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Venue

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Programme

23 May 2024

Time	Title
13:00-14:00	Registration, poster set-up and welcome snack
14:00-14:30	**Welcome** Jesús Requena (U. Santiago de Compostela) and Leticia Lucero (CNB-CSIC)
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14:45-15:30	**Alicia Otero (U. Zaragoza)** **Experimental prion diseases in large animals: The problems of cow-laboration in prion research**
15:30-15:45	Sara Canoyra (CISA-INIA-CSIC) *Evolution of Nor98/Atypical scrapie through transmission in a heterologous and homologous PrPC context*
15:45-16:00	Natalia Fernández-Borges (CISA-INIA-CSIC) *Goat PrP-222K polymorphic variant generates silent carriers for classical scrapie prions*
16:00-16:15	Lucien van Keulen (Wageningen Bioveterinary Research, the Netherlands) *Change in the molecular properties of CH1641 prions after transmission to wild-type mice: evidence for a single strain*
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17:15-17:30	Tomás Barrio (INRAE) *Zoonotic potential of moose-derived chronic wasting disease prions after adaptation in intermediate species*

17:30-17:45	Maria Rebeca Benavente (UTHealth Houston) *Detection of chronic wasting disease prions in processed meats and evaluations of zoonotic potentials*
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18:15-18:30	Jason Bartz (Creighton University) *Alterations in prion mutant spectra precede strain breakdown in animals*
18:30-19:15	**Carsten Korth (U. Düsseldorf) - Frontiers in Prion Research** ***DISC1 protein aggregates define a subset of chronic mental illnesses***
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Animal Prion Diseases

1. Experimental prion diseases in large animals: The problems of cow-laboration in prion research.

Alicia Otero[1], Belén Marín[1], Diego Sola[1], Carlos Hedman[1], Sonia Pérez[1], José Luis Pitarch[1], Helen Raksa[1], Paula A. Marco Lorente[1], Nerea Larrañaga[1], Eloisa Sevilla[1], Bernardino Moreno[1], Eva Monleón[1], Marta Monzón[1], Cristina Acín[1], Juan J. Badiola[1] y Rosa Bolea[1]

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Prion diseases pose significant threats to both human and animal health, with a range of species susceptible to infection. While much research has focused on transgenic models, understanding prion transmission dynamics in large animals, the natural hosts of these diseases, is crucial. In the Research Centre for Encephalopathies and Emerging Transmissible Diseases of the University of Zaragoza, we have been conducting prion transmission experiments in large animals for more than ten years, aiming to elucidate key aspects of prion pathogenesis and transmission kinetics.

Therefore, Classical Bovine Spongiform Encephalopathy (BSE) has been inoculated in goats to demonstrate if the genetic background of this species could protect against this strain; goat BSE and sheep BSE have been inoculated in goats and pigs respectively to evaluate the effect of species barrier; classical scrapie was inoculated in cow and atypical scrapie (Nor98) has been inoculated in a variety of species, with surprising results.

Through these experiments, we gained invaluable insights into the transmission barrier, tissue tropism, and host susceptibility factors associated with prion diseases in large animals.

However, conducting these type of experiments presents numerous challenges. These include the ethical considerations surrounding animal experimentation, logistical complexities in maintaining large animal colonies, and the need for specialized facilities equipped to handle these infected animals safely. Additionally, the length of the incubation periods and the cost of maintaining these experiments can make any researcher think twice before undertaking this venture.

Despite these challenges, large animal models can closely mimic the natural course of prion diseases, allowing for more accurate assessment of disease progression, pathogenesis, and therapeutic interventions. Furthermore, insights gained from large animal studies have the potential to inform public health policies and strategies for prion disease management and control.

2. Detection of chronic wasting disease prions in processed meats and evaluations of zoonotic potentials

Rebeca Benavente[1], Fraser Brydon[2] Francisca Bravo[1,3], Paulina Soto[1,3], J. Hunter Reed[4], Mitch Lockwood[4], Glenn Telling[5], Marcelo Barria[2], Rodrigo Morales[1,3]

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Keywords: Chronic Wasting disease, processed meats, zoonotic potential.

Aims: Identify the presence of CWD prions in processed meats derived from elk and assess the zoonotic potential of these samples by means of the protein misfolding cyclic amplification (PMCA).

Materials and Methods: In this study, we analyzed different processed meats derived from a CWD-positive (pre-clinical) free-ranging elk. Products tested included filets, sausages, boneless steaks, burgers, seasoned chili meats, and spiced meats. The presence of CWD-prions in these samples were assessed by PMCA using deer and elk substrates. The same analyses were performed in grilled and boiled meats to evaluate the resistance of the infectious agent to these procedures. Furthermore, the ability of these samples to induce the misfolding of human 129M prion protein substrate was assess by PMCA.

Results: Our results show positive prion detection in all the samples analyzed using deer and elk substrates. Surprisingly, cooked meats displayed increased seeding activities. However, none of them showed seeding activity in human PrP substrate after one round of PMCA.

Conclusions: These results suggest that CWD prions are accessible to humans through meats, even after processing and cooking. Importantly, the specific prion present in the meats included in this study exhibit a strong species barrier with human PrP. Considering the fact that these samples were collected from already processed specimens, the availability of CWD prions to humans is probably underestimated.

Funded by: NIH and USDA

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Acknowledgement: We would like to thank TPWD personnel for providing us with valuable samples.

3. CWD prions in the environment. New insights on how infectivity spreads in natural settings.

Paulina Soto[1,2], Francisca Bravo-Risi[1,2], Rebeca Benavente[1], J. Hunter Reed[3], Jennifer Malmberg[4], Vienna Brown[4], Tracy Nichols[5] and Rodrigo Morales[1].

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Keywords: chronic wasting disease (CWD); natural transmission; ecology; protein misfolding cyclic amplification (PMCA); prion infectivity.

Chronic wasting disease (CWD) is an infamous prionopathy affecting free-ranging and farmed cervids. Multiple modes of transmission have been identified in CWD, including direct animal contact, horizontal transmissions, and exposure to contaminated environmental fomites. However, it is still unclear which of these mechanisms is mainly responsible for perpetuating the spread of this disease. Recent research from our laboratory has identified the presence and infectivity of prions from multiple previously undescribed natural sources and humanmade materials. These include parasites (ticks and nasal bots), plants, scavengers (wild pigs), soils, insects (flies, dermestids), and cervid processing equipment. Importantly, the identification of infectious prions from the above-mentioned sources was achieved using prion amplification assays (PMCA or RT-QuIC) which are known to detect prions with sensitivities beyond those required to induce infectivity. To further understand infectious doses, some of these prion-contaminated components were tested in transgenic mouse bioassays to evaluate infectivity titers. Our results so far show that while wild pig tissues and naturally contaminated plants carry non-infectious prion quantities, nasal bots carry infectious quantities of prions and therefore have the potential to act as vectors of CWD. Current investigations in our laboratory will help to understand relationship between detection and infectivity, which will inform the role and significance of different fomites and potential vectors in the spread of CWD. In summary, our recently published and current research efforts are significantly helping to better connect the results of prion amplification assays to risk of transmission, and therefore to the ecology and epidemiology of CWD. In parallel, we expect that the data generated by our group will help to clarify the specific role that different components of the environment and ecosystem play in the natural transmission of this disease.

4. Development of a high-throughput system for the screening of anti-prion molecules

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Keywords: Protein misfolding cyclic amplification, drug screening, prion strains

PrP^{Sc} (a misfolded form of the physiological prion protein, PrP^{C}) is the main effector of prion diseases. Extensive evidence suggests that preventing or decreasing PrP^{Sc} replication is a promising target for therapeutic intervention. Unfortunately, the existence of different PrP^{Sc} strains provide challenges for the identification of anti-prion compounds as molecules modifying a particular prion agent may not be effective on a second one. The search of appropriate anti-prion therapies is limited by the lack of platforms allowing the screening of compound libraries in a high throughput manner. Although cell-based systems have been adapted for such purpose, they are not available for any relevant prion strains. We have shown that the Protein Misfolding Cyclic Amplification (PMCA) technology faithfully replicate the conformational properties of prions, maintaining all relevant biological features such as their infectivity and particular strain properties.

Results: We were able to reduce PMCA times to 24 hours (compared to conventional 48 or 72 hours per PMCA round) and volumes to 50 µL (compared to conventional 100 µL) without losing sensitivity. Dot blots were adapted in replacement of conventional western blotting, fact that substantially reduced the time to visualize PrP^{Sc} signals. Other materials and reagents were also reduced. We tested these optimizations with previously reported inhibitors of prion replication, as well as with molecules that interfere with the aggregation of other misfolded proteins. We observed strain-specific patterns of prion misfolding interference with specific compounds. Specifically, our data shows that while some drugs were active for some prion strains, they were inactive in others. Our system showed great reproducibility as judged by replicates run in different plates. In addition, we analyzed a small anti-neurodegeneration compound library (n=204) against chronic wasting disease prions. AS a result of this screening, we identified 7 hits.

Conclusions: Our PMCA system is suitable to test chemical libraries for putative anti-prion molecules. The data presented in this work demonstrate the use of the PMCA technique in the selection of prion strain-specific anti-prion compounds. Future studies focused in disease-relevant prion strains may help to identify most needed therapies against these fatal diseases.

Funded by: Creutzfeldt-Jakob disease Foundation

5. Prion-induced seeding is inactivated by incineration, but not vaporous hydrogen peroxide

Allen Herbst[1], Harrison Lamb[1], Emily Smith[2], Grace Hareid[2], Lauren Gritzmacher[2], Hannah Faber[2]

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Prions are widely regarded as the most resistant pathogen to chemical and physical inactivation. The aim of this work was to evaluate the capability of the real-time quaking-induced conversion (RT-QuIC) assay to monitor chronic wasting disease (CWD) prion inactivation after combustion in an incinerator or after exposure to vaporous hydrogen peroxide (VHP). Both incineration and VHP-exposure are recognized as effective for prion disinfection. White-tailed deer heads were tested for CWD, and batches of negative or positive heads were incinerated at a primary combustion temperature of about 1400°F. The resultant incinerator ash from each batch was collected and assayed by RT-QuIC. CWD positive brain tissue homogenate was dried onto plastic surfaces and treated in an encapsulated biological safety cabinet with vaporized hydrogen peroxide generated using a spinning disk. The seeding activity of the CWD-contaminated VHP-treated surfaces was compared to replica plated controls that were not exposed. Similarly, residual prion protein immunoreactivity from the CWD-contaminated VHP-treated surfaces was measured by western blotting and compared with replica plated non-exposed controls.

We were unable to detect seeding activity in incinerator ash samples generated by the combustion of either CWD+ or CWD- deer heads. By contrast, when ash from CWD- heads was spiked with CWD+ brain homogenate, we were able to detect seeding down to 10^-6 dilution.

VHP treatment was ineffective at destroying RT-QuIC seeding activity. Seeding was observed in VHP treated wells with even low concentrations of CWD prions. Western blotting data indicate that VHP treatment sensitized CWD prions to proteinase K. Incineration is effective at decontaminating and disinfecting prions, and RT-QuIC is a suitable method to evaluate residual CWD contamination in incinerator ash. Published bioassay data indicate that VHP treatment is effective at inactivating prions, and this is confirmed by our western blot data. VHP treatment is effective at disinfecting prions, but not decontaminating prions.

