11th Iberian Congress on Prions Barcelona 2023

BOOK OF ABSTRACTS

Hosted by

UAB Universitat Autònoma de Barcelona

ibb Institut de Biotecnologia i de Biomedicina

IRTA

CReSA Centre de Recerca en Sanitat Animal

Societat Catalana de BIOLOGIA

Sponsored by

IDEXX

matachana

biomolecules an Open Access Journal by MDPI

Cultek

BioLab

WELCOME

Over 20 years have passed since the first case of BSE was diagnosed in Spain at the beginning of the century, an event that shaped many of our professional careers. Unfortunately, no treatment exists yet to fight the devastating fate of patients suffering from prion diseases, but since the "mad cow" crisis, formidable breakthroughs have happened in prion science. It is a field that has always embraced the one Health approach: bringing together multidisciplinary scientists to tackle one of the great unsolved challenges in science, to cure prion diseases, which share molecular mechanisms with other protein misfolding disorders of high prevalence in our aging society.

With this in mind, Barcelona is proud to host the Iberian prion congress for the second time, a unique opportunity to establish and strengthen interdisciplinary collaborations between medical doctors, biologists, veterinarians, biotechnologists, biochemists, and other specialists from the Iberian Peninsula and beyond with a particular emphasis on providing a forum for young scientists to present and discuss their research. It is only through collaborative work among all of us that we will reach our joint goal.

Welcome to Barcelona!

The organizing committee

Universitat Autònoma de Barcelona

ibb Institut de Biotecnologia i de Biomedicina

IRTA CReSA Centre de Recerca en Sanitat Animal

Societat Catalana de BIOLOGIA

ORGANIZING AND SCIENTIFIC COMMITTEE

LOCAL ORGANIZING COMMITTEE

- Nuria Busquets, IRTA-CReSA
- Samanta Giler, IRTA-CReSA
- Michał Burdukiewicz, IBB-UAB
- Carlos Pintado-Grima, IBB-UAB
- Irantzu Pallares, IBB-UAB
- Salvador Ventura, IBB-UAB
- Enric Vidal, IRTA-CReSA
- Eduard Cecília, IRTA-CReSA

SCIENTIFIC COMMITTEE

- Olivier Andreoletti, INRAE - ENVT
- Juan José Badiola, UNIZAR
- Vincent Beringue, INRAE
- Rosa Bolea, UNIZAR
- Miguel Calero , ISCII
- Joaquin Castilla, CICbioGUNE
- José Antonio del Río, IBEC
- Hasier Eraña, CICbioGUNE
- Natàlia Fernandez, CISA-INIA-CSIC
- Rafael Giraldo, CNB-CSIC
- Carmen Guerrero, Hospital Universitario Fundación Alcorcón
- Eduardo Melo, UALG
- Catarina Oliveira, U. Coimbra
- Leonor Orgue, INIAV
- Alicia Otero, UNIZAR
- Irantzu Pallares, UAB-IBB
- Jesús Requena, USC
- Human Rezaei, INRAE
- Raimon Sabaté, UB
- Raquel Sanchez, Hospital Clínic Barcelona
- Juan Maria Torres, CISA-INIA-CSIC
- Salvador Ventura, UAB-IBB
- Enric Vidal, IRTA-CReSA

