPase-dependent vacuolar swelling associated with altered endosomal acidification.

Although epithelial in origin, the RovP2FJ6 model recapitulates key features of prion-induced endocytic disruption and provides a tractable system to dissect prion–host interactions and membrane dynamics relevant to prion pathogenesis.

10. The non-Octa-Repeat region is the main Structural Determinant of Prion Conversion

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Keywords: PrP, PrP^{C}, PrP^{Sc}, non-octarepeat (OR) copper-binding site, mutant PrP

Prion diseases are rare neurodegenerative disorders caused by a change in conformation of the prion protein from the cellular form (PrP^{C}) to a misfolded isoform (PrP^{Sc}). PrP^{C} is a copper binding protein via histidine residues in the octapeptide repeats (OR) and the non-OR region located at the N-terminus. Although the functional implication of copper binding to PrP^{C} is still under continuous investigation, altering copper coordination may play a role in prion disease.
Previously, we have hypothesized that histidine 95 and 110 may play a key role in prion conversion and structural studies have supported such hypothesis (1).
We have recently described transgenic mice expressing mouse PrP with histidine 95 replaced by tyrosine (PrP H95Y) to modify the non-OR copper-binding site. Transgenic mice overexpressing this mutant PrP H95Y show clinical signs and die at about 100 days with spongiform degeneration, gliosis and PK-resistant PrP. Passaging brain homogenate from these mice overexpressing PrP H95Y to mice expressing wild-type PrP also cause lethal, spongiform encephalopathy, suggesting effective prion transmissibility (2).
These findings suggest that the H95Y substitution could promote PrP^{C}-PrP^{Sc} conversion and induce spontaneous prion disease *in vivo*, highlighting a critical role of the non-OR copper-binding site in regulating PrP conformational stability and driving prion pathogenesis.

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11. Prion Protein Deficiency Results in Synaptic, Neural Network and Behavioral Alterations

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The cellular prion protein (PrP^C) is known for its involvement in the pathogenesis of prion diseases. Recent research implicates this physiological isoform in neuronal development, excitability, synaptic plasticity and other biological processes. However, its precise function in the development and function of neurons remains poorly understood. Here, we investigated its role during different developmental stages, both *in vitro* and *in vivo*, using different PrP knock-out (KO) mouse lines (*Prnp*$^{-/-}$). PrP KO neurons cultured on microelectrode arrays (MEAs) displayed altered network dynamics compared to wild type cultures, comprising reduced burst frequency, desynchronization, and abnormal spike patterns, indicative of impaired maturation of the synaptic circuitry. These functional alterations were associated with reduced expression of key presynaptic and postsynaptic proteins, including elements of the SNARE complex and regulators of excitation-inhibition balance. Similar molecular changes were found in a second *Prnp*$^{-/-}$ model, suggesting that PrP^C is directly involved in these mechanisms regardless of genetic backgrounds. Alterations in neuronal networks were traceable into adulthood: *in vivo* recordings in adult *Prnp*$^{-/-}$ mice revealed increased neuronal responses to visual danger stimuli, which correlated with behaviorally increased fear responses to those stimuli. Together, our findings support a critical role for PrP^C in the establishment and maintenance of functional neuronal networks, from early developmental stages *in vitro* to behaviorally mature relevant circuits *in vivo*, beyond genomic background.

These results have direct implications for strategies aiming to lower PrP^C to treat or prevent prion disease. Genetic deletion of PrP^C protects against prion replication and confers resistance to scrapie, and several pharmacological and genetic approaches to reduce PrP^C expression are under active development as disease-modifying therapies. Our data indicate, however, that long-term absence of PrP^C is not physiologically neutral: it delays network maturation, promotes late-emerging instability and alters the processing of survival-relevant stimuli. While targeting PrP^C remains a promising approach to counteract prion diseases, our data reveal that chronic loss of PrP^C could cause network impairments, especially at maturation and adulthood. Consequently, researchers working on therapeutic strategies should consider the risk of destabilizing normal synaptic and network functions, while balancing the benefits of PrP^C removal, particularly in the context of aging/neurodegeneration.

12. A BiFC-based CRISPR screening platform for the identification of early genetic regulators of PrP biogenesis

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Keywords: PrP Biogenesis, BiFC Assay, Crispr-screening & Genetic Perturbation

Prion diseases are rare, fatal neurodegenerative disorders. Because the cellular prion protein (PrP) is notoriously difficult to target directly, identifying druggable genetic regulators of its expression represents a critical therapeutic strategy [1]. This project describes a novel cellular assay based on Bimolecular Fluorescence Complementation (BiFC) [2] designed to focus on the earliest steps of PrP biogenesis, with the specific goal of uncovering genes that regulate this process. By targeting these initial stages, it prioritizes factors that influence PrP production before it reaches downstream cellular compartments. Its architecture enables efficient genetic perturbation screens, allowing systematic identification of genes whose disruption alters PrP expression.
Using this platform, we screened a focused RNA-binding protein library (Yeo library) and a genome-wide library (Brunello library). To handle the large-scale datasets generated, a dedicated software tool was developed to streamline analysis. Application of this pipeline revealed that top-ranking hits were strongly enriched for components of the translation machinery, consistent with the endoplasmic reticulum-focused design of the BiFC system. Importantly, candidates identified in the targeted Yeo screen were independently validated as positive regulators in the genome-wide Brunello dataset, highlighting the robustness and reproducibility of the approach. Among the most significant hits, HSPA5 stood out, in agreement with recent findings by Supattapone et al. (2026) [3], further supporting the biological validity of the system. In summary, this work combines an innovative biological sensor with an accessible, no-code platform for the analysis of CRISPR screening data. Building on these findings, a comprehensive validation strategy has been established to prioritize the most promising targets. Secondary genetic validation will involve individual sgRNAs directed against candidates that most effectively reduce PrP expression. In parallel, we will test approved drugs known to target our validated hits to evaluate their ability to lower PrP levels, thereby accelerating the identification of potential therapeutic interventions.

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13. Exploring the unfolding of the folded domain of PrP^C at 37 °C in the context of prion propagation

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Keywords: PrP^C unfolding, PrP^{Sc}, PrP^{Sc} propagation, NMR.

PrP^{Sc} is an essentially inert scaffold. It can easily trap the ~90-120 disordered domain of an approaching PrP^C molecule and template it to adopt a rigid, β sheet-rich PIRIBS conformation. But it does not seem to have any capacity to coerce its ~121-231 (bank vole numbering) folded domain (FD) to follow in adopting the PrP^{Sc} conformation. We have recently proposed that the FD must first unfold before it can refold to such conformation on the PrP^{Sc} surface [1]. By using solution NMR-tracked thermal unfolding, we mapped the initial steps of unfolding of the FD and proposed a plausible timeline of unfolding/refolding.

In this context, we aimed at exploring the unfolding of the FD of PrP^C at a physiologically relevant temperature. We incubated a solution of recombinant BVPrP90-231 at 37 °C and tracked changes in its conformation by acquiring a series of HSQC spectra, which are very sensitive reporters of structural changes, over time. The spectra show a very high stability of the sample, with no appreciable changes over many days of incubation. This means that over that period, partial unfolding, that has been documented [1], is fully reversible, not leading to any permanent structural collapse. We propose two non-exclusive hypotheses to interpret these results: 1) Reversible conformational fluctuations occurring at physiological temperature over many days might become irreversible in the presence of the PrP^{Sc}, that would trap fleeting unfolded segments; 2) co-factors are critical to help partial unfolding of the FD under physiological conditions. It should be noted that PrP^{Sc} propagation *in vitro* has been demonstrated in the absence of any co-factor, albeit with substantially lower efficiency compared with its presence [2].

We are currently experimentally exploring both possibilities. In this respect, when sulphated dextran, a known facilitator of PrP^{Sc} propagation was added in an equimolar concentration to BVPrP90-231, it induced aggregation and precipitation of the protein, likely a consequence of a profound conformational effect.

Our ultimate goal is to expand these findings and to integrate them in an atomistic model of PrP^{Sc} propagation that should explain how prions propagate.

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14. Glycosidic polyanionic cofactors as triggers of spontaneous PrP misfolding

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Prion diseases are fatal neurodegenerative disorders caused by the misfolding of the cellular prion protein (PrP) into a pathogenic conformer. Although best known for their infectious nature, prion diseases can also arise de novo in sporadic and genetic forms. Because spontaneous PrP misfolding is rare and unpredictable in vivo, robust in vitro models are needed to dissect the molecular events that initiate this process.

Here, we investigated the role of glycosidic polyanionic cofactors in triggering spontaneous PrP misfolding. We adapted Protein Misfolding Cyclic Amplification (PMCA), a well-established technique for prion propagation, to reproducibly model de novo PrP misfolding in a brain-like environment in the presence of dextran sulfate, a sulfated glucose polymer. This system enabled the systematic analysis of protein–cofactor interactions associated with the initiation of misfolding.

Using a rational design strategy, we examined the structural features required for glycosidic cofactors to induce spontaneous PrP misfolding. In addition to dextran sulfate, compounds sharing similar physicochemical properties, including molecules resembling components of the mammalian brain extracellular matrix, were also able to trigger this event. Nuclear Magnetic Resonance (NMR) studies further identified specific residues involved in the interaction with these negatively charged compounds. Altogether, the data support a model in which these cofactors promote a crowding-like environment that facilitates intermolecular PrP interactions and aggregation.

In summary, this work establishes a reproducible model to study the contribution of glycosidic polyanionic cofactors to spontaneous PrP misfolding and identifies candidate molecular features that may be relevant to this process in vivo.

Keywords: prion, spontaneous misfolding, glycosidic cofactor, PMCA

15. Rep-WH1 prions from the phytopathogen *Xylella fastidiosa* and their potential for controlling plant disease

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Keywords: Bacterial prions, Rep-WH1, plasmids, *X. fastidiosa*, phytopathogen

Xylella fastidiosa globally is the bacterial pathogen having the highest impact on woody plants of economic interest. It proliferates in the xylem of the infected hosts to the point of occluding their vases, thus leading to the irreversible drying of plants. We aim to explore novel approaches to combat *X. fastidiosa*, since there is no solution for the infected plants apart from uprooting.
We have found orthologues of the Rep-WH1 prion domain of the *Pseudomonas* plasmid pPS10 (reviewed in [1]) in the replication proteins of several plasmids in *X. fastidiosa*. The expression of two of these Rep-WH1 prions decreased the culturability of *E. coli* and *X. fastidiosa in vitro* by driving bacteria into dormancy. In *E. coli*, while the pXF64 prion forms multiple globular amyloid aggregates, alike those for the archetypical pPS10 prion, fluorescence recovery after photobleaching (FRAP) reveals that the pXF51 prion forms a cytosolic hydrogel and converts cells from their normal bacillary shape into cocci [2].
Expression of the Rep-WH1 pPS10 and pXF51 prions decreased the virulence of the *X. fastidiosa* IVIA5235 strain upon experimental infection of *Nicotiana* plants, as revealed by net reductions in the symptoms of withering, in the bacterial load (qPCR) and by an anomalous senescent morphology of bacteria within the xylem vases (TEM of sections across plant petioles). Interestingly, qPCR showed that the expression plasmids were quickly lost after inoculation but yet, months after the infection (i.e. after many bacterial generations), bacteria exhibited *in planta* the senescent phenotype associated to prion expression. Thus, we are now exploring the hypothesis of cross-seeding/templating of the toxic prion conformation from the pPS10 and pXF51 Rep-WH1 proteins on their orthologue from the resident plasmid in the *X. fastidiosa* strain (pXFAS5235) [3]. This would provide an *in vivo* demonstration for the vertical propagation of bacterial prions in plants.

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16. Development of genetic tools to dissect host-microbiome amyloid interactions in *Caenorhabditis elegans*

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Keywords: bacterial amyloids, *Caenorhabditis elegans*, host-microbiome interactions, gut-brain axis

The intestinal microbiota modulates human physiology, including brain function. Recently, correlations have been described between microbiota composition and neurodegenerative diseases (e.g., Alzheimer's and Parkinson's). A hallmark of these pathologies is amyloid aggregation. Amyloids self-propagate by converting soluble molecules of the same protein into the aggregated state through protein–protein interactions (self-nucleation). However, what triggers this aggregation cascade remains unknown. Since different members of the microbiota produce functional amyloids, it has been proposed that these may initiate the aggregation of human amyloids (HAs) through *in vivo* cross-seeding, thereby causing disease *[1]*. Supporting this idea, bacterial amyloids (BAs) such as curli from *E. coli [2-4]*, FapC from *Pseudomonas [5]*, and amyloid sequences from BAP proteins of *S. aureus* increase the aggregation of HAs in animal models of neurodegeneration *[6]*. Moreover, it has recently been shown in *C. elegans* that intestinal curli can reach neurons to mediate this effect [4].

However, it remains unknown: (i) whether *in vivo* cross-seeding (protein–protein interaction) between BAs and HAs is the mechanism driving this increased aggregation; (ii) whether such interactions initiate HAs aggregation; (iii) whether other BAs also reach neurons to modulate HAs aggregation; (iv) whether this occurs in a protein-specific manner to promote particular diseases and (v) whether BAs are neurotoxic themselves.

To address these questions, we are developing a bimolecular fluorescence complementation (BiFC)-based system in *C. elegans* to determine if, when, and where direct interactions between different HAs and BAs occur, using a combinatorial approach that will allow us to uncover the protein–protein specificity of these interactions. Another open question in the field is whether BA can be neurotoxic on their own once they reache the nervous system. To determine BAs neurotoxic effects, we are building optogenetic switches to drive light-dependent oligomerization of different BAs inside *C. elegans* neurons to identify BA specific changes in neuronal physiology that could reveal novel links to disease.

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17. Cross-Seeding Between Prion Protein and α-Synuclein: Modulation of Aggregation Kinetics and Structural Outcomes

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Keywords: Amyloid aggregation, prion protein, α-synuclein, cross-seeding

The coexistence of multiple amyloidogenic proteins is a hallmark of several neurodegenerative diseases, suggesting cross-interactions between distinct aggregation pathways. Here, we investigate how prion protein (PrP) aggregates influence the aggregation behavior of α-synuclein (α-syn), a key protein in Parkinson's disease.

Kinetic analyses show that both PrP^{Sc} and PrP^{C} significantly modulate α-syn aggregation in a concentration-dependent manner. PrP seeds reduce the nucleation rate, resulting in an extended lag phase, while slightly increasing elongation. Despite this, the overall formation of mature α-syn fibrils is diminished, indicating a shift in the aggregation pathway.

Structural characterization by transmission electron microscopy and chemical denaturation assays reveals that α-syn aggregates formed in the presence of PrP exhibit altered morphology and reduced stability, particularly with PrP^{Sc}. These results support the formation of alternative or off-pathway aggregation states, likely involving less ordered or intermediate species.

Overall, our findings demonstrate that PrP not only reshapes the kinetics of α-syn aggregation but also alters the structural properties of the resulting assemblies. This cross-seeding behavior underscores a complex interplay between amyloid systems and provides insight into the molecular basis of overlapping neurodegenerative pathologies, highlighting potential targets for therapeutic intervention.

18. Bidirectional Cross-Seeding Between Amyloid-β and Prion Protein: Kinetic Interplay and Modulation of Aggregation Pathways

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Keywords: Amyloid aggregation, prion protein, amyloid-β peptide, cross-seeding, oligomer

Growing evidence supports mechanistic links between neurodegenerative diseases through cross-interactions among amyloidogenic proteins. Building on previous findings that prion protein (PrP) fibrils modulate amyloid-β (Aβ40) aggregation, we investigate the reciprocal effect—how Aβ assemblies influence PrP aggregation kinetics and pathways.

Using kinetic assays, we show that Aβ species significantly alter PrP aggregation in a concentration- and state-dependent manner. Preformed Aβ aggregates modulate both nucleation and elongation phases of PrP fibrillization, resulting in changes in lag time and growth rates. Notably, Aβ promotes the formation of alternative aggregation intermediates, including soluble oligomeric species that may exhibit enhanced biological activity and toxicity.