6. RT-QuIC substrate preparation by diffusion dialysis

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Real-time quaking induced conversion (RT-QuIC), a fluorescence-based amyloid seeding assay, is improving prion detection as the assay is several orders of magnitude more sensitive that other prion detection techniques. RT-QuIC substrate preparation is a crucial step that typically involves immobilized metal affinity resin and fast protein liquid chromatography (FPLC). The role of various parameters to produce substrate by FPLC are not well understood and substrate production by this approach is time intensive, expensive, and the yields are low.

To better understand RT-QuIC substrate production, improve substrate yield and decrease complexity and cost, we conducted production experiments that involved the ordered refolding of prion proteins from inclusion bodies using stepwise dialysis. The performance of substrates produced by a basic stepwise dialysis was not different from those produced by FPLC, and the yield was superior (~6x higher). After successful production of substrate from stepwise dialysis, additional modifications were made to test the bounds of this approach. We manipulated pH, introduced various concentrations of arginine, examined the effect of extending the refolding time, and explored the nature of the precipitation formed during refolding. In one experiment, 14.8% of the input inclusion body material was recovered as usable substrate with seeding times around 8 hours for 10^{-5} brain homogenate. Some substrates had a 10^{-7} sensitivity for detecting Chronic Wasting Disease prions from brain tissue with a false seeding rate of <1%. In other experiments, the higher yield from stepwise dialysis was accompanied by a slower time to threshold or an increased false seeding rate.

If substrate could be prepared at lower cost and higher yield and did not require chromatographic capability it would increase accessibility to RT-QuIC. Specifying the minimum conditions necessary to produce quality RT-QuIC substrate and optimizing refolding to increase yield will help to deepen our understanding of RT-QuIC technology.

7. Degradation of environmental prions by soil-dwelling detritivores

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Keywords: CWD, RT-QuIC, Earthworms

Chronic Wasting Disease (CWD) prions are shed into the environment via feces, urine, saliva, and ultimately carcasses. The persistence of prions has led to the hypothesis that the environment is a reservoir for CWD. To test this hypothesis, we constructed environmental macrocosms that contained different species of detritivores and measured the destruction of CWD prions. We constructed model systems for five species of worms (*Eisenia fetida*, *Lumbricus terrestris*, *Amynthas spp.*, and *Lumbriculus variegatus*) from three different annelid families across two phylogenic orders. The effect of constituent components in the model systems were tested to ensure acceptable RT-QuIC assay performance. We exposed the worms to white-tailed deer PrP^{CWD} collected from southern Wisconsin. The rate of PrP^{CWD} degradation was evaluated in macrocosm homogenates at various timepoints across 14 days. RT-QuIC was used to measure the abundance of prions in each sample.

Evaluation of system components determined that worm bedding did not affect RT-QuIC substrate performance. By contrast, at high concentrations (10%) worm castings caused false seeding and worm homogenate suppressed seeding. This was resolved by dilution. When we added CWD prions to the macrocosms, we observed decreased seeding over time with all the species of worms tested. For *Lumbricus terrestis* systems, the amount of seeding decreased the quickest and showed the largest decline among tested species. For *E. fetida systems*, the decrease in positive seeding occurred between 7- and 14-days while for systems containing A*mythas and Lumbricus* the rate of positive seeding decreased after 7 days. These data show that earthworms reduce RT-QuIC seeding, and this indicates the potential destruction of CWD prions in the environment by annelids.

8. Change in the molecular properties of CH1641 prions after transmission to wild-type mice: evidence for a single strain

Lucien van Keulen[1], Corry Dolstra[1], Ruth Bossers-de Vries[1], Alex Bossers[1], Jorg Jacobs[1], Thierry Baron[2], Juan Maria Torres[3], Jan Langeveld[1]

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Keywords: classical scrapie; protease resistant prion; PrP^{Sc}; strain typing; transgenic mice

Background. CH1641 was discovered in 1970 in the UK as a scrapie isolate that was unlike all other classical strains of scrapie isolated sofar. Not only did CH1641 behave differently in sheep transmission experiments, it also did not transmit to wild-type mice.
Methods. We inoculated the original CH1641 isolate into wild -type mice (RIII-$Prnp^{a}$ and VM-$Prnp^{b}$), ovine PrP transgenic mice (VRQ-Tg338 and ARQ-Tg59) and bovine PrP transgenic mice (Tg110). In addition, we performed cross- and back passages between the various mouse lines to examine if one prion strain was isolated in all mouse lines or whether multiple prion strains exist in CH1641.
Results. We report the first successful transmission of the original CH1641 isolate from the UK to wild-type RIII mice and via RIII mice to wild-type VM mice. Unexpectedly, analysis of the protease resistant prion protein (PrP^{res}) in wild-type mice showed a classical scrapie banding pattern differing from the banding pattern of the original CH1641 isolate and its passages in ovine and bovine PrP transgenic mice. Cross- and back passages of CH1641 between the various mouse lines confirmed that the same prion strain had been isolated in all mouse lines (Figure 1).***Conclusions***. The CH1641 isolate consists of a single prion strain but its molecular banding pattern of PrP^{res} differs between wild-type mice and PrP transgenic mice. Consequently, molecular banding patterns of PrP^{res} should be used with caution in strain typing since they do not solely depend on the properties of the prion strain but also on the host PrP.

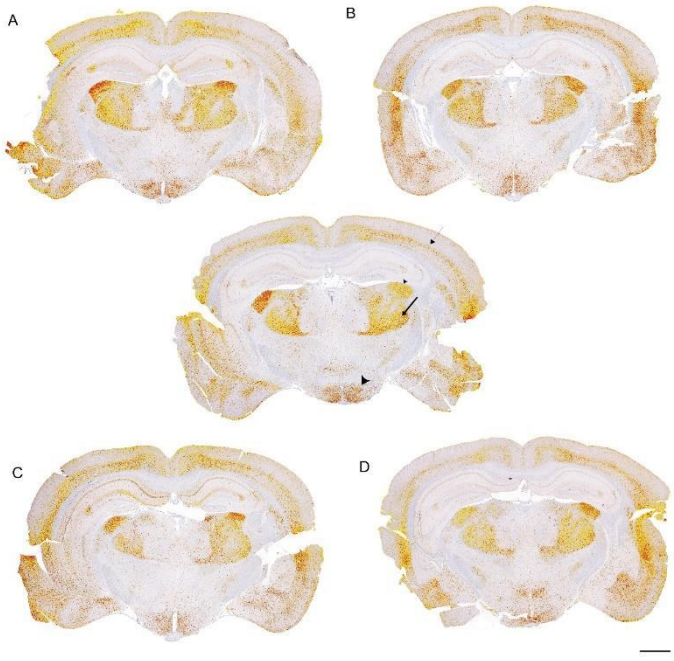

Figure 1. CH1641 was first passaged through the various mouse lines. CH1641 passaged through Tg338 (A), Tg110 (B), RIII (C) and VM (D) mice was then cross passaged to Tg59 mice and the PrP^{Sc} depositions in the brain of the Tg59 mice were immunostained. All PrP^{Sc} profiles at the level of the thalamus were similar to the PrP^{Sc} profile of the homologous passage of CH1641 in Tg59 mice (center). In addition, incubation periods in Tg59 mice were not significantly different after stabilisation, indicating that the same strain was isolated.

Reference

[1] Change in the molecular properties of CH1641 prions after transmission to wild-type mice: Evidence for a single strain. Keulen LJM, Dolstra CH,
de Vries RB, Bossers A, Jacobs JG, Baron T, Torres JM, Langeveld JPM. Neuropathol Appl Neurobiol. 2024 Feb;50(1):e12963. doi: 10.1111/nan.12963.

9. Adaptive events in peripheral and intracerebral compartments of gene-targeted mice result in the selective propagation of CWD strains

Joseph P. DeFranco[1], Jifeng Bian[1,2], Sehun Kim[1], Jenna Crowell[1], Tomás Barrio[1,3], Bailey K. Webster[1], Zoe N. Atkinson[1], and Glenn C. Telling[1]

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Keywords: prions, chronic wasting disease, strains, peripheral pathogenesis

Prion strain phenotypes, which are enciphered by the conformations of their constituent infectious proteins, are subject to adaptation in response to selective pressures. While certain prions, including those causing chronic wasting disease (CWD), replicate in both peripheral and central nervous system tissues, their strain properties are thought to remain constant when propagated by these different routes of infection. To examine this notion in the context of CWD, we inoculated gene-targeted GtE and GtQ mice expressing physiologically accurate levels of elk or deer PrP by peripheral or intracerebral routes. Whereas oral and intraperitoneal transmissions preserved the convergent conformations of elk and deer prions and produced identical neuropathological outcomes in cognate GtE and GtQ mice, intracerebral transmissions generated divergent conformers with strain properties that were unrelated to their native counterparts. While CWD replication kinetics remained unchanged during iterative peripheral transmissions and brain titers reflected those found in native hosts, intracerebral transmissions produced 10-fold higher titers and accelerated incubation times during serial passages. Our findings that peripherally- and intracerebrally-challenged Gt mice propagate distinct CWD strain conformers reveal that prions adapt in response to varying routes of transmission. This discovery is consistent with the selection of optimized conformers from strain mixtures by tissue-specific factors. Since peripheral transmissions in Gt mice preserved the natural features of elk and deer prions whereas adaptive events during intracerebral propagation produced distorted strain estimates, our findings underscore the importance of experimental characterizations in hosts that not only abrogate species barriers but also recapitulate natural transmission routes of native strains.

10. Alterations in prion mutant spectra precede strain breakdown in animals

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Keywords: Prion strain quasispecies, prion strain breakdown, conformational stability, proteinase K

Objectives
Prions are composed of PrP^{Sc}, the disease specific conformation of the host encoded prion protein. Treatment of rodents with anti-prion drugs results in the emergence of drug-resistant prion strains, suggesting prions are comprised of a dominant strain and substrains. While considerable experimental evidence supports this hypothesis, direct observation of substrains has not been observed. The objective of this study was to directly investigate if prion strains contain substrain diversity.

Methods
We investigated the presence of substrain diversity in a biologically stable hamster-adapted prion strain, DY TME and a biologically unstable hamster-adapted prion strain 139H that, following serial passage at high titer, can breakdown into a strain with a shorter incubation period. Both strains were probed for substrains by selectively reducing the abundance of the dominant strain PrP^{Sc} using either extended digestion with proteinase K or with chaotropic treatment or susceptibility to RK13 cell infection. The remaining PrP^{Sc} was subjected to protein misfolding cyclic amplification to probe for substrains.

Results
Selective reduction of DY or 139H PrP^{Sc} using a combination of biochemical methods resulted in the emergence of strains with properties that differed from DY or 139H and were consistent with the selection criteria. Substrains identified in 139H-infected brains were similar to the strains that emerged in animals following strain breakdown.

Conclusions
The substrain selection methods occurred outside of prion formation, providing direct evidence for the preexistence of substrains. Furthermore, we hypothesize that substrains are a common feature of prions as they were identified in a stable, biologically-cloned prion strain. The identification of preexisting substrains may contribute to the ability of prions to rapidly adapt to new replication environments such as transmission to a new species or replication in the presence of anti-prion drugs.

11. Detection of prions in oocytes and ovaries of ewes naturally infected with classical scrapie

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Keywords: scrapie, transmission, oocytes, ovaries, sheep.

