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Protein Structure and Biology

P.01: A semisynthetic approach to investigate the influence of posttranslational modifications on prion pathogenicity

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Keywords: Expressed Protein Ligation, Prion Protein, PTM, semisynthesis, solid phase peptide synthesis

Transmissible spongiform encephalopathies (TSEs) are a large group of neurodegenerative diseases including BSE, FFI and CJD with a sporadic, inherent or spontaneous etiology.¹ The hallmark of many TSEs is the conformational conversion of the cellular prion protein (PrP^C) into pathogenic PrP^{Sc}. This occurs on the outer leaflet of the membrane where PrP is attached via a glycosylphosphatidylinositol (GPI)-anchor. Hence, the GPI-anchor plays an important role in PrP pathogenicity and its role in disease progression is well studied.²⁻³

Besides the GPI-anchor, N-glycosylation is an important posttranslational modification (PTM) of PrP. The impact of N-glycosylation on prion pathogenicity is not well defined, mainly due to the lack of access to homogenous glycosylated PrP variants. To fill this gap, we recently develop a semisynthetic approach to generate different variants of homogenous posttranslationally modified PrP.⁴⁻⁵ We successfully expressed two recombinant truncated PrP variants comprising aminoacids 23-178 and 90-178 fused to intein protein in order to generate protein- α -thioester upon intein cleavage. Furthermore, we will combine recently developed strategies for peptide- α -thioester synthesis with N-glycosylation strategies.⁶⁻⁷

Our current focus is on testing different ligation conditions for both variants mentioned above. Once the ligation conditions are optimized, the access to multimilligrams of homogenous posttranslationally modified PrP variants is expected and the biophysical/biochemical characterization will be carried out.

References

1. Sakaguchi S. Molecular biology of prion protein and its first homologous protein. *J Med Invest* 2007; 54:211-23; PMID:17878669; <http://dx.doi.org/10.2152/jmi.54.211>
2. Baron GS, Caughey B. Effect of glycosylphosphatidylinositol anchor-dependent and -independent prion protein association with model raft membranes on conversion to the protease-resistant isoform. *J Biol Chem* 2003; 278:14883-92; PMID:12594216; <http://dx.doi.org/10.1074/jbc.M210840200>
3. Priola SA, McNally KL. The role of the prion protein membrane anchor in prion infection. *Prion* 2009; 3:134-8; PMID:19786843; <http://dx.doi.org/10.4161/pri.3.3.9771>
4. Chu NK, Becker CF. Semisynthesis of membrane-attached prion proteins. *Methods Enzymol* 2009; 462:177-93; PMID:19632475; [http://dx.doi.org/10.1016/S0076-6879\(09\)62009-7](http://dx.doi.org/10.1016/S0076-6879(09)62009-7)
5. Olschewski D, Seidel R, Miesbauer M, Rambold AS, Oesterhelt D, Winklhofer KF, Tatzelt J, Engelhard M, Becker CF. Semisynthetic murine prion protein equipped with a GPI anchor mimic incorporates into cellular membranes. *Chem Biol* 2007; 14:994-1006; PMID:17884632; <http://dx.doi.org/10.1016/j.chembiol.2007.08.007>

6. Ollivier N, Vicogne J, Vallin A, Drobecq H, Desmet R, El Mahdi O, Leclercq B, Goormachtigh G, Fafeur V, Melnyk O. A one-pot three-segment ligation strategy for protein chemical synthesis. *Angew Chem Int Ed* 2012; 51:209-13; PMID: 22095761; <http://dx.doi.org/10.1002/anie.201105837>
7. Fang GM1, Li YM, Shen F, Huang YC, Li JB, Lin Y, Cui HK, Liu L. Protein chemical synthesis by ligation of peptide hydrazides. *Angew Chem Int Ed* 2011; 50:7645-9; PMID: 21648030; <http://dx.doi.org/10.1002/anie.201100996>

P.02: Prion and shadoo in nuclear speckles: Intermediaries between heparan sulfate template biosynthesis and non-coding nucleic acid editing

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The function of cellular prion protein remains unknown, however its affinity for proteins, nucleic acids and heparan sulphate (HS) is established. Previously we reported on the immunofluorescent detection of shadoo in speckles (an interchromatin region where non-coding RNA processing has been demonstrated), and the high affinity of shadoo N-terminal domain for nucleic acids in G-quadruplex conformation (a four-stranded nucleic acid secondary structure with complex topology, abundant in telomeres).

In the present study we used immunofluorescence to further study the endogenous distribution of prion-ligands in the nuclei of cultured synoviocytes. Our approach was two strand: In the first we studied the nuclear distribution of HS and its biosynthetic enzymes. Like PrP, HS has been detected throughout the cell including the nucleus. HS has many functions including axonal guidance. In the second strand we studied non-coding RNA and the RNA -editing enzyme ADAR1 which have been implicated in various prion-related neuropathologies.

PrP was detected in speckles in a subpopulation of cells by using the nuclear speckle marker SC-35. We also observed the HS biosynthetic enzymes EXT1 and HS1 desulfatase, nucleic acid quadruplexes and the RNA-editing enzyme ADAR1 in speckles of interphase cells.

We conclude that prp/shadoo heterodimers in nuclear speckles bring together both the information encoded in the complex structure of PrP-bound HS chains and genetic information contained in shadoo-bound non-coding nucleic acids (eg SINES), their ADAR1 edited subspecies and reverse transcribed sequences forming quadruplex structures.

Based on known and predicted conformational data, we present a model of PrP/shadoo heterodimer function in which (1) ligand-induced conformational changes have allosteric effects on the conformation of the dimer interface, (2) by push-pull thermodynamics, allosteric effects constrained in the dimer interface have reciprocal effects on ligand binding domains in both units of the heterodimer, (3) these reciprocal allosteric effects and the

plasticity of the dimer interface enable template transfer of structural information between nucleic acid and HS.

P.03: Calcium-mediated activation of MAP-kinase pathways and prion formation in GT1-1 cells

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Cellular mechanisms play a role in the conversion of the normal prion protein PrP^C to the disease-associated isoform PrP^{Sc}. Cells harbor as yet largely undefined factors required for efficient prion replication. Previously, we had observed that interference with the calcium-activated MEK/ERK MAP kinase pathway affects the phosphorylation of the Elk-1 transcription factor and the formation of PrP^{Sc} in GT1-1 cells infected with the RML strain of scrapie (ScGT1-1 cells).¹ In order to obtain a “flow-chart” for the activation of various MAP kinase pathways in relation to PrP^{Sc} accumulation, here we analyze the involvement of JNK/c-Jun in this process. Calcium channels are activated by high [KCl] and their role is determined by the use of inhibitors of voltage-sensitive calcium channels and N-methyl-D-aspartate glutamate receptors.

We observed that depolarization of GT1-1 cells with high [KCl] increases PrP^{Sc} accumulation and causes phosphorylation of both JNK and c-Jun in parallel with that of MEK/ERK and Elk-1. Notably, PrP^{Sc} accumulation in ScGT1-1 cells is promoted by JNK inhibitors, and opposed by MEK/ERK inhibitors. These results indicate that during depolarisation the stimulatory effects on PrP^{Sc} accumulation exerted by activated MEK/ERK signaling play a preponderant role, thereby prevailing over activated (JNK) MAPkinase signaling.

Altogether, our studies indicate that prion protein conversion is under the control of opposing signaling pathways and suggest that, under certain conditions, the balance between these pathways may be tilted, leading either to clearance of PrP^{Sc} or to an irreversible response that could lead to progressive neurodegenerative diseases.

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References

1. Allard EK, Grujic M, Fisone G, Kristensson K. Prion formation correlates with activation of translation-regulating protein 4E-BP and neuronal transcription factor Elk1. *Neurobiol Dis* 2013; 58:116-22; PMID:23742760; <http://dx.doi.org/10.1016/j.nbd.2013.05.014>

P.04: The absence of the cellular prion protein impairs copper metabolism and copper-dependent ferroxidase activity, thus affecting iron mobilization

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Neurodegenerative pathologies are characterized by metal ion imbalance, oxidative/nitrosative stress and aggregation of an endogenous metal-binding protein that undergoes a conformational change. In prion disorders, the metal-binding protein involved is the cellular prion protein (PrP^C). PrP^C binds with high affinity copper, both Cu(I) and Cu(II), and with lower affinity other cations. Although many data suggest a role for PrP^C in metal homeostasis, the mechanism through which it exerts this function has not been described. In prion disorders, PrP^C is aggregated in the brain and also in other organs, such as the spleen, thus its function in metal metabolism is likely lost. Elucidating PrP^C function in metal homeostasis is crucial to understand the mechanisms underlying the disease pathogenesis and progression.

To this aim, we measured copper and iron content, expression levels of proteins involved in copper and iron metabolic pathways, and the activity of copper-dependent ferroxidases in serum, liver, spleen, brain and hippocampus of wild-type (*Prnp*^{+/+}) and PrP^C-null (*Prnp*^{0/0}) mice. To determine whether the influence of PrP^C on metal metabolism changes according to the developmental stage, we performed the analyses at different ages, from early postnatal days to 1 year.

In the sera of *Prnp*^{0/0} mice, we found the same total amount of copper as in *Prnp*^{+/+} mice, but lower copper bound to ceruloplasmin (Cp). Indeed, we detected lower Cp ferroxidase activity and ensuing lower serum iron levels. Results observed in serum are likely related to what happens in *Prnp*^{0/0} mouse liver and spleen. Though copper content is unaltered in *Prnp*^{0/0} mouse liver, its delivery to Cp is likely impaired since Cp activity is lower, resulting in iron accumulation and increased hepcidin production. These observations are supported by the different expression levels of proteins involved in copper and iron metabolism in *Prnp*^{0/0} mouse liver. In *Prnp*^{0/0} mouse spleen, copper content is lower, while iron content is higher compared to wild-type. Moreover, lower iron level in *Prnp*^{0/0} mouse serum induces splenomegaly. These alterations in copper and iron metabolic pathways result in copper and iron deficiency in the central nervous system, also confirmed by the different expression levels of iron and copper metabolism proteins.

In light of our results, we propose a mechanism in which PrP^C passes copper ions to transporters, thus promoting its uptake. The PrP^C-mediated copper entry into the cell facilitates its loading on copper-dependent ferroxidases, thus allowing iron efflux from ferroportin and ensuing oxidation by Cp and loading on transferrin.

P.05: S3 PrP with a variant octarepeat region has conditional sensitivity to misfolding

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Introduction. The C-terminal globular domain of cellular PrP is preceded by a flexible N-terminus that can bind copper and zinc via four histidine-containing octarepeats. Alternative conformations of the PrP^C N-terminal octarepeat region (OR), denoted components 1, 2 and 3, occur in the presence of different concentrations of copper ions.

Hypothesis. Since conformation is a crucial parameter in prion disease we hypothesized that different N-terminal PrP conformations might be associated with different phenotypic properties.

Work Plan. Repetitive peptide mutagenesis was performed to produce octarepeat variants with discrete, component 1 or 3 copper-binding geometries with metal binding stoichiometries at saturation of 4 and 1 copper ions per polypeptide molecule, respectively. DNA sequences encoding tandem variant OR's with "geometry-locked" conformations were then engineered into a full-length mouse PrP DNA coding region to generate novel lines of transgenic mice and stable cell-lines.

Results. While studies in transgenic mice have shown that the conformation-locked PrP alleles S1 with component 1 geometry and S3 with component 3 geometry are not toxic and able to support prion infections (Lau et al, in preparation), striking divergence was noticed for the same alleles stably transfected into RK13 cells and then challenged by *de novo* infection. Notably, and unlike S1 and wild type PrP, the S3 allele was not able to support replication of the RML isolate of mouse-adapted scrapie prions. However, S3 PrP programmed the production of a full-length protease-resistant PrP species in uninfected cells; subsequent to denaturation and treatment with PNGaseF the S3 allele engendered a chemical signature of simultaneous production of 17 and 21 kDa proteinase K-resistant fragments. Moreover, assessment of the S3 allele in RK13 cells grown in different conditions defined an important role for the use of different culture media. Analyses to define the composition of media followed by mixing experiments have allowed us to assign the crucial nutritional components conferring the ability of S3 PrP to form protease-resistant species.

Conclusion. Our data establish that (i) different conformations of the PrP OR can be associated with different phenotypic attributes, (ii) the OR's are sensitive to nutritional factors and (iii) that binding of nutritional factors to the OR's transduces effects in *cis* to affect folding of the PrP C-terminal domain in vivo.

P.06: Seeding PrP amyloid formation in the absence of TSE disease

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Human P102L Gerstmann-Sträussler-Scheinker (GSS) disease is a familial TSE which is associated with a single amino acid mutation at codon 102 in human PrP. However, two distinct phenotypes of disease are associated with this single amino acid mutation. In classical P102L GSS (PrP-21) amyloid plaques and diffuse PrP deposition are accompanied by spongiform degeneration of the brain and the presence of PrP 27-30. In atypical forms of P102L GSS (PrP-8), amyloid deposition occurs in the absence of any spongiosis, and only an 8kDa PrP-res band is observed on immunoblot. While inoculation of PrP-21 can transmit TSE disease to transgenic mice homozygous for the equivalent mutation in murine PrP (101LL; courtesy of Prof. J. Manson, Edinburgh), inoculation of 101LL mice with PrP-8 results mainly in the production of further PrP amyloid without any associated clinical signs or spongiform degeneration. Subsequent subpassages produced further amyloid seeding but no disease. These data indicate that PrP amyloid may be seeded in the brain of 101LL mice in the absence of TSE disease or replication of infectivity. To further investigate this seeding mechanism we have utilised recombinant PrP (Prnp^a and Prnp^{a-101L}) refolded into three different conformations (α -monomeric, β -oligomeric or fibril amyloid) and inoculated this material into both 101LL and WT mice. No PrP aggregation was observed following inoculation of α -monomeric or β -oligomeric PrP isoforms; however PrP amyloid inoculations exhibited seeding of amyloid plaques in 101LL but not Wt mice with both 101L and Wt fibril amyloid preps. We clearly demonstrate here that abnormal forms of PrP can exist in the brain without causing a spongiform encephalopathy. PrP misfolding can therefore be separated from propagation of TSE infectivity as PrP amyloid accumulation can be induced in 101LL transgenic mice in the absence of infected inoculum.

P.07: In-vitro models utilised to enhance our understanding of early mechanisms of misfolded protein formation, accumulation and clearance

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The relationship between misfolded prion protein, infection and neurotoxicity is still not clearly understood. Previous work from our group demonstrated that inoculation of refolded

recombinant PrP into transgenic mice expressing a proline to leucine mutation at PrP codon 101 (101LL) resulted in the presence and seeding of PrP amyloid plaques in the brain in the absence of TSE disease or replication of infectivity. Subsequent subpassages produced further amyloid seeding but no disease. Here we demonstrate clearly that abnormal forms of PrP can exist in the brain without causing a spongiform encephalopathy. PrP misfolding can also be separated from propagation of TSE infectivity as PrP amyloid accumulation can be induced in 101LL transgenic mice in the absence of infected inoculum. Interestingly throughout these experiments we observed that WT control mice were all negative for plaque deposition following inoculation. We could therefore hypothesise that in the absence of this point mutation the “healthy” brain can maintain homeostasis and efficiently clear any abnormal protein present. Further analysis of these intricate mechanisms is crucial to fully understand amyloid interactions within a healthy brain and whether elements such as age for example would be an important factor regarding homeostasis. To address these issues our investigations focus primarily on early time point mechanisms of cellular response after challenge with fluorescently labelled amyloid fibrils in both WT and PrP^{101L} models utilising both in vivo and in vitro models. We have currently developed two in vitro systems one with primary hippocampal cells isolated from E17 embryos and the second using organotypic brain slices. These systems are now fully developed and optimised and provide easily assessable models for investigating the trafficking and interactions of fluorescently labelled misfolded protein in vitro.

P.08: The effect of gamma irradiation on atypical scrapie

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The transmissible spongiform encephalopathies (TSEs) are resistant to treatments expected to inactivate nucleic acids, including irradiation, and these characteristics contributed to the development of the prion hypothesis, now widely accepted. The biochemical hallmark of TSE is the misfolded protein PrP^{Sc} which is usually deposited in infected tissues such as brain and lymph nodes and is formed in a misfolding conversion reaction from the normal cellular protein PrP^C. PrP^{Sc} also shows high resistance to digestion with enzymes and to decontamination procedures. Previous studies by colleagues have demonstrated that irradiated classical scrapie, transmitted to mice, shows little alteration in phenotype.

Atypical scrapie has not been fully tested for its resistance to inactivation although the disease-related PrP is known to be much more sensitive to proteinase K than PrP^{Sc} from classical TSEs. We reasoned therefore that it was possible that atypical scrapie would also be sensitive to gamma irradiation treatment. In this project gamma (Isotron 25kGy) irradiated atypical scrapie

was transmitted to tg 338 mice which were assessed for changes in incubation period and lesion profile compared with the same inoculum injected before irradiation. Incubation period, lesion profiles, immunocytochemistry, and Western blot analysis were undertaken to determine if irradiation inactivated or modulated the neuropathological characteristics of infection or biochemical characteristics of atypical scrapie.

There was no significant difference observed in incubation periods or lesion profiles between irradiated and non-irradiated samples. Following immunohistochemistry, neuroanatomical distribution of PrP^{Sc} was not modulated and Western blot analysis also demonstrated no differences - all samples displayed a low molecular weight band at ~8-11 kDa, consistent with the glycoform of atypical scrapie. It was concluded that 25 kGy of γ -irradiation does not markedly alter the infection characteristics of atypical scrapie and that inactivation of atypical scrapie contamination should be carried out with the same rigorous procedures used for the classical TSEs.

P.09: Investigating the structure of PrP^{Sc} using an antibody screen

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Prion diseases are neurodegenerative illnesses that are caused by an aberrantly folded form of the prion protein, PrP. It can be folded into the cellular form, PrP^C, and is a membrane-anchored protein with a globular C-terminal domain and a flexible, unstructured N-terminal domain. The C-terminal domain of PrP^C is mostly α -helical with only a small amount of β -sheet structure. Either spontaneously or through infection, the prion protein can fold into the disease-causing form, known as PrP^{Sc}. The structure of PrP^{Sc} is significantly altered with a high β -sheet content, which is in contrast to the non-infectious form. This altered protein structure renders most forms of PrP^{Sc} greatly resistant to proteases and induces their polymerization into amyloid fibrils. PrP^{Sc} is highly insoluble, which has inhibited structural research. The knowledge of the structure of PrP^{Sc} comes mainly from low-resolution methods, such as Fourier-transform infrared spectroscopy and X-ray fiber diffraction. A multitude of models have been proposed for the structure of PrP^{Sc}, but the low-resolution data are most consistent with a β -solenoid architecture. Higher-resolution details, such as which residues participate in the β -sheets that make up the β -solenoid structure, are, however, still not known.