Structural analyses indicate that cross-seeding does not merely accelerate fibril formation but redirects the aggregation landscape toward distinct conformational states. This suggests a complex bidirectional interplay in which Aβ and PrP mutually influence each other's aggregation behavior.

Overall, our findings support a model of reciprocal cross-talk between amyloid systems, providing new insights into the molecular basis of overlapping neurodegenerative disorders and highlighting potential therapeutic strategies aimed at disrupting pathological protein–protein interactions.

19. Structural Determinants of Prion Protein Misfolding: Functional Analysis of the non-Octa-Repeat region

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Keywords: PrP non-OR domain, Scrapie Conversion Assay, RT-QuIC

Prion diseases are fatal neurodegenerative disorders caused by the conformational conversion of the cellular prion protein (PrP^{C}) into a misfolded, aggregation-prone isoform (PrP^{Sc}) [1]. Despite extensive research into understanding prion propagation, the molecular determinants triggering early PrP^{C} misfolding remain elusive.
Recent findings implicate the non-octa-repeat (non-OR) region as a critical determinant for the genesis of metastable intermediates, although the underlying molecular mechanisms remain poorly defined.

This project focuses on the non-OR region of the prion protein N-terminus, which lies between the polybasic segment and the octa-repeat domains. This region has been implicated in metal binding, intramolecular interactions, and long-range conformational regulation of the protein conversion [2–4]. Increasing evidence suggests that it may influence the structural stability of PrP^{C} and play a role in modulating its susceptibility to pathological misfolding [3–5].
To investigate the functional role of this region, we generated a panel of targeted mutants within the non-OR domain and characterized them through a scrapie conversion assay. PrP^{C} mutant constructs have been transiently expressed in chronically infected mammalian cells to assess their conversion propensity. The impact of these mutations on prion seeding and aggregation propensity has been also evaluated using Real-Time Quaking-Induced Conversion (RT-QuIC), enabling quantitative comparison of fibrillation kinetics and seeding efficiency across different variants.

Results showed that mutants within the non–octa-repeat region display a wide spectrum of conversion propensity, depending on the physicochemical properties of the substituted amino acid. Notably, substitutions at the same residues can lead to markedly different outcomes, ranging from near-complete suppression to several-fold increases in conversion. These findings identify the non–octa-repeat region as a critical hotspot regulating early prion conversion and highlight its key role in modulating PrP^{C} misfolding and its potential as a target to control prion propagation.

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Prion Diseases in Animals

20. Microglia regulate the rate of prion neuropathogenesis

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Keywords: Prion Diseases, Neuropathology, Microglia, Prion-like spreading, Neuropathogenesis

Prion diseases are devastating neurodegenerative diseases with no effective cure. The role of microglia during prion disease is becoming increasingly clear through our investigations of prion disease in microglia-deficient *Csf1r*-FIRE knockout mice [1]. These mice reveal that prion disease is accelerated in the absence of microglia despite no difference observed in the kinetics of prion accumulation. Following direct intracerebral delivery of prions we observed accelerated spread through the brain resulting in increased astrocyte activation and targeting of neuropathology to other brain regions resulting in shorter disease survival times.

To confirm the protective roles of microglia we undertook microglial repopulation via the delivery of *Csf1r*-wild type bone marrow to *Csf1r*-FIRE knockout mice. In this model the vacant microglia niche is repopulated completely from donor bone marrow. Microglial reconstitution post prion infection in *Csf1r*-FIRE knockout mice was capable of delaying neuropathological changes and extending disease survival times back to the equivalent of prion infected wild type mice. Partial or unilateral repopulation of microglia further highlighting the specific neuropathological changes including sequestration of prions and prevention of astrocyte activation and vacuolation in the condition of prion disease in the presence of microglia. This study also highlighted the toxic impact of prion disease on reconstitution and potentially naturally on resident microglia during prion disease.

Further studies investigating peripheral intraperitoneal prion infection of *Csf1r*-FIRE knockout mice have also revealed altered prion spread through the brain with less brain regions affected compared to wild type mice. These data suggest that efficient prion-like spreading between brain areas through neural connections is much less efficient in the absence of microglia and instead prions spread more locally via astrocyte-astrocyte connections. The result was a much more minor reduction in disease survival time than anticipated and highlighted the contradictory activities of microglia during neurodegeneration.

We have observed that microglia are protective during early disease. Microglia play critical roles in the spreading of prions through the brain, sequestering and aggregating extra-cellular prions but also facilitating efficient inter-neuronal prion-like spreading. These activities also impact on astrocyte activation and propagation of prions and thereby regulate the rate of prion neuropathogenesis.

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21. Publishing prion research in *Veterinary Research*: from fundamental mechanisms to population-level

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Keywords: host-pathogen interaction, scientific publication**,** open access

Veterinary Research is an international, fully open-access journal owned by INRAE and published by *Springer Nature*, dedicated to advancing knowledge in infectious diseases affecting animals, with a strong emphasis on host–pathogen interactions, immunology, and epidemiology. The journal provides a natural home for animal prion disease research, particularly studies that go beyond descriptive approaches and address mechanistic insights, transmission dynamics, or host responses.

This presentation will introduce the scope and editorial vision of *Veterinary Research*, highlighting what constitutes a strong contribution in the context of prion biology. Particular attention will be given to the types of studies actively encouraged by the journal, including:

* mechanistic studies of prion replication and cell biology,
* investigations of strain diversity and interspecies transmission,
* host genetic susceptibility and immune responses,
* environmental and epidemiological aspects of prion spread.

As a selective journal, *Veterinary Research* prioritizes originality, conceptual advances, and robust experimental design, and actively avoids incremental or purely descriptive studies. The editorial process is handled by active scientists in the field, ensuring fair and expert evaluation.

Finally, the role of *Veterinary Research* in supporting the prion community will be discussed, notably through high visibility provided by open access, rapid dissemination, and a strong editorial commitment to scientific rigor and relevance.

22. Updates on the properties of emergent CWD prions

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Chronic wasting disease (CWD) is a burgeoning and ineradicable epizootic affecting deer, elk, and other members of the family *Cervidae*. In North America, CWD is efficiently propagated by contagious transmission among wild and captive cervid populations. At the time of writing, cases have been identified in 36 American states and five Canadian provinces. Commercial exportation of sub-clinically diseased elk from Canada inadvertently introduced CWD to South Korea in 2001, where it subsequently spread to other farmed cervid species. Since 2016, CWD has emerged in free-ranging cervids from Norway, Finland, and Sweden. Insights into how strain CWD prion phenotypes are enciphered by distinct PrP^{Sc} conformations derive, in large measure, from the development of experimentally tractable, genetically-modified mouse models that recapitulate the diverse properties of naturally-occurring prion strains. Using a refined approach we created gene-targeted (Gt) mice in which expression of cervid PrP^{C} coding sequences is accurately regulated by the mouse *Prnp* gene. Here we provide an overview of our studies in Gt mice that illustrate the properties of emergent strains of CWD prions from moose, reindeer, and red deer from Norway, Finland, and Sweden. An emerging picture shows that these isolates are comprised of diverse and unstable prion strains, with properties distinct from a dominant, established straing causing CWD in North American and Korean cervids.

23. Growing Evidence for Dust-Borne, Environmental, Chronic Wasting Disease Prions: Insights from Northern Minnesota

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Keywords: RT-QuIC, environmental, dust, swab

Chronic wasting disease (CWD) prions (PrP^{Sc}) persist within environmental settings for years, contaminating soil and vegetation and posing significant challenges to effective CWD management. Following confirmation that multiple CWD-positive white-tailed deer carcasses had been disposed on forested land in northern Minnesota, USA, our team established a long-term ecological study site to investigate the environmental persistence of CWD prions. We implemented recent advancements in the real-time quaking-induced conversion assay (RT-QuIC) to conduct a variety of environmental-PrP^{Sc} detection efforts including investigations of both soil and water. In light of 1) historical research indicating that PrP^{Sc} can bind to dust particles and 2) our confirmation of CWD prions in soil and water at the study site, we hypothesized that PrP^{Sc} was also circulating in dust. We conducted RT-QuIC testing of personal protective equipment (PPE) worn in the site and of stainless-steel sentinels deployed throughout the site and exposed to local microenvironmental conditions. Swab-based sampling protocols for each sample were refined for RT-QuIC testing of dust, and the sampling design included two negative control locations. Our results revealed statistically significant RT-QuIC seeding activity associated with the PPE swabs and sentinels at the CWD-positive site compared to the CWD-negative site, as well as elevated seeding activity along the study site perimeter compared to the negative site. Collectively, these findings support the hypothesis that PrP^{Sc} is likely circulating on dust particles in the study site with significant implications for natural environments where CWD is endemic.

24. Oral inoculation of sheep with reindeer CWD results in prion amplification in the gut-associated lymphoid tissues and the central nervous system

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Keywords: CWD, reindeer, Norway, sheep, prion

Abstract:

Chronic wasting disease (CWD) is a prion disease affecting wild and captive cervids. Contagious CWD was first described in the USA over 50 years ago. The contagious disease appeared on the European continent in 2016 in a wild reindeer in Norway. Later, multiple cases of non-contagious sporadic CWD have been detected in moose across Fennoscandia and red deer in Norway [1].

In Norway, reindeer and sheep share summer pasture in several mountainous areas, and we have previously documented spatiotemporal overlap between sheep and CWD-infected reindeer. Shedding and environmental persistence of prions raise concern for potential spillover to grazing livestock. We previously demonstrated intracerebral transmission of Norwegian reindeer CWD to sheep with prions detectable in CNS and lymphoid tissues, primarily by amplification assays [2].

To investigate transmission via the natural route, six newborn VRQ/VRQ lambs were orally inoculated with brain and spleen homogenates from CWD-infected reindeer; two lambs served as controls. Animals were monitored for up to 70 months with repeated neurological examinations and rectal biopsy collections. At necropsy, a panel of tissues was examined using histopathology, immunohistochemistry (IHC), ELISA, Western blot (WB), and amplification assays (Protein Misfolding Cyclic Amplification (PMCA) and Real Time Quaking Induced Conversion (RT-QuIC)).

Three sheep were euthanized early due to intercurrent disease. Most inoculated sheep showed inconsistent neurological signs that increased in frequency in the later stages of the study. No ante-mortem rectal biopsies were positive by IHC or PMCA. However, prions were detected by RT-QuIC and/or PMCA in gut-associated lymphoid tissues from all inoculated sheep and peripheral lymph nodes of some animals, indicating uptake via the alimentary tract. Histologically, inoculated animals showed sparse to moderate vacuolation, particularly in some white matter regions. All the inoculated sheep had amplifiable prions in one or more regions of the CNS, especially in the brainstem and thoracic spinal cord. In one of the longest living sheep, prions were detectable in several CNS regions with WB.

These findings demonstrate that Norwegian reindeer CWD can be transmitted experimentally to sheep via the oral route, with important implications for future management of areas where these species share habitat.

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25. When Prions Travel South: Gastrointestinal Dysfunction as a Route- and Strain-Dependent Feature of CWD

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Keywords: Chronic wasting disease, prion strains, gastrointestinal dysfunction, gut-brain axis

Chronic wasting disease (CWD) is a transmissible prion disease of cervids characterized by early peripheral prion replication, prominent lymphoreticular involvement, and efficient environmental shedding. Following oral exposure, prion amplification in gut-associated tissues precedes neuroinvasion, establishing the gastrointestinal (GI) tract as a primary site of entry and early replication. Beyond this role, however, whether the GI tract constitutes a site of sustained and functionally relevant pathology during prion disease progression remains largely unexplored.

We assessed GI function and tissue organization in knock-in cervidized mouse models following intracerebral (i.c.) and oral inoculation with reindeer-derived CWD prions. Across both routes of exposure, prion infection was associated with reproducible and region-specific alterations in GI physiology.

In i.c.-inoculated mice, prion disease resulted in a significant prolongation of whole-gut transit time and delayed distal colonic bead expulsion, consistent with impaired colonic neuromuscular function, while small intestinal transit remained unchanged. These functional alterations were accompanied by increased fecal pellet output and altered fecal water content under stress conditions. Anatomically, cecal enlargement and increased distal colon length were observed, in the absence of changes in small intestinal length, pointing to selective involvement of the distal gut.

In contrast, orally inoculated mice exhibited a distinct physiological profile characterized by accelerated GI transit and increased intestinal permeability, indicating that the route of exposure, and thus the spatial and temporal dynamics of prion replication, shapes GI functional outcomes.

Notably, these alterations were not uniformly observed across all prion isolates tested. While cervid CWD isolates consistently induced GI dysfunction, a Norwegian moose-derived isolate did not produce detectable changes in gut physiology under comparable conditions. This divergence suggests that GI involvement is not an intrinsic consequence of prion infection *per se*, but may instead reflect isolate-specific properties, consistent with strain- or conformer-dependent effects.

Together, these findings demonstrate that the GI tract is not only an entry and amplification site for CWD prions, but also a functionally affected system during disease. This challenges a strictly brain-centric view of CWD and supports a more integrated model in which GI dysfunction represents a variable and strain-dependent component of prion disease biology.

26. Of Mice and Deer: understanding prion conformational variability and transmission barriers in chronic wasting disease

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Keywords: chronic wasting disease, *Prnp* polymorphisms, gene-targeted mouse models, prion strains, neuroinvasion

Chronic wasting disease (CWD) affects deer, elk and moose and is the only prion disease found in both farmed and wild animals. CWD is endemic in 36 US states, 5 Canadian provinces, and has been detected in wild reindeer, red deer and moose in Norway, Finland and Sweden. In North America, the efficient transmission and significant spread of CWD is linked to extensive extraneural prion distribution including lymphatic and muscle tissue, and shedding of infectious prions in urine, saliva and feces. This contributes to direct, animal-to-animal, and indirect transmission via prion-contaminated environments.

CWD raises concerns about decline of deer populations, transmission to endangered caribou populations in Canada. Zoonotic potential cannot be excluded and is fueled by the fact that distinct strains of CWD exist. Among cervids, the prion protein gene is highly conserved; however, species-specific polymorphisms exist that modulate CWD pathogenesis. We have generated gene-targeted mouse models expressing wildtype deer, S138N caribou, or A116G white-tailed deer PrP^{C} in order to study genetic resistance and the impact of inoculation route, tissue environment of prion replication, and CWD strain-host genotype combinations on PrP^{Sc} conformational heterogeneity and CWD pathogenesis. Infection of the A116G model with two distinct CWD strains caused fast or slow pathogenesis. In mice heterozygous for the A116G polymorphism, the generation of two PrP^{Sc} types could provide insights into recruitment of PrP variants into fibrils. In the S138N model, infection with a variety of North American CWD isolates, independent of the strain, resulted in subclinical infection, whereas infection with Norwegian moose CWD induced clinical disease, highlighting the lack of absolute resistance and the strain dependence of transmission barriers. Subclinical infection was characterized by sustained fecal prion shedding and detectable seeding activity in spleens and brains. Passage of spleen and brain homogenates revealed higher attack rates and conformational variability in mice inoculated with spleen homogenates, indicating that tissue origin of prions
might modulate host range.

Overall, our studies provide insights into transmission dynamics of different CWD strains and PrP genotypes and the emergence of conformational CWD prion variants, which is critical to understand the transmission barrier to non-cervid species including humans.

27. When Scrapie Masks BSE in Co-infected Sheep: Limitations of Standard Discriminatory Tests

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Keywords: BSE, Scrapie; co-infection; Discriminatory tests; PMCA

Classical scrapie has circulated in sheep populations for centuries without apparent harm to humans, while exposure to BSE during the cattle epidemic led to zoonotic transmission cases in humans. This raised concerns about the potential presence of BSE in small ruminants. Detecting BSE in sheep is challenging due to similarities with scrapie, prompting the implementation of specific tests/procedures within the EU to differentiate between scrapie and BSE in TSE-affected sheep.
Recent experimental work demonstrated that BSE can propagate in sheep co-infected or pre-infected with scrapie and that a dominant scrapie phenotype may mask the presence of BSE in standard discriminatory analyses.
This study assessed the performance of current discriminatory methods in sheep intracerebrally co-infected with ovine BSE and three distinct natural classical scrapie isolates from French field cases.
All co-infected sheep developed clinical TSE. Standard discriminatory Western blot procedures failed to detect BSE in brain and spleen, whereas bioassays in bovine PrP transgenic mice and a BSE-oriented PMCA assay consistently demonstrated its presence in both tissues.
Using a tg650 substrate that did not amplify the scrapie isolates examined here, PMCA reached maximal analytical sensitivity after three rounds (72 h) and was approximately 900-fold more sensitive than tgBov bioassay for the reference ovine BSE isolate.
Together, these data independently validate and extend those earlier observations and support the use of BSE-oriented amplification methods as complementary tools in small-ruminant TSE surveillance.