SPONSORED BY

IDEXX

matachana

biomolecules
an Open Access Journal by MDPI

Cultek

BioLab

PROGRAMME – 11th May 2023

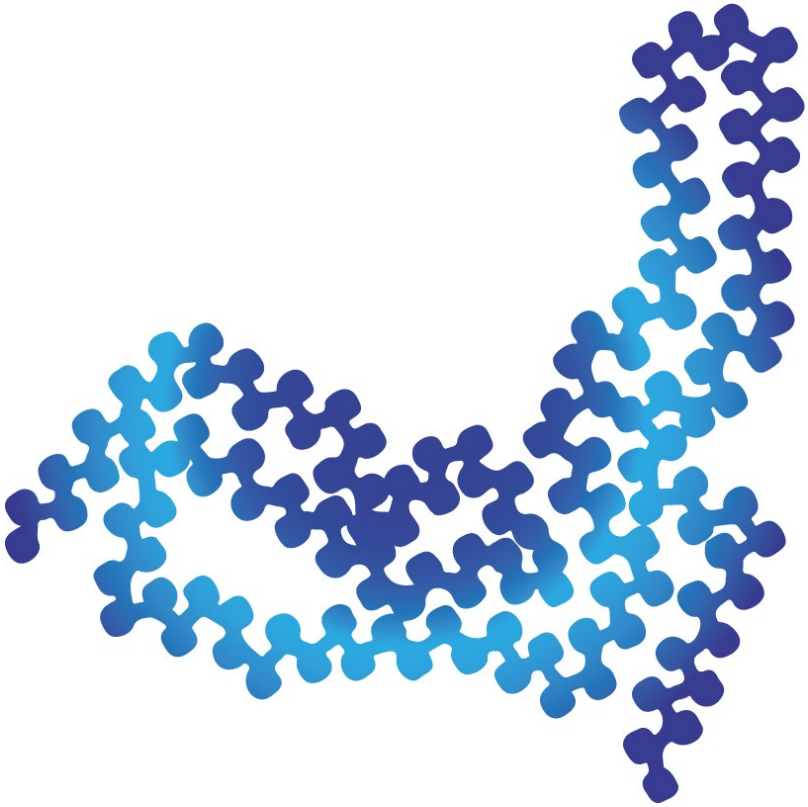
	08:45 - 09:30	Registration and posters set-up
	09:30 - 10:00	Welcome
Prion diseases in animals Chairs: Juan Carlos Espinosa (CISA) + Alicia Otero (UNIZAR)	10:00 - 10:45	**KEYNOTE 1** - Camel prion disease: a new emerging disease in North Africa. *Dr. Laura Pirisinu, Istituto Superiore di Sanita, Italy*
	10:45 - 11:00	**ORAL 1** - Assessment of the goat I/M142 polymorphism in the susceptibility to atypical scrapie prions. *Natalia Fernández-Borges, CISA-INIA-CSIC*
	11:00 - 11:15	**ORAL 2** - Innate immune status of glia and prion replication, *Sang-Gyun Kang, University of Alberta*
	11:15 - 11:30	**ORAL 3** - sPMCA conversion efficacies of Norwegian prions, *Cecile Ersdal, Norwegian University of Life Sciences.*
	11:30 - 12:00	Coffee break & Posters
Prion diseases in animals Chairs: Leonor Orge (UTAD) + Tomás Barrio (ENVT)	12:00 - 12:15	**ORAL 4** – Atypical scrapie evolution during propagation in different PrPC subtract both in vitro and in vivo, *Sara Canoyra Sánchez, CISA-INIA-CSIC*
	12:15 - 12:30	**ORAL 5** - In vitro modelling of cervid prion strain emergence and evolution considering all the described polymorphic variants, *Carlos M. Díaz-Domínguez, CIBERInfec, CIC bioGUNE*
	12:30 - 12:45	**ORAL 6** - Vaccines for chronic wasting disease, *Hermann Schatzl, University of Calgary*
	12:45 - 13:30	**KEYNOTE 4** - Interventional clinical trials in human prion diseases: is now the time?, *Dr. Raquel Sánchez-Valle, Hospital Clínic de Barcelona, Spain* – (Prion and prion-like diseases in humans)
	13:30 - 15:00	Lunch & Posters & Group photo
Prion and prion-like diseases in humans Chairs: Jokin Castilla (CIC bioGUNE) + Natalia Sanchez de Groot (UAB)	15:00 - 15:45	**KEYNOTE 3** - Strain-specific lesioning patterns in sporadic CJD - a neuropathological perspective, *Dr. Ellen Gelpi, Medical University of Vienna, Austria*
	15:45 - 15:50	Genetic CJD Biobank, *Alice Ananne, CJD Foundation Israel*
	15:50 - 16:05	**ORAL 7** - Human prion strain discrimination by RT-QuIC, *Alba Marín-Moreno, Université Paris-Saclay, INRAE*
	16:05 - 16:20	**ORAL 8** - Gene therapy for sCJD in a humanized mouse model, *Qingzhong Kong, Case Western Reserve University*
	16:20 - 16:35	**ORAL 9** - A robust method for evaluation of prionicide treatments, *Angélique Igel, INRAE*
	16:35 - 17:05	Coffee break & Posters
Prion and prion-like diseases in humans Chairs: Jesús Requena (CiMUS USC) + Michał Burdukiewicz (UAB)	17:05 - 17:20	**ORAL 10** - Neuronal networks in absence of the prion protein, *Anna Burato International School for Advanced Studies - SISSA*
	17:20 - 17:35	**ORAL 11** - The small aromatic compound SynuClean-D inhibits the aggregation and seeded polymerization of multiple α-synuclein strains, *Samuel Peña Díaz, Universitat Autònoma de Barcelona (UAB)*
	17:35 - 17:50	**ORAL 12** - The structural architecture of an α-synuclein toxic oligomer, *Jaime Santos, Universitat Autònoma de Barcelona*
	17:50 - 18:35	**KEYNOTE 2** - Thirty years of cohabitation with prion diseases: my personal experience, *Dr. Martí Pumarola, Universitat Autònoma de Barcelona, Spain* - (Prion diseases in animals)
	21:00 - 00:00	Congress dinner at bullfighting arena