We are limited mostly to low-resolution methods to study the structure of PrP^{Sc} and one technique of interest is antibody mapping. Since antibodies bind to highly specific protein sequences and structures, mapping the epitope of an antibody can give highly localized and detailed structural information. Consequently, our project focuses on screening PrP^C, PrP^{Sc}, and

denatured PrP with a series of monoclonal antibodies using dot blotting. In our screen, brain homogenates of infected and non-infected animals were treated with proteinase K and either denatured or non-denatured. These treatments allow us to determine if antibodies bind to epitopes that are surface-exposed or buried within the protein structures of PrP^C, PrP^{Sc}, or denatured PrP. We have identified a number of antibodies that recognize PrP^C and denatured forms of PrP, but fail to bind protease-resistant PrP^{Sc}. We have been unable to test protease-sensitive PrP^{Sc} using this assay. Currently we are mapping the epitopes of these antibodies. The information acquired will be used to generate a more accurate model for the structure of PrP^{Sc}.

P.10: A loss of gene function approach to better understand the role of cholesterol in prion replication

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Keywords: cholesterol, Dhcr24, Hmgcr

The conversion of the prion protein, PrP^C to self-aggregating, protease-resistant conformers is a pathological hallmark of prion diseases. By virtue of its glycosylphosphatidylinositol (GPI) anchor, PrP^C is incorporated into lipid rafts of the outer cell membrane. Whilst cholesterol constitutes a key component of these membrane microdomains, its role in prion replication remains controversial. Cellular cholesterol levels are tightly regulated by transcriptional and posttranscriptional feedback mechanisms. To scrutinise the role of cholesterol we are perturbing expression of key genes associated with biosynthesis, import, trafficking and export of cholesterol whilst concomitantly monitoring changes in the levels of cholesterol and prions, respectively.

Gene silencing of *Dhcr24* which encodes the 3 β -hydroxysteroid- Δ 24 reductase enzyme, required for the conversion of desmosterol to cholesterol, the final step in *de novo* cholesterol synthesis, markedly decreased cellular cholesterol levels and significantly increased the rate of prion propagation. Knockdown of 3-hydroxy-3-methylglutaryl-CoA reductase (*Hmgcr*), a key regulatory gene of cholesterol synthesis and HMGCR inhibition by preincubation of cells by lovastatin showed similar effects, suggesting that cholesterol inhibits prion propagation. Experiments are now in progress to examine the role of cholesterol import, trafficking and export of cholesterol on the rates of prion replication.

P.11: Peptide aptamers interfering with PrP^C-PrP^{Sc} interaction inhibit prion protein misfolding

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Keywords: prion, peptide aptamer, misfolding

Transmissible Spongiform Encephalopathies (TSEs) are fatal neurodegenerative diseases affecting both humans and animals. They include Creutzfeldt-Jakob disease (CJD) in humans, bovine spongiform encephalopathy (BSE) in cows, scrapie in small ruminants and chronic wasting disease (CWD) in cervids. The causative agent is the prion, a pathogen consisting of an abnormally folded isoform (PrP^{Sc}) of the cellular prion protein (PrP^C). The steric interaction between these two isoforms leads to misfolding of the soluble and protease-sensitive PrP^C into an insoluble, protease-resistant form, which displays the same primary sequence but different secondary structure. This process is still poorly understood and no therapeutic nor prophylactic tools are available yet.

In order to identify anti-prion compounds, we aim to inhibit prion conversion by interfering with the PrP^C-PrP^{Sc} interplay. We have previously described a peptide aptamer (PA8) with high affinity for PrP^C which reduces prion propagation in prion infected cultured cells.¹ Peptide aptamers consist of a peptide inserted in a scaffold protein, here the *Escherichia coli* thioredoxin A (trxA). To improve binding affinity and anti-prionic activity of PA8, its binding to PrP^C has been modelled *in silico*. New mutant clones of PA8 with an expected higher binding affinity will be created by selected amino acid replacements in the peptide moiety. These new peptide aptamers are created by site directed mutagenesis, expressed in *E. coli*, purified via His₆-Tag and employed for treatment of prion-infected cells. An improvement of their binding property is expected, as well as an enhanced inhibition of the endogenous PrP^{Sc} conversion in treated cells. The anti-prion effects of the most promising candidates will be further evaluated in murine bioassays. In future, our results will be useful for identifying a compound by structure-based drug design with anti-prionic activity, able to counteract prion conversion *in vivo*.

References

1. Gilch S, Kehler C, Schätzl HM. Peptide aptamers expressed in the secretory pathway interfere with cellular PrP^{Sc} formation. *J Mol Biol* 2007; 371:362-73; PMID:17574575; <http://dx.doi.org/10.1016/j.jmb.2007.05.052>

P.12: Myotube cultures as a prion source for structural investigations

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Prion diseases are fatal neurodegenerative diseases caused by a misfolding of the prion protein (PrP^C). Although the structure of PrP^C is well characterized, there is limited information available regarding the structure of the infectious isoform of PrP (PrP^{Sc}) beyond partial resistance to proteinase K digestion and the presence of a complex β -sheet architecture. Most techniques used to decipher the structure of proteins are hampered by the insoluble nature of PrP^{Sc} and the need for substantial amounts of highly purified PrP^{Sc}. Currently, all structural biology approaches that target PrP^{Sc} rely on brains of infected and sick animals. Attempts to develop other sources of PrP^{Sc}, such as mammalian cell cultures or recombinant protein, have limited utility as they do not provide sufficient amounts of PrP^{Sc} (cell cultures) or the prion protein (recombinant sources) may not be in the right conformational state.

The aim of this study is to explore a newly developed system of producing prions by infecting cultured muscle cells¹ as a source for PrP^{Sc} structural studies. Myotubes are non-proliferative cells that upon infection can generate high levels of PrP^{Sc} and infectivity, about ten times more than other mammalian cells in culture (e.g. N2a cells). Murine C2C12 myoblast cells differentiated into myotubes were incubated with 0.01% RML brain or with normal brain (mock-infected). These myotubes were harvested at 15-20 days post-inoculation and lysed using different buffers (RIPA, buffers containing sarkosyl in concentrations from 0.5 to 5%, and non-EDTA based buffer containing sarkosyl) to examine their efficacy in PrP^{Sc} extraction. To obtain highly purified PrP^{Sc}, a number of modifications of the purification protocol have been explored, which is mainly based on proteinase digestion (Pronase E and proteinase K) followed by precipitation with sodium phosphotungstate (NaPTA) in the presence of sarkosyl. Variables we tested to optimize the protocol included the number of centrifugation steps, centrifugation speed, and concentrations of proteinases, sarkosyl, and NaPTA. The purified samples were evaluated by SDS-PAGE followed by silver staining or Western blotting. Further analyses will be performed by electron microscopy to confirm sample purity and suitability for structural studies.

References

1. Herbst A, Baner P, Velasquez CD, Mays CE, Sim VL, Westaway D, Aiken JM, McKenzie D. Infectious prions accumulate to high levels in non proliferative C2C12 myotubes. *PLoS Pathog* 2013; 9:e1003755; PMID:24244171; <http://dx.doi.org/10.1371/journal.ppat.1003755>

P.13: Prion protein gamma-cleavage: A novel proteolytic event

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Keywords: PrP^C endoproteolysis

The cellular prion protein (PrP^C) is known to undergo two well-described endoproteolytic cleavage events, alpha- and beta-cleavage, producing the fragments C1/N1 and C2/N2, respectively. After the introduction of c-myc tagged murine PrP^C, previously shown to behave as wild-type PrP^C in vivo and in vitro,¹ into various cell lines, we observed another PrP^C cleavage fragment, 'C3'. Our pilot studies indicated C3 was GPI-anchored and not glycosylated, and the cleavage site was most likely within the highly conserved PrP^C far c-terminus.² We postulated C3, due to its small size and the likely position of its cleavage site, has been previously overlooked.

We have considerably extended our previous study to further characterise this novel cleavage event, which we have coined PrP "γ-cleavage". We have detected the C3 fragment in un-transfected cultured cells, as well as human and animal brain tissue, confirming that this is a real in vivo cleavage event and not an artefact of the PrP^C myc-tag engineering. Our data suggests γ-cleavage occurs after PrP^C translocation from the ER, and that C3 may be preferentially cleaved from an unglycosylated substrate by matrix metalloproteinase/s. Further, at steady-state levels, C3 is not primarily located at the cell surface, and that compared to other PrP^C species, is highly proteinase K resistant. The relevance of PrP γ-cleavage to normal PrP^C function, as well as prion and other neurodegenerative diseases, is still under investigation.

References

1. Rutishauser D, Mertz KD, Moos R, Brunner E, Rulicke T, Calella AM, Aguzzi A. The comprehensive native interactome of a fully functional tagged prion protein. *PLoS One* 2009; 4:e4446; PMID:19209230; <http://dx.doi.org/10.1371/journal.pone.0004446>
2. Lewis, et al. Poster No.: PO-216. Prion 2012 from Prion 2012; 6(S): 122-123.

P.14: How to shape the future of prion research

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Keywords: atypical TSE, chronic intoxication, Ivermectin, GABA receptor, Nodding Syndrome, prion trigger mechanism and biological function

At PRION 2010 in Salzburg my poster was completely ignored, at PRION 2012 in Amsterdam it was given the poster award for style and originality! Here I present my poster again

in a new context for better understanding: The red smiley “PrP^c ready to convert” in my poster corresponds to the “prion with flexible tail” of Aguzzi et al.¹

That is exactly the symbol of a switch with deactivated child-proof lock! According to my hypothesis (<http://www.inter-uni.net/Komplementaerheilkunde#becker>) the deactivating factors are advancing age, copper deficiency, oversupply of manganese, oxidative stress, genetic mutations and all GABA receptor stimulating agents like alcohol, bromine, macrocyclic lactones, benzodiazepines and others.

Considering this basic trigger mechanism each prion disease is a real TSE, a **triggered** spongiform encephalopathy. There is no future for prions as infectious agents; it is only a simple triggering with PrP^{Sc}.

For the first time since 2007, a case of atypical (L-type) bovine spongiform encephalopathy (BSE) was identified in a beef cow in the German state of Brandenburg in January 2014 as part of the German targeted BSE surveillance system. The cow was slaughtered at the age of over ten years without clinical signs of disease. The source of outbreak was unknown or inconclusive. But the cow and most likely her mother were treated with Ivermectin every year! Treating female animals and women with Ivermectin is extremely dangerous because of toxic residues in their colostrums.

In South Sudan and Uganda Nodding Syndrome² is a mysterious illness that affects the brains and nervous system of children, primarily between the ages of 5 and 15. To me Nodding Syndrome is similar to Chronic Wasting Disease in game farms. Yet the World Health Organization (WHO) and Center for Disease Control (CDC) are not fully sure what is causing the illness. Theories have linked the disease to *Onchocerca volvulus* (the organism that causes River Blindness), environmental toxins, vitamin B6 deficiency, vaccine reaction, a fungi found in bush meat... but nothing has yet been conclusively proven. River blindness is treated twice a year with Ivermectin (Mectizan); the epileptic seizures are treated with Phenobarbital. Both medications together with alcohol cumulate their effects on the GABA receptor.

What is the biological function of the prion protein?

The trigger mechanism is the only way to create the highest quantity of cells in a limited capacity, important for the function of brain and bone marrow.

References

1. Sonati T, Reimann RR, Falsig J, Baral PK, O'Connor T, Hornemann S, Yaganoglu S, Li B, Herrmann US, Wieland B, et al. The toxicity of anti-prion antibodies is mediated by the flexible tail of the prion protein. *Nature* 2013; 501:102-6; PMID:23903654; <http://dx.doi.org/10.1038/nature12402>
2. Dowell SF, Sejvar JJ, Riek L, Vandemaele KA, Lamunu M, Kuesel AC, Schmutzhard E, Matuja W, Bunga S, Foltz J, et al. Nodding syndrome. *Emerg Infect Dis* 2013; 19:1374-84; PMID:23965548; <http://dx.doi.org/10.3201/eid1909.130401>

P.15: Heparin binding confers prion stability and impairs its aggregation

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Keywords: inhibition of amyloid aggregation, prion conversion, glycosaminoglycan, neurodegeneration

The conversion of the prion protein (PrP) into scrapie PrP is a central event in prion diseases. Several molecules work as cofactors in the conversion process, including glycosaminoglycans (GAGs). GAGs exhibit a paradoxical effect as they convert PrP into PrP-res, while they also exert protective activity. We asked whether LMWHep increases murine prion protein stability and thus changes its ability to aggregate. We compared the stability and aggregation propensity of PrP and the heparin-PrP complex through the application of different in vitro aggregation approaches, including real-time quaking-induced conversion (RT-QuIC). Transmissible spongiform encephalopathy-associated forms from mouse and hamster brain homogenates were used to seed RT-QuIC-induced fibrillization. We show that interaction between heparin and cellular prion protein (PrP^C) increases thermal PrP stability, leading to an eight-fold decrease in the temperature-induced aggregation. The interaction of LMWHep with the PrP N- or C-terminal domain affects not only the extent of PrP fibrillization but also its kinetics, lowering the reaction rate constant from 1.04 s⁻¹ to 0.29 s⁻¹ and increasing lag phase from 12 to 19 hours in RT-QuIC experiments. Our findings explain the protective effect heparin has in different models of prion and other prion-like neurodegenerative diseases and establish the groundwork for the development of therapeutic strategies based on GAGs.

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P.16: Crosslinking B2-H2 loop with C-terminal region of PrP retains convertibility into PrP^{Sc} in cultured cells

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Prions propagate by refolding host-encoded PrP into the PrP^{Sc} isoform. Since the properties of prions are postulated to be enciphered in the structure of PrP^{Sc}, its identification is crucially important for understanding prion biology. However, the structure of PrP^{Sc} is yet to be identified in full detail. Recently, we reported that the region between the first and the second alpha helix (H1-H2) of PrP is an interaction interface for PrP with deletions in H1-H2 (Δ PrP) to PrP^{Sc}.¹ Data from these experiments also suggested that there is some cooperation between H1-H2 and the region C-terminal to it for Δ PrP-PrP^{Sc} interactions. Since H1-H2 is located close to the C-terminal region of H3 (C_{trm}), we hypothesized that their cooperation might also contribute to interactions between full-length PrP with PrP^{Sc} and consequently prion conversion. To test this hypothesis, we created a series of mutant PrPs with two cysteine (Cys) substitutions: one at the residue 166 in H1-H2 and the other scanning C_{trm}, namely from 220 to 229, so that a disulfide crosslink would be formed between those regions when in sufficient proximity. Mutant PrPs were expressed with banding patterns consistent with complex-type N-glycans and localized at the cell surface, despite that the two cysteine residues in H1-H2 and C_{trm} form an additional disulfide crosslink. We transfected these constructs into 22L-scrapie infected N2a cells and observed their conversion efficiencies to assess the influence of such disulfide crosslinks. Interestingly, 166C;220 to 166C;224C did not convert, but 166C;225C to 166C;229C showed substantial efficiencies of prion conversion. The tolerance of PrP^C-PrP^{Sc} conversion reaction to the structural constraint posed by the disulfide crosslinks implies that the region around the residue 166 and C_{trm} might undergo relatively small structural changes in the conversion process, even when adjacent regions extensively change structures. Besides, the protease-resistant cores of the mutant PrPs showed unique glycoform ratios distinct from that of wild-type PrP, suggesting a possible origin of variation in glycoform ratios among different prion subpopulations. The series of two-Cys mutant PrPs might become a useful tool for investigation of structures and/or structure-property correlations of PrP^{Sc}.

References

1. Taguchi Y, Mistica AM, Kitamoto T, Schätzl HM. Critical significance of the region between Helix 1 and 2 for efficient dominant-negative inhibition by conversion-incompetent prion protein. *PLoS Pathog* 2013; 9:e1003466; PMID:23825952; <http://dx.doi.org/10.1371/journal.ppat.1003466>

P.17: Metabolism of PrP^{Sc} differs depending on prion strains

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Keywords: prion strain, autophagy, Fukuoka-1 (FK) strain

Prion diseases are neurodegenerative disorders caused by the aggregation and accumulation of abnormal prion protein (PrP^{Sc}). With the aim of elucidating the accumulation mechanism of PrP^{Sc}, we focused on the mechanism of clearance of PrP^{Sc} and investigated whether the degradation system of the PrP^{Sc} has a difference among strains. Intriguingly, a significantly increment of PrP^{Sc} was observed upon autophagy-inhibitor 3-methyladenine (3MA) treatment in GSS-derived persistently prion-infected cells (N2a-FK cells) but not in scrapie-derived persistently prion-infected cells (N2a-22L cells). Conversely, a substantial reduction of FK-derived PrP^{Sc} was observed upon autophagy-accelerator rapamycin treatment but not 22L-derived PrP^{Sc} by western blotting and immunofluorescent staining, indicating that FK-PrP^{Sc} is more prone to be degraded by autophagic system than 22L-PrP^{Sc}. Furthermore, we assessed whether the proteasome inhibitors (MG132 and Epoxomicin) and subcellular signal transduction inhibitors (such as LY294002 inhibiting phosphatidylinositol 3-kinase (PI3K) and PD98059 inhibiting mitogen-activated protein kinase/extracellular signal-regulated kinase (MEK)) have any effect on the PrP^{Sc} degradation in N2a-FK and -22L cells. As a result, the FK-derived PrP^{Sc} was specifically degraded by autophagic system mediating PI3K and MEK signal pathway rather than proteasome system compared with 22L-derived PrP^{Sc} cells by pharmacological approach. These results suggest that PrP^{Sc} might be degraded by the prion strain individual system.

P.18: Role of cellular prion protein in myelin formation and maintenance in the central nervous system

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The cellular form of the prion protein (PrP^C) is involved in many physiological processes in the nervous system. One is the formation and maintenance of peripheral nervous system myelin. Evidence reported in literature shows ambiguous results

concerning a similar role in the central nervous system (CNS). Myelin formation and maintenance in the CNS are influenced by metal homeostasis and oxidative stress. In particular, copper and iron are crucial for a correct myelination. Interestingly, PrP^C is involved in both the protection against oxidative stress and the regulation of metal homeostasis. Hence, we decided to investigate the role of PrP^C in CNS myelination by comparing wild-type (WT) and PrP^C-null mice at different developmental stages, from early postnatal days to aging. We adopted different biochemical approaches. Considering the high lipid content of CNS myelin, we decided to perform a total lipid composition analysis by Fourier Transformed Infrared Spectroscopy and to measure membrane cholesterol levels. Moreover, the expression of different myelin proteins, proteolipid protein and myelin basic protein, was evaluated and compared in WT and PrP^C-null mice. Early findings suggest some differences in brain lipid composition between WT and PrP^C-null mice during the early postnatal stages. Even the myelin protein expression results altered between the two genotypes at different ages. Taken together, these preliminary data support the hypothesis that PrP^C could play a role also in CNS myelin formation and maintenance. Further experiments will be performed to investigate the mechanism by which PrP^C can play this role.