28. Rapid generation of prion disease models using AAV-delivered PrP variants in knockout mice

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Keywords: Prion disease, AAV, Transgenic mice: AAV-mediated PrP delivery, RML, Gerstmann-Sträussler-Scheinker (GSS).

The study of prion biology has traditionally relied on transgenic mouse models, which, while valuable, require significant time and resources to develop. Our aim was to find a rapid and flexible alternative using adeno-associated virus (AAV) vectors to express modified prion proteins in PrP-knockout (PrP-KO) mice. Through systematic evaluation of multiple AAV constructs in vivo, we optimized vector design by comparing different CNS-specific promoters and regulatory elements to generate prion disease models capable of faithfully propagating the inoculated prion strain.

We identified an optimized AAV construct incorporating the human synapsin promoter, MVM enhancer, and WPRE posttranscriptional regulatory element encapsidated in the AAV9P31 serotype to drive neuron-specific expression of modified mouse PrP (W144Y epitope) and bank vole I109 PrP (W145Y epitope). Following intravenous administration, we achieved brain-wide expression at levels comparable to or even exceeding endogenous PrP in some regions. When challenged with mouse-adapted RML prions or human Gerstmann-Sträussler-Scheinker (GSS-A117V) disease-causing prions, AAV-PrP mice developed characteristic signs of prion disease with accelerated kinetics (58-106 days post-inoculation for RML; 105-112 dpi for GSS-A117V), displaying features typical of each strain. Serial transmission of AAV-generated RML prions to wild-type mice confirmed preservation of strain-specific properties (165 ± 4 dpi), validating the authenticity of prion propagation in this system. This approach provides a versatile platform for rapidly generating and studying prion variants in an authentic brain environment. By reducing model generation time from months to weeks, this system enables accelerated investigation of prion structure-function relationships, strain properties, and therapeutic strategies, with potential applications extending to other protein misfolding diseases.

29. A Comparative Analysis of Animal Prion Strains around the Globe

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Keywords: Strain typing, Scrapie, BSE, CWD, prion strain evolution

Bovine Spongiform Encephalopathy (BSE) in cattle, scrapie in small ruminants and Chronic Wasting Disease (CWD) in cervids represent animal prion diseases, caused by misfolding of the cellular prion protein (PrP^{C}) into an infectious conformer (PrP^{Sc}). Animal prion diseases are endemic in most parts of the world, often introduced through import of subclinically infected animals. PrP^{Sc} occurs in so-called prion strains, dictating disease phenotypes. However, the mechanisms of prion strain evolution are yet to be fully determined.

Therefore, this study aimed to (a) unravel the global diversity of field prion strains and (b) get new insights into prion strain evolution. Using the transgenic ovinized Tgshp IX mouse model (ARQ-genotype), an in-depth characterization of atypical and classical BSE, classical sheep and goat scrapie isolates from Europe, Canada and Libya, and Canadian and European CWD isolates was performed. This analysis allowed evaluation of the interspecies transmission potential of BSE and CWD isolates. After intracerebral inoculation, the lesion and PrP^{Sc}-profile in mouse brains was comparatively analyzed using histopathology and immunohistochemistry.

While classical BSE induced a 100% attack rate in Tgshp IX mice, indicating a high interspecies transmission potential, transmission of the atypical BSE isolates remained incomplete. CWD isolates of Canadian elk, moose and red deer, as well as two Swedish moose isolates, transmitted after first passage, suggesting a moderate interspecies transmission potential. Classical scrapie isolates easily transmitted, however, with differences in incubation periods and attack rates. While in all isolates, the histopathologic lesion-profile alone provided insufficient data for strain discrimination, combining it with the immunohistochemical PrP^{Sc}-profile, particularly in corpus callosum and cerebellum, enabled an in-depth evaluation. Taking attack rates, incubation periods and geographical metadata into account, clear discrimination of multiple prion strains was possible considering the different continents involved.

In this study, the Tgshp IX mouse model, provided reliable prion strain typing data, revealing a global diversity of field prion isolates, some with interspecies transmission potential. Thus, besides host genetics, the geographical origin might influence prion strain evolution. Therefore, this studies' results highlight the importance of a continuous active surveillance of animal prion diseases and a consequent in-depth evaluation of novel field prion isolates.

30. Emergence of minor prion strains following heat treatment

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The emergence of bovine spongiform encephalopathy (BSE) is caused the feeding of ruminant meat and bone meal (MBM) to cattle. Changes in the rendering process of MBM are thought to allow for the survival of prions. Consistent with this observation is the relatively high heat resistance of murine adapted BSE. Subsequent studies on heat inactivation profiles of ME7 murine-adapted scrapie suggested the emergence of a heat adapted prion strain. In the current study, we explored the hypothesis that prions exist as a dynamic mixture of strains by further investigating the consequences of incomplete heat inactivation on emergence of minor prion strains. To test this hypothesis, we exposed biologically cloned DY TME to temperatures ranging from 85-105°C for 24 hours, amplified residual prions with protein misfolding cyclic amplification (PMCA), and probed for the emergence of minor strains that we term, the heat strain selection assay (HSSA). As a negative HSSA control, uninfected hamster brain homogenate was incubated at 85, 95 or 105°C. Western blot analysis of the second round of PMCA failed to detect PrP^{Sc}. Next, we probed DY TME for the presence of minor strains with HSSA. We defined the identification of a non-DY minor strain as PrP^{Sc} that is immunoreactive with both 12B2 and 3F4 in contrast with the parental DY PrP^{Sc} that only immunoreacts with 12B2. Treatment of DY TME with HSSA at 85, 95 or 105°C either i) failed to detect PrP^{Sc} ii) detected PrP^{Sc} with DY migration properties or iii) detected PrP^{Sc} that differed from DY TME (i.e., 12B2 and 3F4 positive). Intracerebral inoculation of these minor strains into hamsters resulted in incubation periods and clinical signs that differed from DY TME. Minor strain PrP^{Sc} had greater thermostabilities and intraspecies PMCA conversion efficiencies compared to the parental strain, DY TME. Significantly, in contrast to DY TME, all minor strains were lymphotropic. Overall, these observations suggest minor prion strains can emerge following heat exposure with greater transmission efficiency compared to the parental strain and may contribute to the emergence of lymphotropism.

Keywords: Prion strain, Prion evolution, Environmental prions

31. Scrapie strain 87V mutates into CH1641 on transmission to ovine PrP transgenic mice

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Keywords: Prion Protein (PrP) , scrapie, 87V, PrP transgenic mice, prion mutation

The scrapie strain 87V was originally isolated in the UK in the 1970s from a natural case of scrapie in a crossbred Cheviot and Border Leicester sheep through multiple intracerebral (i.c.) passages in VM mice (homozygous for the *Sinc^{p7}/ Prnp^{b}* allele).

We inoculated cloned murine 87V into ovine and bovine PrP transgenic mice and compared the incubation periods, immunohistochemical PrP^{Sc} profiles and biochemical PrP^{res} profiles in the brain of these mice with that of other scrapie strains from our reference database.[1] After transmission of 87V to both VRQ and ARQ ovine PrP transgenic mice, the resulting strain showed the same immunohistochemical PrP^{Sc} profile and biochemical PrP^{res} profile as CH1641 in ovine PrP transgenic mice. In addition, stabilized incubation periods in ovine PrP transgenic mice were not statistically different from those of CH641. Passage from ovine PrP transgenic mice at stabilization back into VM mice did not result in transmission, indicating to an inability to re-isolate 87V and to a complete mutation of 87V into CH1641 which itself does not transmit to VM mice.[2]

Sisó et al. inoculated sheep homozygous for the VRQ or the ARQ allele with cloned murine 87V.[3] Oral inoculation of 87V in sheep was not successful and combined oral/s.c./i.c. inoculation only led to infection of the central nervous system but not of the lymphoid system. Studies of PrP^{Sc} from the sheep brain showed an immunohistochemical profile, truncation site and biochemical profile of PrP^{Sc} that was similar to CH1641. Inoculation of the infected sheep brain directly back into VM mice resulted in re-isolation of 87V on primary passage with prolonged incubation periods and low attack rates, while inoculation into VRQ ovine PrP transgenic mice showed a 100% attack rate and an incubation period on primary transmission similar to that of ovine CH1641.[4]

The finding that 87V is not present in lymphoid tissues of sheep and mutates into CH1641 in both VRQ and ARQ ovine PrP transgenic mice as well as in homozygous VRQ and ARQ sheep, raises the question whether 87V is truly a naturally occurring scrapie strain in sheep or merely a mouse adapted/ mutated prion strain.

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32. TGVOLE(I109)1X FOR PRION BIOASSAYS: BASELINE DEFINITION OF SPONTANEOUS NEUROPATHOLOGY TO ENSURE ACCURATE INTERPRETATION

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Keywords: prion, Bank Vole, transgenic mouse model, bioassay, phenotype

Bank voles are highly permissive hosts for prion propagation and act as near-universal acceptors of prions, markedly reducing transmission barriers (1,2). Transgenic mice expressing bank vole prion protein (BV PrP^C) further enhance this utility by combining broad strain susceptibility with shorter incubation times and the practical advantages of a mouse model (3,4). However, TgVole(I109)1x mice may also develop spontaneous prion disease at older ages, representing an important limitation for bioassay interpretation and a potential confounder in serial passage studies.

Here, we performed a comprehensive characterization of the spontaneous phenotype in TgVole(I109)1x mice, including age at onset, penetrance, biochemical profile, and neuropathological features. Brain tissue was divided sagittally, with one hemisphere analysed by Western blot and the other processed for histopathology and immunohistochemistry to assess spongiform degeneration, PrP deposition, and neuroinflammatory responses using GFAP and IBA1 staining. We also evaluated the transmissibility of the spontaneous strain on second passage within the same model, compared spontaneous strains arising in TgVole mice expressing BV PrP^C at different levels (1x versus 4x), and assessed the outcome of co-propagation with a classical prion strain (CWD-vole).

Together, these analyses define the baseline spontaneous phenotype of TgVole(I109)1x mice and provide a framework for distinguishing true transmission events from background spontaneous disease. This work strengthens the reliability of TgVole(I109)1x mice as a model for prion bioassays and should improve interpretation of both primary and serial passage experiments.

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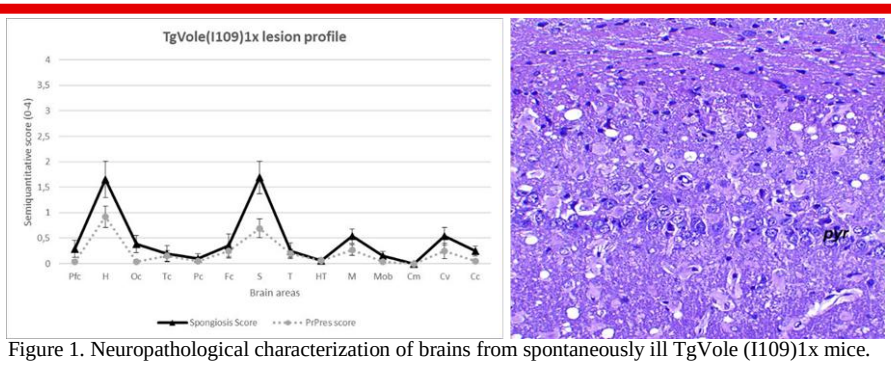

Figure 1. Neuropathological characterization of brains from spontaneously ill TgVole (I109)1x mice. Graph indicates mean semiquantitative scores ± standard error of the mean (s.e.m.) (0 = absence, 4 = maximum intensity) of spongiform change (solid black line) and PrPres immunohistochemical deposition (dashed grey line). Fourteen brain regions were assessed: piriform cortex (Pfc), hippocampus (H), occipital cortex (Oc), temporal cortex (Tc), parietal cortex (Pc), frontal cortex (Fc), striatum (S), thalamus (T), hypothalamus (HT), mesencephalon (M), medulla oblongata (Mob), cerebellar nuclei (Cm), cerebellar vermis (Cv), and cerebellar cortex (Cc). Image shows the hippocampus region stained with hematoxylin and eosin (H&E) for lesion assessment. Note moderate spongiosis, evident gliosis and loss of pyramidal cell layer (*pyr*).

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33. Validation of RT-QuIC and Nano-QuIC for the Antemortem Detection of Chronic Wasting Disease in Experimentally Infected White-Tailed Deer (*Odocoileus virginianus*)

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Keywords: white-tailed deer (WTD; *Odocoileus virginianus*), chronic wasting disease (CWD), RT-QuIC, Nano-QuIC, validation

Background: The continued spread of chronic wasting disease (CWD) among white-tailed deer (WTD; *Odocoileus virginianus*) requires the development of highly sensitive, antemortem diagnostic tools. The University of Minnesota Center for Prion Research and Outreach (MNPRO) is validating real-time quaking-induced conversion (RT-QuIC) and nanoparticle-enhanced RT-QuIC (Nano-QuIC) using samples from an ongoing longitudinal USDA study involving experimentally CWD-inoculated WTD.
Methods: Thirty WTD were group-housed (one inoculated, two non-inoculated per room; n=10 rooms) at USDA facilities. Rectoanal mucosal-associated lymphoid tissue (RAMALT) samples were collected at regular intervals and at the time of death. MNPRO laboratory researchers remained blinded to inoculation status during testing and analysis. RAMALT tissues were tested by RT-QuIC and Nano-QuIC at two dilutions each (10^{-2} (1:10 w:v homogenate with an additional 1:10 dilution in buffer) and 10^{-3} (1:10 w:v homogenate with an additional 1:100 dilution in buffer)). Several assay metrics were analyzed (area under the RT-QuIC curve (AURC), maxpoint ratio (MPR), and max slope (MS)) to statistically determine if seeding activity was detected in each sample.
Results: Using ROC analysis, both assays showed high diagnostic accuracy at the 10^{-3} dilution, with AUC values ranging from 0.915-0.966 for RT-QuIC and 0.881-0.978 for Nano-QuIC when assessing AURC, MPR, and MS. While RT-QuIC consistently outperformed Nano-QuIC at the 10^{-2} dilution, there was a marked decrease in sensitivity and specificity for both assays at this dilution, with AUCs decreasing to 0.723-0.867 for RT-QuIC and 0.546-0.647 for Nano-QuIC. Preliminary results suggest that both assays can detect seeding activity in antemortem RAMALT samples from inoculated and contact animals as early as 6 months post exposure.
Conclusion: These results demonstrate that seeded amplification assays provide reliable and sensitive methods of CWD detection. In WTD antemortem RAMALT homogenates at a 10^{-3} dilution, RT-QuIC and Nano-QuIC provide a diagnostically accurate method for CWD detection. By validating these tools against known infection status and ELISA-confirmed samples, this work highlights the reliability of RT-QuIC and Nano-QuIC for antemortem CWD surveillance.

34. *In vitro* evaluation of Jamaican fruit bat as a potential vector of prion diseases

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Keywords: Bats, Interspecies transmission, Transmission barrier, CWD, PMCA

Prion diseases are fatal neurodegenerative disorders affecting mammals caused by PrP^{Sc}, the misfolded version of PrP^{C}, the cellular prion protein, which is capable of self-propagation and occasional cross-species transmission. Among them, chronic wasting disease (CWD), which affects cervids, continues to expand geographically in North America, with new forms of the disease are emerging in Europe, raising concerns about host range and zoonotic potential. Bats are highly mobile mammals that frequently interact with diverse ecosystems and species, yet their susceptibility to prion infection remains largely unexplored.