PROGRAMME – 12TH MAY 2023

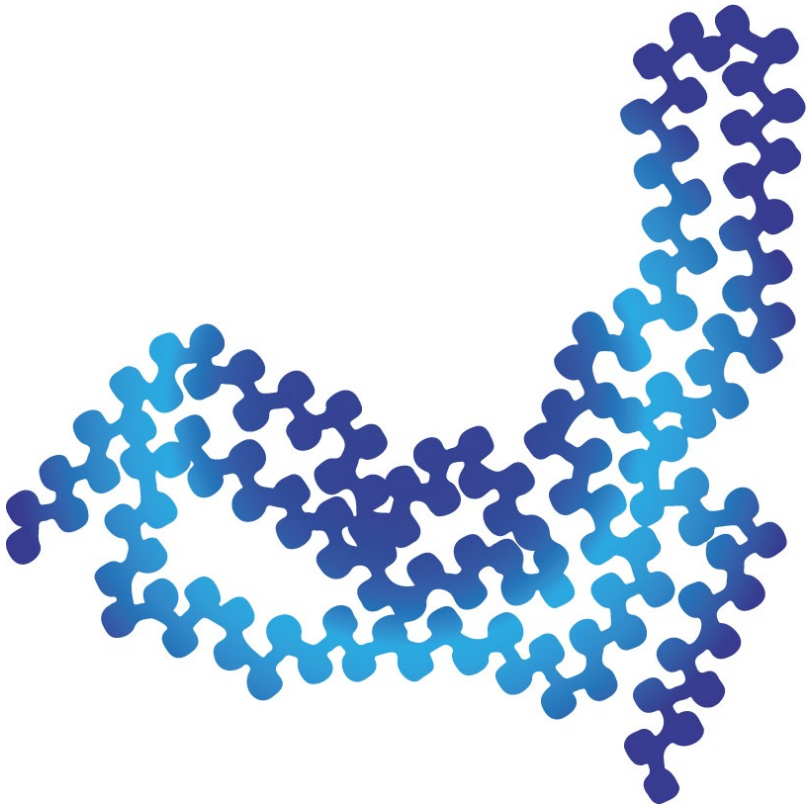
Prion structure and biology Chairs: Raimon Sabaté (UB) + Nuria Lopez Lorenzo (CiMUS)	10:00 - 10:45	**KEYNOTE 5** - Protein Folding Pathways Across Physiology & Therapy, *Dr. Emiliano Biasini, University of Trento, Italy*
	10:45 - 11:00	**ORAL 13** - Antibody binding modulates PrPC dynamics, *Ioana Ilie, University of Amsterdam*
	11:00 - 11:15	**ORAL 14** - Prion protein-based phylogeny inferred from hundreds of species of the class Mammalia shows overall conservation with canonical mammalian phylogenetic tree, *Cristina Sampedro, CIC bioGUNE*
	11:15 - 11:30	**ORAL 15** - Isolation of oligomeric prions from AS, *Ilaria Vani, Istituto Superiore di Sanità*
	11:30 - 12:00	Coffee break & Posters
Prion structure and biology Chairs: Hasier Eraña (CICbioGUNE) + Alba Espargaro (UB)	12:00 - 12:15	**ORAL 16** – A porphyrin with dual anti-prion activity, *Roberto Chiesa, Istituto di Ricerche Farmacologiche Mario Negri*
	12:15 - 12:30	**ORAL 17** - Spectroscopic heterogeneity in recombinant PrPSc prion strains, *Sanaz Sabhezei, Universidade de Santiago de Compostela*
	12:30 - 12:45	**ORAL 18** - Tau Dynamics and Cooperative Interactions: possible relevance for its role as a Microtubule Associated Protein and for its pathological Solid Transition in Neurodegenerative Disease, *Amayra Hernández-Vega, Institute for BioEngineering of Catalonia (IBEC)*
	12:45 - 13:30	**KEYNOTE 6** - Kinetic stabilization of biomolecular condensates by heterotypic interactions, *Dr. Xavier Salvatella, Institute for Research in Biomedicine, Spain*
	13:30 - 13:45	Best poster/oral communication prize
	13:45 - 14:00	Next Meeting anouncement
	14:00 - 15:00	Lunch
	15:00 - 16:00	Human castles workshop
	16:00 - 16:30	Farewell