P.19: Characterization of goats naturally devoid of the prion protein

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Several studies have shown that prion protein (PrP) knock-out animals are viable and without any major physiological abnormalities. We have recently discovered that animals of the Norwegian Dairy Goat breed naturally lack PrP due to a nonsense mutation in the *Prnp* gene. Goats with this specific genotype have a stop mutation at codon 32 that blocks the synthesis of PrP. As development of prion diseases is dependent on endogenous cellular PrP, goats homozygous for this mutation are expected to be scrapie resistant. As far as we know, this line of goats is the first species identified that is naturally devoid of PrP, and thus provides a unique opportunity to study the normal function of the protein in a naturally receptive host. Preliminary results of genetic testing to establish the frequency of the 32 stop allele in a number of Norwegian herds, have revealed that 11% (n=192) of the goats are carrying the mutation. All heterozygous and homozygous animals are in good health and have not shown any abnormal behavior or physiology. Further genetic testing is in progress and will be reported. Additional investigations will include transcriptomic profiling of brain tissues to investigate potential responses to loss of PrP. Hetero- and homozygous kids born this spring will be monitored for growth, general health

status and production traits. Blood samples and fecal samples will give insights on hematology, clinical chemistry, immunological status and parasite burden compared with matched controls. Goats will also be subjected to a thorough clinical and neurological examination, and some will be necropsied to investigate gross and histopathology.

P.20: Murine-adapted strains of prions differ in the amount of infectivity associated with PrP-res and PrP-sen fractions

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Introduction. Major feature of the pathological prion protein (PrP^{TSE}) is its partial resistance to proteolysis by proteinase K (PK). However, the existence of PK-sensitive pool of PrP^{TSE} (PrP-sen) is well recognized and the role of PrP-sen in pathogenesis of prion diseases is under intense debate. The aim of our study was to elucidate the contribution of PK-resistant PrP^{TSE} (PrP-res) and PrP-sen pools towards the infectivity of three murine-adapted prion strains in in vitro cell model.

Materials and Methods. The presence of PrP^{TSE} in brain homogenates of mice infected with murine-adapted scrapie (RML), Gerstmann-Sträussler-Scheinker syndrome (Fukuoka-1, Fu) or variant Creutzfeldt-Jakob disease (mvCJD) was evaluated by western blot and the proportion of PrP-sen was assessed by conformation-dependent immunoassay. Brain homogenates were digested by PK at 250 µg/ml for 0, 30, 90 or 270 min at 45°C and used for the infection of susceptible CAD5 cells. The cells were cultivated for 30 days and their infection was periodically monitored by cell blot and scrapie-cell assay.

Results. Brain homogenates demonstrated similar levels of PrP-res, but the level of total PrP and the proportion of PrP-sen were higher in mvCJD. Digestion of brain homogenates with PK led to the time-dependent decrease of PrP signal which was comparable for all prion strains tested. Also, the amount of residual PrP-res after extensive 270 min PK digestion was similar. In contrast, the infection of CAD5 cells with PK-digested RML and Fu brain homogenates was more effective than infection with mvCJD. Both scrapie-cell assay and cell blot demonstrated significant drop of prion infectivity in mvCJD brain homogenate in comparison to RML and Fu homogenates after PK treatment at all time intervals tested demonstrating higher dependence of mvCJD infectivity on PrP-sen.

Conclusions. Our study indicates that prion strains may differ not only by the content of PrP-res and PrP-sen, but also by the amount of infectivity associated with PrP-res and PrP-sen fractions.

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P.21: Modification of [PSI⁺] prion properties by combining amino acid changes in the N-terminal domain of Sup35 protein

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The prion [PSI⁺] is an amyloid isoform of the translation termination factor eRF3 encoded by the *SUP35* gene in *Saccharomyces cerevisiae* yeast. Naturally occurring amyloid complexes have been studied for a long time, yet their structural organization is still not well understood. The formation of amyloid forms of the wild-type Sup35 protein (Sup35p) is directed by its N-terminal portion, which forms a superpleated β -sheet structure. We previously constructed a set of *sup35* mutant alleles bearing QQ or YQ to KK substitutions (*sup35^{KK}*) and characterized their effects on [PSI⁺] propagation.¹ Two of these *sup35^{KK}* alleles led to prion elimination in different manner and other mutations influenced [PSI⁺] properties.

In the current study, we investigated the contribution of each mutant peptide to the stability of the prion and aggregation properties, and compared the effects of single mutations and combinations of different mutant alleles. Studies were carried out in yeast strains designed to bear single mutation or a combination of different *sup35* alleles. Based on this analysis, we propose a model that clarifies the 3D organization of the β -sheets within the prion. We also provide evidence that *sup35-M2* (Q61K/Q62K) and *sup35-M4* (Q80K/Q81K) mutations change the structure of prion complexes. We propose that the destabilization of prion complexes in these mutants is due to the decreased efficiency of the fragmentation of the prion aggregates by chaperone complexes.

References

1. Bondarev SA, Shchepachev VV, Kajava AV, Zhouravleva GA. Effect of charged residues in the N-domain of Sup35 protein on prion [PSI⁺] stability and propagation. *J Biol Chem* 2013; 288:28503-13; PMID:23965990; <http://dx.doi.org/10.1074/jbc.M113.471805>

P.22: Interaction and disruption of model lipid vesicles by the bacterial RepA-WH1 prionoid

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We have recently reported that engineering RepA-WH1, a bacterial DNA-toggled protein conformational switch

(dWH1 \rightarrow mWH1) sharing some analogies with nucleic acid-promoted PrP^c \rightarrow PrP^{Sc} replication,¹ constitutes a suitable synthetic model system to study protein amyloidosis in bacteria.^{2,3} Although amyloidogenesis has been the focus of intense research, the origin of the amyloid toxicity remains unclear. One proposed mechanism of cytotoxicity is lipid membrane permeabilization.

To understand how membranes affect amyloid formation we are studying the aggregation of the bacterial RepA-WH1 prionoid in the presence of cytomimetic model systems (large and giant unilamellar lipid vesicles, LUVs, and GUVs respectively).⁴ Confocal microscopy images of protein encapsulated into GUVs show association and aggregation of the protein preferentially to lipid vesicles containing acidic phospholipids. We have also observed that RepA-WH1 elicits membrane disruption using a dye release assay on LUVs.⁵ The extent of leakage was dependent on protein concentration.

We have been able to directly measure the process of membrane permeation and leakage by time-elapsing imaging of dye filled GUVs upon the addition of protein. This process is fast and over the course of the experiment most of the vesicles remain intact, suggesting the assembly of defined pores by RepA-WH1.

References

1. Silva JL, Lima LM, Foguel D, Cordeiro Y. Intriguing nucleic-acid-binding features of mammalian prion protein. *Trends Biochem Sci* 2008; 33:132-40; PMID:18243708; <http://dx.doi.org/10.1016/j.tibs.2007.11.003>
2. Giraldo R, Moreno-Díaz de la Espina S, Fernández-Tresguerres ME, Gasset-Rosa F. RepA-WH1 prionoid: a synthetic amyloid proteinopathy in a minimalist host. *Prion* 2011; 5:60-4; PMID:21293179; <http://dx.doi.org/10.4161/pri.5.2.14913>
3. Fernández-Tresguerres ME, de la Espina SM, Gasset-Rosa F, Giraldo R. A DNA-promoted amyloid proteinopathy in *Escherichia coli*. *Mol Microbiol* 2010; 77:1456-69; PMID:20662778; <http://dx.doi.org/10.1111/j.1365-2958.2010.07299.x>
4. Butterfield SM, Lashuel HA. Amyloidogenic protein-membrane interactions: mechanistic insight from model systems. *Angew Chem Int Ed* 2010; 49:5628-54; PMID: 20623810; <http://dx.doi.org/10.1002/anie.200906670>
5. van Rooijen BD, Claessens MM, Subramaniam V. Lipid bilayer disruption by oligomeric alpha-synuclein depends on bilayer charge and accessibility of the hydrophobic core. *Biochim Biophys Acta* 2009; 1788:1271-1278; PMID: 19328772; <http://dx.doi.org/10.1016/j.bbamem.2009.03.010>

P.23: Unraveling the pathways of toxicity of the RepA-WH1 prionoid in bacteria

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The complexity of the processes leading to the development of amyloid proteinopathies is counterbalanced by their universal structural basis: the amyloid crossed β -sheets.¹ This has fuelled the quest for disease-unrelated model systems suitable to study protein amyloidosis under quasi-physiological conditions in vitro and in simpler organisms in vivo. We had reported that RepA-WH1, a bacterial protein conformational switch (dWH1 \rightarrow mWH1) functional in plasmid replication², when it is fused to a red fluorescent protein, causes an amyloid proteinopathy in *Escherichia coli* which prevented cell proliferation.³ RepA-WH1 aggregates are not infectious (*horizontally*

transmissible), although enable conformational templating by cross-seeding in vitro.³ It is thus the first bacterial prionoid.⁴ We have recently found that DnaK, the Hsp70 chaperone in *E. coli*, contributes to RepA-WH1 amyloidogenesis in vivo, but the Hsp104 chaperone ClpB does not have a major effect on the vertical spread of the amyloid aggregates.⁵ We have now studied the pathways of toxicity of the prionoid combining genomic, proteomic and physiological approaches. Preliminary results indicate that cell damage is produced by ROS-dependent mechanisms.

References

1. Giraldo R. Amyloid assemblies: protein legos at a crossroads in bottom-up synthetic biology. *Chembiochem* 2010; 11:2347-57; PMID: 20979077; <http://dx.doi.org/10.1002/cbic.201000412>
2. Giraldo R, Fernández-Tornero C, Evans PR, Díaz-Orejas R, Romero A. A conformational switch between transcriptional repression and replication initiation in the RepA dimerization domain. *Nat Struct Biol* 2003; 10:565-71; PMID:12766757; <http://dx.doi.org/10.1038/nsb937>
3. Fernández-Tresguerres ME, de la Espina SM, Gasset-Rosa F, Giraldo R. A DNA-promoted amyloid proteinopathy in *Escherichia coli*. *Mol Microbiol* 2010; 77:1456-69; PMID:20662778; <http://dx.doi.org/10.1111/j.1365-2958.2010.07299.x>
4. Giraldo R, Moreno-Díaz de la Espina S, Fernández-Tresguerres ME, Gasset-Rosa F. RepA-WH1 prionoid: a synthetic amyloid proteinopathy in a minimalist host. *Prion* 2011; 5:60-4; PMID:21293179; <http://dx.doi.org/10.4161/pri.5.2.14913>
5. Gasset-Rosa F, Coquel AS, Moreno-Del Álamo M, Chen P, Song X, Serrano AM, Fernández-Tresguerres ME, Moreno-Díaz de la Espina S, Lindner AB, Giraldo R. Direct assessment in bacteria of prionoid propagation and phenotype selection by Hsp70 chaperone. *Mol Microbiol* 2014; 91:1070-87; <http://dx.doi.org/10.1111/mmi.12518>; PMID:24417419

P.24: Addressing thermal and mechanical stability of human prion protein with single molecule and ensemble measurements

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Keywords: atomic force microscopy, calorimetry, prion stability

Prion disease is a complex pathology in which the main mechanism is associated with the conversion of the endogenous prion protein into a transmissible, pathogenic isoform. The crucial role of this misfolding event is nowadays widely assessed, but there is still a little consensus on the details of the underlying mechanism. In particular, the role (and to some extent the existence) of partially or fully unfolded intermediates toward the structure of the pathological state is not clear. Difficulty in addressing this question probably arises from the intrinsic complexity of the energy landscape experienced by the protein in its native dynamics.¹ This putative peculiar characteristic was very recently highlighted by means of thermodynamic measurements on two variants of murine prion protein (moPrP) in which the native state appeared to undergo substantial fluctuations in enthalpy and hence, in structure.² Moreover, switching from ensemble to

single molecule measurements, it was possible to show the presence of several unstable intermediate states in moPrP unfolding, which probably play a relevant role in the functionality of the protein system.³

Here we present a study on two variants of the human prion protein (hPrP) whose conformational transition intermediates have been characterized by means of single molecule force spectroscopy and calorimetry. The difference in the conformational states between the two forms and their relationship with the respective biological activities will allow to obtain a deeper insight into the dynamics of hPrP and highlight a link to its pathological role.

References

1. Yu H, Dee DR, Woodside MT. Single-molecule approaches to prion protein misfolding. *Prion* 2013; 7:140-6; PMID:23357831; <http://dx.doi.org/10.4161/pri.23303>
2. Moullick R, Udgaonkar JB. Thermodynamic characterization of the unfolding of the prion protein. *Biophys J* 2014; 106:410-20; PMID:24461016; <http://dx.doi.org/10.1016/j.bpj.2013.11.4491>
3. Yu H, Liu X, Neupane K, Gupta AN, Brigley AM, Solanki A, Sosova I, Woodside MT. Direct observation of multiple misfolding pathways in a single prion protein molecule. *Proc Natl Acad Sci U S A* 2012; 109:5283-8; PMID:22421432; <http://dx.doi.org/10.1073/pnas.1107736109>

P.25: Real-time quaking induced conversion assay for prion seeding

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Keywords: prion, seeding assay, microplate reader

Previously, prions were studied using lengthy bioassays where infected animals were studied over long periods of time (1-6 months). This was both time consuming and costly. Researchers at Rocky Mountain Laboratories (Hamilton, Montana) have developed a new prion seeding assay called Real-Time Quaking-Induced Conversion Assay (RT-QuIC) that gives end-point quantitation for measuring prion levels in infected samples.¹ For the development of the assay, Rocky Mountain Laboratories used BMG LABTECH's Omega series of microplate readers. The assay measures serial dilutions of samples, and statistically estimates the loss of seeding activity (SD50). The advantage of the RT-QuIC assay is faster measurement times than previous tests: an assay can be completed in as little as 20 hours. Moreover, RT-QuIC is equally sensitive, if not more so, and has a higher throughput than previous methods using whole animal models.

BMG LABTECH's Omega series of readers have the ability to shake and incubate microplates over long periods of time and are robust to withstand extended shaking at high speeds. Omega series readers allow researchers to continuously measure RT-QuIC samples at 42°C for days (20-68 hours), with reads every 15 minutes while alternately shaking and resting for one minute.

Some of the transmissible spongiform encephalopathies that have been shown to work using RT-QuIC include hamster and

sheep scrapie, chronic wasting disease in deer, Creutzfeldt-Jakob Disease (CJD),¹ and Bovine Spongiform Encephalopathy (BSE).²

The RT-QuIC assay coupled with the Omega series of plate readers is both faster and more sensitive than past bioassays. This means that prion seeding assays can now be measured with a higher throughput using the RT-QuIC assay and BMG LABTECH's Omega microplate readers.

References:

1. Wilham JM, Orrú CD, Bessen RA, Atarashi R, Sano K, Race B, Meade-White KD, Taubner LM, Timmes A, Caughey B. Rapid end-point quantitation of prion seeding activity with sensitivity comparable to bioassays. *PLoS Pathog* 2010; 6:e1001217; PMID:21152012; <http://dx.doi.org/10.1371/journal.ppat.1001217>
2. Atarashi R, Wilham JM, Christensen L, Hughson AG, Moore RA, Johnson LM, Onwubiko HA, Priola SA, Caughey B. Simplified ultrasensitive prion detection by recombinant PrP conversion with shaking. *Nat Methods* 2008; 5:211-2; PMID:18309304; <http://dx.doi.org/10.1038/nmeth0308-211>

P.26: Glycosaminoglycan mimetic and sulfation inhibitor do not prevent the initial uptake of prions but impair the establishment of productive infections

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Transmissible spongiform encephalopathies are caused by prions, unconventional infectious agents predominately composed of misfolded host-encoded prion protein (PrP^{Sc}). PrP^{Sc} is formed by the conformational conversion of the cellular prion protein, PrP^C. Alternative heritable conformations of PrP^{Sc} most likely encipher different prion strains. The precise mechanisms and cellular requirements for PrP^{Sc} uptake, the initial PrP^{Sc} formation and the persistent PrP^{Sc} propagation still remain unknown. Glycosaminoglycans (GAGs), highly-sulfated unbranched polysaccharides, present on the cell surface and within endocytic vesicles, might act as co-factors for prion infection and propagation. So far, comparative analysis of the role of GAGs during the individual stages of infection by different prion strains have not been performed. We examined the effect of the GAG mimetic, DS-500, and the sulfation inhibitor, NaClO₃, on prion infection by scrapie strains RML and 22L in L929 cells and in prion-infected cerebellar brain slices. Neither the treatment with DS-500 nor NaClO₃ inhibited the uptake of RML and 22L PrP^{Sc} by L929 cells, arguing for a sulfated GAG-independent uptake of PrP^{Sc}. Treatment during the early infection stages impaired the establishment of prion infections in cell culture and organotypic cerebellar slice cultures, suggesting that sulfated GAGs are required for the establishment of productive prion infections. Both treatments also reduced PrP^{Sc} levels in persistently infected L929 cells and infected cerebellar brain slices. In conclusion, our

findings suggest that different prion strains depend on sulfated GAGs during acute and chronic infections, but sulfated GAGs are not essential for prion uptake.

P.27: Interaction of PrP and nucleic acids: Comparative effects of DNA and RNA molecules

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Keywords: prion, nucleic acid, DNA, RNA, aggregation, misfolding, toxicity

Prion diseases are triggered when the cellular prion protein (PrP^C) is enriched in β -sheet-rich secondary structure, generating PrP^{Sc}, the main component of the infectious prion particle. The main hypothesis for prion diseases proposes that conversion of PrP^C into PrP^{Sc} occurs without the participation of any other molecule. For decades, it was believed that prion proteins were the only entity necessary to generate misfolding, aggregation and toxicity. However, there are evidences that an adjuvant factor might play a role in PrP^{Sc} formation, lowering the energy barrier between PrP^C and PrP^{Sc}. In this context, nucleic acids have aroused as an interesting group of prion protein ligands. Both DNA and RNA interact with PrP and catalyze the misfolding of the cellular PrP (PrP^C) into a scrapie-like isoform (PrP^{Sc}). Also, it has been observed that interaction of PrP with nucleic acids can be toxic to cultured cells. Here we investigate whether different aggregation, stability, and toxicity effects are detected when non-related DNA and RNA sequences interact with recombinant PrP constructions, using a spectroscopic approach and cell toxicity studies. Our results show that DNA and RNA bind in different regions of murine PrP, inducing secondary structure changes, aggregation and toxicity at different intensities. Composition and size of these molecules can also interfere in these effects. We believe that nucleic acids can be suitable candidates for prion cofactor, facilitating PrP^{Sc} formation.

P.28: Modeling prion species barriers and the new host effect using RT-QuIC

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The propensity for trans-species prion transmission is related to the structural characteristics of the enciphering and heterologous PrP, but the exact mechanism remains mostly mysterious. Studies of the effects of primary or tertiary prion protein

structures on trans-species prion transmission have relied upon animal bioassays, making the influence of prion protein structure vs. host co-factors (e.g. cellular constituents, trafficking, and innate immune interactions) difficult to dissect. As an alternative strategy, we are using real-time quaking-induced conversion (RT-QuIC) to investigate the propensity for and the kinetics of trans-species prion conversion. RT-QuIC has the advantage of providing more defined conditions of seeded conversion to study the specific role of native PrP:PrP^{RES} interactions as a component of the species barrier.