In this study, we evaluated the capacity of 24 distinct natural prion isolates and 14 synthetic prions to adapt to Jamaican fruit bats (*Artibeus jamaicensis*) using protein misfolding cyclic amplification (PMCA) with bat brain homogenate as substrate. Only three natural isolates successfully adapted to the Jamaican fruit bat substrate, suggesting a substantial species barrier with selective permissiveness. Interestingly, all 14 synthetic prions showed a high propensity to misfold bat PrP *in vitro*, independently of the amino acid sequence of the seed.

To assess potential public health implications, we further evaluated the ability of bat-adapted prions to propagate in substrates from gene-targeted mice expressing the prion protein of different species. The resulting *in vitro* transmissibility to substrates from distinct species differed from the original isolates, suggesting a complete adaptation to Jamaican fruit bat PrP. Together, our findings contribute to a better understanding of cross-species prion adaptability and the potential role of bats in prion disease ecology.

35. *In vitro* detection of Caprine BSE in asymptomatic PrP humanized mice after inoculation with negative goat tissues

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Keywords: BSE; PRNP; Codon 222; PMCA

Abstract

Transmissible spongiform encephalopathies (TSEs) are fatal neurodegenerative diseases caused by misfolded prion protein (PrPSc). Naturally occurring bovine spongiform encephalopathy (BSE) has been reported in goats [1], raising concerns about zoonotic risk and the influence of caprine PRNP polymorphisms such as codon 222 (Q/K). Here we evaluated the infectivity of goat tissues and fluids from animals intracerebrally challenged with caprine BSE (second passage) or bovine BSE, including Q222Q, Q222K and K222K genotypes.

Human PrP transgenic mice homozygous for Met129 (Tg340) were inoculated intracerebrally with obex, skeletal muscle, liver, whole blood, white blood cells and selected milk fractions. Transmission was assessed by clinical follow-up and detection of PrPres by immunohistochemistry and Western blot. To explore low-level infectivity, brains from clinically healthy and conventionally negative mice were analysed by protein misfolding cyclic amplification (PMCA) using TgBov substrate.

Obex-derived inocula transmitted efficiently, and caprine BSE obex isolates showed higher transmission capacity than bovine BSE obex isolates as previously described in other studies [2] (attack rate 80% [14/18] vs 40% [4/10]). Within caprine BSE obex groups, Q222Q donors yielded higher attack rates than K-containing donors (6/6, 5/6 and 3/6 for Q222Q, Q222K and K222K, respectively). Neuropathological and molecular features in Tg340 mice were consistent with a vCJD-like phenotype (florid plaques and a characteristic PrPvCJD banding pattern). While peripheral tissues and fluids remained negative by conventional assays, PMCA detected prion seeding activity in brains from subclinical mice inoculated with caprine BSE-derived semitendinosus muscle, triceps brachii muscle, liver and whole blood, but not in mice inoculated with bovine BSE-derived peripheral inocula.

These findings support genotype-dependent transmission efficiency and peripheral prion distribution in goats, and highlight PMCA as a sensitive approach for detecting low-level prion infectivity relevant to food-safety risk assessment.

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36. From survival extension to neuroprotection: New findings on AAV9-mediated gene therapy

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Keywords: Gene therapy, scrapie, AAV9, G127V

Developing an effective treatment for prion diseases remains a major challenge, as conventional pharmacological approaches have proven ineffective. We propose gene therapy as a novel strategy to counteract prion propagation. Specifically, our approach involves introducing a mutation that confers dominant-negative resistance to prion protein misfolding, previously described in humans [1,2], by means of an adeno-associated virus type 9 (AAV9) vector capable of crossing the blood–brain barrier. This mutation consists of replacing glycine (G) with valine (V) at codon 130 of the ovine PrP sequence.

In the initial phase of this study, prion-infected Tg338 mice were treated with the AAV9 vector via various routes and regimens (single or multiple doses). Survival analysis revealed an extension ranging from 6.23% to 47.29% compared with corresponding positive controls. The greatest effect was observed in orally infected mice treated intravenously with two doses. However, neuropathological analyses at the terminal stage showed no significant differences in prion deposition or neuroinflammatory markers between groups.

These findings prompted a second phase focused on characterizing early pathological events. In this follow-up study, treated mice and their corresponding controls were analyzed during preclinical stages to assess whether the therapeutic effect is associated with altered neurodegeneration onset. Preliminary histopathological evaluation indicates that treated animals display reduced spongiform degeneration compared with controls, suggesting a potential delay in disease progression prior to clinical onset.

Overall, our results reinforce the potential of AAV9-mediated delivery of the G127V (V130 in ovine sequence) PRNP mutation as a promising gene therapy approach for prion diseases. Ongoing work will further characterize molecular markers of prion replication and neuroinflammation during asymptomatic stages to elucidate the underlying mechanisms of resistance.

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37. Modeling Chronic Wasting Disease Spillover into Livestock Using PrP-Transgenic Mice

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Keywords: chronic wasting disease, species barrier, prion strains, livestock species.

Chronic wasting disease (CWD) is a transmissible prion disease of cervids that has expanded across North America in recent decades and has more recently emerged in Europe. Its environmental persistence and strain diversity raise concerns about its ability to cross species barriers. Potential exposure of livestock, such as cattle and sheep, highlights a plausible risk of adaptation to new hosts, with important implications for animal health and for humans in close contact with these species.

In this study, we evaluated the ability of a panel of CWD prion isolates, derived from various cervid species, to infect livestock species using transgenic mouse models overexpressing either bovine PrP (BoPrP-Tg110) or ovine PrP (Ov_{VRQ}-Tg338 and Ov_{ARQ}-Tg501). Mice were intracranially inoculated with CWD isolates originating from North America and Europe. In parallel, the *in vitro* propagation potential of these isolates was assessed using protein misfolding cyclic amplification (PMCA).

Our preliminary findings, with several experiments still ongoing, indicate that some CWD isolates can overcome species barriers and establish infection in livestock-PrP contexts, albeit with variable transmission efficiency. While some isolates failed to propagate even after serial passage, others adapted, showing differences in attack rates, survival times, PrP^{res} biochemical properties, and histopathology. These results suggest that adaptation to new hosts is dynamic and strain-dependent, and is driven by host-PrP compatibility.

Consistent with these findings, PMCA results demonstrated selective and efficient amplification in certain livestock-PrP substrates, supporting the capacity of certain isolates to undergo conformational adaptation in these heterologous environments.

Taken together, these results support the possibility that CWD prions are not static entities but can evolve through interactions with new host-PrP contexts, enabling certain variants to breach interspecies barriers and adapt to livestock species. Given the central role of cattle and sheep in the food chain, their close interaction with humans, and the possibility that passage through a second species may modify their ability to infect humans, these adaptations represent a plausible risk for animal and public health. These findings underscore the need for further investigation into the mechanisms governing CWD adaptation, transmission dynamics, and its potential zoonotic implications.

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38. Neurofilament Light Chain Quantification for *In Vivo* Diagnosis of Animal TSEs

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Keywords: Neurofilament light chain (NfL), Prion diseases, Bovine spongiform encephalopathy (BSE), Goats, RT-QuIC, Ante-mortem diagnosis

Transmissible spongiform encephalopathies (TSEs) are fatal neurodegenerative diseases characterized by prolonged incubation periods during which infected animals remain clinically asymptomatic, severely limiting the effectiveness of surveillance and control strategies in livestock. Current diagnostic approaches rely primarily on post-mortem detection of pathological prion protein in the central nervous system [1]. Although prion amplification assays such as real-time quaking-induced conversion (RT-QuIC) enable ultrasensitive detection of prion seeding activity in cerebrospinal fluid (CSF) [1], their application in field surveillance remains constrained by the invasiveness of CSF sampling and the lack of scalable blood-based diagnostics.

In this study, we evaluated whether neurofilament light chain (NfL), a biomarker of neuroaxonal injury [2], could provide a minimally invasive indicator of prion-associated neurodegeneration in goats experimentally challenged with classical (n=4 via i.c.) and atypical bovine spongiform encephalopathy (BSE) (n=5 i.c., n=3 o.s.). NfL concentrations were quantified longitudinally in serum and CSF using ultrasensitive single-molecule array (Simoa) technology [2] and interpreted alongside prion seeding activity detected in CSF by RT-QuIC.
RT-QuIC confirmed the presence of prion seeding activity in CSF during the asymptomatic phase of infection, demonstrating early central nervous system involvement. In parallel, NfL concentrations progressively increased in infected goats and showed a strong correlation between serum and CSF levels. Temporal analyses revealed route and strain-dependent dynamics of neuroaxonal damage, with earlier and more pronounced NfL elevations following intracranial infection. ROC analyses demonstrated high diagnostic accuracy, with serum NfL achieving >83% sensitivity and 100% specificity at the optimal cut-off.
Collectively, these results represent one of the first longitudinal integration of prion seeding assays with fluid biomarkers of neuronal injury in a goat BSE model, linking prion replication dynamics with downstream neurodegeneration [3]. They support a complementary diagnostic framework in which blood-based NfL functions as an early screening marker to identify animals undergoing prion-associated neurodegeneration. Confirmation of infection could subsequently rely on prion-specific assays such as RT-QuIC. This integrated approach may offer a practical pathway toward scalable ante-mortem surveillance of TSEs in small ruminants.

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39. Detection of PrP^{Sc} in extra-neural tissues from cattle with natural L-BSE by RT-QuIC.

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Keywords: Prion diseases, BSE, RT-QuIC, L-BSE, BASE.

Three BSE strains are now known: Classical BSE (C-BSE) and two atypical forms called High BSE (H-BSE) and Low BSE (L-BSE or BASE). C-BSE is the best known,also with regards to its zoonotic potential, while there is a number of missing information on the atypical ones, including the possible peripheral distribution of PrP^{Sc} in cattle. To investigate this aspect experimental transmissions were performed in the past by different research groubut no information is available on naturally occurring cases. Epidemiological data also suggest that cases of atypical BSE arise spontaneously and therefore their eradication is impossible.

As stated in the EFSA Opinion [1], investigations, using highly sensitive methods, into the distribution of pathological prion protein (PrP^{Sc}) and infectivity in peripheral tissues from cattle affected by atypical BSE is needed to confirm the experimental data and to evaluate the risk of contaminated meat entering the food chain. The methods reccomended by EFSA include: the Protein Misfolding Cyclic Amplification (PMCA) and the Real-Time Quaking Induced-Conversion (RT-QuIC).

The purpose of this study was to develop and standardize suitable protocols to investigate the distribution of PrP^{Sc} in the peripheral tissues from cattle with L-BSE using RT-QuIC assay.

RT-QuIC is a method based on the in vitro amplification of PrP^{Sc} using recombinant PrP substrates and revealing the seeding activity by a fluorescent stain with Thioflavin T, able to intercalate in the amyloid fibrils.

In this study, RT-QuIC analyses were carried out on the brain, several peripheral tissues and edible organs collected from Italian cattle with L-BSE.

The PrP^{Sc} presence, defined as "seeding activity", was observed in the skeletal muscles of cattle with L-BSE, while no activity in other tissues and organs was detected, suggesting a neuromuscular tropism of L-BSE agent.

According to our previous experimental studies, although it has not been possible to quantify the pathological marker, it is presumable that the skeletal muscles are infectious in L-BASE cases.

These findings confirm the need to apply very accurate surveillance in the bovine population to promptly identify atypical BSE cases and eliminate them from the food chain.

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40. Evolution of Nor98/atypical scrapie through transmission in a homologous PrP^{C} context

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Keywords: Nor98/atypical scrapie, prion evolution, c-BSE, species barrier

Nor98/atypical scrapie (AS) is an idiopathic prion disease that occurs sporadically in sheep and goats. Previous studies have shown that transmission of AS to heterologous species can result in the emergence of classical PrP^{res} prion strains, including classical BSE (c-BSE)-like strains in cattle and pigs and classical scrapie-like strains in bank voles. The emergence of c-BSE in bovine hosts is driven by the conformational shift of the AS conformers. Although the emergence of distinct scrapie strains during intra-species transmission has been reported, the potential evolution of AS prions within the same species remains poorly understood.

To address this, we investigated the transmission of a diverse panel of AS isolates from sheep and goats, differing in genotype and geographical origin, in a homologous ovine PrP-context. Using ARQ-ovPrP-Tg501 mice, we performed both *in vivo* transmission studies and *in vitro* PMCA analyses. All isolates efficiently infected ARQ-ovPrP-Tg501 mice, showing complete attack rate and homogeneous survival times. Remarkably, some transmissions led to the emergence of distinct prion strains, including 19 kDa (BSE-like), 21 kDa (classical scrapie-like), and mixed PrP^{res} profiles. Consistently, PMCA propagation in ARQ-ovPrP-Tg501 substrate also resulted in the emergence of classical PrP^{res} prions.

The appearance of classical prions following AS transmission in an ovine-PrP context may reflect either the conformational shift of AS prions during propagation or the coexistence of multiple strains within the original isolates, which could have evolved within the original host. These findings support the hypothesis that atypical prions may constitute a source of prion diversity with a propensity to evolve into classical forms. Understanding this process is essential for assessing the risks associated with AS transmission in livestock and its implications for prion disease surveillance and control.

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41. Assessment of Prion Distribution in Reproductive and Peripheral Tissues of Scrapie-Infected Mice

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Keywords: Scrapie, Oocytes, Vertical transmission, VRQ genotype, PMCA

The transmission dynamics of classical scrapie in small ruminants involve complex pathways, particularly regarding maternal transfer [1–6]. While horizontal dissemination is considered the primary route of infection, vertical transmission represents a significant gap in current epidemiological knowledge. Investigating these mechanisms in the highly susceptible VRQ/VRQ genotype is frequently hindered by the scarcity of natural cases resulting from strict disease eradication policies [7]

This research utilized Tg338 transgenic mice (which overexpress the ovine VRQ/VRQ PrP^{C}) as a high-fidelity model to evaluate prion accumulation within the female reproductive system and other peripheral tissues. Following intracerebral inoculation with the Dawson (PG127) scrapie strain, various tissues were harvested at the terminal stage of the disease. To identify minute concentrations of the pathological prion protein (PrP^{Sc}), the study employed Protein Misfolding Cyclic Amplification (PMCA), an ultrasensitive tool capable of detecting titers below the threshold of standard assays such as immunohistochemistry or western blot.

The findings demonstrate a consistent presence of prions within the reproductive tract, with PrP^{Sc} detected in 100% of oocyte samples and 66.7% of analyzed ovaries. Densitometric analysis using Mean Gray Values (MGV) revealed that uterine tissue harbors a significantly higher prion load than the ovaries and oocytes, reaching levels comparable to those found in skeletal muscle. These observations indicate that while the germline is a viable reservoir for infection, the uterus may serve as a major focal point for maternal spread. Additionally, PrP^{Sc} was detected in all other peripheral organs analyzed, with intermediate accumulation in the spleen and liver, while the kidney exhibited the lowest levels.

In conclusion, the detection of prions in oocytes and reproductive tissues supports the hypothesis of a potential germline route for scrapie transmission. This study highlights how tissue composition and innervation influence peripheral prion distribution, although lower signals in blood-rich organs such as the kidney or spleen might be attributed to PMCA inhibition. Our findings underscore the critical need for ultrasensitive diagnostic methodologies to track peripheral distribution and the relevance of reproductive pathology in disease control programs

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42. BIOASSAY OF A CLASSICAL SCRAPIE OUTBREAK IN THE UK FROM 2019

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Keywords: Scrapie, Strain, Bioassay, Outbreak, IHC profile.