Classic scrapie is a transmissible spongiform encephalopathy (TSE) that naturally affects goats and sheep and is mainly transmitted horizontally [1]. However, infection can also occur via the vertical or maternal route, although the exact moment and route by which it occurs remains unclear. Three possibilities are currently being considered: prenatal transmission (from oogenesis to parturition) [2], during parturition (by contact with the placenta or fluids) [3, 4], or after birth (by ingestion of contaminated colostrum or milk or by contact with the contaminated environment) [5–7]. To better understand these vertical transmission routes, several studies have been conducted on the presence of prions in placentas [3, 8, 9], uterus [10], amniotic fluid, fetuses [11, 12], milk [5, 7] and even sperm [13], thus raising the possibility of vertical transmission through the germ line. However, the presence of prions in female reproductive tissues and oocytes has barely been studied; therefore, whether and to what extent they could contribute to the vertical transmission of scrapie is unknown.

For this reason, we evaluated the presence of prions in the oocytes of ewes of ARQ/ARQ or VRQ/VRQ genotypes naturally infected with classical scrapie by Protein Misfolding Cyclic Amplification (PMCA) and subsequent Dot Blot and Western Blot techniques. Positive results were obtained for oocytes from most of the ARQ/ARQ ewes (especially those at a more advanced stage of the disease), but hardly any positivity was observed in the case of oocytes from VRQ/VRQ ewes, which could suggest a possible difference in the accumulation of prions in oocytes depending on the genotype. On the other hand, the presence of PrPSc deposits and associated lesions in the ovaries was also analyzed by hematoxylin-eosin staining and immunohistochemistry, finding some PrPSc-like deposits mainly in the ovaries of ARQ/ARQ ewes whose oocytes had showed a higher positivity, suggesting again differences depending on the genotype.

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12. Development of potential gene therapies for prion diseases in animal models

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Keywords: prions, scrapie, gene therapy, AAV9

Transmissible spongiform encephalopathies (TSEs) or prion diseases are fatal neurodegenerative processes with a long incubation period for which there is no treatment. All of them involve in their pathogenesis the accumulation of abnormal isoforms (PrP^{Sc}) of the cellular prion protein (PrP^{C}) in the central nervous system (CNS), leading to neuronal dysfunction, cell death and the appearance of vacuoles in the CNS. Many different molecules have been tested to find a suitable treatment for these diseases, however, their usefulness is limited due to their means of administration, or because they are only effective if administrated before neuroinvasion [2,3]

Therefore, in this study we suggest an innovative gene therapy for prion diseases: using an Adeno-Associated Virus 9 (AAV9) to deliver a negative dominant mutation into the neurons. The selected mutation is the substitution of glycine (G) for valine (V) in codon 127 of human PrP, which confers resistance to human prion diseases [4,5].

Our hypothesis is that this mutation may grant the same resistance to scrapie. Thus, for this experiment, transgenic mice expressing ovine PrP^{C} have been inoculated with scrapie isolates and then treated with an AAV9 containing coding transgene for this mutation (G130V in ovine PrP sequence), using different routes of administration. Several treated and non-treated mice have developed signs of prion disease. These animals have been euthanized and their brains collected and examined using Immunohistochemistry (IHC) and Western Blot (WB) techniques.

We have confirmed that the AAV9 is capable of reaching the CNS when injected peripherally thus crossing the blood-brain barrier and that there is a prolongation in the incubation period of treated animals.

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13. Neuropathological characterization of the prion disease induced by synthetic prions from different mammal families (gerbil, mink, rabbit and bat)

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Keywords: recombinant, prion, PMSA, neuropathology, TgVole(1x)

A new protein misfolding shaking amplification-based method (PMSA) has been recently published allowing the production *in vitro* of *bona fide* recombinant prions *de novo* from hundreds of different species [1]. This method not only has permitted to establish each species' PrP^{c} misfolding proneness but also has been proven to yield, driven by the addition of specific cofactors, different stable conformers with distinguishable pathobiological features that will help understand the prion strain phenomenon [2]. To confirm the infectivity of these synthetic, spontaneously misfolded recombinant PrP molecules, bioassays were conducted in a well characterized, highly susceptible transgenic mouse model, the TgVole (1x) mice which express 1x levels of the bank vole PrP^{c} and exhibit high susceptibility to many prion strains. In this communication, we present preliminary results analysing the outcome of these first passage bioassays to determine the phenotypic variability elicited in this model by a selection of synthetic prions. These include recombinant prions from mink (*Neovison vison*), mongolian gerbil (*Meriones ungulaticus*), rabbit (*Oryctolagus cuniculus*), egyptian fruit bat (*Rousettus aegyptiacus*), and bank vole (*Myodes glareolus*) for comparison [2]. A semiquantitative evaluation of the spongiform change and PrP^{res} deposition has been performed using H/E staining and immunohistochemistry on formalin fixed, paraffin embedded TgVole (1x) mouse brain tissue sections. We present data along with incubation periods and biochemical profiles of proteinase K-resistant PrP. While certain level of variability was observed among individuals within each inoculated group of mice, common features were also identified among groups.

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14. Extraneural infection restricts prion conformational variability and attenuates impact of quaternary structure on infectivity

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Keywords: animal prion disease, prion biology, chronic wasting disease, strain selection, prion quaternary structure,

Prions can exist as different strains that consist of conformational variants of the misfolded, pathogenic prion protein isoform PrP^{Sc}. Defined by stably transmissible biological and biochemical properties, strains have been identified in a spectrum of prion diseases, including chronic wasting disease (CWD) of wild and farmed cervids. CWD is highly contagious and spreads via direct and indirect transmission involving extraneural sites of infection, peripheral replication and neuroinvasion of prions.

Here, we investigated the impact of infection route on CWD prion conformational selection and propagation. We used gene-targeted mouse models expressing deer PrP for intracerebral or intraperitoneal inoculation with fractionated or unfractionated brain homogenates from white-tailed deer, harboring CWD strains Wisc-1 or 116AG. Upon intracerebral inoculation, Wisc-1 and 116AG-inoculated mice differed in extent, distribution, and conformational stability of PrP^{Sc}. In brains of mice infected intraperitoneally with either inoculum, PrP^{Sc} propagated with identical conformational stability and fewer PrP^{Sc} deposits. For either inoculum, PrP^{Sc} conformational stability in brain and spinal cord was similar upon intracerebral infection but significantly higher in spinal cords of intraperitoneally infected animals. Inoculation with fractionated brain homogenates resulted in lower variance of survival times upon intraperitoneal compared to intracerebral infection.

In summary, we demonstrate that extraneural infection mitigates the impact of PrP^{Sc} quaternary structure on infection and reduces conformational variability of PrP^{Sc} propagated in the brain. These findings provide new insights into the evolution of stable CWD strains in natural, extraneural transmissions.

15. Goat PrP-222K polymorphic variant generates silent carriers for classical scrapie prions

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Keywords: classical scrapie, goats, resistant genotypes, silent prion carriers.

Classical scrapie is a transmissible spongiform encephalopathy affecting sheep and goats, caused by various prion strains. To control its prevalence in sheep, the European Union initiated breeding programs targeting resilient genotypes. Although certain polymorphisms, such as $Q_{222}K$, have been linked to resistance in goats, specific breeding programs favoring these genotypes have not yet been implemented.

Hemizygous transgenic mice carrying the goat K_{222}-PrP^{c} allele (K_{222}-Tg516) have demonstrated resistance to several classical scrapie isolates [1]. Furthermore, experimental studies involving heterozygous and homozygous K_{222} goats revealed varying levels of resistance to classical scrapie, ranging from partial to complete [2-5].

In this study, homozygous K_{222}-Tg516 and Q_{222}-Tg501 mice were intracranially inoculated with several isolates representing the four categories of classical scrapie strains circulating in Europe [6]. Surprisingly, homozygous K_{222}-Tg516 mice reached the end of their lifespan without exhibiting clinical signs of the disease; however, they displayed brain PrP^{res} accumulation, albeit lower when compared to Q_{222}-Tg501 mice. Histologically, K_{222}-Tg516 mouse brains showed an absence of prion-related lesions, except for the presence of few isolated PrP^{Sc} plaques in certain prion isolates highly adapted to the K_{222}-PrP^{c} environment through subsequent passages.

Our findings caution against the inclusion of this polymorphism in breeding programs, as it may lead to the emergence of asymptomatic silent prion carriers of classical scrapie among goat populations.

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16. Sleep disturbance in clinical and preclinical scrapie-infected sheep measured by polysomnography

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Prion diseases share common neurodegenerative features with other neurodegenerative protein misfolding diseases like Alzheimer's disease (AD), Parkinson's disease (PD), dementia with Lewy bodies and Huntington's disease (HD) among others [1]. These neurodegenerative diseases are characterised by neuronal loss and abnormal deposition of proteins (phosphorylated α-synuclein, tau, β-amyloid, huntingtin or PrP^{Sc}) in the nervous system, mainly in the central nervous system [2, 3].

A variety of neurological symptoms and signs, such as dementia, parkinsonism, motor weakness, ataxia, cerebellar syndrome, dysautonomia or oculomotor abnormalities, are typical of these diseases, which often have a chronic and progressive course [4,5]. In addition to all of these, sleep disturbances are very common and may be the first manifestation of disease and a crucial diagnostic sign for predicting disease progression [6]. It has become increasingly evident that sleep dysfunction commonly accompanies chronic neurodegenerative diseases and may appear several years before the main symptoms of these disorders [7].

Currently, one of the main objectives in the study of TSEs is to try to establish an early diagnosis, as clinical signs do not appear until the damage to the central nervous system is very advanced, which prevents any therapeutic approach. In this study, we provide the first description of sleep disturbance caused by classical scrapie in clinical and preclinical sheep using polysomnography compared to healthy controls. Fifteen sheep classified into three groups, clinical, preclinical and negative control, were analysed. The results show a decrease in total sleep time as the disease progresses, with significant changes between control, clinical and pre-clinical animals. The results also show an increase in sleep fragmentation in clinical animals compared to preclinical and control animals. In addition, sheep with clinical scrapie show a total loss of Rapid Eye Movement sleep (REM) and alterations in Non Rapid Eyes Movement sleep (NREM) compared to control sheep, demonstrating more shallow sleep. Although further research is needed, these results suggest that prion diseases also produce sleep disturbances in animals and that polysomnography could be a diagnostic tool of interest in clinical and preclinical cases of prion diseases.

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17. Zoonotic potential of moose-derived chronic wasting disease prions after adaptation in intermediate species

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Keywords: chronic wasting disease, European moose, transmission barrier, enhanced zoonotic potential, transgenic model

Chronic wasting disease (CWD) is an emerging prion disease in Europe. To date, cases have been reported in three Nordic Countries and in several species, including reindeer (*Rangifer tarandus*), moose (*Alces alces*) and red deer (*Cervus elaphus*). Cumulating data suggest that the prion strains responsible for the European cases are distinct from those circulating in North America. The biological properties of CWD prions are still poorly documented. In this study, we aimed at characterizing the interspecies transmission potential of Norwegian moose CWD isolates by experimental transmission in a panel of PrP expressing transgenic models. On first passage, one moose isolate propagated in ovine PrP^{c}-expressing model (Tg338). After adaptation in this host, the moose CWD prions were able to transmit in mice expressing either bovine or human PrP^{c} with high efficacy. These results suggest that CWD prions can acquire zoonotic properties following passage in an intermediate species.