We are comparing chronic wasting disease (CWD) and bovine spongiform encephalopathy (BSE) prions by seeding each prion into its native host recPrP (full-length bovine recPrP, or white tail deer recPrP) vs. into the heterologous species. Upon establishing the characteristics of intra-species and inter-species prion seeding for CWD and BSE prions, we will evaluate the seeding kinetics and cross-species seeding efficiencies of BSE and CWD passaged into a common new host—feline—shown to be a permissive host for both CWD and BSE. We hypothesize that both BSE prions and CWD prions passaged through felines will seed human recPrP more efficiently than BSE or CWD from the original hosts, evidence that the new host will dampen the species barrier between humans and BSE or CWD. The new host effect is particularly relevant as we investigate potential means of trans-species transmission of prion disease.

P.29: A transgenic luciferase-green fluorescence protein reporter to monitor prion promoter activity in vivo and in primary cell culture

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Keywords: colitis, inflammation, PrP^C, bioluminescence, macrophage

Background. The loss or the overexpression of PrP^C has shown to exacerbate or diminish the extent of tissue damage in response to cytotoxic insults. As visualization of gene expression changes could advance the understanding of disease progression in vivo, we hypothesized that transgenic mice having a GFP-luciferase fusion protein under the control of the prion gene promoter would be useful to investigate stimuli and signalling pathways that positively or negatively regulate *Prnp* expression in mice.

Materials and Methods. Using pronuclear microinjection, transgenic mouse lines using the MoPrP.Xho transgene vector backbone, were constructed so to direct the expression of a cDNA encoding an EGFP-luciferase (Luc2) fusion protein. To assess in vitro utility, splenocytes and bone marrow-derived macrophages (BMDMs) from these transgenic mice were isolated and

stimulated with PMA/ionomycin or LPS respectively. To examine the effects of an inflammatory insult in vivo, transgenic mice were treated with 4% dextran sulphate sodium to induce colitis and then prion promoter activity was assessed during different stages of inflammation using bioluminescent imaging (BLI).

Results. Splenic cells activated with PMA and ionomycin, as well as macrophages stimulated with LPS, had increased GFP signal (detected by FACS); the kinetics of which were similar to PrP^C changes in protein expression. We also found that in the DSS model of murine colitis, increased PrP^C expression levels measured by Western blot, mirrored BLI level changes in the *Prnp*-EGFP-Luc2 mice. Of note, the brains from colitic *Prnp*-EGFP-Luc2 mice showed increased luciferase activity.

Conclusion. Activation of macrophages and splenocytes in vitro, as well as in vivo imaging of *Prnp*-EGFP-Luc2 mice following DSS administration, resulted in a dramatic up-regulation of Luc2-dependent photon emission rates likely in response to pro-inflammatory stimuli capable of activating *Prnp* transcription. In addition, as the brains of *Prnp*-EGFP-Luc2 mice showed increased luciferase activity following an inflammatory insult, this suggests that systemic inflammatory mediators released into the blood may increase *Prnp* promoter activity in the CNS.

P.30: Transition between PrP^C and PrP^{Sc} to wild type and mutants studied by normal mode analysis and activated molecular dynamics simulations

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Introduction. The process of formation of amyloid fibrils and their aggregation is not well understood. A possible theory proposes that some proteins, under certain physiological conditions or mutations, are partially or completely denatured triggering these processes. Among the proteins classes that can aggregate are prions. These proteins can coexist in two forms: cellular prion protein (PrP^C) and infectious form PrP^{Sc} scrapie (PrP^{Sc}).

Material and Methods. In this study, we propose a new protocol to study the conformational transition between the PrP^C and its isoform PrP^{Sc} and their mutants using Normal Modes Analysis (NMA), and carrying out Molecular Dynamics simulations (MD) in which the normal mode motions are kinetically activated (ANMMD). This is achieved through a script used with the CHARMM program in which different random linear combinations of low frequency modes are generated, and which are kinetically excited by assigning velocities along them. The normal modes are first calculated for the our model of PrP^C (built by molecular modelling) using the CHARMM program. The MD simulations for PrP^C are the carried out at different temperatures in the normal mode space, and at room temperature in the

Cartesian space, using Generalized Born at constant temperature and constant pressure conditions, at acidic and neutral pH. A series of simulations with different excitation energies in the normal mode space were carried out and statistically analyzed.

Results. The results showed an increase of the length of β -sheet which corresponds to the conformational transition between the PrP^C and PrP^{Sc}, and the results of acidic pH were better than neutral pH. The D178N mutant showed many number of structures with large β -sheet.

Conclusion. The obtained structures may be the intermediate states of PrP^{Sc}. These results showed that the simulation method that we developed could be used for studying such conformational changes very efficiently.

P.31: Over-expression of PrP^C prevents the development of ileitis in the $TNF^{\Delta ARE/+}$ murine model of Crohn's disease

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Keywords: colitis, Crohn's disease, inflammation, TNF, PrP^C

Background. The prion is best known for its central role in the pathogenesis of the transmissible spongiform encephalopathies (TSEs), a set of inevitably lethal neurodegenerative diseases that affect humans and a variety of animals. The inflammatory bowel diseases (IBD), comprised of both Crohn's disease and ulcerative colitis, are chronic and relapsing inflammatory disorders of the small intestine and colon that affect millions of people worldwide. In this regard, previously we found that the expression level of PrP^C regulates the severity of experimental colitis in mice. TNF α appears to be an important mediator in the pathogenesis of idiopathic IBD, for example, anti-TNF α antibody therapy often results in the induction of clinical remission in treated patients. As we previously found that the level of PrP^C expression modulated the severity of colitis, we hypothesized that the level of PrP^C expression would also exert modulatory effects in the TNF α -driven model of ileitis seen in the $TNF^{\Delta ARE/+}$ transgenic mice.

Materials and Methods. $TNF^{\Delta ARE/+}$ mice, having a deletion in a region within the AU-rich element (ARE) of the TNF gene that stabilizes TNF α mRNA, have systemic TNF overproduction and the development of spontaneous chronic inflammation localized to the terminal ileum and synovial joints. We crossed these $TNF^{\Delta ARE/+}$ mice either with (i) hemizygous Tga20 mice that

have approximately 7-fold over-expression of PrP^C or (ii) with $Prnp^{-/-}$ mice. All mice were on a C57BL/6 background. Mice were weighed weekly, then at 12 weeks of age, ileal tissue was harvested and analyzed for histological damage and cytokine changes. As these mice develop arthritis between 20-24 weeks of age, joint regions within the ankle were analyzed by micro-computed tomography and histology.

Results. Both gross and histological ileal damage was markedly reduced in $TNF^{\Delta ARE/+}; Tga20$ mice, whereas damage was exacerbated in the $TNF^{\Delta ARE/+}; Prnp^{-/-}$ mice. In addition, several cytokines were increased in the sera of $Prnp^{-/-}; TNF^{\Delta ARE/+}$ mice. Interestingly, PrP^C over-expression did not appear to prevent the development of inflammatory arthritis in the $TNF^{\Delta ARE/+}$ model.

Conclusion. Herein we report that PrP^C over-expression greatly attenuated the severity of ileitis in $TNF^{\Delta ARE}$ mice, while mice lacking the prion gene exhibited increased disease severity compared to wild-type controls.

P.32: The extent of protease resistance of misfolded prion protein is highly dependent on the salt concentration

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Keywords: prion, proteinase K resistance, misfolding

Transmissible spongiform encephalopathies are neurodegenerative diseases caused by prions in mammals. An aberrant folded form (PrP^{Sc}) of the cellular nonpathogenic PrP protein (PrP^C) is the main component of these proteinaceous infectious particles. PrP^{Sc} is able to confer its structural conformation to PrP^C, and with it, its pathogenic and biochemical properties. Prions exhibit strong resistance to protease digestion, a property that is typically used for biochemical discrimination from PrP^C. This classical feature has been partially challenged by the isolation of sizeable amounts of protease-sensitive PrP^{Sc} isoforms that self-propagate in vivo.

Here, we report that the degree of PrP^{Sc} protease resistance is highly dependent on the concentration of salt in the solution. Similar changes were observed in PrP^{Sc} obtained from different strains and species. Strikingly, the effect of salt on protease resistance is reversible and is associated with changes on the size of PrP^{Sc} aggregates, but surprisingly, the more protease-sensitive species consists of a larger size. These findings shed light on the mechanistic aspects of prion proteolysis resistance and should be

considered when assessing samples of biomedical relevance or purification procedures.

P.33: In vitro isolation of a defective prion mutant from a natural prion isolate

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Vole brain homogenates represent an ultra-efficient substrate for homologous and heterologous PMCA replication.¹ While studying the sensitivity of detection of sheep scrapie by vole PMCA (vPMCA), we recovered a PK-resistant PrP^{Sc} core (PrP^{res}) similar to that of the sheep inoculum from all isolates but one, which repeatedly resulted in a deviant PrP^{res} phenotype only when seeded at very high dilutions (10⁻⁵-to-10⁻⁷). We thus studied the biochemical and biological features of vPMCA-derived faithful (named 18K) and deviant (named 14K) PrP^{Sc} types by vPMCA, biochemical techniques and bioassay in voles.

We selected 2 independently derived deviant PrP^{Sc} populations, 14K/1 and 14K/2, along with 18K, all originating from the same sheep isolate (SS21). When subjected to further vPMCA rounds 14K/2 and 18K were faithfully reproduced, while 14K/1 gradually shifted to a 18K phenotype. By end dilution experiments, we determined that 14K/1 initially contained very low levels of 18K, while a "pure" 14K population was derived from 14K/2 and named 14K/3. The amplification rates of 18K and 14K/3 were 10⁶ and 10⁴, respectively, after a single PMCA round. Biochemical analyses showed that the deviant 14K/3 was conformationally stable and characterized by C-terminal PrP^{res} spanning a.a. ~155-232. In contrast, 18K had a PrP^{res} similar to the starting sheep inoculum (a.a. ~80-232), but shifted to a lower conformational stability. By vole bioassay, in vitro vole-adapted 18K and 14K/1 were highly infectious and converged to the same vole-adapted strain as sheep SS21, implying that in vitro adaptation and strain preservation had occurred. In contrast, 14K/3 did not transmit to voles, although deriving from an homologous PrP sequence. Prompted by this unexpected finding, we looked for 14K seeding activity in brains of i.c. inoculated voles at different time points and found that 18K and 14K PrP^{Sc} types had similar kinetics of clearance. However, no converting activity was detected in 14K-inoculated voles from 14 d.p.i. until old age, implying that 14K/3 was unable to replicate in vivo at levels sufficient to self-sustain.

In conclusion, we derived a mutant PrP^{Sc} from natural scrapie, which was autocatalytic in vitro but unable to replicate in

vivo. The emergence of the defective mutant from highly diluted seeds, i.e. through bottleneck passages, is consistent with the hypothesis that prions are subject to mutation and selection² and behave as quasispecies.³ Finally, the isolation of a defective prion provides new insight for dissecting the pathogenetic steps which differentiate autocatalytic PrP^{Sc} amplification from in vivo prion replication.

References

1. Cosseddu GM, Nonno R, Vaccari G, Bucalossi C, Fernandez-Borges N, Di Bari MA, Castilla J, Agrimi U. Ultra-efficient PrP(Sc) amplification highlights potentialities and pitfalls of PMCA technology. PLoS Pathog 2011; 7:e1002370; PMID:22114554; <http://dx.doi.org/10.1371/journal.ppat.1002370>
2. Li J, Browning S, Mahal SP, Oelschlegel AM, Weissmann C. Darwinian evolution of prions in cell culture. Science 2010; 327:869-72; PMID:20044542; <http://dx.doi.org/10.1126/science.1183218>
3. Domingo E, Sheldon J, Perales C. Viral quasispecies evolution. Microbiol Mol Biol Rev 2012; 76:159-216; PMID:22688811; <http://dx.doi.org/10.1128/MMBR.05023-11>

P.34: A new anti-prion strategy: modulation of Prnp transcriptional activity

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Keywords: novel anti-prion strategy, novel transgenic reporter mouse, Prnp-EGFP-Luc2 transgenic mouse, high-throughput screening, chemical compound library

Prions diseases are infectious fatal neurodegenerative disorders with no treatments or cures. The diseases occur when the normal cellular prion protein (PrP^C) converts into the infectious isoform PrP^{Sc}. It is our goal to develop anti-prion treatments by disrupting the prion conversion process. Interfering with PrP^C expression has no apparent side effects and genetically modified knockout animals are immune to the diseases. Therefore, we hypothesize that compounds that reduce PrP^C expression can be used as therapeutic or prophylactic treatments against prion diseases. We will develop targeted and high-throughput screens (HTS) of natural and chemical compounds using cells from a novel reporter mouse line. This line has been engineered to express GFP and luciferase under control of the *Prnp* promoter (Prnp-EGFP-Luc2). This novel strategy has the advantages of (1) no major side effects, since PrP^{0/0} mice do not exhibit a pronounced phenotype, (2) this should be effective against different prion strains, and (3) we can screen uninfected cells and animals. We will test luciferase expression of macrophages and primary neurons treated with growth factors and cytokines we have previously shown to alter PrP^C expression. We will develop a HTS using the INCell2000 imaging system to examine EGFP fluorescence in primary neurons and astrocytes treated with our in-house libraries of about 7000 compounds. Compounds causing a >2-fold decrease in

Prnp expression will be evaluated in vivo by administering candidates to transgenic mice, then examining PrP^C expression in brain homogenates and spleen using immunoblot and qRT-PCR. Lastly, we will test candidate compounds on prion infected cell cultures to validate our hypothesis. We expect to identify novel candidates useful for treating prion diseases, gain insight into pathways controlling *Prnp* in various cell types and in vivo, and the research may have relevance to Alzheimer's disease since PrP^C has been shown to transduce A β toxicity.

P.35: Mutant PrP sensitizes neurons to excitotoxicity by impairing membrane delivery and function of glutamate receptors

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Genetic prion diseases are fatal neurodegenerative disorders linked to mutations in the gene encoding the cellular prion protein (PrP^C). PrP^C mutations favor the conformational conversion of the protein into a pathogenic misfolded isoform that kills neurons through an unknown mechanism.

We have recently found that mutant PrP^C is retained in the endoplasmic reticulum where it interacts with the $\alpha_2\delta$ -1 subunit of voltage-gated calcium channels.¹ This impairs the correct delivery of the channel complex to the cell surface, impacting synaptic transmission. Based on this observation, we hypothesized that mutant PrP^C might alter the secretory trafficking of other PrP^C-interacting proteins essential for synaptic function, such as AMPA and NMDA glutamate receptors. For this purpose, we assessed whether expression of mutant PrP altered localization and function of AMPARs and NMDARs, thus disrupting synaptic transmission and leading to enhanced susceptibility to excitotoxic cell death.

We found that mutant PrP selectively interacts with AMPAR GluR2 subunit and sequesters it in intracellular compartment, resulting in surface exposure of GluR2-lacking AMPARs, which have increased calcium permeability and altered functional properties. Furthermore, we observed that mutant PrP increases neuronal susceptibility to AMPA toxicity. Our data indicate that impairment of secretory protein trafficking may be a major cause of synaptic dysfunction and neurodegeneration in genetic prion diseases.

References

1. Senatore A, Colleoni S, Verderio C, Restelli E, Morini R, Condliffe SB, Bertani I, Mantovani S, Canovi M, Micotti E, et al. Mutant PrP suppresses glutamatergic neurotransmission in cerebellar granule neurons by impairing membrane delivery of VGCC $\alpha(2)\delta$ -1 Subunit. *Neuron* 2012; 74:300-13; PMID:22542184; <http://dx.doi.org/10.1016/j.neuron.2012.02.027>

P.36: Supramolecular complexes of natural humic substances and prion proteins investigated by solution-state NMR studies: environmental implications

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Scrapie in sheep and chronic wasting disease in cervids can be horizontally transmitted through environmental routes. Increasing evidence suggests that soil may serve as a natural reservoir of prion infectivity and that transmission of prions through infected soils is largely facilitated during grazing, when ruminants routinely ingest or inhale soil particles. Organic matter, including humic substances (HS), is mainly present in the soil surface layer, which therefore constitutes the early site where prion contamination is likely to occur after PrP^{Sc} shedding from diseased animals. The potential of HS in affecting prion replication and infectivity has not been fully clarified. Interaction of prions with HS might strongly impact their stability in the environment, possibly affecting also their availability and infectivity in grazing lands. This study, financed by the Italian Ministry of Agricultural, Food and Forestry Policies (SCRASU Project), reports the results of NMR spectroscopy investigation of the direct interaction between recombinant murine PrP^C (MoPrP, here used as surrogate for PrP^{Sc}) and HS (humic and fulvic acids) of different origins. We found that HS formed insoluble adducts with MoPrP. The remaining protein in solution appeared in its native folding. NMR analysis revealed that the interaction between HS and MoPrP mainly occurs through the N-terminal region located between residues 89 and 125. PrP^{Sc} interaction with HS may be environmentally relevant in soils. We conclude that prions are likely to be strongly retained in soil organic matter, thus reducing the odds of infectivity in grazing lands.

P.37: Solution-state NMR studies on human prion protein mutants

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The post-translational conversion of the ubiquitously expressed cellular form of the prion protein, PrP^C, into its misfolded and pathogenic isoform, known as prion or PrP^{Sc}, plays a key role in prion diseases. These maladies are denoted transmissible spongiform encephalopathies (TSEs) and affect both humans and animals. A prerequisite for understanding TSEs is unraveling the molecular mechanism leading to the conversion process whereby most α -helical motifs are replaced by β -sheet secondary structures. Importantly, most point mutations linked to inherited prion diseases are clustered in the C-terminal domain region of PrP^C and cause spontaneous conversion to PrP^{Sc}. Structural studies with PrP variants promise new clues regarding the proposed conversion mechanism and may help identify “hot spots” in PrP^C involved in the pathogenic conversion. These investigations may also shed light on the early structural rearrangements occurring in some PrP^C epitopes thought to be involved in modulating prion susceptibility. Here we present a detailed overview of our solution-state NMR studies on human prion protein carrying different pathological point mutations and the implications that such findings may have for the future of prion research.

P.38: Identification of small molecule ligands for the cellular prion protein

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Keywords: prion, in silico modeling, virtual screening, PrP^C ligands

Background. Prion diseases are inevitably fatal neurodegenerative disorders linked to the misfolding of the cellular prion protein (PrP^C), a cell-surface glycoprotein of uncertain function, into an aggregated form (prion or PrP^{Sc}) that self-propagates by imposing its abnormal conformation onto PrP^C molecules.¹ Several previous drug discovery approaches have been focused on blocking prion replication by targeting PrP^{Sc}. However, emerging evidence suggest that PrP^{Sc} may not be the primary pathogenic form in prion diseases. Instead, PrP^C has been shown to play a dual role in the pathogenesis of prion diseases by serving as a

substrate for PrP^{Sc} propagation, and by acting as a transducer of neurotoxicity. Thus, an alternative strategy to prevent prion diseases could be to target PrP^C, with the dual objective of inhibiting its misfolding and blocking its potential transducing activity.^{2,3,4}

Material and Methods. We employed molecular modeling and virtual screening to identify novel, small ligands for PrP^C. Multiple NMR conformations of the C-terminal domain of murine PrP^C were used as input to search for potential binding sites for small molecules. The highest ranking and most frequently recurrent pocket across all conformations was selected as the target site for a novel virtual screening paradigm which used molecular dynamics calculation to rank the hit molecules.