Introduction: Classical Scrapie (CS) in the UK has become a rare disease following the National Scrapie Plan efforts during the 2000s. Since 2010, only few outbreaks have taken place, with the most recent occurring in May 2025. Here we report on the bioassay characteristics of an outbreak that happened in 2019, where from a total of 820 animals, 9 (7 ARQ/VRQ and 2 ARH/VRQ) were found to be scrapie positive via ELISA, IHC and/or Western Blot.
Materials: Inocula was prepared from CNS of the 9 positive animals and was inoculated under general anesthesia to transgenic mice tg338 (ovine VRQ/VRQ) or tgShpXI (ovine ARQ/ARQ). Once clinically positive for TSE, they were euthanized, and brains gathered in fixative (2/3 via parasagittal cut) and frozen (remaining 1/3 of brain).
Survival time, and PrPSc deposition patterns were analysed from the mice to determine the CS strain present during this outbreak. Lesion profiles are being processed and will be reported with the poster. TgShpXI bioassay is ongoing so only the survival times are reported.
Results: The inocula produced 2 clearly different groups as shown in Figure 1. Group 1, where attack rates were high in tg338 (87.5-100%) and low for tgShpXI (40%); survival time was 449±63 days in tg338 mice, and 653.5±126.1 days in tgShpXI. Group 2 had much lower attack rates (25-50%) and higher survival times (686.1±131.6 days post-inoculation) in tg338, and no tgShpXI were affected.
PrP^{Sc} deposition was diffuse in group 1, with deposits extending throughout the neuraxis, with coarse punctate deposits predominantly; while the group 2 showed a much more restricted distribution, affecting medulla, cerebellar nuclei, midbrain and thalamus with a mostly granular and coalescing pattern.
Discussion: The current study suggests presence of 2 strains of CS in the 2019 UK outbreak of CS. Both genotypes (ARQ/VRQ and ARH/VRQ) are found in each group, so differences cannot be attributed to PrP genetics. The survival time and IHC patterns for these strains do not match historical data for CS cases from the UK [1].

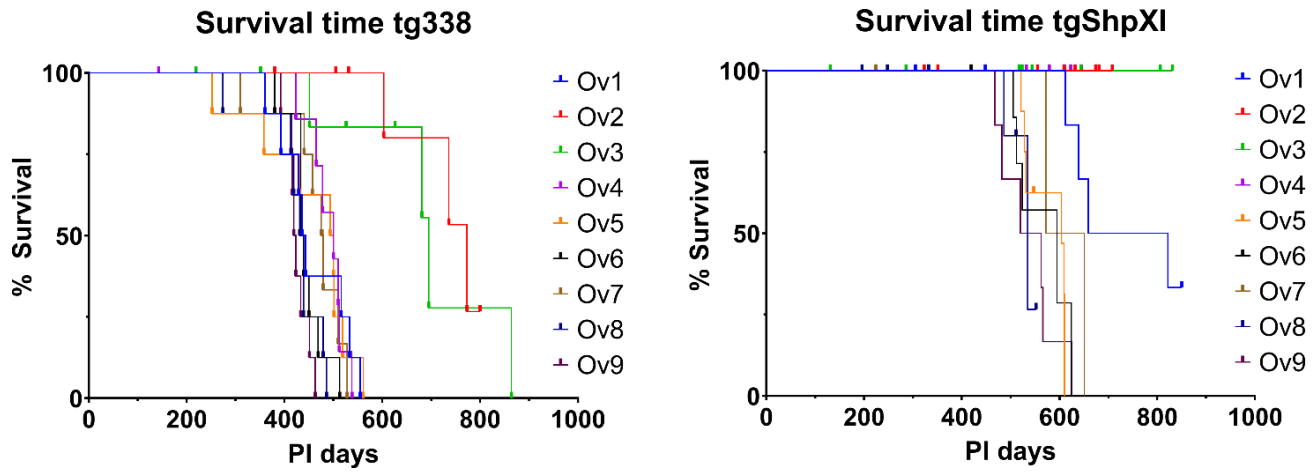

Figure 1. Survival time tg338 vs tgShpXI

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43. Evaluation of rapid tests and western blot confirmatory methods to detect PrP^{Res} in TSE-positive dromedary camels

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Keywords: TSEs, PRION DISEASES, CAMEL PRION DISEASE, RAPID TEST, WESTERN BLOT

Transmissible Spongiform Encephalopathies (TSEs) have been identified in several animal species (cattle, sheep, goats, deer) and, in the past few years, also in dromedary camels in Algeria[1]. With regards to this species, a recent diagnostic study performed on animals with neurological symptoms has revealed six cases in Tunisia as well. The detection of the pathological prion protein (PrP^{Res}) in the central nervous system and lymphoid tissues of these animals further suggests that the disease is highly likely to be transmitted to other species, as it has been observed in classical Scrapie and Chronic Wasting Disease. These findings raise several epidemiological and diagnostic issues, in the latter case it is important to evaluate the ability of diagnostic methods to identify PrP^{Res} with a view to the implementation of possible active surveillance systems.
Our goal was to evaluate the performance of rapid and confirmatory methods to detect PrP^{Res} in terms of analytical sensitivity in TSE-positive dromedary camels.
Three EC-approved rapid tests for cattle and small ruminants, commercially available, were evaluated: the HerdChek BSE-Scrapie Antigen Test (Idexx); the TeSeE SAP Combi Kit (Bio-Rad) and the BetaPrion BSE EIA Test (Roboscreen). Their analytical sensitivity was compared with two confirmatory western blot (WB) methods, TeSeE™ Western Blot (Bio-Rad) and a SAF-Immunoblot developed at the Italian Reference Laboratory. The brain samples from six natural TSE cases were included in this study and the analyses with the above commercial kits were performed according to the manufacturer's manual.
Idexx and Bio-Rad rapid tests were able to detect the presence of PrP^{Res} although with a different analytical sensitivity, higher for the former and lower for the latter test. No PrP^{Res} was detected in the samples analyzed by the Roboscreen test. Both confirmatory western blot methods were able to show the PrP^{Res} signals in all dilutions of the samples detected by the screening methods. The results obtained from this study revealed that the diagnostic methods applied for the active surveillance of BSE and Scrapie in Europe were also able to identify the presence of PrP^{Res} in TSE-positive dromedary camel cases with high analytical sensitivity except one screening test.

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44. Epidemiological situation of scrapie in Spain (2020–2024) in the framework of the European Union

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Keywords: classical scrapie, atypical scrapie, Spain, prion, strain.

Scrapie remains an endemic transmissible spongiform encephalopathy in small ruminants within the European Union (EU), despite long-standing surveillance and control measures. This work analyses the epidemiological situation of scrapie in Spain from 2020 to 2024 and places national findings in the EU context.

In Spain, data from 2020–2024 show a declining circulation of classical scrapie (CS), alongside a low and stable occurrence of atypical scrapie (AS). In sheep, CS cases decreased from 244 (2020) to 151 (2024), although annual figures fluctuated. In goats, CS occurred at lower levels and declined from 29 cases (2020) to 14 cases (2024). By contrast, AS showed a similar epidemiological pattern in both species, characterised by low and relatively stable numbers of sporadic index cases and no evidence of sustained intra-herd transmission.

At EU level, CS cases in sheep and goats declined from 589 ovine and 319 caprine cases (2020) to 380 ovine and 65 caprine cases (2024) (−35% and −80%, respectively). AS maintained stable numbers in both species. In 2024, CS accounted for 84% of all scrapie cases in small ruminants, whereas AS represented 16%. CS cases remained highly concentrated geographically in a few countries, with Spain reporting between 20 to 40% of ovine CS cases and 10 to 20% of caprine CS cases over the 5 year period.

In conclusion, although the Spanish scrapie situation broadly reflects EU-wide trends, Spain continues to contribute substantially to the EU's overall burden, underscoring the need for sustained surveillance and genetic resistance programmes.

45. TSEs Surveillance in Europe: Evaluation of NRL Performance in Immunohistochemistry

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Keywords: EURL, BSE, scrapie, immunohistochemistry, brainstem

Integration of active surveillance, through systematic testing of target animal populations, and passive surveillance, via notification of suspect clinical cases, is fundamental for comprehensive Transmissible Spongiform Encephalopathies (TSEs) monitoring and early detection across the EU. Within this EU surveillance framework, the European Reference Laboratory (EURL) for TSE, designated in 2017 as a consortium of the Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta (IZSPLVA, Turin) and the Istituto Superiore di Sanità (ISS, Rome), coordinates and harmonizes TSE diagnostics across EU Member States and oversees reference material management, strain characterization, epidemiological monitoring, outbreak support, and laboratory training.

As part of its quality assurance activities, the EURL organizes an annual external quality assessment (EQA) to evaluate the technical performance of immunohistochemical (IHC) assays across NRLs operating under their respective validated in-house protocols.

Here we present the results of the last EQA held in 2025. Overall, 19 NRLs from 19 different countries participated. The technical round included samples from small ruminants with classical scrapie (obex and lymph node) and atypical scrapie (obex and cerebellum), as well as cattle with classical BSE (obex) and negative controls. Staining intensity was scored on a semi-quantitative scale from 1 (weak) to 3 (strong) using the EURL reference scoring as benchmark. Submission, reporting, and collation of results were centrally managed by the EURL.

All EQA participants except one correctly immunolabeled the samples, indicating satisfactory performance. Across laboratories, mean and median values were calculated to summarize results. Staining intensity was consistently high (mean 2.4–2.7; median 3.0), with a slight decrease observed for atypical scrapie at the obex (median 2.5). One NRL failed to immunolabel the atypical scrapie obex and the lymph node. Additionally, eight laboratories exhibited a higher level of background staining. Taken together, these findings provide further insight into inter-laboratory variability and highlight areas for ongoing harmonization and technical refinement.

The 2025 EQA round confirmed a high level of concordance and consistency among NRLs, demonstrating continued harmonization of TSE diagnostics across EU Member States. Overall, most laboratories met performance criteria, supporting the effectiveness of the EURL scheme in maintaining diagnostic quality and guiding ongoing training efforts.

46. Surveillance and characterization of Atypical Bovine Spongiform Encephalopathy cases in the United Kingdom between 2020 and early 2026

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Keywords: Atypical BSE, H-Type & L-Type

Classical Bovine Spongiform Encephalopathy (C-BSE) emerged in the 1980s, causing a global epidemic linked to the consumption of contaminated meat and bone meal in cattle. This prion disease demonstrated zoonotic potential, resulting in variant Creutzfeldt–Jakob disease (vCJD) in humans. Following its identification, enhanced surveillance led to the detection of atypical BSE forms (H- and L-type) in 2004, which are considered to arise spontaneously in older cattle.

In Great Britain, BSE surveillance comprises passive and active components, with active surveillance focused on fallen stock and emergency slaughter cattle over 48 months of age. This study describes five atypical BSE cases identified between 2020 and early 2026. During this period, 634,699 cattle were tested, yielding four H-type and one L-type case. All were detected between 2023 and early 2026 following the introduction of a different rapid screening test, whereas none were identified between 2019 and 2022, suggesting improved detection rather than increased incidence. Cases were identified through fallen stock surveillance and confirmed by discriminatory Western blot analysis. Affected animals were aged 8–17 years and predominantly presented with non-specific clinical signs, most commonly recumbency or difficulty rising. Genotyping of the PRNP open reading frame identified no unusual polymorphisms, including absence of the E211K mutation, a variant analogous to the E200K mutation associated with hereditary Creutzfeldt–Jakob disease in humans.

Although atypical BSE is not currently considered a public health risk, important gaps remain in understanding its pathogenesis. Continued surveillance, particularly in fallen stock with a history of recumbency, is essential to monitor changes in prevalence. Increasing awareness of clinical presentation will support case detection through passive surveillance, particularly in the context of potential reductions in active surveillance. Collection of additional material from naturally occurring atypical cases will be critical to improve understanding of pathology, prion protein distribution, and disease pathogenesis.

47. Genetic Diversity of *PRNP* Gene in Portuguese Goats: Baseline Data for Breeding Resistance to Classical and Characterization of Atypical Scrapie

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Keywords: *PRNP* polymorphisms, Classical scrapie, Atypical scrapie (Nor98), Scrapie resistance breeding, Goats

Prion diseases, collectively termed transmissible spongiform encephalopathies (TSEs), result from conformational conversion of the cellular prion protein (PrP^{c}) into a pathogenic, β-sheet-rich, partially protease-resistant isoform designated PrP^{sc}. PrP^{sc} is propagated by templating the misfolding of PrP^{c}, causing spongiform neurodegeneration. These invariably fatal disorders affect numerous mammalian species, including humans (Creutzfeldt-Jakob disease), cattle (bovine spongiform encephalopathy, BSE), cervids (chronic wasting disease), and small ruminants. In goats, TSEs manifest primarily as classical scrapie (CSc), a contagious form, and atypical scrapie (ASc, also known as Nor98), a putative variant with distinct strain properties, biochemical signatures, and low transmissibility. Cross-species transmission is limited, yet experimental evidence indicates ASc prions may evolve into classical BSE under specific experimental conditions, underscoring potential risks of strain adaptation.

Portugal maintains ≈305,000 goats, represented in part by native breeds, mainly used in the Northern, Central, and Alentejo, generating €28–34 million annually from milk and meat production. Between 2003 and 2024, the scrapie surveillance programme tested 100,564 goats nationwide identifying 22 cases of ASc (16 Portuguese goats, 6 imported from Spain), plus one indigenous case of CSc. Confirmatory testing [histopathology, immunohistochemistry (2G11), Western immunoblotting] revealed Nor98-like PrP^{sc} deposition and electrophoretic profiles (3–5 bands between 12–30 kDa) for ASc, while the CSc case showed classical three-banding distinguishable from BSE.

Comprehensive surveys of prion protein gene (*PRNP*) diversity in European goat populations remainlimited, with data available for <10% of recognized breeds. Under the PeXPTPrionGoat project, we sequenced the complete *PRNP* open reading frame in 150 TSE-negative goats (randomly selected from various production regions) and 10 goats from CSc-affected flocks. This first survey of *PRNP* diversity in Portuguese caprine populations determined allele frequencies for key polymorphisms (including codons 146 and 222) and compared their distribution between TSE-positive and negative animals.

These baseline data fill a critical gap, supporting design of targeted genetic breeding programs incorporating resistance alleles (*e.g.*, K222, D146, S146) while preserving breed diversity. Combined with ongoing surveillance, these findings will enhance scrapie control and protect sustainability of the goat industry in Portugal.

48. Alkaline Hydrolysis of Animal By-Products: Perspectives and Challenges for the Circular Economy

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Keywords: Animal By-Products, transmissible spongiform encephalopathies, alkaline hydrolysis, Real-Time Quaking-Induced Conversion assay

Animal by-products (ABPs) represent a strategic resource within the circular economy, contributing to waste reduction and the sustainable recovery of non-edible animal materials in line with the Sustainable Development Goals of the 2030 Agenda. Alkaline hydrolysis has recently been recognized by European legislation as an alternative method for treating ABPs of all categories [1]. The process involves alkaline solutions at temperatures ≥150 °C and an absolute pressure ≥4 bar for 3 hours for materials derived from animals untested or negative for transmissible spongiform encephalopathies (TSEs) or for 6 hours for animals suspected of infection or subject to eradication measures. The resulting hydrolysate can be used for biogas production. However, previous studies have shown that although alkaline hydrolysis significantly reduces pathological prion protein (PrPSc), a residual infectivity risk may remain [2]. Moreover, the 2021 EFSA opinion [3] emphasized that a reduction of at least 6 log of the PrPsc titre is required for the process to be considered equivalent to traditional inactivation methods, highlighting the need to rigorously assess the safety of alkaline hydrolysis and the management of the derived biogas.
This study aims to evaluate the feasibility of treating Category 1 ABPs contaminated with prions through alkaline hydrolysis and to assess its efficacy in reducing PrPSc levels.
Category 1 ABP samples will be experimentally contaminated with different prion strains and subjected to alkaline hydrolysis under suboptimal conditions (130 °C and 3.0 bar for 3 and 6 hours). PrPSc reduction will be evaluated using Western blot analysis and Real-Time Quaking-Induced Conversion assays.
Preliminary analyses have focused on identifying equipment and containment boxes suitable for processing high-risk materials. Early experiments on non-infected ABPs have provided optimization data of NaOH concentration for autoclave-based hydrolysis and helped define key operational parameters, ensuring controlled and reproducible conditions for subsequent tests on prion-contaminated samples.
The results will be compared with European regulatory benchmarks, providing baseline data for further in vitro and in vivo studies and supporting potential regulatory updates on alkaline hydrolysis and biogas utilization. The analysis of TSE-risk ABPs will enhance technical and regulatory knowledge while supporting evidence-based policymaking.