18. Emergence of a novel TSE Strain Following Passage of Classical BSE in VRQ/VRQ Sheep

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Keywords: BSE, TSE, strain, VRQ, bioassay

The natural host of classical BSE (C-BSE) is cattle. Currently, only a single C-BSE strain has been identified. Experimental transmissions in sheep showed that, in contrast to ARQ or AHQ alleles, the VRQ allele confers resistance to C-BSE. However, this resistance is not absolute, as occasionally VRQ/VRQ sheep can succumb to C-BSE albeit with prolonged incubation periods [1]. As the properties of TSE agents are more likely to change when they cross transmission barriers, we analysed the biological properties of the agent isolated from a VRQ/VRQ sheep, which was challenged orally with bovine C-BSE, by bioassay in RIII, tg338 (VRQ ovine transgene), tgShpXI (ARQ ovine transgene) and tg110 (bovine transgene) mice. We observed that in tg338 mice two strains were isolated. One with long survival time in tg338 murine line (muLST) which was indistinguishable from the strain that is typically isolated from C-BSE affected cattle or ARQ/ARQ sheep. A novel strain characterised by short survival time in tg338 mice (muSST) was also isolated. In the other mouse lines only the muLST strain was isolated. Interestingly, the muSST strain could only be maintained by serial passaging in tg338 mice as after serial passaging in other transgenic mouse lines only the muLST strain was isolated. Initial analysis suggested that this novel strain had been developed in the sheep, and it was not generated by the tg338 mice. To further study this phenomenon, bioassays in transgenic mice from the remaining 5 C-BSE affected VRQ/VRQ sheep were also initiated, and similar data were obtained. Second passages in sheep, guided by tg338 bioassay data obtained from the donor animals, produced two distinct phenotypes in the recipient ovine animals, one with long and one with short incubation period (ovLST and ovSST respectively). From ovSST sheep only the muSST strain was isolated in tg338 mice. From ovLST sheep a mixture of muSST and muLST strains was propagated in tg338 mice. These data suggest that muSST and muLST are associated with the ovSST and ovLST respectively. The zoonotic potential of the SST strain or if it exists, in any form, in cattle are yet to be determined.

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19. Chronic Wasting Disease in Bovine-PrP Transgenic Mice Propagates with Different Prion Strain Features

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Keywords: prion strains, species barrier, chronic wasting disease, cervids

Chronic wasting disease (CWD) is an infectious prion disease that affects cervids. Diverse strains of CWD prions have been detected in various cervid species across North America and Europe. These properties of the infectious prion isolates are influenced by amino acids changes and polymorphisms in the PrP sequences of different cervid species [1]. This research aimed to evaluate the ability of a panel of CWD prion samples from various cervid species across North America and Europe to infect bovine species, while also exploring the traits of the prion strains after adapting to the bovine-PrP context. BoPrP-Tg110 mice overexpressing the bovine-PrP sequence [2] were inoculated by intracranial route with the panel of CWD prion isolates from both North America and Europe.

Our findings reveal distinct behaviours in the transmission of the CWD samples to the BoPrP-Tg110 mouse model. A few of these isolates failed to transmit even upon the second passage. Those that did transfer exhibited differences in terms of infection rate, survival times, biochemical properties of brain PrP^{res}, and histopathological characteristics.

Overall, these results demonstrate the range of CWD strains within the collection of CWD samples, as well as the ability of at least certain CWD isolates to infect cattle species. Given that these are among the most significant livestock species, this ability poses a potential risk to both animal and human health, thus demanding further investigation.

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20. Blood microRNA alterations in prion disease progression

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Keywords: microRNA, minimally invasive biomarker, neurodegeneration, scrapie, prion diseases.

MicroRNAs are small non-coding RNA molecules that regulate gene expression at the post-transcriptional level. Since microRNAs regulate more than half of the protein-coding genes and are mostly expressed in the brain, alterations in their expression have been associated with neurodegenerative disorders, as expected, including prion diseases. These fatal diseases are spread worldwide and still no reliable *in vivo* diagnostic method is available. To address this problem, high throughput massive sequencing techniques are of great importance. Given the marked stability of microRNAs in body fluids, they can be considered as potential minimally invasive biomarkers of these diseases.

In this study, we have performed smallRNA sequencing in blood due to its easy accessibility. For this purpose, we have analysed naturally infected sheep with classical scrapie, as a natural model of prion diseases. These animals were divided in groups depending on their stage of the pathogenesis of the scrapie infection: 5 preclinical sheep before the onset of clinical signs, 10 clinical scrapie sheep and 10 healthy animals used as controls. Then, a bioinformatic analysis was performed and most promising microRNAs were validated using real-time quantitative PCR. Additionally, we are investigating their expression in the central nervous system to infer a possible correlation between their blood levels and the neurodegeneration.

Specifically, 136 microRNAs were significantly dysregulated in blood from clinical animals compared to healthy controls. From these, 69 microRNAs were downregulated in clinical animals whereas 67 were upregulated. However, only 1 microRNA was significantly altered in preclinical individuals compared to healthy ones.

Our results suggest that microRNAs seem to alter their expression when clinical signs appear. Anyway, these results are promising, and the alterations observed in the clinical stage could reflect their important role in the pathogenesis of these neurodegenerative diseases and their potential usefulness as biomarkers of prion diseases.

21. Evolution of Nor98/Atypical scrapie through transmission in a heterologous and homologous PrP^C context

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Keywords: prion evolution, Nor98/atypical scrapie, C-BSE, prion strain diversity

Intra- or cross-species transmission of prions can result in the emergence of new prion strains due to changes in their conformational characteristics. Two non-mutually exclusive theories explain this process of prion strain emergence: the 'deformed templating' or conformational mutation model, and the 'conformational selection' model. The conformational mutation model suggests that the host's inability to replicate the prion forces a shift in the PrP^{Sc} conformation, leading to the emergence of new prion strains *de novo*. Conversely, the 'conformational selection' theory postulates that prion isolates consist of a conglomerate of PrP^{Sc} conformations, with a predominant energetically favorable conformation. During cross-species transmission, the species barrier acts as a filter that selects other PrP^{Sc} conformers.

In this work, we studied the evolution of Nor98/Atypical scrapie (AS), a prion disease that causes sporadic cases in sheep and goats, as a paradigm of prion strain emergence during the transmission of this prion to different PrPs. In the bovine and porcine PrP sequence, there has been reported the emergence of classical Bovine Spongiform Encephalopathy (C-BSE) prions [1–3] and in the bank vole PrP, classical scrapie-like prion strain emerges [4]. We analyzed the evolution of the AS prion through inter-species transmission to examine the evolutionary dichotomy by the transmission in the bovine PrP^C context. We used the differential strain characteristics of thermostability and *in vitro* prion amplification by the Protein Misfolding Cyclic Amplification (PMCA) for that purpose. Our results suggest that conformational mutation serves as the primary mechanism driving the emergence of C-BSE. In addition, we investigated the possible evolution of AS prions within the same species by modelling the transmission in a homologous ovine ARQ-PrP context both *in vivo* and *in vitro* by PMCA. Beside propagating AS *in vivo,* classical forms emerge as BSE-like and 21kDa scrapie according to the brain-PrP^{res} profile by Western Blot. This emergence could be due to either the coexistence of strains in the isolate or the evolution of AS through propagation in the ovine PrP context. Altogether, these findings shed light on the potential origin of prion diversity, suggesting that atypical prions may give rise to classical forms.

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22. Atypical scrapie features can be reproduced through intracerebral inoculation of sheep with prions spontaneously originated in the ShTgI112 transgenic mouse model

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Keywords: atypical, scrapie, spontaneous, prion disease.

Transmissible spongiform encephalopathies or prion diseases are a group of fatal neurodegenerative disorders caused by the conversion and misfolding of the physiological prion protein (PrP^{C}) into the pathological isoform (PrP^{Sc}), leading to its aggregation and accumulation in the central nervous system. Scrapie belongs to this group of diseases and affects sheep and goats. Atypical forms of scrapie have been reported that differ from the classical, more abundant, strains and, due to the epidemiological characteristics of its presentation, with isolated cases in small ruminant flocks, many researchers have suggested that it is a spontaneously occurring disease, without the need for contact with external sources of prions.

Recently, the creation of a transgenic mouse model overexpressing ovine PrP^{C} with the I112 polymorphism (TgShI112) has been published, and it has been described that these mice develop a spontaneous prion disease with similar properties to atypical scrapie [1]. An inoculum was prepared with brain samples from these mice (named ShTgSPON) which was intracerebrally inoculated in four Churra Tensina sheep to assess the reproducibility of the spontaneous disease reported in the murine model in its natural host. All sheep exhibited clinical signs and have been euthanised with terminal nervous system disease.

In this study, an in-depth analysis through immunohistochemical and histopathological assessment of different areas of the central nervous system was performed in two of these animals, assessing PrP^{Sc} deposition and comparing the neuropathological profile and morphology of the immunostaining patterns with those of sheep intracerebrally inoculated with an atypical scrapie isolate. Additionally, an exhaustive immunohistochemical study of all peripheral organs was conducted to search for possible PrP^{Sc} accumulation.

These preliminary results indicate a striking similarity between the neuropathological features of sheep inoculated with the ShTgSPON inoculum and sheep inoculated with an isolate of ovine atypical scrapie,

further confirming that the strain generated spontaneously in the TgShI112 model is identical to the atypical scrapie cases.

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Prion Structure and Biology

23. Prion replication – from structure to mechanism and back

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Prion diseases are caused by the structural conversion of the prion protein from its native form (PrPC) to the prion fold, which presents as insoluble, denaturation and protease resistant fibrils (PrPSc). Replication of prion fold follows the mechanism of nucleated polymerization, initially thought to be unique, but now confirmed to underly a large number of neurodegenerative and systemic diseases. Yet ,it still unknown what makes prions infectious in organisms when compared to other amyloid.

Recent advances in cryo-EM yielded the prion structure as well as structures of many other amyloids. These results confirmed that prion strains are encoded in the fold of their amyloid fibrils, but also revealed the multitude of possible amyloid conformers. This suggests that prions can evolve on the structural level: strain adaption, environmental adaption and that prion evolution follows the same principles as genetic Darwinian evolution.
Structural analysis and single particle kinetic assays now allow the analysis of the evolution of prion conformer populations and to identify the necessary components for infectious prion formation.

24. Fluorescent prions in live cells: from real-time monitoring to nano-scale structural snapshots

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Keywords: genetic code expansion, unnatural amino acid, bio-orthogonal labeling, fluorescent prions, cryo-CLEM

Although numerous prion protein structures before and after conversion into infectious prion fibrils of various strains have now been determined, the cellular whereabouts and mechanisms of prion replication are still unclear. To advance our studies beyond understanding the “what” and towards understanding the “how and where” we engineered prion-susceptible cells to incorporate unnatural amino acids into the prion protein that can be rendered fluorescent. This enables precise spatiotemporal tracking of prion infection in live cells. Cryo-correlated fluorescence and electron microscopy enables unambiguous localisation of prion assemblies at defined time points and their precise structural characterisation within highly resolved cellular nano-environments.

25. Characterization of WH1 amyloid domains in Rep proteins from *Xylella fastidiosa* plasmids

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Keywords: *Escherichia coli*, *Xylella fastidiosa*, Rep-WH1, amyloid.

In our group we had thoroughly characterized RepA, a protein involved in the DNA replication from a plasmid belonging to *Pseudomonas savastanoi* [1]. This allowed us to prove that its WH1 dimerization domain is a functional amyloid [2]. Furthermore, we were able to trigger the formation of RepA-WH1 amyloid aggregates in *Escherichia coli* [3], which turned out to be cytotoxic by forming pores in the bacterial lipid membrane, thus hampering the proton motive force and impeding membrane trafficking, reducing ATP synthesis and triggering the generation of ROS [2,4].