Results. We identified several new putative ligands of PrP^C, with the aim to confer thermodynamic stability to the globular domain of the protein. The interaction of some of these compounds with PrP^C was then validated in vitro by biophysical techniques.

Conclusions. We describe a novel in silico workflow to identify ligands for PrP^C. Our data support the notion that PrP^C is a druggable target, and lay the groundwork for the rational design of novel PrP^C-directed, anti-prion compounds.

References

1. Colby DW, Prusiner SB. Prions. *Cold Spring Harb Perspect Biol* 2011; 3:a006833; PMID: 21421910; <http://dx.doi.org/10.1101/cshperspect.a006833>
2. Nicoll AJ, Collinge J. Preventing prion pathogenicity by targeting the cellular prion protein. *Infect Disord Drug Targets* 2009; 9:48-57; PMID:19200015; <http://dx.doi.org/10.2174/1871526510909010048>
3. Biasini E, Harris DA. Targeting the cellular prion protein to treat neurodegeneration. *Future. Med Chem* 2012; 4:1655-8; PMID: 22924502; <http://dx.doi.org/10.4155/fmc.12.114>
4. Kuwata K, Nishida N, Matsumoto T, Kamatari YO, Hosokawa-Muto J, Kodama K, Nakamura HK, Kimura K, Kawasaki M, Takakura Y, et al. Hot spots in prion protein for pathogenic conversion. *Proc Natl Acad Sci U S A* 2007; 104:11921-6; PMID:17616582; <http://dx.doi.org/10.1073/pnas.0702671104>

P.39: Analysis of mechanism for PrP^{Sc}-specific detection by anti-PrP monoclonal antibody mAb132

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The monoclonal antibody (mAb) 132 recognizing mouse PrP amino acid 119-127 facilitates PrP^{Sc}-specific detection from cells or frozen tissue sections by immunofluorescence assay even though treatment of fixed cells or tissue sections with chaotropic agents such as guanidinium salts is prerequisite. Despite the benefit of this mAb, the mechanism of the PrP^{Sc}-specific detection is still unclear. Therefore, we produced mono- and bi-valent recombinant mAb132 (rFab132 and rIgG132) to analyze the mechanism of PrP^{Sc}-specific detection by mAb132. We also produced rFab132 and rIgG132 conjugated with enhanced green fluorescent protein (EGFP) that enable a direct detection of PrP^{Sc}. The reactivity of recombinant antibodies produced in HEK293F cells was assessed by ELISA using recombinant mouse PrP (rMoPrP)

as an antigen. Compared to parental mAb, both of bivalent rIgG132 and rIgG132-EGFP showed similar reactivity to rMoPrP in density dependent manner. However, the facts that binding of rFab132 to rMoPrP was less efficient than rIgG132 and more rFab-EGFP molecules than rIgG132-EGFP were required to detect PrP^{Sc} in prion-infected cells, suggesting the bivalent form conferred a higher binding avidity. To analyze the effect of valency of antibody or density of antigen more detail, we measured the binding kinetics of rFab132 and mAb132 to rMoPrP by surface plasmon resonance (SPR). The mAb132 showed the lower dissociation constant (K_D) than rFab132 by two orders of magnitude. This indicates that the bivalent binding to the antigen confers a higher avidity to mAb132. Interestingly, mAb132 showed the similar K_D value similar to pan-PrP mAb31C6 in binding to immobilized rMoPrP; however, the association rate constants (k_a) of mAb132 was ten-fold lower than that of mAb31C6, suggested that mAb132 requires more antigen than does mAb31C6 in binding. The k_a value decreased to ten-fold if mAb132 attached on the surface of sensor chip as ligand and rMoPrP used as analyte, suggesting that the reactivity of mAb132 was also affected by conformation of the epitope. Taken together, the mechanism of PrP^{Sc}-specific detection by mAb132 may be explained by the combined effect of (i) enhancement of binding affinity in bivalent binding, (ii) requirement of a higher antigen density in efficient binding, and (iii) conformation of epitope.

P.40: Prion protein signaling and trafficking cell model

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Keywords: cell model, prion protein knockout, signaling, CAD cells, CRISPR

Prion diseases are fatal, neurodegenerative disorders and are characterized by the accumulation of an abnormally folded isoform of the cellular prion protein (PrP^C), denoted PrP^{Sc}, which represents the major component of infectious scrapie prions. Understanding both the pathogenesis of TSEs and physiology of cellular prion protein require the use of knock-out model systems. In mice there are several PrP knock-out and PrP transgenic strains available.¹ Nevertheless there is still a need in a neuronal cell line model in which we can regulate PrP^C expression.

We decided to edit the genome of CAD (cath.a differentiated) cells.² CAD are a variant of the central nervous system catecholaminergic line cath.a. CAD cells endogenously express high level of PrP^C. Their infectibility with RML and ME7 has been reported previously.³

In order to create PrP knock-out CAD cells we applied CRISPR technology.⁴ We have inserted gentamicin resistance

cassette in the first exon of PrP and then selected a clone with both PrP alleles disrupted. Then we reintroduced PrP^C under regulated promoter. Here we characterize PrP^C signaling and trafficking in genome edited CAD cells compare to CAD wt and to primary neurons and astrocytes.

References

1. Weissmann C, Flechsig E. PrP knock-out and PrP transgenic mice in prion research. *Br Med Bull* 2003; 66:43-60; PMID:14522848; <http://dx.doi.org/10.1093/bmb/66.1.43>
2. Qi Y, Wang JK, McMillan M, Chikaraishi DM. Characterization of a CNS cell line, CAD, in which morphological differentiation is initiated by serum deprivation. *J Neurosci* 1997; 17:1217-25; PMID:9006967
3. Mahal SP, Baker CA, Demczyk CA, Smith EW, Julius C, Weissmann C. Prion strain discrimination in cell culture: the cell panel assay. *Proc Natl Acad Sci U S A* 2007; 104:20908-13; PMID:18077360; <http://dx.doi.org/10.1073/pnas.0710054104>
4. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* 2012; 337:816-21; PMID:22745249; <http://dx.doi.org/10.1126/science.1225829>

P.41: Rescuing the drug hypersensitivity phenotype of PrPΔ105-125 by the co-expression of the Shadoo protein

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Keywords: PrPΔ105-125, Shadoo, DBCA, Zeocin, neurotoxicity

Prion protein is famous due to its involvement in transmissible spongiform encephalopathies. Prion protein constructs with deletions in their N-terminal flexible domains involving the Central Region (Δ105-125) have a neurotoxic phenotype when expressed in PrP knockout transgenic mice. However, when wild type PrP is co-expressed, this neurotoxic phenotype is rescued in a dose dependent manner. Expressing PrPΔ105-125, or other N-terminally truncated PrP constructs in mammalian cell lines was shown to cause hypersensitivity to certain antibiotics. This drug hypersensitive phenotype can be also rescued by co-expression of wild type PrP.

The Shadow of Prion protein, or Shadoo is the newest member of the prion protein family. The physiological function of this protein is not really understood, but various models suggest that Shadoo may have overlapping functions with the prion protein. Like wild type PrP it also ameliorates the excitotoxicity caused by glutamate, in cultured cells. Furthermore, it rescues cells from spontaneous cell death caused by a toxic deletion mutant of PrP in transiently transfected primary cell cultures as well as in a neuroblastoma cell line.

Here we examined if the hypersensitivity to Zeocin caused by PrPΔ105-125 could also be rescued by the Shadoo protein.

P.42: Deletion of the C-terminal part of helix alpha 2 does not prevent the prion conversion

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The mechanistic insights of prion conversion remain highly controversial. Several regions of PrP have been postulated as critical in this process; however, few attempts have been done to determine to what extent we can modify PrP sequences maintaining its properties. Recently, our laboratory progressed on these issues by showing that a PrP with eight extra amino acids inserted in the middle of the helix-2-helix-3 domain remains convertible into prion. These insertion mutants displayed no significant loss of the alpha helix content suggesting a replacement by the amino acids inserted. To determine whether a full size of H2 is required for prion conversion we performed series of deletions by site-directed mutagenesis in the ovine PrP sequence (VRQ haplotype). Mutants of PrP were then stably transfected in RK13 cells where protein expression and distribution of the mutants were monitored. The secondary structure of the recombinant proteins with similar deletions was analyzed by circular dichroism, showing no significant differences. The efficiency of infection was then tested through determination of the amount of protease resistant PrP accumulated in cells several passages after infection. Using 3 different prion strains for infection, we demonstrated that deletion of two turns of the H2 end does not prevent prion conversion indicating that this portion of the protein is not critically involved in the conformational change. We also probed that the susceptibility to infection was not altered in the mutants when compared to the wild type protein. The cells did propagate bona fide prions, infectious not only for naive homologous cell cultures but also for wt-expressing cells and for tg338 mice expressing the ovine PrP. This work demonstrates that the end of H2 is dispensable for prions and extends our previous conclusions that the amino acid specificity of this region was not required for the conversion.

To our best knowledge, most deletions in the protease-resistant domain that were reported so far remained unconvertible into prions with the exception of PrP106 produced by two large deletions in the first moiety of PrP^C. Our work demonstrates the possibility to produce prions with short internal deletions inside the protease-resistant core.

P.43: Cloning the prion and the Shadoo proteins: Restriction enzyme Body Doubles and PCR cloning

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Here we demonstrate a procedure that solves a frequent problem in genetic engineering experiments that we faced working with the cDNA of the Prion and Shadoo proteins. This procedure allows the cloning of PCR fragments containing a recognition site of the restriction endonuclease (Type IIP) used for cloning in the sequence of the insert. A Type IIS endonuclease - a Body Double of the Type IIP enzyme - is used to generate the same protruding palindrome. Thus, the insert can be cloned to the Type IIP site of the vector without digesting the PCR product with the same Type IIP enzyme. We achieve this by incorporating the recognition site of a Type IIS restriction enzyme that cleaves the DNA outside of its recognition site in the PCR primer in such a way that the cutting positions straddle the desired overhang sequence. Digestion of the PCR product by the Body Double generates the required overhang. Hitherto the use of Type IIS restriction enzymes in cloning reactions has only been used for special applications, the approach presented here makes Type IIS enzymes as useful as Type IIP enzymes for general cloning purposes. To assist in finding Body Double enzymes, we summarised the available Type IIS enzymes which are potentially useful for Body Double cloning and created an online program (http://group.szbk.u-szeged.hu/welkergr/body_double/index.html) for the selection of suitable Body Double enzymes and the design of the appropriate primers. We routinely use this procedure to bypass this frequently arising problem.

P.44: Conformational properties of prion strains can be transmitted to recombinant prion protein fibrils in real-time quaking-induced conversion

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The phenomenon of prion strains with distinct biological characteristics has been hypothesized to be involved in the diverse structures of abnormal prion protein (PrP^{Sc}). However, the molecular basis of the strain diversity, including how to transmit the strain properties, remains uncertain. Real-time quaking-induced conversion (RT-QUIC) is a cell-free system that uses *E. coli*-derived recombinant PrP (rPrP) for the sensitive detection of PrP^{Sc}. To investigate whether properties of various prion strains can be transmitted to amyloid fibrils consisting of

rPrP (rPrP-fibrils) using RT-QUIC, we examined the secondary structure, conformational stability and infectivity of rPrP-fibrils seeded with PrP^{Sc} derived from either the Chandler or 22L strain. In the first round of the reaction, there were differences in the secondary structures, especially in bands attributed to β -sheets, as determined by infrared spectroscopy, and conformational stability between Chandler-seeded (1st-rPrP-fib^{Ch}) and 22L-seeded rPrP-fibrils (1st-rPrP-fib^{22L}). Of note, specific identifying characteristics of the two rPrP-fibrils seen in the β -sheets resembled those of the original PrP^{Sc}. Furthermore, the conformational stability of 1st-rPrP-fib^{Ch} was significantly higher than that of 1st-rPrP-fib^{22L}, as with Chandler- and 22L-PrP^{Sc}. The survival periods in mice inoculated with 1st-rPrP-fib^{Ch} or 1st-rPrP-fib^{22L} were significantly shorter than those of seed-only control mice. In contrast, these biochemical characteristics were disappeared in subsequent rounds, suggesting that nonspecific rPrP-fibrils without prion infectivity became predominance probably because of their rapid growth rate. Together, these findings show that some strain-specific conformational properties can be transmitted to rPrP-fibrils and unknown cofactors or environmental conditions in the RT-QUIC may be required for further conservation.

P.45: Designing small molecule ligands for the cellular prion protein

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Keywords: prion diseases, PrP^C, PrP^{Sc}, pharmacological chaperones

Background. Prion diseases are rare neurodegenerative disorders characterized by dementia, motor dysfunction, and cerebral amyloidosis. The key pathogenic event underlying all forms of prion diseases is the conversion of the cellular prion protein (PrP^C) into an aggregated form (PrP^{Sc}) that self-propagates by imposing its abnormal conformation onto PrP^C molecules.¹ PrP^{Sc} has represented the main therapeutic target in several pharmacological approaches for prion diseases. However, such attempts have generally been unrewarding, and emerging evidence suggests that preventing PrP^C monomers from undergoing misfolding would be a more promising avenue.^{2,3} Unfortunately, with very few exceptions, this strategy has so far remained unexplored.^{4,5} Here, we report the identification of small ligands for PrP^C that block its aggregation by stabilizing the native fold of the protein.

Material and Methods. We combined *in silico* techniques of binding site detection, virtual screening and molecular dynamics with several biochemical and biophysical assays, to identify small molecules that bind tightly to PrP^C. We then tested the ability of these compounds to prevent PrP^C misfolding by stabilizing its native conformation.

Results. We report the identification and characterization of several small molecules that bind PrP^C with low- or sub-micromolar affinity. Computational, biochemical and biophysical analyses reveal that some of these ligands exert a robust stabilization effect on PrP^C folding. Importantly, these compounds efficiently inhibit PrP^{Sc} replication in cell cultures.

Conclusions. We describe a novel experimental paradigm to identify high affinity ligands for PrP^C. Our data provide strong biological evidence that prion propagation can be counteracted by stabilizing the native conformation of PrP^C with small ligands. Our compounds represent a new generation of pharmacological chaperones for PrP^C, potentially active against prion diseases.

References

- Colby DW, Prusiner SB. Prions. *Cold Spring Harb Perspect Biol* 2011; 3:a006833; PMID: 21421910; <http://dx.doi.org/10.1101/cshperspect.a006833>
- Nicoll AJ, Collinge J. Preventing prion pathogenicity by targeting the cellular prion protein. *Infect Disord Drug Targets* 2009; 9:48-57; PMID:19200015; <http://dx.doi.org/10.2174/1871526510909010048>
- Biasini E, Harris DA. Targeting the cellular prion protein to treat neurodegeneration. *Future. Med Chem* 2012; 4:1655-8; PMID: 22924502; <http://dx.doi.org/10.4155/fmc.12.114>
- Nicoll AJ, Trevitt CR, Tattum MH, Risse E, Quartermann E, Ibarra AA, Wright C, Jackson GS, Sessions RB, Farrow M, et al. Pharmacological chaperone for the structured domain of human prion protein. *Proc Natl Acad Sci U S A* 2010; 107:17610-5; PMID:20876144; <http://dx.doi.org/10.1073/pnas.1009062107>
- Kuwata K, Nishida N, Matsumoto T, Kamatari YO, Hosokawa-Muto J, Kodama K, Nakamura HK, Kimura K, Kawasaki M, Takakura Y, et al. Hot spots in prion protein for pathogenic conversion. *Proc Natl Acad Sci U S A* 2007; 104:11921-6; PMID:17616582; <http://dx.doi.org/10.1073/pnas.0702671104>

P.46: PrP^C negatively controls neuronal GSK3 β and serotonin 1B receptor via a caveolin-Lyn complex

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Despite much recent progress, our knowledge of the physiological role of PrP^C is still far from complete. Previously, taking advantage of 1C11^{5HT} serotonergic neuronal cells, we documented that PrP^C can instruct signal transduction events.¹ We identified a neurospecific PrP^C-dependent signaling cascade, which requires the association of PrP^C, the transmembrane protein caveolin1 and the Fyn tyrosine kinase within a signaling platform, selectively implemented on the neurites of differentiated cells.

Here, we report that GSK3 β , a multifunctional kinase whose inhibition is neuroprotective, is a downstream effector of PrP^C in 1C11^{5HT} neuronal cells. This coupling, which was evidenced by antibody-mediated PrP^C ligation, is induced by the PrP^C endogenous ligand STI-1, known to trigger neuroprotection.² Our

data show that PrP^Cinduced GSK3 β inactivation is independent from Fyn and, instead, is relayed by the Lyn tyrosine kinase, another member of the Src kinase family, via caveolin-1. Our cell fractionation experiments further indicate that this cascade is restricted to the cell bodies of 1C11^{5HT} neurons, supporting the idea that PrP^C signalling complexes are subject to tight spatial control. Besides, we provide evidence that the coupling of PrP^C to GSK3 β potentiates serotonergic signalling by altering the subcellular distribution and the activity of the serotonin 1B receptor, an autoreceptor known to limit serotonin release.³

Finally, the in vivo relevance of the observations obtained with 1C11^{5HT} neuronal cells is substantiated by increased activity of GSK3 β kinase in the brains of PrP-deficient mice, as well as sustained serotonin 1B receptor activity. As a whole, our data unveil a new facet of PrP^C signalling that strengthens neurotransmission.