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Prion Prion and Prion-like Diseases in Humans

49. Prion detection in the urine of individuals at genetic risk for fatal familial insomnia

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Keywords: FFI, biomarker, PMCA, prions, urine.

Fatal Familial Insomnia (FFI) is a rare, rapidly fatal genetic prion disease caused by the D178N *PRNP* pathogenic variant. Despite genetic testing identifying at-risk individuals decades before symptom onset, no validated biomarker exists to detect preclinical prion pathology, monitor disease activity longitudinally, or assess therapeutic interventions.

Here, the first demonstration of prion seeding activity in urine from D178N pathogenic variant carriers is reported using the ultrasensitive protein misfolding cyclic amplification (PMCA) assay optimized with transgenic mice expressing bank vole PrP substrate.

Blinded longitudinal analysis was conducted on urine samples from 28 D178N carriers (24 asymptomatic and 4 symptomatic at baseline) and 37 non-carrier relatives from Italian and Spanish families, with follow-up spanning up to 5 years. Multiple aliquots per collection were tested to maximize analytical sensitivity. Parallel blood samples underwent exploratory PMCA analysis when available.

Prions were detected in urine from 22/28 carriers (78.6%; 95% CI 59–91%) but in none of the 37 non-carrier relatives, yielding 100% specificity (95% CI 91–100%) against familial controls sharing similar backgrounds and lifestyle. Detection was higher among codon 129 MV carriers (8/8, 100%) than MM carriers (14/20, 70%). Once established, urine prion positivity showed remarkable longitudinal stability, persisting across all subsequent collections, including through clinical phenoconversion. All 4 carriers who developed symptoms during follow-up had tested urine-positive beforehand (median 8 months prior; range 0-12 months), whereas 18 of 24 urine-positive carriers remained asymptomatic during observation.

Blood PMCA signals from parallel collections were inconsistent longitudinally. Italian carriers had previously received doxycycline prophylaxis in the context of a prevention study, yet in vitro analyses indicated that the corresponding levels were

insufficient to inhibit PMCA amplification [1]. Normal kidney function across participants excluded renal impairment as a confounding factor.

These findings establish urine as the first robust, non-invasive biofluid for detecting FFI-associated prion seeding activity from the presymptomatic phase. The longitudinal stability of urine PMCA supports its potential role as a validated biomarker to monitor clinical progression for genetic prion diseases such as FFI.

Reference:

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50. The perfect match for RT-QuIC: FLUOstar Omega microplate reader from BMG LABTECH

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Keywords: RT-QuIC, Prion, ThT, Microplate reader, Alpha-Synuclein

Real-time quaking-induced conversion (RT-QuIC) has emerged as a highly sensitive and rapid assay for detecting protein aggregation associated with neurodegenerative disorders, including prion diseases, Alzheimer's disease, and Parkinson's disease. The assay relies on the amplification of misfolded protein seeds and their real-time detection using Thioflavin T (ThT) fluorescence, requiring precise control of temperature, shaking, and long-term kinetic fluorescence measurements. These stringent technical demands put exceptional stress on microplate readers, making instrument robustness and performance critical for reliable RT-QuIC assays.

This presentation highlights the FLUOstar® Omega microplate reader (BMG LABTECH) as an optimized platform for RT-QuIC applications. The system supports long-term kinetic measurements exceeding 70 hours with alternating shaking and incubation cycles at controlled temperatures of up to 45 °C (or 65 °C optional). Its dedicated transport system, reinforced microplate carrier, and specialized components are designed to withstand continuous high-speed shaking, ensuring mechanical stability and reproducible performance throughout extended assay runtimes.

The FLUOstar Omega offers flexible shaking modes (linear, orbital, and double-orbital), high shaking speeds, bottom-reading fluorescence detection, and compatibility with multi-well formats up to 384 wells, enabling high-throughput RT-QuIC analyses. Integrated data analysis using the MARS software allows automated processing of ThT kinetic curves, including time-to-threshold determination, baseline calculations, and user-defined formulas, facilitating standardized and reproducible assay evaluation.

Originally used in the development of the RT-QuIC assay, the FLUOstar Omega has become one of the most widely cited microplate readers for prion and amyloid seeding research. Its proven robustness, sensitivity, and suitability for demanding aggregation assays make it a reliable and trusted solution for laboratories performing RT-QuIC and related protein misfolding studies.

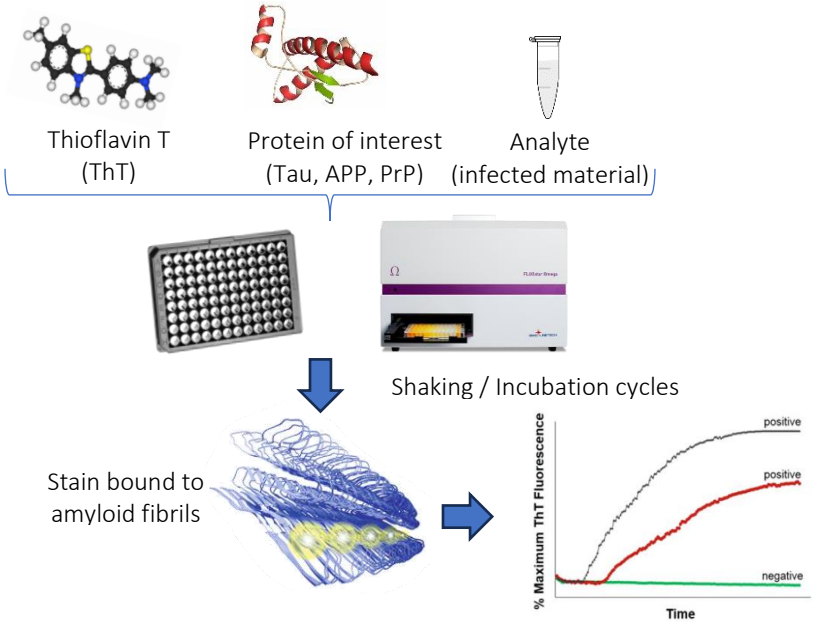

Figure 1: Schematic overview of the real-time quaking-induced conversion (RT-QuIC) assay. Recombinant protein substrate (e.g., prion protein PrP, tau, or amyloid precursor protein) is incubated with Thioflavin T (ThT) in the presence of potentially seeding-competent analyte material. Alternating cycles of shaking and incubation in a microplate format promote seed-dependent misfolding and fibril elongation. Binding of ThT to newly formed amyloid fibrils results in an increase in fluorescence signal, which is monitored kinetically in real time, enabling discrimination between positive and negative samples based on aggregation kinetics.

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51. ALTERED CIRCADIAN AND CLOCK GENE PROFILES AS POTENTIAL BIOMARKERS FOR FATAL FAMILIAL INSOMNIA

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Keywords: fatal familial insomnia; circadian rhythms; actigraphy; cortisol; melatonin

Familial Fatal Insomnia (FFI) is a rare, fatal prion neurodegenerative disorder caused by a mutation at codon 178 of the prion protein gene (PRNP) [1,2]. FFI is clinically characterized by progressive, severe insomnia, alongside neurological, motor, and psychiatric symptoms, as well as circadian rhythm disruption [2]. Circadian rhythm disturbances are common in neurodegenerative disorders, even during early stages, and could be used as potential disease biomarkers for predicting disease onset. Given the critical role of clock genes in regulating circadian rhythms and their relevance to various neurological disorders, we hypothesize that FFI mutation carriers exhibit disrupted clock gene expression, which could provide insights into disease onset and progression. Here, we aim to characterize circadian alterations (clinical, hormonal and molecular) in PRNP codon 178 mutation carriers.
We evaluated circadian rhythm changes in four carriers (three in a preclinical condition and one symptomatic) and seven healthy controls. Assessments included a one-night at home polysomnography (PSG), two-weeks of continuous actigraphy monitoring and at four timepoints across the day assessment of plasma cortisol levels, body temperature, and clock gene expression via qRT-PCR in peripheral blood mononuclear cells (PBMCs). This study received approval from the Ethical Committees of the Faculty of Medicine, University of Coimbra (CE-162/2021) and the Coimbra Hospital and University Centre (ULSC, OBS.SF.55-2021) in Coimbra, Portugal.
Compared to controls, FFI patients showed higher levels of **cortisol** (Controls: 46.55±4.32; FFI patients: 68.76±5.38; $p<0.01$) and **melatonin** (Controls: 77.16±2.33; FFI patients: 94.17±4.51; $p<0.05$) and significant alterations in the clock genes expression: ***BMAL1*** in the morning timepoint (Controls: -0.50±0.11; FFI patients: 0.42±0.30; $p<0.05$), ***PER2*** (Controls: -0.34±0.20; FFI patients: 0.57±0.45; $p<0.05$) and ***CRY2*** (Controls: -0.90±0.32; FFI patients: 1.19±0.25; $p<0.01$). Notably, these changes were detectable even in presymptomatic individuals, suggesting early circadian disruption prior to overt symptom onset.
These findings suggest potential of integrating circadian markers to monitor disease progression, identify the onset of FFI, and enhance our understanding of its cellular mechanisms.

Acknowledgments: LA/P/0058/2020 [DOI: 10.54499/LA/P/0058/2020]; UID/04539/2025; UID/PRR/4539/2025 [DOI: 10.54499/UID/PRR/04539/2025]; UID/PRR2/4539/2025 [DOI: 10.54499/UID/PRR2/04539/2025] and Portuguese national funds via FCT (2020.04499.BD; 2023.17896.ICDT [DOI https://doi.org/10.54499/2023.17896.ICDT]).

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52. Prodromal detection of skin α-Synuclein and clinical relevance of Seed Amplification Assay

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Introduction

Seed amplification assays (SAA) for detecting misfolded α-synuclein (αSyn) is a highly sensitive biomarker for α-synucleinopathies. Skin biopsies represent a minimally invasive alternative to lumbar puncture with potential applicability in early and prodromal disease stages. Beyond binary diagnostic classification, SAA-derived kinetic parameters may also reflect disease stage or severity. In this three-study investigation, we first implemented aαSyn-SAA in skin biopsies in comparison to CSF, in a single cohort. We then investigated whether αSyn-SAA in skin could detect pathological αSyn in a cohort with idiopathic olfactory dysfunction (iOD), a prodromal symptom for α-synucleinopathies. Lastly, we analyzed if skin αSyn seeding kinetics differentiate between iOD and clinical *Lewy*-Body dementia (LBD).

Methods

Study 1) 31 clinical LBD and 25 Alzheimer´s Disease patients were tested using αSyn-SAA in CSF (25) and skin (31), and diagnostic performance was evaluated. **Study 2)** 44 iOD and 50 healthy controls (HC) were tested using skin αSyn-SAA. **Study 3)** 49 αSyn-SAA-negative HC, 18 αSyn-SAA-positive iOD patients, and 25 αSyn-SAA-positive (CSF and skin) LBD patients were tested using skin αSyn-SAA, and kinetic parameters (Time to threshold, maximum fluorescence, time to half maximum fluorescence (T50), area under curve, and quenching rate) were evaluated.

Results

Study 1) αSyn-SAA demonstrated high diagnostic accuracy for clinical LBD, reaching 87% (95% CI: 77–98%) in CSF and 85% (95% CI: 75–98%) in skin. These findings establish skin αSyn-SAA as a robust alternative to CSF-based testing. **Study 2)** 41% of iOD became SAA positive and with 98% specificity for the non-anosmia HC group. **Study 3)** A significant difference in αSyn seeding kinetics was observed between iOD and clinical LBD. Clinical LBD exhibited faster aggregation kinetics, reflected by shorter T50 ($p=0.0017$) and time to threshold ($p=0.029$) compared to iOD. These results could indicate stage-dependent differences in peripheral αSyn accumulation.

Conclusion

αSyn-SAA performed in both CSF and skin provides high diagnostic accuracy for LBD and reliably distinguishes LBD from AD. Furthermore, skin αSyn-SAA in iOD cohort revealed high prevalence of aSyn pathology, suggesting iOD as a prodromal stage of LBD. Finally, different αSyn-SAA kinetics between clinical LBD and iOD propose that αSyn seeding dynamics reflect disease stage or severity.

Keywords: α-Synuclein, Seed Amplification Assay, skin, anosmia

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53. ANTI-PRION DRUGS REDUCE ENDOPLASMIC RETICULUM STRESS AND PROTECT HUMAN DOPAMINERGIC NEURONS FROM DEATH

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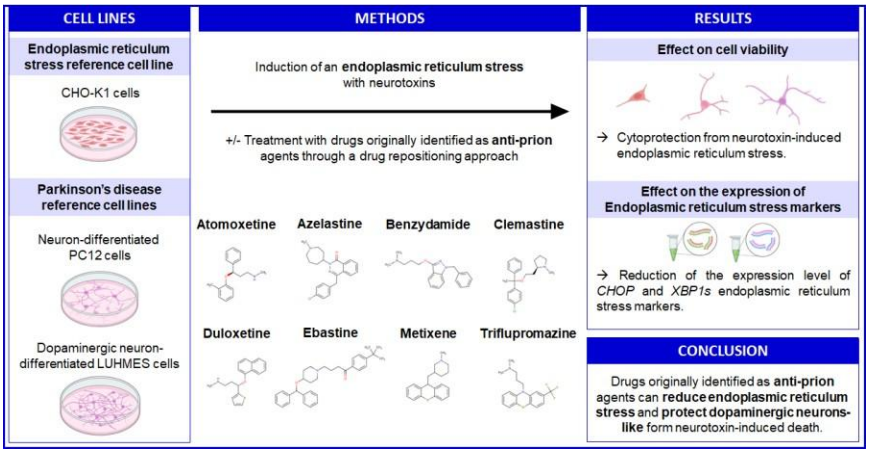
Keywords: Anti-prion drugs, drug repurposing, endoplasmic reticulum stress, Parkinson's disease, LUHMES cells differentiated into dopaminergic-like neurons

Parkinson's disease (PD) is characterized by the pathologic aggregation of α-synuclein, which induces endoplasmic reticulum (ER) stress and activates the unfolded protein response (UPR), ultimately leading to the death of dopaminergic neurons. This study investigated whether drugs known to mitigate protein aggregation in prion disease models would attenuate the stress response and cell death in PD models. Flunarizine and ten of its structural analogues, previously identified through a drug repositioning approach to reduce the aggregation of PrP^{Sc} prion protein [1], were evaluated in a Chinese hamster ovary (CHO-K1) cell model. First, UPR was induced in CHO-K1 cells using tunicamycin, revealing that several of these drugs conferred protection and also reduced the expression of the UPR marker CHOP. Subsequently, we tested these anti-prion drugs in PD relevant cellular models. Neuronally-differentiated PC12 cells were employed, and all 11 drugs exhibited protective effect. Finally, human dopaminergic neurons (LUHMES cells) were exposed to 1-methyl-4-phenylpyridinium (MPP+), a compound commonly used to induce parkinsonian-like pathology. In this model, all 11 drugs demonstrated cytoprotective properties and attenuated UPR. Notably, several compounds - benzydamine, duloxetine, flunarizine, metixene and trifluprom azine – protected LUHMES cells from MPP+-induced ER stress at nanomolar concentrations [2]. These findings provide the proof of concept that drugs approved for other indications, may be repurposed to ameliorate PD and related pathologies linked to ER stress.



Flunarizine analogs were originally identified through a drug repositioning approach for their capacity to reduce PrP^{Sc} prion protein aggregation. These anti-prion drugs protect neuronally-differentiated PC12 cells from ER stress and also preserve human dopaminergic neurons from MPP+ and mitigate UPR activation. These data are a proof of concept that anti-prion drugs could be repurposed for Parkinson's treatment, and that ER stress-related pathologies may also benefit from these anti-prion therapies.