Here, we report the use of homologous Rep-WH1 domains from plasmids belonging to the phytopathogen *Xylella fastidiosa* (*spp. pauca* and *spp. fastidiosa*) to generate a synthetic amyloid proteinopathy in bacteria. For that, we separately expressed both proteins in *E. coli*, thus generating amyloid aggregates with different characteristics (according to Th-S staining) that drastically changed bacterial morphology, growth rate and viability. Chaperone DnaK (Hsp 70) overexpression, which showed to promote a shift in the aggregation pattern of RepA-WH1 from *P. savastanoi* [5,6], does the same when co-expressed with the two *X. fastidiosa* Rep-WH1. To further understand the physicochemical aspects of these two proteins, we are currently performing Circular Dichroism, Transmission Electron Microscopy and phase transition studies. Our final aim is to characterize the aggregation dynamics of both variants *in vitro* and explore their use as a control agent against *X. fastidiosa in planta*.

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26. Understanding the interaction between the polybasic regions of PrP and polyanionic cofactors in spontaneous misfolding

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Keywords: cofactor, polybasic region, recombinant PrP, PMSA, spontaneous misfolding

The polyanionic cofactors have been identified as significant contributors to the misfolding process of the PrP protein, playing a pivotal role in modulating its conformational changes. These cofactors are commonly employed for in vitro prion production to enhance the efficiency of both spontaneous misfolding and seed-mediated prion propagation. Examples of such cofactors include RNA, lipids, glycosaminoglycans (GAGs), and a variety of other polyanions, which encompass polysaccharides among others. The intricate interplay between these cofactors and the PrP is relevant for the initiation and propagation of the misfolding cascade. Furthermore, the N-terminal (residues 23-27, in bank vole PrP numbering) and central (101-110) polybasic regions of the protein have been recognized as potential binding sites for these polyanionic cofactors to PrP^{c}, suggesting an important role in the misfolding process. These regions may act as focal points for the interaction between the PrP and cofactors, facilitating the formation of misfolded conformers and ultimately leading to prion propagation. To further explore the underlying mechanisms of this phenomenon, we have used a set of recombinant PrP variants derived from bank voles (*Myodes glareolus*). These variants feature deletions and substitutions within the aforementioned regions. Our aim was to investigate whether these alterations affect cofactor binding by assessing their propensity for spontaneous misfolding using the Protein Misfolding Shaking Amplification (PMSA) technique.

27. Characterization of wild-type and mutant PrP-HaloTag chimaeras

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Keywords: PrP, HaloTag, HEK293, trafficking, genetic prion disease.

Background: Genetic prion diseases, including genetic Creutzfeldt-Jakob disease (gCJD), Fatal Familial Insomnia (FFI), and Gerstmann-Sträussler-Scheinker (GSS) syndrome, are neurodegenerative disorders linked to gain-of-function mutations in the *PRNP* gene encoding the cellular prion protein (PrP^{C}). *PRNP* mutations favor PrP^{C} misfolding and aggregation, eventually leading to formation of the infectious PrP^{Sc} isoform, or prion. To study how pathogenic mutations influence cellular PrP^{C} trafficking and metabolism we generated mouse PrP constructs containing a HaloTag (PrP-Halo) derived from a bacterial haloalkane dehalogenase, which can be fluorescently labeled with various cell-permeant and -impermeant ligands.

Methods: Mouse PrP wild-type (WT-Halo), D177N/129M (FFI-Halo), D177N/129V (CJD-Halo) and PG14 (GSS-Halo) constructs were generated by inserting the HaloTag after amino acid 230 of mouse PrP and cloned in pCDNA3.1. HEK293 cells were transfected with Fugene HD (Promega). PrP-HaloTag expression was analyzed by western blot (WB) or SDS-PAGE after HaloTag labeling. The cellular localization of PrP-HaloTag was studied by confocal and super-resolution microscopy. After labeling with fluorescent cell-permeant or -impermeant substrates (Promega), cells were fixed and immunostained with antibodies against the lysosomal-marker LAMP1 (Abcam), the endoplasmic reticulum-marker BAP31 (Proteintech) and the Golgi-marker GM130 (BD-biosciences).

Results: WT-Halo, like untagged PrP, is glycosylated, GPI-anchored, undergoes α-cleavage processing, and is degraded via the lysosomal pathway[1]. FFI-Halo, CJD-Halo, and GSS-Halo constructs expressed by HEK293 cells localize abnormally within intracellular compartments and are detergent insoluble, similar to their untagged or EGFP (Enhanced Green Fluorescent Protein) tagged counterparts[2]. Thanks to the possibility to carry out pulse-chase experiments, simultaneously labelling intracellular and cell surface PrP using specific HaloTag substrates, we demonstrated that the mutated PrP-Halo constructs are slowed down in their trafficking to the plasma membrane and become insoluble before reaching the cell surface.

Conclusions: The HaloTag technology facilitates precise examination of the cellular distribution, trafficking, and metabolism of wild-type and mutant PrP, providing an invaluable tool to elucidate the molecular and cellular mechanisms underpinning genetic prion diseases.

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28. ELECTROPHYSIOLOGICAL CHARACTERIZATION OF PRIMARY CORTICAL NEURONS DEVOID OF THE PRION PROTEIN

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Keywords: prion protein, primary cultures, microelectrode arrays, bursts, synapses

The cellular form of the prion protein (PrP^{c}) is a ubiquitously expressed protein extensively studied in the progression of prion diseases. However, it also exerts a plethora of physiological functions: we hypothesize that the absence of this protein could modulate neuronal excitability and synaptic transmission along network development. Our goal is to observe and quantify these foreseen electrical activity alterations in *ex vivo* primary cultures of PrP^{c} knock out cells.
We employed substrate-integrated MicroElectrode Arrays (MEAs) that, compared to conventional techniques, allow the simultaneous monitoring of multiple neurons, providing a high-resolution view of network activity, by non-invasive means.
Indeed, MEAs were exploited to detect the extracellular electrical activity of cultured primary neurons, dissociated from the murine cortex (FVB, P0/P1; WT, $PrP^{+/+}$, KO, $PrP^{-/-}$).
Electrophysiological results reveal a significant difference between $PrP^{+/+}$ and $PrP^{-/-}$ when the culture reached maturity (~23 days *in vitro*, DIVs), both in terms of duration and occurrence rate of bursts, defined as episodic spontaneous synchronous network electrical events. At earlier time-points (10 and 17 DIVs, during *ex vivo* maturation) no significant differences were found, even if a trend similar to 23 DIVs is detectable at 10 DIVs. This may suggest the existence of compensatory mechanisms arising specifically in the phase of neuronal pruning. Finally, to dissect whether PrP^{c} involvement in neuritogenesis or synaptogenesis might explain our electrophysiological data, molecular techniques such as western blot and immunofluorescence are exploited upon different neuronal and synaptic markers.
The difference in burst activity between $PrP^{+/+}$ and $PrP^{-/-}$ mice is consistent with our hypothesis on the physiological role of PrP: at later stages of network maturation, the electrical activity seems altered when PrP is knocked out. The molecular explanation of this disruption might reside in a synaptic unbalance between the two genotypes or in structural differences across neuronal development.
Unravelling these aspects will give a better insight on the physiological role of PrP, while it may shed light on the implications of PrP^{c}-designed therapies for prion diseases.

29. Potential impact of SUMO on Prion-related diseases

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Keywords: PrP^{C}, PrP^{Sc}, SUMO, SUMOylation

The cellular prion protein PrP^{c} is a cell surface protein that has been implicated in neuroprotection, cell proliferation, differentiation or cell migration, among others, and plays a role in physiological and pathological processes, including different types of cancer and prion diseases. The elucidation of PrP^{c} protein regulation can help us to modulate its levels and/or function. Here we have evaluated the potential regulation of PrP^{c} as well as of its pathogenic conformer PrP^{Sc} through their interaction with the small ubiquitin-like modifier (SUMO) protein. The attachment of SUMO to lysine residues in a target protein or SUMOylation is considered as a key regulatory mechanism modulating the functional properties of a large number of proteins. The consequences of SUMO conjugation vary greatly depending on the substrate. It can affect their solubility, stability, localization or activity, influencing many different processes such as cell cycle, DNA repair, tumor progression, and inflammation. SUMOylation is essential for proper neuronal function and its dysregulation has been proposed to be implicated in various protein misfolding diseases. Therefore, targeting SUMOylation has been anticipated as a promising treatment for neurodegenerative disorders. However, the exact role of SUMO in these diseases remains unknown in many cases. Our results indicate that SUMO may be an important regulator of prion protein PrP^{c}. We show that PrP^{c} is modified by SUMO1 and SUMO2 *in vitro* and in the mouse neuroblastoma N2a cell line, suggesting a regulatory role for SUMO on PrP^{c}. Interestingly, we observed that downmodulation of global SUMOylation in a N2a cell line persistently infected with PrP^{Sc}, ScN2a, triggers the translocation of PrP from the cellular membrane to intracellular aggregates, suggestive of aggregation. This hypothesis is further supported by our results showing an increase in the levels of PK-resistant PrP in ScN2a cells upon treatment with a SUMOylation inhibitor, as well as by the increased stability of PrP^{c} upon global SUMOylation inhibition. Altogether our results suggest that SUMOylation inhibition may contribute to PrP^{c}-based diseases and strategies that modulate PrP-SUMO interaction may offer therapeutic potential in several neurodegenerative pathologies.

30. The dynamics of prion spreading is governed by the interplay between the non-linearities of tissue response and replication kinetics

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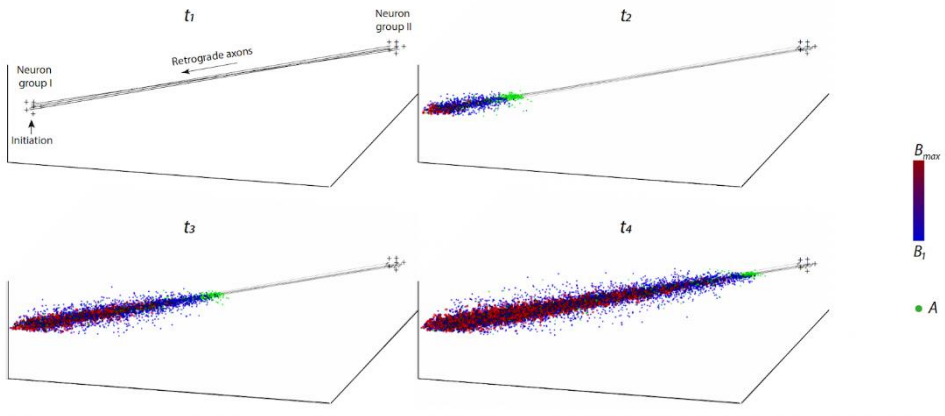
Keywords: prion, neuro-invasion, connectome, non-linearity, reaction-diffusion, stochastic modeling.

Prion diseases, or Transmissible Spongiform Encephalopathies (TSE), are neurodegenerative disorders caused by the accumulation of misfolded conformers (PrP^{Sc}) of the host-encoded cellular prion protein (PrP^{C}). During the pathogenesis, the PrP^{Sc} seeds disseminate in the central nervous system and convert PrP^{C} by changing their conformation leading to the formation of extracellular pathogenic insoluble assemblies. As for conventional infectious diseases, variations in the clinical manifestation define a specific prion strain which correspond to different PrP^{Sc} structures. Yet, the current understanding of the prion replication process fails to explain the link between structure and phenotypical differences such as incubation times, lesion profiles or tissue tropisms. Additionally, while the spatiotemporal progression of other proteinopathies such as Alzheimer's and Parkinson's diseases follows the neuronal connectome in a well-established manner called Braak's staging [1, 2], the contribution of the connectome, if any, to the neuro-invasion process of prions has yet to be defined [3].