References

1. Mouillet-Richard S, Ermonval M, Chebassier C, Laplanche JL, Lehmann S, Launay JM, Kellermann O. Signal transduction through prion protein. *Science* 2000; 289:1925-8; PMID:10988071; <http://dx.doi.org/10.1126/science.289.5486.1925>
2. Zanata SM, Lopes MH, Mercadante AF, Hajj GN, Chiarini LB, Nomizo R, Freitas AR, Cabral AL, Lee KS, Juliano MA, et al. Stress-inducible protein 1 is a cell surface ligand for cellular prion that triggers neuroprotection. *EMBO J* 2002; 21:3307-16; PMID:12093732; <http://dx.doi.org/10.1093/emboj/cdf325>
3. Sari Y. Serotonin1B receptors: from protein to physiological function and behavior. *Neurosci Biobehav Rev* 2004; 28:565-82; PMID: 15527863; <http://dx.doi.org/10.1016/j.neubiorev.2004.08.008>

P.47: Logical design of medical chaperone for prion diseases

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Keywords: logical drug design, medical chaperone, quantum chemistry, NMR

We established a logical design strategy of the medical chaperone for prion diseases.^{1,2} Medical chaperone is defined as a material which interacts with the target assisting its physiological function or stabilizing its functional structure. Native prion protein has a characteristic major groove on its surface, which fluctuates slowly on a timescale of milliseconds called 'hot spot'.¹ In contrast to the enzyme inhibitor, the medical chaperone stabilizes the PrP^C conformation along the pathway from PrP^C to PrP^{Sc} (Class I).³ Some anti-prion compounds, such as quinacrine, nonspecifically bind with PrP^C (Class II).³ We experimentally

confirmed many strong binding compounds, such as Congo red or pentosan polysulfate (PPS), reduced the solubility of PrP and actually precipitate it, thereby reducing the effective concentration of PrP^C involved in the conversion process (Class III).³

Logical design of medical chaperone is feasible when the following conditions are fulfilled. 1. Localizability of the instability, which triggers the misfolding of the whole molecule. If unstable regions are diffusely distributed all over the protein, it might be hard to regulate its stability. 2. Regulability of the hot spot by a small compound. Reaction pathway must be regulated by the medical chaperone in terms of the static structure as well as its dynamics. Thus unlike enzyme inhibitors, the simple discussion on K_D cannot be applied. Interactions between structural and dynamical variables in the non-commutative geometrical space must be properly treated.⁴ 3. Synthesizability of the computationally designed compounds. Quantum chemical calculation generates the covalent bonds for small compound under the boundary conditions of the protein surface curvature.⁵ But there is no guarantee of the existence of the synthesis root for the designed compounds.⁶ 4. Specificity of the compounds once administered to the human. IC₅₀ is not essential for medical chaperone because compounds designed ab initio according to the prion surface geometry have less toxicity in general and their pharmacological effects must be discussed in relation to their toxicity⁷. We will also discuss further progress of medical chaperone in the pre-clinical examination under the ICH regulations.

References

1. Kuwata K, Nishida N, Matsumoto T, Kamatari YO, Hosokawa-Muto J, Kodama K, Nakamura HK, Kimura K, Kawasaki M, Takakura Y, et al. Hot spots in prion protein for pathogenic conversion. *Proc Natl Acad Sci U S A* 2007; 104:11921-6; PMID:17616582; <http://dx.doi.org/10.1073/pnas.0702671104>
2. Kuwata K. Logical design of medical chaperone for prion diseases. *Curr Top Med Chem* 2013; 13:2432-40; PMID:24059338; <http://dx.doi.org/10.2174/15680266113136660171>
3. Kamatari YO, Hayano Y, Yamaguchi K, Hosokawa-Muto J, Kuwata K. Characterizing antiprion compounds based on their binding properties to prion proteins: implications as medical chaperones. *Prot Sci* 2013; 22-34; PMID: 23081827; <http://dx.doi.org/10.1002/pro.2180>
4. Kuwata K. [Non-commutative geometrical drug discovery--the principle of geometrical regulation]. *Yakugaku Zasshi* 2012; 132:873-9; PMID:22864344; <http://dx.doi.org/10.1248/yakushi.132.873>
5. Ishikawa T, Ishikura T, Kuwata K. Theoretical study of the prion protein based on the fragment molecular orbital method. *J Comp Chem* 2009; 2594-601; PMID: 19408278; <http://dx.doi.org/>
6. Kimura T, Hosokawa-Muto J, Asami K, Murai T, Kuwata K. Synthesis of 9-substituted 2,3,4,9-tetrahydro-1H-carbazole derivatives and evaluation of their anti-prion activity in TSE-infected cells. *Eur J Med Chem* 2011 46:5675-9; PMID: 21906853; <http://dx.doi.org/10.1016/j.ejmech.2011.08.039>
7. Hosokawa-Muto J, Kimura T, Kuwata K. Respiratory and cardiovascular toxicity studies of a novel antiprion compound, GN8, in rats and dogs. *Drug Chem Toxicol* 2012; 35:264-71; PMID:21999415; <http://dx.doi.org/10.3109/01480545.2011.598533>

P.48: Abeta dimers are sufficient to cause cognitive impairments in the absence of plaques

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Keywords: Alzheimer, Abeta, dimer, mouse model, disulfide engineering

A hallmark of neurodegenerative diseases is the deposition of microscopic intra- or extracellular aggregated proteins, which also play a key role in causing these conditions. Beyond these highly structured aggregates, recent evidence points to low-n oligomeric transition states of the corresponding proteins as playing major roles in synapto- and neurotoxicity. In Alzheimer's disease, the dimeric assembly of the Abeta peptide, a cleavage product of the amyloid precursor protein (APP), has been found to be highly synaptotoxic and to correlate best with the course of the disease in comparison with Abeta monomers or Abeta plaque load. However, analysis of the biological functions of Abeta dimers in vivo has been hampered by their low abundance and weak conformational stability.

We overcame this problem by using intermolecular disulfide engineering. A conservative serine to cysteine mutation was introduced at position 8 of Abeta (Abeta-S8C), allowing the covalent coupling of two Abeta monomers and resulting in the secretion of high levels of natural derived dimeric Abeta in a cell culture system¹. Abeta-S8C dimers that had been purified by size exclusion chromatography turned out to be highly synaptotoxic at low picomolar concentrations as tested by whole cell patch clamp analysis of primary cortical mouse neurons. In order to study Abeta dimers in vivo, we generated a mouse model (tgDimer) that expresses APP-Abeta-S8C under the control of the neuron-specific Thy1 promoter.

Strikingly, in brain homogenates of tgDimer mice, exclusively dimeric, but not monomeric or other oligomeric Abeta, was detected throughout their lifetime. Despite high amounts of Abeta dimers being expressed over the whole live span of these mice, there was a complete absence of insoluble Abeta and Abeta plaque pathology at age 24 months, demonstrating the inability of Abeta-S8C stabilized dimers to further aggregate into fibrils. Investigation of spatial learning ability using the Morris water maze test revealed a significant cognitive deficit phenotype in tgDimer mice.

Our tgDimer mouse model demonstrates that Abeta dimers are sufficient for causing cognitive deficits and thus presents a

model for early events during Alzheimer's disease. It therefore represents a potential basis for future testing of drugs that specifically target the synaptotoxic dimeric Abeta species.

References

1. Müller-Schiffmann A, Andreyeva A, Horn AH, Gottmann K, Korth C, Sticht H. Molecular engineering of a secreted, highly homogeneous, and neurotoxic a β dimer. *ACS Chem Neurosci* 2011; 2:242-8; PMID: 22778868; <http://dx.doi.org/10.1073/10.1021/cn200011h>

P.49: Mutations in helix 3 promote cross-seeding of PrP-WT and neurotoxicity

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Prion diseases encompass a diverse group of neurodegenerative conditions characterized by vacuolar degeneration and accumulation of misfolded conformers of the Prion protein (PrP). Over 40 pathogenic mutations in the globular domain of PrP lead to dominantly inherited forms of prion diseases. However, the mechanisms by which these mutations induce disease are mostly unknown. To better understand how mutations in the globular domain of PrP lead to the accumulation of pathogenic conformations, we analyzed the consequences of disrupting the hydrophobic domain. Expression of the artificial mutations PrP-M205S and -M205,212S (mouse PrP numbering) in *Drosophila* caused drastic conformational changes on PrP, including aberrant oxidative folding and accumulation of Ctm topologies. These profound structural changes caused neurodevelopmental defects, but did not induce progressive neurodegeneration. Interestingly, co-expression of PrP-M205S or -M205,212S with PrP-WT resulted in accelerated locomotor dysfunction, reduced survival, and very aggressive neuronal degeneration. This increase in neurotoxicity was accompanied by the accumulation of PrP-WT into 15B3-positive conformations in young flies and higher levels in older flies. These results indicate that PrP mutations altering its globular domain can seed PrP-WT, accelerating both protein misfolding and neurotoxicity. If pathogenic mutations in PrP also induce PrP-WT misfolding, this allelic cross-seeding could explain the mechanism mediating pathogenesis in inherited prion diseases. To test the ability of PrP-D202N and -Q212P to seed PrP-WT misfolding, we expressed these pathogenic mutants in flies and confirmed that they induce conformational changes, aberrant oxidative folding, and moderate neurodegeneration. We are currently combining the transgenes to investigate the ability of PrP-D202N and -Q212P to seed PrP-WT. These studies have the potential to uncover a common mechanism (allelic cross-seeding) mediating neuropathology in inherited forms of prion diseases.

P.50: X-ray absorption fine structure studies on human prion protein non-octarepeat copper binding site

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The post-translational conversion of PrP^C into the misfolded, pathogenic form PrP^{Sc} plays a key role in prion diseases or transmissible spongiform encephalopathies. PrP^C interacts with metal ions, in particular copper and zinc, through the octarepeat and non-octarepeat binding sites. One of the crucial questions in prion biology is the identification of the regions on PrP^C that lead to the conversion process, whereby most α -helical motifs are replaced by β -sheet secondary structures. In order to gain insights into the structural determinants involved in PrP^{Sc} formation we investigated the effect of human PrP^C (HuPrP) point mutations linked to the genetic form of prion diseases. Pathological point mutations cause spontaneous formation of PrP^{Sc} in the brain. We carried out a structural investigation to determine the high-resolution NMR three-dimensional structure of the truncated recHuPrP(90-231) carrying both the fCJD-linked V210I and the GSS-causing Q212P mutations. Moreover, we determined the 3D NMR structure of the E219K polymorphism in order to find the structural basis responsible for its protective effect. Such structural studies led to the preliminary conclusion that the structural disorders of the β 2- α 2 loop region, together with the increased spacing between this loop and the C-terminal part of α 3 helix are key pathological features. This observation raises the possibility that the spontaneous formation of prions might start with the disruption of the hydrophobic core present in the structured HuPrP domain. We then evaluated the effect of the pathological mutations on the N-terminal unstructured domain. We used synchrotron-based X-ray absorption fine structure (XAFS) technique to study the coordination geometries of Zn²⁺, Cu²⁺ and Cu⁺ at two pH values (5.5 and 7) in different HuPrP constructs carrying pathological mutations. We clearly showed that mutations and pH exchanges cause a dramatic modification on the non-OR copper binding site in the presence of metal ions. These findings provide a structure-function relationship that lays a biological basis for understanding the spontaneous generation of PrP^{Sc} in inherited prion diseases.

P.51: Extensive autophagy in cerebellar organotypic cultured cells exposed to anti-PrP antibodies

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We describe here extensive autophagy in cerebellar organotypic cultured slices (COCS) exposed to an antibody targeting the α 1 and α 3 helices of the globular domain of the cellular prion protein PrP^C (POM1) for 1 – 7 days. Extensive autophagy was observed in COCS and increased from day 1 until day 7 after exposure. All typical elements of autophagy were seen: phagophores, autophagic vacuoles, autophagolysosomes. Additional abundant dystrophic neuritis containing those structures were seen and their number dramatically increased at day 7 after exposure. A large number of multivesicular bodies were also seen. In conclusion, autophagy is an important feature of the ultrastructural pictures of COCS exposed to POM1 antibodies.

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P.52: Characterizing the structure of GPI-anchorless PrP^{Sc} by cryo-electron microscopy

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Introduction. The structure of the infectious prion protein (PrP^{Sc}) is poorly understood. Recent technological breakthroughs have improved the resolution that can be achieved by electron microscopy (EM), and cryo-EM has become a suitable technique for the structural analysis of prions. The propensity of PrP^{Sc} to polymerize into amyloid fibrils, and the regular helical structure that defines most amyloid fibers, can be exploited to study its molecular architecture. Therefore, a combination of EM and computational approaches allowed us to gain insights into the structure of GPI-anchorless PrP^{Sc}.

Materials and Methods. To collect high-resolution structural data we have used cryo low-dose electron microscopy, which preserves the specimen free of drying or staining artifacts and avoids radiation damage. Initially, a preliminary 3D reconstruction was generated from cryo-EM images of individual GPI-less PrP^{Sc} fibrils to visualize their 3D structure. The individual fibrils were segmented into boxes along the helical axis and the repeat distance of the helix allows an estimation of the angular orientation of each box. The underlying assumption is that each box is an identical, rotated view of the fibril. By assigning the angles of each box, a 3D volume was generated. To improve and extend the information obtained from the individual fibrils, a single-particle analysis of 15,942 non-overlapping PrP^{Sc} fibril segments was carried out. The fibril segments were centered, aligned and classified. The averaged particles were processed by Fourier transform (FT) analysis to determine periodic features. The EMAN and SPIDER software packages were used for the image processing.

Results. The 3D reconstructions of individual fibrils show two protofilaments coiled around a common axis with 0.48 nm striations perpendicular to the fibrils axis, suggesting that they represent the β -strands of PrP^{Sc}. In support of this interpretation, FT analyses of the raw images also show a distinct signal at 0.48 nm. Furthermore, some 2D averages from the single-particle analysis show a repeating pattern of ~ 4 nm densities with ~ 2 nm sized subunits. This observation suggests a head-to-head assembly of the PrP^{Sc} subunits. Also, averaged FT analyses of aligned fibril segments show a reflection at 0.48 nm, confirming that the β -structure of PrP^{Sc} is being visualized.

Conclusions. Our data provide increasing support for the hypothesis that the structure of PrP^{Sc} and PrP 27-30 consists of a four-rung β -solenoid with a central β -strand-rich core. Furthermore, the results suggest that individual monomers may stack in a head-to-head arrangement along the fibril axis.

P.53: Gene targeting approaches to assess the role of cervid PrP residue 226 on CWD pathogenesis and prion strain selection

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Our previously published and current studies point to a crucial role for residue 226, which encodes the singular primary structural difference between Rocky mountain elk (E) and deer (Q) PrP, in the susceptibilities of these species to prion disease. Furthermore, our data provides functional support to structural models suggesting residue 226, and the adjacent residue 225, participate in a tertiary structural epitope formed by α helix 3 and the $\beta 2$ - $\alpha 2$ region which influences PrP^C to PrP^{Sc} conversion and strain propagation. To precisely investigate the effects of residue 226 on prion pathogenesis, we generated gene-targeted (Gt) mice expressing elk or deer PrP (GtE and GtD) in which transgene expression recapitulates native host PrP^C expression. We will

present our preliminary characterizations of GtE and GtD mice including their susceptibility to prions by a variety of inoculation routes, and compare their responses with syngeneic transgenic mice expressing the same primary structures produced by conventional means.

P.54: Prions with deletions or insertions inside their protease-resistant core

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Identification of the PrP^C regions critical for prion conversion remains a challenging task. The problem is not only to define the domain(s) that undergo a conformational change, it is also to identify regions that favour prion replication and that have a dramatic effect on the susceptibility to the infection. Toward this goal we introduced insertions and performed deletions in the central part of the H2-H3 domain of PrP, in which the two α helices are separated by a short and flexible unstructured loop harbouring the second glycosylation site.

Using the RK13 cell model expressing ovine PrP^C (VRQ allele), we previously reported that it was feasible to introduce eight additional amino acids at the end of the loop without losing the susceptibility to prion infection. We further introduced the octapeptide insert in the last two turns of H2 and *bona fide* prions were also generated upon infection. Structural analysis of recombinant mutant PrP indicated that some of the amino acids introduced might have replaced the original ones at the end of H2. To determine whether a full length H2 helix is mandatory for replication, we performed sequential deletions and we challenged cells expressing the mutant PrPs by three distinct prion strains. RK13 cells expressing PrP^C deleted in the C-terminus of H2 were permissive to the three strains and generated prions that were maintained for months in cell culture. Their inoculation to mice expressing wild-type ovine PrP induced a *bona fide* prion disease. While PrP mutants with deletions further upstream in H2 were normally expressed at the cell surface, cells were refractory to the infection, whatever the strain used. The conversion potency of the different mutant PrPs was also assayed by PMCA. The effect of the deletions on PrP structure was investigated by analysis of mutant recombinant PrPs produced in *E. coli*.

To conclude, our results show that either a suppression of H2 end or an insertion in the last turns of H2 are compatible with prion replication, indicating that this region is not essential for prion conversion. However deletions or insertions further upstream in the helix dramatically reduced the efficiency of the replication or did not fit anymore the conversion. These observations also demonstrate that it is possible to propagate prions with

noticeable internal modifications, even in a central place of the protease-resistant core.

P.55: The role of PrP present on myeloma cells for efficient fusion with *Prnp*^{0/0} lymphocytes

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One of the roles of prion protein (PrP) on the cell membrane is the transmission of apoptotic signal to the cell.^{1,2} Apoptotic signal might be generated upon dimerisation of two PrP molecules by a PrP-specific antibody.^{3,4} It has been hypothesised that this feature might hamper the fusion of myeloma cells, expressing normal PrP^C, to *Prnp*^{0/0} lymphocytes which produce anti-PrP antibodies.^{5,6}

With the aim to produce stable anti-PrP monoclonal antibodies secreting hybridoma, we intended to select a fusion partner that would be most resistant to PrP-induced apoptosis among cell lines which are available in our lab. We tested three myeloma cell lines, Sp2/0, P3X63Ag8.653 and NS1, for the presence of PrP on the membranes as well as for the susceptibility to the addition of polyclonal antisera of *Prnp*^{0/0} mice, immunized with recombinant PrP. Simultaneous cell fusions were performed with two selected myeloma cell lines. We have demonstrated that NS1 cells were superior fusion partner to Sp2/0, although the latter express much less PrP. Furthermore, we were able to isolate potent monoclonal antibodies specific for native mouse PrP. Our results demonstrate that PrP expression on myeloma cells is not a limiting factor for successful production of hybridoma secreting anti-self PrP mAbs.

References

1. Spielhauer C, Schätzl HM. PrP^C directly interacts with proteins involved in signaling pathways. *J Biol Chem* 2001; 276:44604-12; PMID:11571277; <http://dx.doi.org/10.1074/jbc.M103289200>
2. Wechselberger C, Wurm S, Pfarr W, Höglinger O. The physiological functions of prion protein. *Exp Cell Res* 2002; 281:1-8; PMID:12441124; <http://dx.doi.org/10.1006/excr.2002.5655>
3. Mouillet-Richard S, Ermonval M, Chebassier C, Laplanche JL, Lehmann S, Launay JM, Kellermann O. Signal transduction through prion protein. *Science* 2000; 289:1925-8; PMID:10988071; <http://dx.doi.org/10.1126/science.289.5486.1925>
4. Solfrosi L, Criado JR, McGavern DB, Wirz S, Sánchez-Alavez M, Sugama S, DeGiorgio LA, Volpe BT, Wiseman E, Abalos G, et al. Cross-linking cellular prion protein triggers neuronal apoptosis in vivo. *Science* 2004; 303:1514-6; PMID:14752167
5. Rubenstein R, Kascsak RJ, Papini M, Kascsak R, Carp RI, LaFauci G, Meloen R, Langeveld J. Immune surveillance and antigen conformation determines humoral immune response to the prion protein immunogen. *J Neurovirol* 1999; 5:401-13; PMID:10463862; <http://dx.doi.org/10.3109/13550289909029481>
6. Williamson RA, Peretz D, Smorodinsky N, Bastidas R, Serban H, Mehlhorn I, DeArmond SJ, Prusiner SB, Burton DR. Circumventing tolerance to generate autologous monoclonal antibodies to the prion protein. *Proc Natl Acad Sci U S A* 1996; 93:7279-82; PMID:8692983; <http://dx.doi.org/10.1073/pnas.93.14.7279>

P.56: New monoclonal antibodies against central and C-terminal part of prion protein

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As prion protein (PrP) can exist in many different conformations and the existence of different truncated forms of PrP has also been demonstrated, there is an urge to study this protein by a panel of monoclonal antibodies (mAbs) instead of observing only one epitope. We here present the characterization of a panel of new anti-PrP monoclonal antibodies that were obtained after immunization of wild type BALB/c mice with C-terminal part of recombinant human PrP (amino acid residues 90-231). We have determined their relative binding affinities to the original antigen, their subclasses and epitopes. Reactivity of mAbs against PrP of different species was tested, as well.