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54. Evaluation of different substrates for the optimization of an amyloid propagation assay for the diagnosis and monitoring of proteinopathies

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Keywords: α-synuclein; RT-QuIC; α-synucleinopathies

α-Synucleinopathies are a group of neurodegenerative diseases characterized by the misfolding and aggregation of α-synuclein, a process that impairs its physiological activity and causes progressive neurodegeneration[1]. Seed amplification assays such as Real-Time Quaking-Induced Conversion (RT-QuIC) enable the detection of minute amounts of pathological or misfolded α-synuclein in biological samples, facilitating early diagnosis[2]. However, broader implementation of RT-QuIC is limited by interlaboratory variability, largely due to the structural heterogeneity of recombinant α-synuclein substrates[3]. Evaluating recombinant α-synuclein variants from different mammalian species aims to identify alternative substrates that improve RT-QuIC assay robustness, sensitivity, interlaboratory standardization, and overall assay performance for the reliable early detection and discrimination of synucleinopathies. In this study, 14 recombinant α-synuclein variants derived from different mammalian species, together with human K23Q α-synuclein as the reference substrate, were designed based on *SNCA* sequences, cloned into plasmids, expressed in *Escherichia coli*, and recovered by osmotic shock prior to purification by immobilized metal affinity chromatography and anion-exchange chromatography. RT-QuIC reactions were then seeded with brain homogenates from different synucleinopathies, while other neurodegenerative diseases served as negative controls. Among the 14 recombinant α-synuclein substrates evaluated, two exhibited robust aggregation kinetics and emerged as promising candidates. These substrates selectively aggregated in samples from Parkinson´s disease and dementia with Lewy bodies, but not from multiple system atrophy. One substrate demonstrated the best overall performance, detecting seeding activity at dilutions as low as 10^{-5}, with 100 % sensitivity for Parkinson´s disease and 66,7 % for dementia with Lewy bodies, and 90 % specificity. Notably, this substrate exhibited a lag phase approximately half that of the human K23Q reference substrate (~10 h). These findings support alternative α-synuclein substrates to enhance RT-QuIC robustness and improve the reliability of early detection of synucleinopathies.

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55. Generation of a G114V humanized transgenic mouse model and phenotypic characterization of the spontaneous disease

Sara Canoyra[1,2], Ilaria Vanni[3], Juan Carlos Espinosa[1], Michele A. Di Bari[3], Alba Marin-Moreno[1], Patrizia Ratano[3], Natalia Fernández-Borges[1], Claudia D'Agostino[3], Belén Pintado[4], Barbara Chiappini[3], Matteo Giovannelli[3], Stefano Marcon[3], Geraldina Riccardi[3], Jessica Del Bravo[3], Laura Pirisinu[3], Umberto Agrimi[3], Emiliano Biasini[5], Juan María Torres[1], and Romolo Nonno[3]

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Keywords: Gerstmann-Straussler-Scheinker disease, inherited prion disease, human transgenic mouse model

The G114V mutation in the human prion protein (PrP) leads to an early-onset, inherited form of prion disease. The phenotypic features of this disease have been reported to resemble those of either genetic Creutzfeldt-Jakob disease (gCJD) or Gerstmann–Straüssler–Scheinker syndrome (GSS). It is located within the central hydrophobic domain of PrP, within the palindromic sequence and immediately upstream of the glycine-rich region. In this study, we generated and characterized a transgenic mouse line expressing human-PrP carrying the $G_{114}V$ mutation (HuV_{114}PrP-Tg741), with expression levels comparable to transgenic lines expressing wild type (wt) human-PrP. This model was developed to investigate the role of central domain perturbations to prion formation and disease.
HuV_{114}PrP-Tg741 mice exhibited increased proteolytic processing, including enhanced α-cleavage and shedding, both of which progressed with age. These mice developed a spontaneous neurological disease after prolonged incubation periods (onset >550 days), with incomplete and age-dependent penetrance.
Neuropathological analysis revealed spongiform degeneration, neuronal loss, and diffuse PrP^{Sc} deposition in affected brain regions. Biochemical analysis of brains from diseased HuV_{114}PrP-Tg741 mice showed a predominant low-molecular-weight ~7 kDa PrP^{res} fragment, consistent with a GSS-associated profile. Diseased mice also showed increased accumulation of insoluble PrP species, including full-length PrP, which accumulated with aging, and N-terminal fragments specifically associated with disease.
Transmission studies demonstrated seeding activity of the spontaneous disease when transmitted within the same transgenic line, with reduced incubation times and complete attack rate upon passage but showed no transmission to other prion models. The lack of transmissibility to humanized transgenic mice mimics the features reported for G114V human cases. Heterozygous mice ($HuV/G_{114}M_{129}$ and $HuV/G_{114}V_{129}$) also developed disease, although with reduced prevalence, and PrP^{res} was derived exclusively from the mutant allele.
Overall, HuV_{114}PrP-Tg741 mice reproduce key biochemical and neuropathological features of $G_{114}V$-associated inherited prion disease, providing a robust platform for mechanistic studies and for preclinical testing of therapeutic strategies targeting early misfolding events.

Funding Information
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56. Evaluation of PrP endoproteolytic processing in a humanized G114V transgenic mouse model

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Keywords: PrPC, proteolysis, proteoforms, genetic prion disease, aging

We recently generated a transgenic mouse line (HuV114PrP-Tg741) expressing human PrP carrying the G114V mutation, a mutation associated with an inherited prion disease presenting as an early-onset phenotype. After prolonged incubation periods, these mice develop a spontaneous prion disease characterized by an incomplete, age-dependent penetrance and a PrP^{Sc} with a ~7 kDa N- and C-terminally cleaved protease-resistant core. We thus aimed to evaluate the interplay between PrP^{C} endoproteolytic processing, the accumulation of insoluble PrP species, and disease onset.

We first compared PrP^{C} endoproteolytic processing in young healthy tgHuV114PrP-Tg741 and tgHuG114PrP-340 mice, the latter expressing wild-type human PrP. Despite comparable total PrP^{C} levels and overall PrP^{C} proteoform patterns, young tgHuV114PrP-Tg741 mice showed increased PrP^{C} proteolytic processing, with a slight yet significant increase in α-cleavage and shedding.

We then evaluated PrP^{C} proteolytic processing across aging. Although total PrP^{C} levels remained stable, an age-dependent increase in proteolytic processing was observed, with β-cleavage showing the most pronounced increase at advanced ages. Moreover, while shedding increased up to mid-life and plateaued thereafter, α- and β-cleavage continued to rise, suggesting distinct regulatory mechanisms governing these pathways.

To investigate whether these proteolytic differences were paralleled by changes in PrP aggregation, we performed solubility-based fractionation across different ages and disease statuses. Aged mice exhibited a progressive accumulation of insoluble PrP species, with the highest amounts observed in spontaneously ill mice, indicating both age- and disease-related effects. Further analysis showed that while insoluble full-length PrP already increased with aging, insoluble N-terminal PrP fragments (~12-15 kDa) specifically accumulated in spontaneously ill mice, suggesting a disease-related phenomenon.

Overall, these findings suggest that the G114V mutation might increase PrP^{C} propensity to misfold not only by directly influencing its conformational stability but also by inducing an imbalance of PrP^{C} proteoforms. Interestingly, changes in PrP^{C} processing and accumulation of insoluble PrP species were also observed in healthy animals and progressively increased with age, providing evidence of age-related PrP alterations. Collectively, these observations highlight the importance of investigating PrP^{C} processing alterations associated with PRNP mutations or polymorphisms, as well as with aging, which represent main risk factors for prion diseases in humans.

57. PATIENT-DERIVED IPSC-GENERATED HYPOTHALAMIC NEURONS AS A MODEL FOR FATAL FAMILIAL INSOMNIA

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Keywords: fatal familial insomnia; circadian rhythms; iPSCs; hypothalamic neurons

Familial Fatal Insomnia (FFI) is an ultra-rare genetic prion disease, clinically characterized by progressive insomnia (slow wave sleep loss with disruption of physiological sleep-wake cycle) with autonomic and motor hyperactivation, and by episodes of oneiric stupor [1]. FFI patients have a very short life expectancy after the first symptoms. There is no cure neither experimental models to investigate this fatal disorder. The lack of experimental tools for studying FFI poses significant challenges in understanding the cellular and molecular pathways underlying the disease and identifying potential interventions.

Here, we aimed to establish a patient-specific *in vitro* model of FFI using induced pluripotent stem cells (iPSCs) differentiated into hypothalamic neurons as a valuable experimental model to recapitulate this complex disorder, thereby facilitating the identification of disease mechanisms and enabling the investigation of novel therapeutic strategies

Skin biopsy samples were collected from 4 FFI patients, and fibroblasts were isolated and reprogrammed into induced Pluripotent Stem Cells (iPSCs) using integration-free reprogramming episomal vectors via nucleofection. Subsequently, iPSCs were differentiated into hypothalamic neuronal cells using a culture-driven protocol. The resulting cells were characterized based on morphology and expression of hypothalamic, neuronal, and pluripotency markers (NPY, POMC, AgRP, Orexin, MAP2, Ki67, Sox2, Nanog). Furthermore, Ca^{2+} imaging was performed to assess the functionality of the differentiated hypothalamic neurons. Preliminary results demonstrate successful differentiation into hypothalamic-like neurons exhibiting appropriate marker expression and functional calcium signaling activity. Additionally, we established an isogenic FFI iPSC line by employing CRISPR-Cas9 nucleases to correct the PRNP c.532G>A(p.D178N) gene mutation in a FFI patient line by homology-directed repair.

This study received approval from the Ethical Committees of the Faculty of Medicine, University of Coimbra (CE-162/2021) and the Coimbra Hospital and University Centre (ULSC, OBS.SF.55-2021) in Coimbra, Portugal.

Overall, this patient-derived neuronal model represents a promising platform to investigate FFI pathophysiology and may contribute to future drug discovery efforts.

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58. An oral small molecule inhibitor of PrP translocation extends survival by nearly 50% in a transgenic mouse model of human GSS prion disease

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Keywords: prion diseases, signal peptide processing, PrP lowering therapy, signal peptide blockade, oral therapy.

Prion diseases are fatal neurodegenerative disorders caused by the misfolding of the cellular prion protein (PrP^C). Because PrP^C is required for prion propagation, therapeutic strategies aimed at lowering its expression have emerged as promising approaches. Here, we evaluated the small molecule MG-813 in a transgenic mouse model of Gerstmann-Sträussler-Scheinker (GSS) disease. MG-813 belongs to a novel class of small molecule drugs called Molecular Gates; it selectively blocks nascent PrP^C translocation into the endoplasmic reticulum and promotes its cytosolic degradation. In this rapidly progressing model, mock-treated TgMo(L108I)3x mice inoculated with GSS A117V prions succumb at approximately 60 days post-inoculation (dpi). Oral administration of MG-813 from 30 dpi resulted in at least 50% reduction in PrP expression in the brain and a reproducible survival extension of 46% and 48% in two independent studies using slightly different dosing regimens, compared with controls.

Plasma neurofilament light chain measurements revealed significant neuroprotective effects, with treated animals showing a delayed rise in this biomarker relative to controls, consistent with preserved neuronal integrity during the therapeutic window. The survival benefit was not paralleled by a clear reduction in terminal PrP^{res} accumulation, suggesting that efficacy is primarily mediated by substrate depletion rather than by a direct anti-aggregation effect.

These findings provide *in vivo* proof of concept for Molecular Gates as a PrP-lowering therapeutic strategy for prion disease. Our results support further preclinical development of this class of small molecules, particularly for presymptomatic intervention in genetic prion disease.

59. The power of synergy

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Since 2013, the **Spanish Foundation for Prion Diseases** has been working for and on behalf of those affected by any prion disease.

It actively collaborates in research by funding various projects and provides psychological, scientific, and legal support to all patients and their families. They have successfully made prion diseases the first to be included in Spain's ELA LAW (SLA Law), fighting for rights and better quality of life through direct advocacy with government bodies, the Minister of Health, and Queen Letizia of Spain.

60. BiFC-based visualization of CsgA/α-syn interactions: Linking microbiota to neurodegeneration

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Keywords: Amyloid, *Caenorhabditis elegans*, BiFC, CsgA, α-synuclein

Neurodegenerative diseases are associated with amyloid protein accumulation, such as α-synuclein in Parkinson´s disease. Amyloid aggregation follows the seeding-nucleation polymerization model, in which oligomers act as nuclei inducing the aggregation of additional similar or different amyloid proteins, causing cytotoxicity.

The human microbiome produces functional bacterial amyloid proteins (FBAPs) such as Curli in *Eschericia coli*. Previous studies observed that FBAPs increase host amyloid aggregates [1-3]. Co-localization studies [4] suggest cross-seeding between both amyloids is the mechanism implicated in human aggregation increase, but the protein interaction between them is not demonstrated yet. To study interspecies amyloid protein-protein interactions and their specificity, we used a Biomolecular Fluorescence Complementation (BiFC) system in *Caenorhabditis elegans* [5] due to its transparency, well- characterized nervous system, and ease of microbiota manipulation.

BiFC allows to visualize interaction between proteins by the reconstitution of the fluorescent protein (Venus) whose N-ter and C-ter fragments are respectively linked to the studied proteins.

We generated transgenic *C. elegans* (MOP-1) using a pan-neuronal rab-3 promotor to express α-synuclein linked to C-ter fragment of the Venus split reporter in neurons. MOP-1 worms were fed with *E.coli* strain producing CsgA fused to N-ter Venus, and we examined reconstituted Venus's fluorescence in neurons using confocal microscopy. We observed signal at 2 and 8 days of adulthood in few neurons, suggesting anatomic specificity of this interaction that we are currently characterizing. Furthermore, we also tested negative controls (WT worms fed with and without CsgA producing bacteria and MOP-1 fed with a non-producing CsgA *E. coli*) without observing Venus's fluorescence in any of them. These results demonstrate cross-seeding of gut bacterial and neuronal host amyloids occur in vivo. We are now using our BiFC system to characterize the specificity of a wide variety of host-bacteria amyloid interactions to reveal novel links to disease.

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61. Early-Onset MM2-Cortical Sporadic Creutzfeldt-Jakob Disease with 19-Month Survival, Double-Negative CSF Biomarkers and Neuropathological Evidence of PrP-Alpha-Synuclein Cross-Seeding

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Keywords: Early-onset sCJD, RT-QuIC negative, , MM2-cortical, Cross-seeding, Neuropathology

Background: The MM2-cortical (MM2C) subtype of sporadic Creutzfeldt-Jakob Disease (sCJD) is a rare molecular variant often characterized by atypical clinical presentations, prolonged survival, and low sensitivity to current cerebrospinal fluid (CSF) biomarkers. Understanding the neuropathological background and the interaction between different misfolded proteins is crucial for characterizing these elusive phenotypes.

Case Presentation: We report a 46-year-old patient with progressive cognitive and language decline initially manifesting as profound apathy, loss of initiative, and impaired verbal fluency. Overall survival from the onset of symptoms was 19 months.. Despite the extended survival period, both CSF RT-QuIC and 14-3-3 protein assays were repeatedly negative, leading to significant diagnostic uncertainty during the premortem stage. Genetic analysis of the PRNP gene was negative for pathogenic mutations, with methionine homozygosity (MM) at codon 129.

Neuropathological Findings: Post-mortem examination confirmed MM2C sCJD, showing characteristic large confluent vacuoles. Immunohistochemistry (IHC) revealed a mixed pattern of granular, and immunoreactive plaques involving the cerebral cortex, striatum, and the molecular layer of the cerebellum. Notably, alpha-synuclein IHC showed fine, synaptic-like deposits in the cortex, in the absolute absence of Lewy bodies or Lewy neurites.

Discussion: This case illustrates the significant diagnostic challenges associated with the MM2C strain in younger patients, where a prolonged survival and double-negative CSF biomarkers can mimic other neurodegenerative etiologies. The incidental finding of fine cortical alpha-synuclein deposits suggests a molecular cross-seeding effect, where the primary prionopathy may have acted as a template for secondary protein misfolding. This synergistic interaction, facilitated by the 19-month disease duration, highlights the complex protein-handling disturbances and the convergence of neurodegenerative pathways in sCJD. The presence of these deposits in a young patient suggests that cross-seeding is independent of age-related neurodegeneration and is likely driven by the prolonged interaction between protein species.