In the present work, we implemented the recent developments on PrP^{Sc} structural diversity and dynamicity [4, 5, 6] and non-linear tissue response to prion replication, in the form of a regulation of PrP^{C} expression [7, 8, 9], into a stochastic reaction-diffusion model to study the neuro-invasion process using an application of the Gillespie Algorithm [10]. We showed that a combination of non-linearities imposed by a variable PrP^{C} expression level and highly dynamic prion kinetics can make prion propagation behave as a complex system, promoting the emergence of unpredictable behaviors such as specific subpopulation selection, coevolution of multiple strains as well as transient replication. This work provides an alternative to the current paradigm, which does not rely on local cofactors or a hypothetical variable compatibility between strains and local conformers of PrP^{C}, to explain strain specific phenotypes and tissue tropisms while also clarifying the role of the connectome in the neuro-invasion process.



Simulation of the retrograde dissemination process between two groups of neurons unilaterally linked by axons. The simulation is seeded with assemblies at neuron group 1, receiving axons from the other cluster. Replication occurs in the vicinity of axons as it is where PrP^{C} is located. The kinetic scheme features two conformationally distinct types of PrP^{Sc} assemblies undergoing catalytical exchanges: small oligomeric objects PrP^{Sc}A (denoted as A, in green) and assemblies capable of

condensing PrP^{c}-B_i (denoted as B_i, i referring to the size, in blue/red gradient).When sustained, the replication of A, more mobile due to its small size, facilitates the dissemination of assemblies following the axons, where replication can happen. Small B_i assemblies, which are predatory of A, can then condensate forming a trail following the dissemination front.

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31. Zn(II)-BnPyP-loaded organosilica nanocapsules for prion disease treatment

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Keywords: nanoparticles, prion, anti-prion porphyrin.

Background: Prion diseases, including scrapie in sheep and Creutzfeldt-Jakob in humans, are fatal neurodegenerative disorders for which there is no cure. They are due to the conversion of the cellular prion protein (PrP^{c}) into its aberrant counterpart called PrP^{sc} or prion. Inhibiting this conversion is the primary target for therapy. Recently, we have characterized the anti-prion activity of Zn(II)-BnPyP, a tetracationic porphyrin that targets PrP^{c} with a dual mechanism of action: it induces PrP^{c} degradation and blocks its conversion into PrP^{sc} (1). Zn(II)-BnPyP efficiently inhibits the propagation of different prion strains in *in vitro* and *ex vivo* models. However, Zn(II)-BnPyP does not reach the brain efficiently after systemic delivery. To overcome this limitation, we are exploring a strategy involving encapsulation of Zn(II)-BnPyP into breakable hybrid organosilica nano-capsules (NCs) that can be functionalized to efficiently cross the blood-brain barrier (BBB) (2).
Methods: We encapsulated Zn(II)-BnPyP by using breakable organosilica matrices that disintegrate upon exposure to a chemical stimulus (2). Due to the presence of the disulfide groups in the silica framework constituting the shell of the capsules, the capsules are destroyed and release the active compound upon contact with the reducing cell environment. HEK293 expressing mouse PrP wt and mouse primary neurons were treated with Zn(II)-BnPyP alone, Zn(II)-BnPyP encapsulated into breakable nano-capsules (Zn(II)-BnPyP-NC), or empty nanocapsules (NC) at different times and concentrations. The cellular uptake of NC was assessed by immunofluorescence microscopy, and the effect of treatment on PrP^{c} expression was evaluated by western blot.
Results: We demonstrated efficient Zn(II)-BnPyP encapsulation in NCs (~40 μg of Zn(II)-BnPyP per mg of NC) with an average dimension of 45 nm as determined by transmission electron microscopy. The slightly negative surface charge (-13 mV) confirmed the silica network with the presence of organic disulfide bridges. Zn(II)-BnPyP-loaded NCs effectively associated with cells and downregulated PrP^{c} levels like free Zn(II)-BnPyP. Ongoing experiments aim to determine their ability to inhibit PrP^{sc} replication in prion-infected cells and cultured organotypic cerebellar slices.
Conclusions: Zn(II)-BnPyP encapsulated into NCs retains its ability to promote PrP^{c} degradation in cells without detectable toxicity. Future studies will focus on evaluating the efficacy of Zn(II)-BnPyP-loaded NCs in inhibiting PrP^{sc} replication and developing strategies for their functionalization to enhance BBB penetration.

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32. Influence of metal substituents on the dual anti-prion activity of a tetracationic porphyrin

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Keywords: Prion disease therapy, porphyrin, NMR

Aims: We have recently reported that Zn(II)-BnPyP, a photosensitizer porphyrin developed for the treatment of cancer or bacterial infections, binds to distinct domains of native PrP^C, eliciting a dual anti-prion effect [1]. Zn(II)-BnPyP binding to a C-terminal pocket destabilizes the native PrP^C fold, hindering conversion to PrPSc; Zn(II)-BnPyP binding to the octapeptide repeats (OR) through histidine coordination disrupts N- to C-terminal interactions, triggering PrP^C endocytosis and lysosomal degradation, thus reducing the substrate for PrP^{Sc} generation. With its bimodal action on the prion precursor, Zn(II)-BnPyP efficiently inhibits replication of different prion strains in vitro, in neuronal cells and organotypic brain cultures. The aim of this study was to explore how different metal cations inside the BnPyP scaffold influence the porphyrin binding to PrP^C and its dual anti-prion activity.
Materials and Methods: Cu(II)-BnPyP, Co(II)-BnPyP and Fe(III)-BnPyP were synthesized starting from the related metal pyridyl porphyrins, as described [1]. The binding of the porphyrins to recombinant PrP was studied by NMR and CD. Their ability to downregulate PrP^C was tested in different cell lines and primary mouse neurons. The effect on PrP^{Sc} replication was assessed by PMCA, and in prion-infected N2a cells and cultured organotypic cerebellar slices (COCS).
Results: All porphyrins interacted with the PrP^C globular domain, but only Cu(II)-BnPyP maintained the ability to bind the OR region and downregulate PrP^C. Preliminary data indicate that all porphyrins, with the exception of Co(II)-BnPyP, inhibit PrP^{Sc} replication in N2a cells, with Zn(II)- and Cu(II)-BnPyP being the most effective. Evaluation of the porphyrins ability to inhibit PrP^{Sc} replication in PMCA and COCS is under way.
Conclusions: These results indicate that Zn(II)- or Cu(II)-mediated binding to the OR histidines is required for the PrP^C-lowering activity of the porphyrin. Since Cu(II)-BnPyP lacks the photodynamic activity it may be better suited for further development into an anti-prion drug.

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33. Thermal unfolding of bank vole PrP^C studied by solution NMR and Molecular Dynamics

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Keywords: Bank vole, PrP^C, solution NMR, VT study, Molecular Dynamics

Elucidation of the structure of PrP^{Sc} has opened the way to understand its propagation, *i.e.*, PrP^{Sc}-templated conversion of PrP^C into PrP^{Sc}. It is evident that the first step must necessarily be attachment of the ~90-120 intrinsically unfolded tail section of PrP^C to the corresponding β strand-rich stretch of PrP^{Sc}, with concomitant templated conversion driven by the "H-bond avid" unpaired -C=O and -NH groups in the templating surface of PrP^{Sc}. But what happens next? Given the inability of β to template stable conformational elements such as α helices or rigid coils, it follows that the ~121-230 folded domain (FD) of PrP^C must partially unfold before it can be templated/refolded. We aim at constructing an atomistic model of the conversion process. Therefore, we have undertaken studies aimed at understanding how a PrP^{Sc}-trapped PrP^C folded domain might partially unfold.

We chose Bank vole (Bv)PrP^C as our model given that BVPrP^C is a universal propagation substrate, and therefore it might be particularly prone to productive partial unfolding. We subjected BvPrP^C(109I)90-231 to gradual thermal unfolding at pH=5 and followed it by solution NMR. We obtained Variable Temperature (VT) ^{1}H-^{15}N HSQC spectra in the 5°C to 70°C range. Chemical Shift Perturbation (CSP) were analyzed in these spectra for each N-H amide of the backbone and plotted *vs.* temperature. Deviations from the linearity identified residues with higher trend to explore alternative conformations in the pathway to an unfolded state [1]. Such analysis identified residues in the two paired short β strands as being particularly labile. Interestingly, residues in a stretch of α helix 1 opposing these β strands were also seen to be labile, indicating an unfolding hotspot, compatible with separation of the β1-α1-β2 and α2-α3 subdomains, which "flattens" PrP^C. Separation of these subdomains was seen in 100 ns all-atom Molecular Dynamics (MD) simulations at 80 °C carried out in the Finis Terrae II supercomputer (CESGA). Of note, such event was not detected in similar simulations of Human PrP^C. The thermal unfolding of BVPrP^C led to an irreversible conformational change at ca. 50 ºC that promoted the formation of a stable oligomeric state in solution.

Additional NMR-based analyses such as measurement of HSQC peak integrals, DOSY, analysis of secondary chemical shift indexes, VT study of solvent exposed amides, analysis of NOE cross-peaks, relaxation and further MD simulations should help us delineate a pathway of unfolding of BvPrP FD which in turn should provide us the raw materials to build our atomistic model of conversion.

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34. Further insights on “non-PrPSc” full length PrP prions prions

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Keywords: PrP^{Sc}, prions, recombinant, bank vole

Recent cryo-EM studies show that fibrils of different rodent-adapted PrP^{Sc} strains are arranged in a parallel-in-register beta stack (PIRIBS) with a ~90-230 core (also defined as PrP^{27-30}). However, PrP^{Sc} with a PrP^{27-30} core is not the only type of PrP amyloid. Different variants of recombinant (rec)PrP amyloid are easily prepared by simple shaking under denaturing conditions. Such amyloids do not feature a ~90-230 core, as surmised from proteinase K digestion and confirmed by cryo-EM, which has shown that they feature a ~170-230 PIRIBS core. Such simpler PrP amyloids were initially believed not to be infectious, and the ”PrP^{Sc}” sobriquet has typically not been applied to them. Eventually they were shown to infect mice overexpressing PrP (~4-8X range). We have shown that a bank vole (Bv)PrP(109I) recPrP amyloid of such characteristics is infectious to TgBv mice expressing ~1X PrP with an attack rate of 100%. We detected in these animals propagation of a PrP species with a PK-resistant ~150-230 core.

Here we report that this recBvPrP amyloid is also infectious to bank voles. In ongoing experiments, several animals inoculated intracerebrally have succumbed to disease at 400-500 days post-inoculation while others have clear clinical signs, which anticipates a high attack rate. Brains of animals euthanized as a result of overt disease harbor different patterns of PK-resistant PrP cores, mostly including a short C-terminal one, alone or in combination with “classic”
PrP^{27-30} and/or internal 80-150 cores. Second passage experiments are being started from individual voles to investigate if different propagative PrP conformers act independently of each other.

These preliminary findings suggest that Bv(109I) recPrP amyloid prepared by simple shaking under denaturing conditions and devoid of a PrP^{27-30} core is infectious in wild type animals and leads to the propagation of different PrP conformers, *i.e.*, is a *bona fide* prion too. Furthermore, the fact that PrP amyloids with PIRIBS cores of different sizes and shapes can behave as prions perhaps warrants a redefinition of the relationship between the categories “PrP amyloid” and “PrP^{Sc}”. The fact that PrP^{C} can be templated *in vivo* by such a variety of PrP amyloids suggests that it might play a more active role in the propagation process than thought.