All four mAbs, A14, A16, A18 and A19, are high affinity antibodies of IgG1 subclass. They have been shown to react with human PrP from brain homogenates in western blots and with PrP on human neuroblastoma cells as shown by immunocytochemistry. While A14 and A16 seem to be specific for human PrP, A18 and A19 also bind strongly to bovine PrP. Epitopes of A14 and A16 reside C-terminally from amino acid 120. The epitope of A16 was further restricted to the region 214-226, however this mAb is unable to bind to the truncated form of human PrP, PrP226*. Epitopes of A18 and A19 are located between amino acids 90-120, with the epitope of A19 being especially interesting as it partially shares the well-known 3F4 epitope.

P.57: Differences between mouse and human SNCAs may identify amino acids important in SNCA cytotoxicity

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Keywords: alpha-synuclein, mouse models

Alpha-synuclein (SNCA) is linked to a rare familial form of Parkinson's disease as well as the common sporadic form. Human A53T SNCA was the first mutation to be associated with Parkinson's, but in vertebrates, the wild type residue at that position is threonine. Besides residue 53, mouse and human SNCA differ at 6 of 140 amino acids. We hypothesize that one or more of these amino acid differences mitigates the presence of Thr53.

To investigate the effects of differences between mouse and human SNCAs, we generated expression constructs in which

human amino acids were individually substituted into mouse Snca. Controls included mouse and human WT SNCAs, and the human A53T SNCA. In yeast, four of our Snca variants inhibit cell growth to varying degrees. Expression of A53T human SNCA prevented growth, while 3 mouse variants, human WT and mouse WT were innocuous.

Each of the mouse Snca variant proteins, as well as control SNCAs, was purified. After fibrillization in vitro and sonication, all the Snca species were injected into the brains of A53T SNCA transgenic mice (N=8-12 mice per variant). The injected mice express only human mutant SNCA as they are null for endogenous mouse Snca. The A53T SNCA transgene is regulated by the mouse prion protein promoter, with high expression in spinal cord causing late onset limb paresis, at about 18 months. Intracerebral injection of pre-formed fibrils generated from all the Snca variants and controls significantly lowered the age of disease onset, starting at about 3.5 months post-injection. Spinal cord pathology is similar to that seen in uninjected mice that develop the late onset synucleinopathy. Kaplan-Meier lifespan analysis of the different cohorts showed several significant differences. Injection of one of the mouse variants causes a significantly shorter lifespan than injection with A53T human SNCA. This particular mouse variant inhibits growth when expressed in yeast, though not as strongly as A53T human SNCA. As the injected mice express only human A53T SNCA, we have shown that there is no species barrier between WT mouse and A53T human SNCA. We have also shown that regardless of differences in cytotoxicity in yeast, all our Snca variants can cause earlier disease onset in A53T SNCA transgenic mice. We hypothesize that differences found in the yeast assay reflect differences in the propensity of different Snca species to adopt a misfolded conformation in vivo, and we have identified amino acids that may influence that propensity.

P.58: Pleiotropic in vivo effects of PrP octarepeat substitutions

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Introduction. PrP^C's globular domain is preceded by a flexible N-terminus that can bind copper via four histidine-containing octarepeats. Alternative conformations of the PrP^C N-terminal octarepeat region (OR), denoted components 1, 2 and 3, occur in the presence of different concentrations of copper ions.

Hypothesis. Since conformation is a crucial parameter in prion disease we hypothesized that different N-terminal PrP conformations might be associated with different phenotypic properties.

Work Plan. Repetitive peptide mutagenesis was performed to produce octarepeat variants with discrete, component 1 or 3 copper-binding geometry with respective binding stoichiometries at saturation of 4 and 1 copper ions per molecule. DNA sequences encoding tandem variant OR's with "geometry-locked" conformations were engineered into a full-length mouse PrP DNA coding region. We created PrP constructs with component 1 PrP geometry (TgS1), component 3 geometry (TgS3) and control Tg wt mice bearing identical 5' untranslated region leader sequences. Construct DNAs were injected in *Prnp*^{0/0} oocytes (*Zrch1* null allele).

Results. Independent TgS1 and TgS3 mice lines were obtained with expression levels of full-length PrP no greater than 2.5-fold those of the endogenous wt locus. None of the Tg lines succumbed to spontaneous neurologic disease with observation periods of up to 600 days of age. Allelic differences were noted in the ability to rescue the peripheral neuropathy present in uninfected aged *Zrch1.Prnp*^{0/0} mice. Brain PrP^C in uninfected S3 mice, but not in S1 mice, had an increased propensity for C2 cleavage. Both S1 and S3 PrP alleles supported replication of the RML isolate of mouse-adapted scrapie prions, with TgS3 mice exhibiting incubation periods of under 100 days.

Conclusions. Within in vivo contexts, our data establish that (i) different conformations of the PrP OR can be associated with different phenotypic attributes, (ii) conformational plasticity of the ORs has an unexpected relationship to PrP endoproteolysis and (iii) that C2 PrP deriving from cleavage of full-length PrP - rather than a gene construct with translation initiating at codon 90 - can support prion replication.

P.59: Prion infection dysregulates cholesterol metabolism and alters the profile of lipids associated with extracellular vesicles

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Conversion of the cellular prion protein to the disease associated form associated with the transmissible prion is thought to occur in lipid rafts and depends on the availability of cellular cholesterol. We performed functional analyses on the effect of

increased cholesterol transporter (ABCA1) levels in prion infected neurons. Prion infection redistributes ABCA1 from lipid rafts to intracellular compartments such as late endosomes, resulting in a reduction of cholesterol efflux. Furthermore, lipid profiling of mock and prion infected neuronal cells revealed increases in cholesterol esters, free cholesterol, triglycerides and phosphatidylethanolamine, confirming prion infection affects cholesterol homeostasis. We also investigated the molecular requirements of exosomes (cell derived nanovesicles) to efficiently transfer prion infection between cells. Chemical and physical disruption of the structure of prion containing exosomes and the membrane environment using chemical or physical methods significantly decrease levels of prion infectivity. Determination of the lipid composition of control and prion containing exosomes using an LC-MS based lipidomics approach investigated the profile of more than 1000 lipid species in highly purified exosome preparations. This analysis revealed specific changes in both the overall abundance of major classes of phospholipids, ceramides and cholesterol esters between control and prion infected exosomes. Together, these data demonstrate an important connection between cellular cholesterol metabolism and prion infection. Furthermore, there are similarities with other pathogens such as HIV which also is able to displace ABCA1 from lipid rafts and affect cholesterol efflux, and Alzheimer's disease whereby dysregulation of cholesterol metabolism occurs in the brain. Increasing both cholesterol efflux and ABCA1 levels might have therapeutic potential for prion diseases.

P.60: Addressing the propagation and conformational dynamics of a model prionoid in bacteria

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Protein amyloids arise from the conformational conversion and templated assembly of a soluble protein into fibrillar aggregates with a crossed β -sheet backbone, leading to human neurodegenerative and systemic proteinopathies.¹ Besides epigenetic determinants, such as prions in yeast, functional amyloids are assembled in bacteria as extracellular scaffolds but, until recently, no proteinopathic amyloidosis had been found in microorganisms.²

In some bacterial plasmids, RepA protein initiates DNA replication and then, by physically coupling plasmid molecules through its origin-bound WH1 domain, RepA assembles as an amyloid to inhibit further replication rounds.^{3,4} In vitro, RepA-WH1 can assemble into amyloid fibers upon binding to short, plasmid-specific DNA sequences.^{5,6}

RepA-WH1 causes an amyloid proteinopathy in *E. coli*, hampering cell proliferation.⁷ RepA-WH1 amyloidosis is vertically transmissible from mother to daughter cells, but not infectious,

enabling conformational templating by cross-seeding in vitro and in vivo, thus it qualifies as a prionoid.² Through microfluidics, we have directly assessed the dynamics of the RepA-WH1 prionoid in *E. coli* cells.⁸ Bacterial lineages maintain two mutually exclusive types (strains) of RepA-WH1 amyloids: either multiple globular particles that inhibit cell division, or a single elongated aggregate, mildly detrimental to growth. DnaK, the Hsp70 chaperone in *E. coli*, contributes to RepA-WH1 amyloidogenesis in vivo, but the Hsp104 chaperone ClpB does not have a major effect on the vertical spread of the RepA-WH1 amyloid aggregates.

We have engineered repeats of the RepA-WH1 amyloid stretch to replace the Q/N-rich oligopeptide repeats in *Saccharomyces cerevisiae* Sup35p/[*PSI*⁺], thus building chimeric [*REP-PSI*⁺] prions. These are functional as epigenetic determinants, and become independent on Hsp104p for propagation.⁴

References

- Hoffmann C. G protein-coupled receptor activation: amino acid movements caught infra-red-handed. *ChemBiochem* 2010; 11:2247-9; PMID:20922739
- Giraldo R, Moreno-Díaz de la Espina S, Fernández-Tresguerres ME, Gasset-Rosa F. RepA-WH1 prionoid: a synthetic amyloid proteinopathy in a minimalist host. *Prion* 2011; 5:60-4; PMID:21293179; <http://dx.doi.org/10.4161/pri.5.2.14913>
- Giraldo R, Fernández-Tornero C, Evans PR, Díaz-Orejas R, Romero A. A conformational switch between transcriptional repression and replication initiation in the RepA dimerization domain. *Nat Struct Biol* 2003; 10:565-71; PMID:12766757; <http://dx.doi.org/10.1038/nsb937>
- Moreno-del Álamo, et al. Unpublished data, under review 2014.
- Giraldo R. Defined DNA sequences promote the assembly of a bacterial protein into distinct amyloid nanostructures. *Proc Natl Acad Sci U S A* 2007; 104:17388-93; PMID:17959784
- Gasset-Rosa F, Maté MJ, Dávila-Fajardo C, Bravo J, Giraldo R. Binding of sulphonated indigo derivatives to RepA-WH1 inhibits DNA-induced protein amyloidogenesis. *Nucleic Acids Res* 2008; 36:2249-56; PMID:18285361; <http://dx.doi.org/10.1093/nar/gkn067>
- Fernández-Tresguerres ME, de la Espina SM, Gasset-Rosa F, Giraldo R. A DNA-promoted amyloid proteinopathy in *Escherichia coli*. *Mol Microbiol* 2010; 77:1456-69; PMID:20662778; <http://dx.doi.org/10.1111/j.1365-2958.2010.07299.x>
- Gasset-Rosa F, Coquel AS, Moreno-Del Álamo M, Chen P, Song X, Serrano AM, Fernández-Tresguerres ME, Moreno-Díaz de la Espina S, Lindner AB, Giraldo R. Direct assessment in bacteria of prionoid propagation and phenotype selection by Hsp70 chaperone. *Mol Microbiol* 2014; 91:1070-87; <http://dx.doi.org/10.1111/mmi.12518>; PMID:24417419

P.61: Prion infection impairs lysosomal maturation —a possible mechanism to sustain prion propagation

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PrP^C as well as PrP^{Sc} are glycosyl-phosphatidyl-inositol (GPI) anchored proteins that localize to lipid rafts, which are detergent resistant membrane microdomains characterized by a high cholesterol and sphingolipid content. Cellular cholesterol which is essential for the formation of lipid rafts can be acquired by

endogenous synthesis and uptake from external sources, and these processes are tightly regulated by a feedback loop. Inhibition of cellular cholesterol synthesis or its extraction and complexation at the plasma membrane, respectively, interferes with PrP^{Sc} propagation in prion-infected cultured cells. We demonstrated that accumulation of cholesterol in late endosomes/lysosomes induced by drug treatment or siRNA knock-down of the cholesterol transporting NPC1 protein increases the degradation rate of PrP^{Sc}. On the other hand, cellular cholesterol metabolism is modulated by prion infection. Highly controlled microarray studies revealed an up-regulation of genes involved in cholesterol metabolism in prion-infected neuronal cell lines and primary neurons, resulting in elevated levels of free cholesterol.

Our current research focuses on investigating why prion infection induces cholesterol synthesis, and what the consequences of abnormal cholesterol levels are for neuronal cells. We have addressed these questions by analysing the response of prion infected cells to regulators of cholesterol synthesis. Our data indicate that prion infected cells do not respond with transcriptional regulation of genes involved in cholesterol synthesis to treatment with certain drugs, indicating sequestration of cholesterol. Furthermore, we have studied the distribution of marker proteins between lipid raft and non-lipid raft domains, and found a redistribution of the endosomal transmembrane protein NPC-1 to lipid raft domains in prion infected cells. Analysis of the membrane association of selected rab proteins involved in endosomal vesicle trafficking indicate an interference of prion infection with lysosomal maturation.

In summary, we suggest that prion infection leads to an increase of cellular cholesterol levels and, as a consequence, interferes with late endosomal vesicle trafficking in order to escape lysosomal degradation. This interference with host cell metabolism may enable and sustain stable prion propagation in neuronal cells.

P.62: Large scale “in silico” prediction of amyloid aggregating sequences and hot-spots: Can one predict co-aggregating proteins?

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Keywords: protein co-aggregation, amyloid structure, species barrier

The formation of amyloid aggregates upon protein misfolding is related to several devastating degenerative diseases.¹ The propensities of different protein sequences to aggregate into amyloids, how they are enhanced by pathogenic mutations, the presence of aggregation hot spots stabilizing pathological interactions, the establishing of cross-amyloid interactions between co-aggregating proteins, all relies at the molecular level on the stability of the amyloid cross-beta structure.²

Several predictors were developed in recent years, based on different features of input primary sequences, with the aim of

predicting the aggregation propensity into either amyloid fibrils or prefibrillar toxic oligomeric aggregates.³ Predicted pathogenic mutations indeed confirmed their amyloidogenic potential when engineered “in vivo”.⁴

We developed our own aggregation propensity predictor, PASTA, based on evaluating the pairing energy of two equal-length sequence stretches, when they are considered in a putative cross-beta arrangement^{5,6}. In this way, we were able to predict which sequence stretches form the best cross-beta pairings and the corresponding parallel or anti-parallel arrangements, consistently predicting in-register parallel arrangement in most cases, in agreement with known experimental data on amyloid structure.⁵

The method can be easily extended to the evaluation of cross-beta pairings by two different sequences. This opens the way to evaluate and predict on a large scale cross-amyloid interactions, that are increasingly known to play a critical role in protein misfolding diseases, as evidenced by the co-aggregation of different disease-related proteins into heteromeric oligomer structures.² Similarly, one can test the relative ability of two homologous proteins in oligomerizing together, a fact hypothesized to be the molecular basis of the phenomenon of species barrier, in the context of both mammals and yeast prions.⁷

References

1. Chiti, et al. *Annu Rev Biochem* 2006; 14:333-66; <http://dx.doi.org/10.1146/annurev.biochem.75.101304.123901>
2. Eisenberg D, Jucker M. The amyloid state of proteins in human diseases. *Cell* 2012; 148:1188-203; PMID:22424229; <http://dx.doi.org/10.1016/j.cell.2012.02.022>
3. Castillo V, Graña-Montes R, Sabate R, Ventura S. Prediction of the aggregation propensity of proteins from the primary sequence: aggregation properties of proteomes. *Biotechnol J* 2011; 6:674-85; PMID:21538897; <http://dx.doi.org/10.1002/biot.201000331>
4. Luheshi, et al. *PLoS Biol* 2007; 5:2493-500; <http://dx.doi.org/10.1371/journal.pbio.0050290>
5. Trovato A, Chiti F, Maritan A, Seno F. Insight into the structure of amyloid fibrils from the analysis of globular proteins. *PLoS Comput Biol* 2006; 2:e170; PMID:17173479; <http://dx.doi.org/10.1371/journal.pcbi.0020170>
6. Trovato A, Seno F, Tosatto SC. The PASTA server for protein aggregation prediction. *Protein Eng Sel Des* 2007; 20:521-3; PMID: 17720750; <http://dx.doi.org/10.1093/protein/gzm042>
7. Tuite MF, Serio TR. The prion hypothesis: from biological anomaly to basic regulatory mechanism. *Nat Rev Mol Cell Biol* 2010; 11:823-33; PMID:21081963; <http://dx.doi.org/10.1038/nrm3007>

P.63: The spread of diverse prion strains

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In contrast to amyloid- β , tau, or α -synuclein, prions naturally transmit as infectious agents, and human to human transmission has occurred through transfusion of prion-contaminated blood. In Alzheimer's disease, amyloid- β and tau aggregates spread within the CNS, whereas most cases of natural prion transmission start with a peripheral exposure followed by prion spread to the CNS. This ability of prions to spread readily in and out of the CNS is remarkable, however it is not a universal trait of

prions. We and others have found that certain prions do not efficiently invade the CNS, yet readily accumulate in non-neural organs, such as spleen or heart. Intriguingly, all of these poorly neuroinvasive prions form fibrils, which are uncommon structures in prion disease, but common for amyloid- β and tau. We are investigating the molecular basis for the striking differences in the spread of nonfibrillar and fibrillar prions to the brain. We have found that the fibrillar prions were more resistant to enzyme digestion and were extraordinarily stable when exposed to protein denaturing conditions, in mice and in humans. Taken together, these results provide evidence for a striking separation in the biochemical properties of fibrillar and nonfibrillar prions that underlie their differences in neuroinvasion and may provide insight into the spread of other aggregated proteins.