Conclusion: MM2C sCJD must remain a diagnostic consideration in young patients with rapidly progressive cognitive decline, even when modern biomarkers fail. The presence of fine-granular synucleinopathy suggests that cross-seeding is a relevant phenomenon in prion strains with extended survival, revealing a molecular complexity that remains invisible to current diagnostic standards.

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62. Trial readiness for preventive gene therapy in hereditary prion disease: willingness, preferences, and decision-making among confirmed carriers and individuals at genetic risk

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Keywords: hereditary prion disease; gene therapy; clinical trial participation; asymptomatic carriers; trial readiness

Preventive AAV-mediated gene therapy for hereditary prion disease is nearing clinical translation, yet no published data exist on willingness, decision drivers, or protocol preferences among individuals at genetic risk—a critical gap for imminent trial planning. We conducted a community-based online survey among 113 adults from families with pathogenic *PRNP* variants (E200K and D178N), including 40 confirmed asymptomatic carriers and 73 relatives who underwent predictive testing but chose result non-disclosure. After reviewing a detailed first-in-human preventive AAV trial description—including single-dose constraints, lumbar puncture requirements, and durability uncertainty—52.2% expressed high or very high willingness and 86.7% reported at least moderate interest. Willingness was higher among confirmed carriers than non-disclosed participants (67.6% vs 43.8%), with carriers more often reporting definite willingness. Key facilitators were access to human trial data and prior participant testimonials. The study also revealed that family context and family-related decision considerations were of high relevance for trial associated decision making, almost 90% of participants prioritizing participation to contribute knowledge that could benefit their relatives and 50% reporting the responsibility of going first (participating in earlier phases) to help younger relatives. Procedural barriers were uncommon: only 9.0% rated monitoring procedures (including lumbar punctures) as a major/absolute barrier, and 89.0% would accept ≥10 years of follow-up. Critically, only 34.2% of non-disclosed participants would accept mandatory predictive testing for enrollment. These findings provide the first evidence base for realistic recruitment assumptions and patient-centred protocol design for preventive gene therapy trials in hereditary prion disease.

63. TAR DNA-binding protein 43 (TDP-43) translocation upon nutrient deprivation

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Keywords: TDP-43, *TARDBP*, prion-like, nutrient deprivation

Transactive response DNA-binding protein of 43 kDa (TDP-43) is a disease-associated protein involved in Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD) [1]. TDP-43 is a ubiquitous RNA and DNA-binding protein composed of 414 amino acids encoded by the *TARDBP* gene located on chromosome 1 [2].
TDP-43 is a nucleocytoplasmic shuttling protein primarily expressed in the nucleus containing two RNA recognition motifs (RRMs), an N-terminal domain (NTD) which supports physiological oligomerization and harbors a nuclear localization signal (NLS), and a C-terminal, prion-like domain, responsible for mediating protein-protein interactions and phase separation under normal conditions, but also promotes aberrant cytoplasmic aggregation in pathological conditions [3].

Current evidence supports the possibility of an interconnection between TDP-43 pathology and metabolic deficits, involving mitochondrial impairment, altered RNA regulation, and cellular energy dysfunction, thereby contributing to pathogenic pathways [4-5]. However, the precise hierarchical order of these events and whether TDP-43 dysfunction is a primary driver of metabolic failure or a downstream amplifier of cellular stress remains to be investigated.

Based on these considerations, we investigated whether nutrient deprivation affects TDP-43 translocation. We performed immunofluorescence staining to evaluate the translocation of C-terminal TDP-43 from the nucleus to the cytoplasm in cell lines treated with nutrient-deprived medium.
Results showed a significant change in TDP-43 localization, with a depletion of nuclear signal, suggesting that metabolic alteration may contribute to TDP-43 translocation, potentially representing an early event in disease-related pathology.

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64. Optogenetic control of bacterial CsgA amyloid aggregation in *Caenorhabditis elegans* neurons as a model to study its potential neurotoxic effects

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Keywords: *Caenorhabditis elegans*, optogenetics, neurotoxicity and bacterial amyloids

Several bacteria from the human gut microbiota produce functional amyloids, which share structural and biophysical properties with human amyloids involved in neurodegenerative diseases [1]. These bacterial amyloids, such as curli (CsgA) from Escherichia coli, can reach neurons where they promote α-synuclein aggregation in a *Caenorhabditis elegans* Parkinson's disease model diseases [2]. Furthermore, curli has been shown to exacerbate α-synuclein-induced motor and intestinal dysfunction in mice diseases [3]. However, the neurotoxic effects of CsgA alone once it reaches the brain remain unknown.

To address this question, we generated a transgenic *C. elegans* model expressing a *csga::cry-mcherry* under the pan-neuronal promoter *rab-3*, allowing CRY-dependent light-induced oligomerization of CsgA in neurons. The CRY domain is a modified version of the light-sensitive CRY2 protein from *Arabidopsis thaliana* which oligomerizes upon blue light [4,5], enabling temporal control of CsgA aggregation in vivo.

Our lab previously validated that fusing CRY-mCherry to CsgA does not alter its amyloid assembly capabilities using a Congo red (CR) assay. CR is an amyloid-binding dye that detects β-sheet-rich structures, indicating amyloid formation. We observed red colonies in the *BW25113 E. coli ΔcsgA* strain producing the fusion protein with CR, indicating that CsgA-CRY-mCherry undergoes light-dependent aggregation.

Next, we confirmed neuronal expression of the construct in *C. elegans* by fluorescence microscopy. To evaluate the physiological effects of neuronal light-dependent aggregation of CsgA in our *C. elegans* model, we performed survival assays under light and dark conditions. Preliminary results suggest internal hatching in CsgA-CRY-mCherry animals exposed to light. Internal hatching, in which eggs are retained and hatch within the parental body, can lead to early death and reflects egg-laying defects and possible neuromuscular dysfunctions. These findings could indicate neurotoxicity, particularly affecting neurons controlling egg-laying, which we are currently investigating.

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65. Establishing complex microbial communities in *C. elegans* to dissect the role of microbiota in neurodegeneration

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Keywords: Complex microbial communities, bacterial amyloids, *Caenorhabditis elegans*, CeMbio, gut microbiota.

Differences in the gut microbiota have been observed between healthy individuals and patients with neurodegenerative diseases (ND), including altered microbial community structure and changes in the relative abundance of specific bacterial taxa. ND involve the progressive loss of neuronal structure and function and are associated with the accumulation of amyloid proteins. Alterations in gut microbiota could increase the severity of ND by promoting amyloid aggregation[1,2]. Bacterial amyloids (BA) produced by the gut microbiota may trigger human amyloids (HA) aggregation through cross-seeding[3]. Supporting this, some BAs enhance HA aggregation and co-localize with them in animal models suggesting BA-HA interaction[3,4]. However, this phenomenon has only been studied in germ-free animals fed with mono-culture bacterial species, remaining unclear if and how these interactions occur in a complex microbial community[4].

This study aims to establish complex microbial communities to investigate whether and how these amyloid interactions change in this context. To address this, we optimized a protocol to establish and recover a complex microbiota in the bacterivorous nematode *Caenorhabditis elegans*. This community consist of 12 bacterial strains representing its core microbiota called CeMbio[5]. We successfully cultured all strains individually and as a mixed inoculum, without affecting worm development as previously described. Bacteria were recovered from the worm microbiota through lysis and validated by 16S rRNA sequencing. Once established, we will combine it with a genetic tool developed in our laboratory based on BiFC system which allows visualization of BA-HA interactions in vivo by confocal microscopy. Neuronal BiFC patterns will be compared between worms fed with a single amyloid-producing bacteria and those fed also with CeMbio inoculum. This system will dissect BA-HA interactions in complex microbiota communities, offering a powerful platform to discover links to microbiome-dependent ND progression.

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66. Transmission of a spontaneous prion disease from TgSpon mice to sheep of different *PRNP* genotypes

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Keywords: Atypical scrapie, TgSpon, prion

Classical scrapie is the main prion disease affecting sheep and goats, characterized by infectious transmission within flocks. In contrast, its atypical form (AS) is less well described and appears as isolated cases, leading to the hypothesis that it represents a spontaneous rather than contagious prion disorder. To further explore the nature of this condition, a transgenic mouse model (TgSpon) overexpressing ovine PrP with the I112 polymorphism has been shown to develop a spontaneous prion disease with biochemical and neuropathological features remarkably similar to atypical scrapie [1]. In previous work, brain homogenates from TgSpon mice were intracerebrally inoculated into two Churra Tensina sheep (genotype AHQ/AHQ). The affected animals showed a neuropathological and PrP^{Sc} deposition pattern indistinguishable from that observed in natural atypical *scrapie* cases.

In the present study, two additional sheep of the same breed but with genotype ARR/AHQ were included to evaluate whether the ARR allele influences susceptibility or neuropathological expression of the disease. These animals were inoculated following the same experimental procedure and analyzed by comprehensive histopathological and immunohistochemical examination of both the CNS and peripheral tissues.

Results revealed that the ARR/AHQ sheep displayed similar patterns of PrP^{Sc} distribution and lesion profiles as observed in the AHQ/AHQ individuals. The morphology and localization of the deposits were consistent with those found in atypical *scrapie*, and no PrP^{Sc} accumulation was detected in peripheral organs. These findings reinforce that the prion agent generated in the TgSpon model faithfully reproduces the distinctive features of atypical *scrapie* in its natural host. Moreover, the identical pathological outcome in sheep carrying either ARR/AHQ or AHQ/AHQ genotypes supports that the phenomenon observed in this experimental model likely represents a spontaneous prion disease, independent of host genotype.

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67. A novel structure-driven strategy for Parkinson's disease immunotherapy

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Keywords: Parkinson's Disease, alpha-synuclein, immunotherapy, nanobodies

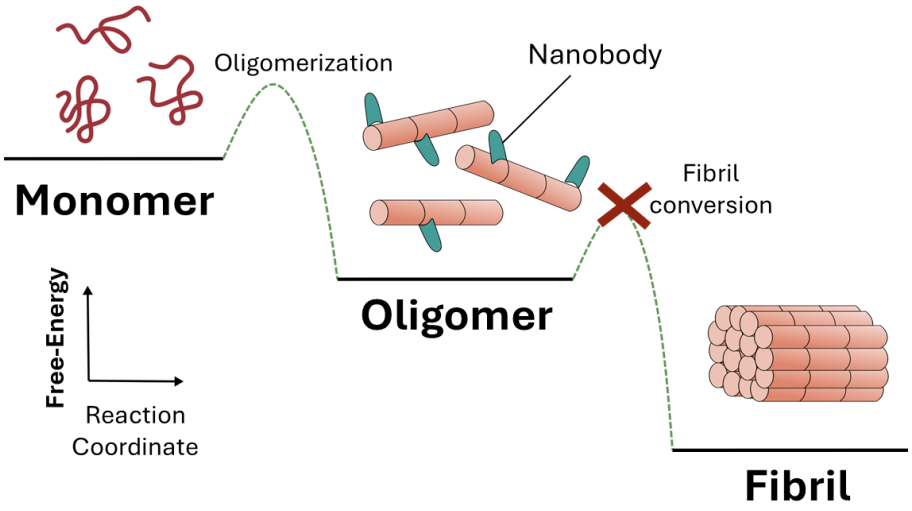
Parkinson's Disease (PD) is the second most common and fastest-growing neurodegenerative disorder, yet no treatments can halt or reverse its progression; current therapies can help manage early symptoms, but they do not slow or stop disease progression. The characteristic loss of dopaminergic neurons in the substantia nigra leads to motor symptoms like bradykinesia, tremors or rigidity. The principal pathological hallmark of PD is the accumulation of insoluble protein inclusions in the brain, named Lewy bodies, primarily composed of aggregated α-Synuclein (αSyn). Of all species formed during amyloid aggregation, αSyn oligomers are considered the main culprits for neuronal homeostasis collapse and disease propagation throughout the brain.

Toxic αSyn oligomers present hydrophobic and anionic surfaces, distinguishing them from αSyn monomers. Our team has worked to identify a structurally defined region only present in oligomeric species that mediates the oligomer-to-fibril conversion [1].

Nanobodies are small, single-domain antibodies capable of binding antigens with high precision. Due to their small size and high stability, they are suitable for a wide range of therapeutic applications, diagnostic procedures and research initiatives [2,3].

Here, we propose a new potential immunotherapeutic approach that combines structurally informed rational design with cutting-edge AI technology to create a series of nanobodies that target this specific region orchestrating the oligomer-to-fibril conversion over any other species.

Our results suggest that these nanobodies can exploit the physicochemical properties of the oligomeric species to impact αSyn aggregation kinetics and achieve selective binding, thus supporting the potential of this new approach.



Schematic representation of the alpha-synuclein aggregation pathway and how the designed nanobodies aim to impact it to avoid oligomer-to-fibril conversion.

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68. A Novel Vaccine for Parkinson's Disease

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Parkinson's Disease (PD) is the second most prevalent and fastest-growing neurodegenerative disorder, marked by progressive dopaminergic neuron loss. The aggregation of α-Synuclein (aSyn) into toxic oligomers and amyloid fibrils is a key driver of neurodegeneration. While current treatments alleviate symptoms, they do neither stop or revert its progression, highlighting the urgent need for novel disease modifying therapies.

Immunotherapy targeting αSyn is a promising approach but lacks specificity for toxic conformations. Using Cryo-electron microscopy (Cryo-EM) and solid-state NMR, we have unrevealed structural information of toxic oligomers with unprecedented resolution. We identified a previously unexplored αSyn region critical for aggregation that is responsible for oligomer to fibril transition. Blocking this region prevents amyloid formation and rescues motor deficits in a PD mouse model. This knowledge enabled the design of NEUROVAX, a virus-like particle (VLP)-based vaccine that mimics the pathological conformation of αSyn, inducing a selective immune response.

VLPs enhance immunogenicity against toxic aSyn species by displaying multiple antigen copies. NEUROVAX presents a conformational epitope that mirrors its pathogenic structure. Mice immunization with NEUROVAX elicits antibodies that selectively recognize toxic αSyn oligomers and fibrils while sparing monomeric αSyn, surpassing existing the affinity of existing antibodies.

By leveraging advances in structural biology, protein engineering, and nanotechnology, NEUROVAX represents a paradigm shift in PD treatment, offering a potential disease-modifying strategy for PD and related disorders.

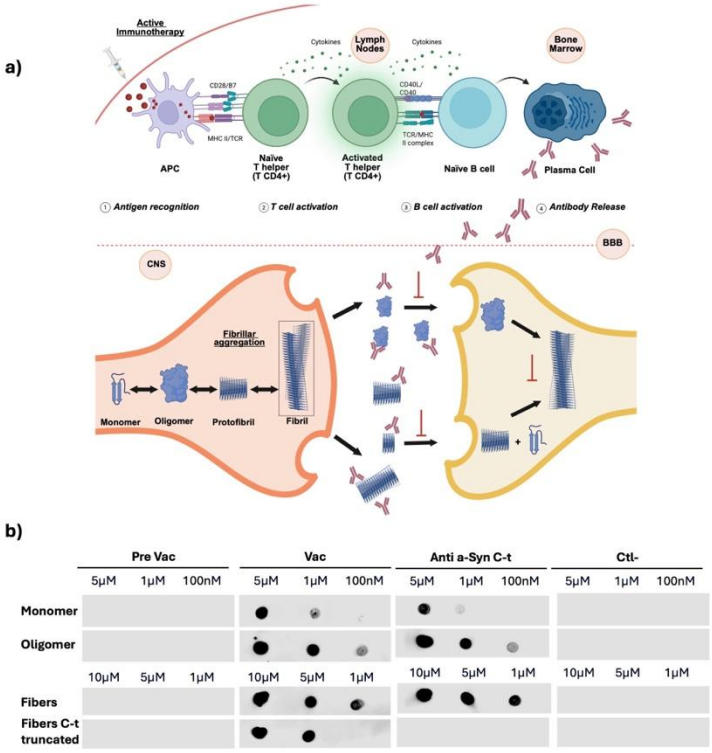

Figure: **Nano-vaccine function and immune response characterization.** (a) Therapeutic immunotherapy intervention in neurodegenerative disorders. (b) Preferential binding of Vac to α-synuclein oligomers and fibrils compared with monomers across various concentrations.

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