Prion and Prion-like Diseases in Humans

35. Biochemical biomarkers for human prion diseases-needs and challenges

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Although the development of aggregation assays has noticeably improved the accuracy of the clinical diagnosis of prion diseases, research on biomarkers remains vital. The major challenges to overcome are non-invasive sampling and the exploration of new biomarkers that may predict the onset or reflect disease progression. This will become extremely important in the near future, when new therapeutics are clinically evaluated and eventually become available for treatment. This talk aims to provide an overview of the achievements of biomarker research in human prion diseases, addresses unmet needs in the field, and points out future perspectives.

36. Optimization of an exosome isolation technique based on nanotechnology to improve Alzheimer's disease diagnose

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Keywords: Exosomes, nanowires, Alzheimer's disease, protocol, mass spectrometry

Extracellular vesicles are defined by the International Society of Extracellular Vesicles as for the vesicles released from the cell. Exosomes are the smallest ones among them, with a size of ~40–160 nm. The fact that exosomes are released from many different types of cells and be found in almost all body fluids, make them approachable for diagnostic purposes. In neurodegenerative disease, neuronal-derived were collected from blood, CSF, saliva and urine and several potential biomarkers were discovered. However, there is a lack of an effective, rapid method to isolate neuronal-derived exosomes. Our aim was to optimize a sensitive and reliable method to isolate neuronal exosomes from plasma sample. We optimized a nanowire-based protocol to isolate neuronal-derived exosomes from plasma samples. For this, antibody-conjugated magnetic nanowires were purchased, coating them with 2 neuronal exosomal markers (L1-CAM & NCAM) and a surface exosomal marker (CD81) as the conjugated antibodies. We validated the protocol using NTA, TEM and immunoblotting approaches. Neuronal-derived exosomes were successfully isolated from plasma samples using a nanowire-based protocol. We characterized the EVs in triplicate using negative staining electron transmission microscopy. Additionally, new potential diagnostic biomarkers for Alzheimer's disease were discovered using mass spectrometry-based analysis. We found extracellular vesicles, with a bi-lipid membrane, and smaller than 250 nm. The immunoblotting results allowed us to detect bands in three different external exosomes markers (CD9, CD63, CD81) and four internal exosome markers according to the International Society for Extracellular Vesicles. Additionally, our EVs were evaluated by NTA in triplicates. In the NTA analysis largest peaks were observed, and they showed a size range from 75 to 160 nm, (average size 116 nm). The presence of T-Tau, P-Tau, and Aβ 1-41 was confirmed by ELISA assays. In mass spectrometry, 319 proteins showed statistical significance. To conclude this new nanowire-based protocol targeting neuronal exosomes is an accurate, rapid, effective, standardized, and reproducible method.

37. DIFFERENT TAU FIBRIL TYPES REDUCE PRION LEVEL IN CHRONICALLY AND *DE NOVO* INFECTED CELLS

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Keywords: neurodegeneration, prion diseases, tau fibrils, tauopathy, prions

The co-deposition of different amyloidogenic proteins, normally associated with distinct proteinopathies, often characterizes neurodegenerative diseases. In prion diseases, for instance, the presence of tau amyloid deposits has been reported together with the classical deposition of PrP^{Sc}. However, the effects of this co-deposition on prion disease progression are still debated. Recently, the cellular form of the prion protein, PrP^{C}, has been reported as binding partner of Aβ, tau and α-synuclein amyloids, suggesting its role in mediating the neurotoxic effects exerted by these aggregated species [1]. Previously, we showed that the treatment of chronically prion-infected cells with tau K18 fibrils reduced PrP^{Sc} levels [2]. Here, we further explored this mechanism by using a distinct tau fibril type, obtained by extending K18 construct to include the sequence forming the core of tau Alzheimer's disease filaments in *vivo* [3].

Firstly, we demonstrated that these two constructs form fibrils characterized by distinct biochemical and biological features. However, both tau fibril types were able to reduce the level of PrP^{Sc} in chronically-infected ScN2a cells. Also, these fibrils were actively internalized by the cells through an energy-dependent mechanism, but the internalization was not necessary for the reduction of PrP^{Sc} levels. Moreover, tau fibril administration was able to hinder the early stages of prion infection and to inhibit the PrP^{C} to PrP^{Sc} pathological conversion event.

These results suggest that the observed prion reduction could derive from the binding of tau fibrils to the PrP^{C} present on the plasma membrane, thus stabilizing the protein and hindering its conversion to PrP^{Sc}. In addition, tau amyloid fibrils impair the initial phases of prion conversion, by preventing PrP^{C}/PrP^{Sc} interaction and/or stabilizing PrP^{C} at the plasma membrane. Accordingly, in prion-infected cells, tau circulating species may protect PrP^{C} from PrP^{Sc} interaction, thus slowing down the conversion and spread of prions.

Overall, our data suggest PrP^{C} as a receptor not only for amyloids of distinct proteins, but also for different aggregated structures of the same protein and point out a role of tau fibrils in preventing PrP^{C} to PrP^{Sc} conversion [4].

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38. Analysis of potential biomarkers in longitudinal plasma samples of carriers of the FFI mutation

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Keywords: biomarker, prion, FFI

Aims:

Fatal familial insomnia (FFI) is a genetic prion disease linked to the D178N-129M *PRNP* mutation. This disease manifests unpredictably during adulthood, leading to death within two years from symptom onset. The lack of prodromal biomarkers leads to diagnosis when neurological damage has already occurred, making the prospect of a cure highly improbable. This study aimed to explore potential plasma biomarkers for early diagnosis and prognosis. We measured neurofilament light chain (NfL), which has been shown to increase prior to disease onset in individuals with *PRNP* mutations linked to slowly progressing genetic prion diseases. Additionally, we examined metalloproteinase-9 (MMP9), a marker of blood-brain-barrier disruption; cyclophilin A (PPIA), a mediator of neuroinflammation; alpha-synuclein (αsyn), a neuronal protein that regulates synaptic vesicle trafficking and neurotransmitter release; and chitinase-3-like protein 1 (CHI3L1 or YKL-40), a protein associated with pathogenic processes related to neuroinflammation. Furthermore, we analyzed a subset of plasma samples using the NUcleic acid Linked Immuno-Sandwich Assay (NULISAseq CNS Panel 120), a multiplex platform designed to measure approximately 120 CNS proteins relevant to neurodegenerative diseases.

Materials and Methods:

Plasma samples were collected longitudinally from 48 individuals, including 22 non-carrier controls and 26 FFI carriers (18 D178N-MM129 and 8 D178N-MV129). Six carriers converted from a non-symptomatic to a symptomatic phase. Plasma samples from four carriers were available only after onset of disease. NfL levels were measured using the SIMOA technology, MMP9 and αsyn by AlphaLISA, CyPA and YKL-40 by ELISA. Analysis with the NULISAseq CNS Panel 120 was outsourced to Alamar Biosciences.

Results:

Analysis on the longitudinal plasma samples found an abrupt increase in NfL levels at the time of disease onset in converters, further increasing during the symptomatic phase. Comparative analysis of all plasma samples found higher NfL levels in healthy FFI carriers compared to non-carrier controls; however, levels were too variable to establish a definitive cut-off value. MMP9, αsyn, CyPA and YKL-40 levels exhibited considerable variability with inconsistent trajectories in the converters.
NULISA™ demonstrated up- or down-regulation of 29 proteins between carriers and non-carrier controls.

Conclusions:
Plasma NfL may serve as a diagnostic/prognostic, but not prodromal FFI biomarker. MMP9, αsyn, YKL-40 and CyPA are not useful due to their highly variable trajectories. Analysis of NULISA data is under way to determine whether any of the differentially regulated proteins may serve as a biomarker of disease proximity.

39. The cellular prion protein mediates tau oligomer-induced synaptotoxicity and memory impairment in mice

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Keywords: Oligomer neurotoxicity, prion protein, tau, long-term potentiation, memory deficits.

Background. The cellular prion protein (PrP^c) has been proposed to mediate the neurotoxicity of amyloid-β, α-synuclein and tau oligomers. We demonstrated that although amyloid-β oligomers (AβOs) bind to PrP^c with high affinity, the memory deficit induced by intracerebroventricular (ICV) administration of AβOs in mice was not mediated by PrP^c [1]. Moreover, we did not confirm the reported interaction between α-synuclein oligomers and PrP^c, and found that their effect on memory did not depend on PrP^c expression [2]. In this study, we examined the interactions between tau oligomers (TauOs) and PrP^c by chemico-physical and functional studies.
Methods. TauOs were prepared in the presence of arachidonic acid and analyzed by atomic force microscopy; the interaction between TauOs and PrP^c was investigated by surface plasmon resonance (SPR); the cellular localization of TauOs was studied in HEK293 cells expressing different levels of PrP^c; the effect of TauOs on memory function was assessed by the novel object recognition test (NORT) after ICV injection (1 µM 7.5 µl) in wild type (wt) and PrP knockout (KO) mice; the effect of TauOs on long-term potentiation (LTP) was analyzed in acute hippocampal slices.
Results. SPR demonstrated a high-affinity binding between TauOs and PrP^c with a K_d in the range of 20-50 nM. Immunofluoresce analysis showed a PrP^c dose-dependent association of TauOs with the plasma membrane of HEK293 cells, and their co-localization with PrP^c. ICV application of TauOs induced memory impairment in wt but not PrP KO mice. Consistently, TauOs inhibited hippocampal LTP in a PrP^c-dependent fashion.
Conclusions. In contrast to previous findings with Aβ and α-synuclein oligomers, we demonstrate that PrP^c interacts with TauOs and this binding has functional consequences. This interaction could represent a promising therapeutic target in Alzheimer's disease and other tauopathies.

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40. Characterization of soluble amyloid beta assemblies

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Keywords: Amyloid-beta, fibrils, lecanemab, Alzheimer's disease, characterization.

Soluble amyloid beta (Aβ) assemblies, present in brains of Alzheimer's Disease (AD) patients since early stages, show both seeding capacity and toxicity. Such assemblies are composed of different Aβ aggregation states like protofibrils or oligomers. Moreover, soluble Aβ assemblies are the target of the antibody lecanemab, a new AD immunotherapy [1,2,3,4].

Here, we analyze synthetic and human soluble Aβ assemblies to identify shared features that might be involved in the mechanism behind anti-Aβ immunotherapy removal of Aβ deposition.

First, we generated synthetic Aβ assemblies of different size and characterized them both biochemically and structurally. We observed the existence of short fibrillar aggregates soluble at 100000 G with features different from insoluble Aβ fibrils. By native PAGE, short fibrils appeared as oligomers whereas insoluble fibrils got stuck in the wells, not entering the gels. Similar results were observed by SDS-PAGE. By transmission electron microscopy (TEM), we saw clear differences between soluble and insoluble fibrils with soluble fibrils being shorter and with a sharp morphology. Interestingly, such short fibrils were almost identical to those showed by Stern et al. that were isolated from human brain and preferentially detected by lecanemab [3].

Additionally, we isolated soluble Aβ assemblies from AD patients and characterized them biochemically. These assemblies appeared as low molecular weight aggregates by SDS-PAGE. Our lab is currently performing structural studies for further comparison with soluble synthetic short Aβ fibrils.