P.64: A proposed mechanism for the promotion of prion conversion involving a strictly conserved tyrosine residue in the β 2- α 2 loop of PrP^C

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Zoonotic prion transmission occurred during the bovine spongiform encephalopathy (BSE) epidemic, with over 200 cases of variant Creutzfeldt-Jakob disease in humans diagnosed to date. Transmission of BSE to humans was not anticipated, and assessing the risk of cross-species prion transmission remains challenging. The transmission of infectious prions into different host species requires compatible PrP primary structures, and even one heterologous residue at a pivotal position can block prion infection. We and others have found that residues within the β 2- α 2 loop region (residues 165-175) markedly impact conversion by sheep scrapie, BSE, mouse-adapted prions, CWD, and hamster-adapted prions. The tyrosine residue at position 169 is strictly conserved among mammals and the aromatic side chain is essential to maintain a 3_{10} -helical turn within the loop. Here we examined the impact of a Y169G substitution together with the S170N, N174T “rigid loop” substitutions on in vivo prion transmission and in vitro conversion. We found that transgenic mice expressing the Y169G, S170N, and N174T substitutions completely resisted infection with two strains of mouse prions and with deer CWD prions. To define how the single amino

acid substitutions at positions 169, 170 or 174 impact conversion, we used variant PrP^Cs having single residue substitutions in the β 2- α 2 loop as a substrate for in vitro prion conversion experiments. PrP^C variants were expressed by transient transfection of immortalized RK13 (rabbit kidney) cells and possessed post-translational modifications including the glycosyl-phosphatidyl inositol (GPI) anchor and glycosylation. The single Y169G substitution strongly inhibited conversion by mouse prions in vitro, and further investigations revealed that substitution of leucine or glutamine at position 169 also reduced conversion. In contrast, replacing the 169 tyrosine with the aromatic residues phenylalanine or tryptophan supported efficient prion conversion. We propose a model based on a requirement for tightly interdigitating complementary amino acid side chains within specific domains of adjacent PrP molecules, known as “steric zippers”, to explain these results. Collectively, these studies suggest that an aromatic residue at position 169 supports efficient prion conversion.

P.65: A Janus face of ADAM10-mediated shedding of PrP in prion disease

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The cellular prion protein (PrP^C) is the substrate for the conversion into its pathogenic isoform PrP^{Sc} and thus a prerequisite for the establishment of fatal prion diseases. In addition, it acts as a neuronal receptor for various β -sheet-rich oligomeric protein species associated with different neurodegenerative proteinopathies (including A β in Alzheimer's and PrP^{Sc} in prion disease) and is thought to mediate their neurotoxicity.

Proteolytic processing of the prion protein influences these pathological roles as well as the physiological functions linked to PrP^C. We and others recently identified the A-disintegrin- and metalloproteinase 10 (ADAM10) as the major sheddase of PrP^C releasing the nearly full-length protein into the extracellular space and thereby regulating PrP^C membrane homeostasis, yet we could not confirm its previously suggested involvement in the α -cleavage within the central part of PrP^C in vivo.

Here, we employed a novel viable ADAM10-knockout mouse model with specific postnatal deletion of the protease in neurons of the forebrain to investigate the role of ADAM10 in prion disease. These mice present with posttranslationally increased PrP^C levels. Upon prion infection, lack of ADAM10 leads to drastically shortened incubation times and spatiotemporal alterations of neuropathological characteristics. Moreover, we find significantly increased PrP^{Sc} production compared to littermate controls and PrP^C-overexpressing (Tga20) mice. While these findings support a protective role of ADAM10-mediated shedding, additional results—on the contrary—also indicate involvement of the protease in the spread of prions to distant brain regions.

Finally, our data on PrP^C-shedding provide a possible explanation for the poor PrP^{Sc} conversion despite high PrP^C content observed in Tga20 mice and further support the notion, that PrP^{Sc} amounts do not directly correlate with prion infectivity.

In summary, ADAM10 seems to play a dual role in prion diseases. However, with regard to incubation time, protective effects by reducing local membrane-bound PrP^C amounts and by producing a protective soluble fragment seem to predominate the disadvantage of increased spread by production of anchorless prions. In other neurodegenerative proteinopathies, where PrP^C-PrP^{Sc} conversion does not play a role, ADAM10-mediated shedding of PrP^C might solely be protective due to the regulation of the receptor as well as the production of a soluble blocker of toxic oligomers.

P.66: Toxic misfolded prion protein causes neuronal death by NAD⁺ starvation

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The mechanisms of neuronal death in protein misfolding neurodegenerative diseases (PMNDs) such as Alzheimer, Parkinson and prion diseases are poorly understood. We used our highly toxic misfolded prion protein (TPrP) model to understand prion protein neurotoxicity. We show that TPrP-induced apoptosis and autophagy are not the primary causes of neuronal demise. On the other hand, TPrP-damaged neuroblastoma cells and primary neurons were completely rescued by treatment with nicotinamide adenine dinucleotide (NAD⁺) or its precursor nicotinamide (NAM). TPrP-exposed cells were severely NAD⁺ depleted and NAD⁺ or NAM treatment restored intracellular NAD⁺ pool. Intracerebral injection of NAD⁺ dose-dependently protected hippocampal neurons from TPrP-induced degeneration. Intranasal NAD⁺ treatment of prion-infected mice starting during established clinical phase significantly delayed motor impairment and reduced the time during which mice were non-ambulatory. We identified for the first time NAD⁺ starvation as a cause of neurodegeneration induced by a misfolded amyloidogenic protein and as an autophagy inducer in a PMND. We propose the development of NAD⁺ replenishment strategies for neuroprotection in prion diseases and possibly other PMNDs.

P.67: Engineering and analysis of antibody fragments for prion disease therapy

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Due to its centrality in the pathogenesis of prion diseases, several antibodies have been developed for therapy that target PrP^C; however these antibodies have shown limited success in animal models when employed in a manner that could be easily translated to humans. We have engineered an array of single chain antibody fragments in order to examine the impact of antibody binding affinity, secretion levels, and epitope on therapeutic potency. We have developed six antibodies targeting the first helix of PrP that vary in affinity from 1 nM to 320 nM and have expression levels that cover two log orders, a characteristic relevant for gene therapy approaches that utilize antibody fragments. Using virally delivered antibody fragments, we find that therapeutic potency in cell culture scrapie models is dependent on both antibody affinity and expression level. We have also isolated scFvs targeting the helix2-helix3 region of PrP^C, a region for which few antibodies are available, and are investigating the potential of these antibodies to halt prion propagation. Finally, we have developed a methodology to provide residue level epitope identification by screening libraries of PrP mutants and identifying mutations that ablate binding by next generation DNA sequencing. We have validated this technology by comparing our findings for ICSM18 to the antibody-complex crystal structure, and applied the new method to identify critical residues that form the epitopes of three additional anti-prion antibodies. By extending this approach to PrP-PrP interactions, we hope to gain molecular level insight into the protein-protein interactions that mediate prion formation and propagation. Such knowledge may lead to the identification of epitopes for more effect therapeutic antibodies for prion diseases. Combined, our work enables a quantitative analysis of the effects of affinity, stability, and epitope specificity on the therapeutic potency of anti-prion antibodies and provides insight into the molecular basis of prion propagation.

P.68: The prion protein: an unexpected link between base excision repair and neurodegeneration

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Keywords: prion protein function, DNA repair

A conformational change in the highly conserved and ubiquitously expressed prion protein (PrP^C) to yield an abnormal form (PrP^{Sc}) is associated with the generation of prions, the infectious agent of transmissible spongiform encephalopathies. Whether the resulting pathology is due to the accumulation of PrP^{Sc} aggregates or to a loss of function of the normal PrP^C is still an open question since, despite decades of investigation, the physiological role of the normal PrP^C remains elusive. A protective effect against oxidative stress has been shown, but the underlying mechanisms have not been determined. Here, by using animal and cellular models, we unveil a key role of PrP in the DNA damage response. We found that exposure of neurons to a genotoxic stress activates *PRNP* transcription leading to an increased amount of PrP in the nucleus where it interacts with APE1, the major mammalian endonuclease essential for base excision repair (BER), and stimulates its activity. Preventing the induction of *PRNP* results in accumulation of abasic (AP) sites in DNA and impaired cell survival after genotoxic treatment. The reduced activity of APE1 in brains from *Prnp*^{-/-} mice is associated with a defect in the repair of induced DNA damage in vivo. Brains from mice at early stages of prion infection also display a reduced APE1 activity, suggesting that loss of the PrP protective function on DNA plays a role in neuronal death and could be implicated in many neurodegenerative processes.

P.69: Membrane lesions of prion disease are not replicated by anchored Aβ and different molecular forms of disease specific PrP are directed to different parts of the cell

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Keywords: prion disease, Aβ, membrane, microgliosis, GPI anchor

All prion diseases of man and animals in which truncation of the protease resistant core of prion protein (PrP^{res}) occurs at approximately 19 or 21 kDa show lesions of cell membranes. These membrane changes co-localise with disease associated forms of prion protein (PrP^d) and consist of bizarre, spiral invaginations of the plasma-membranes of dendrites and neurons. The lesions are caused by the resistance of monomers or oligomers of PrP^d to their excision and endosomal recycling from the membrane. We have further investigated the molecular specificity of these membrane changes in a GPI anchored Aβ peptide and in atypical scrapie in sheep and Tg338 mice.

Using immunogold electron microscopy we found that both anchored and non-anchored forms of Aβ were localised to the plasma-membranes of small diameter neurites but significant morphologic changes to membrane were absent. However, in common with prion diseases all forms of membrane localised monomeric or oligomeric Aβ resulted in rapid activation of glial cells. Thus neither Aβ nor PrP^d require to be released from membrane to elicit rapid gliosis.

PrP^{res} derived from atypical sheep scrapie and their transgenic murine homologues are distinct both in size and protease sensitivity when compared with classical prion sources. Using antibodies directed to different PrP epitopes we first determined whether the protein was truncated in situ. All antibodies, including those to N-terminal epitopes were present in tissues sections suggesting that atypical scrapie PrP^d is present in tissues as a full length protein. Both atypical scrapie affected sheep and Tg338 mice showed prominent accumulations of PrP^d in white matter. On ultrastructural examination these changes were associated with Wallerian-type degeneration and an unusual lesion in which proliferation of the mesaxon (oligodendroglial inner tongue cytoplasm) and invaginations of axonal cytoplasm was found. Immunogold methods showed an association with the latter but not the former change. Dendritic and perikaryonal membrane invagination such as are found in classical forms of prion disease were absent in sheep.

Thus the unusual membrane lesions found in many prion diseases appears to be specific to prion disease and not simply a result of the aggregation of GPI attached amyloidogenic proteins in membranes. Furthermore, strain specific forms of PrP^d may

be directed by different cell sorting processes to different parts of the neuronal membrane where their impaired endocytosis causes plasma membrane specific pathology affecting either the perikaryonal/dendritic or axonal aspects of the cell.

P.70: In-vitro screen of prion disease susceptibility genes using the scrapie cell assay

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The main genetic determinant of human prion disease susceptibility and incubation time in mice is the prion gene, *PRNP*, however, several reports including genome wide association studies and quantitative trait loci mapping have provided evidence for multiple susceptibility genes. To date, few of these genes have been confirmed to influence prion-related phenotypes. Generally, the method of choice for functional validation is to phenotype mouse models such as transgenics and knockouts, however, this is not a feasible option where many novel genes, without pre-existing models, require testing. We have therefore undertaken an in-vitro triage of candidate modifiers to prioritise genes for more detailed future studies which is faster, cost effective and ethical relative to mouse bioassay. The scrapie cell assay (SCA) uses a neuroblastoma derived cell line (PK1) that is susceptible to RML prions and able to propagate prions at high levels indefinitely. In this study, we have generated stably knocked down and/or overexpressing PK1 derived cell lines for 14 candidate genes and tested them in the SCA to obtain a measure of relative susceptibility. No consistent differences were seen for seven genes, however, highly significant changes were detected for *Zbtb38*, *Sorcs1*, *Stmn2*, *Hspa13*, *Fkbp9*, *Actr10* and *Plg* suggesting that they play key roles in the fundamental processes of prion propagation and clearance. Many neurodegenerative diseases involve the accumulation of misfolded protein and prion-like seeding and spread has been implicated in their pathogenesis therefore it is expected that some of these prion-modifier genes may be of wider relevance in neurodegeneration.

P.71: The cellular prion protein contributes to EMT

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Although several phenotypic changes have been observed in *Prnp* null mice, the physiological function of the prion protein (PrP) has remained elusive. The morpholino-based knockdown of PrP-1, one of two PrP orthologs in zebrafish, has been shown to cause developmental arrest at the stage of gastrulation due to a failure to fully execute an epithelial-to-mesenchymal transition (EMT). Interestingly, this developmental phenotype is shared by zebrafish embryos deficient for the ZIP6 zinc transporter, a molecular cousin of PrP. To explore if PrP and ZIP6 play a role in EMT also in mammalian cells, RNA and protein levels of PrP and ZIP6 were monitored in mouse epithelial cells undergoing EMT. Surprisingly, levels of PrP and ZIP6 were more than fivefold increased during the course of EMT. Next, PrP expression was eliminated by CRISPR technology to determine if the increase in PrP levels observed during EMT constitutes a bystander effect or is critical for completing the EMT morphogenetic program. Consistent with observations reported earlier for zebrafish embryos, PrP knockout clones failed to fully execute EMT. It is hoped that further investigation of the molecular biology underlying the involvement of PrP in EMT may provide additional angles for understanding its physiological function.

P.72: β -Hairpin-mediated formation of structurally distinct multimers of neurotoxic prion peptides

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Keywords: prion protein, transmissible spongiform encephalopathies, molecular dynamics, fibrillization, oligomer formation, β -hairpin structure

Protein misfolding disorders are associated with conformational changes in specific proteins, leading to the formation of potentially neurotoxic amyloid fibrils. During pathogenesis of prion disease, the prion protein misfolds into β -sheet rich, protease-resistant isoforms. A key, hydrophobic domain within the prion protein, comprising residues 109-122, recapitulates many properties of the full protein, such as helix-to-sheet structural transition, formation of

fibrils and cytotoxicity of the misfolded isoform. Using all-atom, molecular simulations, it is demonstrated that the monomeric 109-122 peptide has a preference for α -helical conformations, but that this peptide can also form β -hairpin structures resulting from turns around specific glycine residues of the peptide. Altering a single amino acid within the 109-122 peptide ($A_{117}V$, associated with familial prion disease) increases the prevalence of β -hairpin formation and these observations are replicated in a longer peptide, comprising residues 106-126. Multi-molecule simulations of aggregation yield different assemblies of peptide molecules composed of conformationally-distinct monomer units. Small molecular assemblies, consistent with oligomers, comprise peptide monomers in a β -hairpin-like conformation and in many simulations appear to exist only transiently. Conversely, larger assemblies are comprised of extended peptides in predominately antiparallel β -sheets and are stable relative to the length of the simulations. These larger assemblies are consistent with amyloid fibrils, show cross- β structure and can form through elongation of monomer units within pre-existing oligomers. In some simulations, assemblies containing both β -hairpin and linear peptides are evident. Thus, in this work oligomers are on pathway to fibril formation and a preference for β -hairpin structure should enhance oligomer formation whilst inhibiting maturation into fibrils. These simulations provide an important new atomic-level model for the formation of oligomers and fibrils of the prion protein and suggest that stabilization of β -hairpin structure may enhance cellular toxicity by altering the balance between oligomeric and fibrillar protein assemblies.

P.73: Long-term, efficient and reversible gene silencing to study PrP^C functions

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Cellular Prion Protein (PrP^C) appears more complex than initially expected with the description of several proteolytic fragments potentially associated with multiple unclear biological functions. Therefore, development of more powerful tools to study PrP^C and its active fragments for long-term studies now appears as a new challenge for cell biology.

Classical methods to genetically modify target human cells by RNA interference (RNAi) based on transient transfections or infections with lentiviral vectors encompass a number of limitations: saturation of endogenous RNAi machinery caused by high amounts of siRNA duplexes or lentiviral titer, insertional mutagenesis and disruption of endogenous gene expression with lentivirus infection and transcriptional cassette lost during selection pressure with integrative plasmids. As a consequence, long-term maintenance of a modified genotype is riddled with pitfalls.

To overcome these shortcomings, we took advantage of replicative pEBV-based plasmids, which are maintained as extrachromosomal episomes and impose a stable overexpression of a construct of interest. In the RNAi context, we have designed pEBVsiRNA

plasmids driving the in cellulo siRNA synthesis through shRNA sequences, allowing a long-term, efficient, stable and reversible gene silencing (CEA patent 2005, Biard 2007).¹ In our laboratory, more than 220 human genes have been targeted and studies reported in the literature.² In different cell lines, such as HeLa (as control), LAN1 (neuroblastoma) and T98G (glioblastoma) cells, we observed very high extinction rates. For instance, in HeLa cell populations or clones, gene silencing efficiencies currently reaches more than 95% as evidenced by RT-qPCR with no detectable signal in western blot, even after one year of culture. To our knowledge, such level and stability in gene silencing has never been reported in the literature. Importantly, withdrawal of the selective pressure entails recovery of gene expression and the resumption of normal protein contents.

We have also created several pEBV plasmids to overexpress different mutated and normal PrP fragments. Full PrP^C and N-terminal fragments were observed several months after transfection, with levels increased up to several hundred folds in neuroblastoma LAN-1 after 40 days of culture.

As a conclusion, this technical approach allows us to efficiently and stably knockdown and/or overexpress one, two or more genes simultaneously, with minimal time and financial investment. This simplified technique offers an exceptional opportunity to rapidly create a set of stable relevant cellular models usable to study functions of PrP^C or other associated proteins.

References

1. Biard DS. Untangling the relationships between DNA repair pathways by silencing more than 20 DNA repair genes in human stable clones. *Nucleic Acids Res* 2007; 35:3535-50; PMID:17483520; <http://dx.doi.org/10.1093/nar/gkm195>
2. Biard D. DNA Repair 2011, Ed. Inna Kruman, ISBN 978-953-307-697-3, Publisher: InTech.

P.74: Highly sensitive detection of the endogenous cellular human prion protein

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Normal prion protein (PrP^C) function is still poorly understood even if it now appears much more complex than initially expected. Multiple interactions with the metabolism of the precursor of A β (notably as a receptor of A β toxicity) have now been reported and several proteolytic cleavage fragments of PrP^C exhibit a biological activity. In the prion field, the main efforts have been focused on the detection of the abnormal form of the prion protein (PrP^{Sc}) but more sensitive tools are now required to improve the detection of PrP^C and its numerous fragments.

Our preliminary studies have demonstrated a wide range of *PRNP* expression levels (mRNA and protein contents) between several cell lines, in particular neuroblastoma LAN-1 (nearly undetectable) and glioblastoma T98G (strong expression levels).

We have developed a new method to unmask epitopes (5 minutes heat treatment with unmasking buffer) and reduce

the background (2 minutes rapid saturation buffer) in western blot and in immunocytochemistry. Our enhanced western blotting method strongly amplifies the detection of PrP^C in cellular extracts and show interesting differences between cell lines. We are able to increase the immunoreactivity of the samples and strongly improve the signal/background ratio in western blots. Intense signals were detected with 10-fold lower protein extracts, with highly diluted primary and secondary antibodies (respectively 1/10000 and 1/50000). The lower amounts of loaded samples allow a better resolution of the electrophoretic bands visualized.

A similar approach is in progress for immunocytochemical staining. Our preliminary results reveal a strong signal increase (versus classical staining) despite a 10- to 50-fold lower dilution factor of primary antibodies.

We are currently refining these new analytical tools in order to better detect and understand the interactions between APP and PrP^C in normal or pathological (AD) contexts.