Since soluble Aβ fibrils are the target of AD immunotherapies such as lecanemab [4], synthetic soluble Aβ fibrils, much easier to obtain than human soluble Aβ fibrils, could be used as a model to study the mechanism of antibodies targeting the initial stages of Aβ aggregation.

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41. Effect of Formic Acid Treatment in the Reduction of sCJD and vCJD Prion Infectivity

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Keywords: formic acid, infectivity inactivation, bioassay, prion strain

It is considered that formalin-fixed tissues retain prion infectivity for long periods of time, if not indefinitely. Early studies have shown that treatment with formic acid reduces prion infectivity to negligible levels [1]. Based on this, formic acid treatment is widely used to ensure the safety of laboratory staff. However, prion inactivation may vary depending on the prion strain [2]. Therefore, ensuring biosafety for personnel handling different prion strains in laboratories is crucial.

In this study, we evaluated the alteration of type 1 Creutzfeldt-Jakob Disease (sCJD) and variant-CJD (vCJD) prion infectivity in brain after formalin treatment or after one hour in formic acid.

Brains from transgenic mice expressing Met129-human PrP (Hu-Tg340) infected with sCJD or vCJD [3] were sectioned and homogenized after undergoing different procedures: i) untreated, ii) formalin-fixed and iii) formalin-fixed with an additional treatment (inactivation treatment) of 1 hour in 98% formic acid. Hu-Tg340 and Bo-Tg110 (transgenic mice expressing bovine PrP) were intracranially challenged with homogenates after the different treatments from sCJD or vCJD infected brains respectively.

As expected, mice inoculated with homogenates from untreated tissues died with short survival times and 100% attack rates. A slight reduction in survival times was observed in mice inoculated with homogenates from tissues treated only with formalin.
For both vCJD and sCJD, a reduction in the infectious titer of several logarithmic units is observed after formic acid treatment. However, for vCJD, a lower reduction was observed as 100% of the Bo-Tg110 mice tested positive for the disease, although they showed longer survival times. In the case of sCJD, preliminary results indicate that residual infectivity remains, as only some animals are positive after extended survival times.

This work supports the dropping of most, but not all, vCJD and sCJD prion infectivity when brain tissues are treated with 98% formic acid for 1 hour. This data should be carefully considered in the implementation of safety measures for the handling of prion infected samples in the laboratory.

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42. RT- QuIC with universal control fluid for standardized detection of *alpha*-synucleonopathies

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Keywords: RT-QuIC, aSyn RT-QuIC, synucleonopathies, CSF, diagnostics

Introduction: Seeding aggregation assays (SAA´s) and especially Real-Time Quaking Induced Conversion (RT-QuIC) has shown great potential as an early and supplementary biomarker for identifying *alpha*- synucleonopathies (RT-QuIC-αSyn). Multiple groups have demonstrated high sensitivities (>90%) and specificities (>95 %) using cerebrospinal fluid (CSF) and various set-up strategies. We **aim** to establish a RT-QuIC-αSyn protocol that standardize both sample and control reaction set-ups for diagnostic purposes.

Materials and methods: We modified a RT-QuIC-αSyn method adopted from *Acta Neuropath. 2020 Jul 140 (1), Rossi et al*. and evaluated its sensitivity and specificity by a blinded screening of a CSF cohort of clinically classified cases of Parkinson´s (PD) (*N*=12), Dementia with *Lewy*-body (DLB) (*N*=12), Alzheimer´s (AD) (*N*=12), Motor Neuron Disease (MND) (*N*=12), Multiple System Atrophy (MSA) (*N*=18) and normal controls (NC) (*N*=12). We tested quadruple sets of 3 volumes of each CSF sample to assess the fluid and seeding effect. Furthermore, we developed a Universal Control Fluid (UCF) to standardize RT-QuIC reaction environment across all samples and controls by creating and testing various UCF compositions and sample/control set-ups.

Results: Our RT-QuIC-αSyn have a sensitivity of ~92 % for PD and DLB. The specificity was >95 % compared to AD and MND and 100 % compared to NC. No significant detection of MSA was achieved. We have defined a UCF composition that gave 100 % sensitivity and specificity when comparing between neat UCF and seeded with patient material.

Conclusion & perspective: RT-QuIC-αSyn is a potential clinical biomarker for confirming *alpha*-synucleonopathies within 48 hours. RT-QuIC-αSyn with UCF has the potential for a standardized diagnostic protocol.

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43. Chemical exploration of a PrP degrader compound

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Keywords: prion; prion diseases; drug discovery**,** folding intermediate

Although cellular prion protein (PrP) is considered an undruggable protein by classical pharmacology, our previous work shows how it could be possible, by stabilizing a PrP folding intermediate, to send the protein to degradation and halt prion propagation and toxicity. This new approach for selectively reducing the level of target proteins by impairing their folding process rather than targeting their native conformations, called Pharmacological Protein Inactivation by Folding Intermediate Targeting (PPI-FIT), is made possible by computational algorithms allowing the full atomistic reconstruction of folding and misfolding processes of polypeptides. The rationale underlying PPI-FIT is that targeting a folding intermediate with small ligands could promote its degradation by the cellular quality control machinery, which recognizes such artificially stabilized intermediates as improperly folded species. We have applied PPI-FIT to target PrP and identified a pharmacological degrader (named SM875) capable of dose-dependently suppressing the expression of the protein [1].
We have designed a general synthetic plan for SM875 and synthesized more than 40 analogs. These molecules were individually purified and characterized in order to test their biological activity. To improve the testing efficiency, a new image-based screening method was developed, and counter validated by western blotting. Dose-dependent analysis of each molecule allowed us to draw a first structure-activity relationship for SM875, which was used to refine the docking model of the compound-pocket interaction and find other chemical families. In order to improve the process of drug development, it would be desirable to have a high-resolution image of the folding intermediate pocket together with SM875. Different co-crystallization experiments between PrP and SM875 were tried on Earth with unsuccessful results. These drawbacks led us to attempt an experiment on the International Space Station (ISS) to capitalize on its microgravity environment. Collectively, these results represent fundamental steps along the SM875 optimization pipeline and encourage searching for additional analogs and completely new structures with improved pharmacological properties.

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44. Light Identification of Protein Suppressors (LIPS) as a new technology to screen for pharmacological modulators of the cellular prion protein.

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Keywords: prion diseases, HCS, BiFC, Cardiac Glycosides, drug discovery.

We have developed an imaging-based bimolecular fluorescence complementation assay named Light Identification of Protein Suppressors (LIPS) to rapidly quantify protein expression, trafficking, and degradation in real-time. The method was specifically designed to detect early events in the cellular prion protein (PrP) biogenesis, a cell surface glycoprotein mainly expressed in the central nervous system playing a pivotal role in prion diseases and other neurodegenerative conditions. Prion diseases are a class of neurodegenerative disorders manifesting in a sporadic, inherited, or transmissible fashion and are associated with the conformational conversion of PrP into an aberrant form called PrPSc that accumulates in the brain of affected individuals. PrPSc is a proteinaceous infectious particle (prion) capable of multiplying by directly recruiting PrP and causing its conformational rearrangement into new PrPSc molecules. It is becoming increasingly evident that PrP plays a dual role in prion diseases, being both a substrate for PrPSc replication and a mediator of its toxicity. Data revealed that PrP may serve as a receptor for neurotoxicity transduction, particularly for oligomeric forms of amyloid β (Aβ) peptide and alpha-synuclein, key contributors to Alzheimer's and Parkinson's diseases. [1] These observations have important therapeutic implications by directly suggesting that the pharmacological inhibition of PrP expression may provide therapeutic benefits in different neurodegenerative conditions.
With the exception of antisense oligonucleotides recently developed by Ionis Pharmaceuticals, not a single chemical class appears as an immediate candidate for clinical testing in prion diseases soon, supporting the emerging notion that targeting PrP require the exploitation of novel drug discovery paradigms. [2]
LIPS, our new phenotypic high content screening, will allow the rapid screening of PrP-suppressing molecules by high-content imaging platforms addressing precisely such a need.
A pilot screening of approximately 2,000 diverse compounds was conducted and the experimental reliability was assessed by monitoring the Z' factor, resulting in an excellent overall score of 0.85, affirming the system's suitability and reliability for high-throughput screenings. We found 11 PrP-suppressing compounds belonging to the same pharmacological class of cardiac glycosides, subsequently validated through secondary tests.

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45. Development and Validation of a Novel Cellular Assay to Rapidly Monitor Expression and Localization of Intrinsically Disordered Proteins

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Keywords: Bimolecular Fluorescence Complementation, Fragment-based drug discovery, High Content Screening, Intrinsically Disordered Proteins, Parkinson's disease

Intrinsically disordered proteins (IDPs) are characterized by the lack of a well-defined three-dimensional structure under physiological conditions. Despite their structural flexibility, IDPs play critical roles in cellular processes and are implicated in various human diseases. We aim to develop an imaging-based bimolecular fluorescence complementation assay (BiFC) to rapidly quantify the expression, trafficking, and degradation in real-time of a model IDP, the protein alpha-synuclein, a key player in Parkinson's Disease. The opportunity for such an application is provided by the recent development of a superfolder green fluorescent protein (GFP) split into two non-symmetrical halves, one containing the first ten β-strands of the original β-barrel domain (GFP1-10) and the other represented by the single missing β-strand (GFP11) [1]. GFP1-10 and GFP11 re-assemble spontaneously, without the need to be fused to interacting proteins, yielding a highly fluorescent signal, thus allowing easy detection by live microscopy.
By implementing a high-content screening assay while testing a fragment-based compound library, we plan to characterize the expression and subcellular localization of alpha-synuclein under different conditions and treatments. This approach will facilitate the discovery of alpha-synuclein modulators and their further development. Furthermore, once validated, this approach will provide a unique tool in the discovery of modulators of other IDPs.

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Special Talk: Frontiers in Prion Research

46. DISC1 protein aggregates define a subset of chronic mental illnesses

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Aberrant proteostasis is a hallmark feature of chronic brain diseases. Contrary to neurodegenerative diseases, in chronic mental illnesses protein aggregation or misassembly is subtle and not highly neurotoxic, but still likely impairs neuronal functions, eventually restricted to selected cell populations and/or brain regions.

We have extensively characterized subtle protein aggregation of the protein Disrupted-in schizophrenia 1 (DISC1) both in human post mortem brains from patients with schizophrenia or affective disorders vs. healthy controls and in cerebrospinal fluid (CSF). Post mortem biochemical purifications of insoluble DISC1 indicate that a subset (15%) of patients with overlapping clinical diagnoses of schizophrenia and affective disorders display insoluble DISC1 protein. In CSF of first episode psychosis (FEP) patients, in many cases an early stage of schizophrenia, elevated DISC1 aggregates in the fM range could be measured with the highly sensitive surface-based fluorescence intensity distribution analysis (sFIDA).

Causality of aggregated DISC1 protein in non-adaptive behavior was established in a transgenic rat model modestly overexpressing human, non-mutant, full length DISC1 leading to aberrant dopamine homeostasis and behavioral phenotypes with high face validity (as defined by MATRICS), such as subtle memory deficits, social anhedonia, amphetamine supersensitivity or others. To this end, DISC1 aggregates were demonstrated to be cell-invasive and transmissible in vitro. As natural triggers leading to endogenous DISC1 protein aggregation we identify viral infection (influenza virus) or oxidative stress. We conclude that we can establish a triangle of a patient subset, a test to define that subset, and a corresponding animal model, that establish biological underpinnings of a subset of schizophrenia and that enables advancing causal therapies.

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