BOOK OF ABSTRACTS – CONTENT

KEYNOTES AND ORAL PRESENTATIONS



PRION DISEASES IN ANIMALS



KEYNOTE 1

Camel prion disease: a new emerging disease in North Africa

Authors: Laura Pirisinu[3], Amara Abdelkader[1], Babelhadj Baaissa[2], Di Bari Michele Angelo[3], Bruno Rosalia[3], Chiappini Barbara[3], Vanni Ilaria[3], Nonno Romolo[3], Agrimi Umberto[3], Vaccari Gabriele[3], Pirisinu Laura[3]

1 Ecole Nationale de Médecine Vétrinaire de Sidi Thabet, Université Mannouba, Tunis, Tunisia
2 École Normale Supérieure Ouargla, Ouargla, Algeria
3 Istituto Superiore di Sanità, Department of Food safety, Nutrition and Veterinary Public Health, Rome, Italy

Corresponding author: laura.pirisinu@iss.it

In 2018, a new prion disease was identified in dromedary camels in Algeria and later in Tunisia, and named camel prion disease (CPrD).

Evidences obtained from passive surveillance in Algeria as well as the involvement of lymphoid tissue in CPrD pathogenesis concurred in suggesting the contagious nature of this disease, with potential impact on animal and human health.

The world camel population is estimated at almost 39 million heads, 87% of which is found in Africa. Dromedary husbandry is widespread throughout North and Central Africa, the Middle East, Asia and Australia. In some areas intensive camel farming is rapidly increasing. Camels represent vital sources of meat, milk and transportation for millions of people living in the most arid regions of the world.

The emergence of a prion disease in a new species and in new geographical areas requires attention and investigations for understanding the characteristics, the origin and ecology of the disease and the risks in both animals and humans.

The available evidences will be discussed in light of their contribution to understanding the nature of CPrD and developing control strategies to limit its spread in animals and minimise human exposure.

ORAL 1 - Assessment of the goat I/M142 polymorphism in the susceptibility to atypical scrapie prions.

Authors: Natalia Fernández-Borges[1], Sara Canoyra[1], Alba Marín-Moreno[1], Patricia Aguilar-Calvo[1], Enric Vidal[2], Lorenzo González[3], Leonor Orge[4], Sylvie Benestad[5], Romolo Nonno[6], Olivier Andreoletti[7], Juan Carlos Espinosa[1], Juan María Torres[1].

1 Centro de Investigación en Sanidad Animal, CISA-INIA-CSIC, Valdeolmos, Madrid, Spain.
2 Centre de Recerca en Sanitat Animal, Universitat Autònoma de Barcelona (UAB)–Institut de Recerca i Tecnologia Agroalimentàries, Barcelona, Spain.
3 Animal and Plant Health Agency (APHA), Penicuik, Midlothian, UK.
4 Laboratory of Pathology, National Institute for Agrarian and Veterinary Research, 2780-157 Oeiras, Portugal.
5 Norwegian Veterinary Institute, N-0106 Oslo, Norway.
6 Istituto Superiore di Sanitá, Rome, Italy.
7 UMR Institut National de la Recherche Agronomique (INRA)/École Nationale Vétérinaire de Toulouse (ENVT) 1225, Interactions Hôtes Agents Pathogènes, Toulouse, France.

Corresponding author: natalia.fernandez@inia.csic.es

Scrapie is a transmissible spongiform encephalopathy (TSE) naturally affecting sheep, goats, and mouflons that is today endemic in many countries worldwide. Likewise other TSEs, scrapie occurrence is mainly determined by the prion strain and by the host prion protein encoding gene (Prnp). Increased surveillance over the past two decades has led to the identification of a wide variety of scrapie disease phenotypes that are suggestive of a number of scrapie strains and highlight the tremendous heterogeneity of the scrapie disease. In addition, an unusual type of scrapie was identified in 1998 in Norway and was named atypical scrapie Nor-98. Sheep Prnp genotypes V136R154Q171 and A136R154Q171 are associated with a high susceptibility to classical scrapie, while the A136R154R171 genotype is linked to resistance. Interestingly, atypical scrapie more frequently affects sheep carrying classical scrapie resistant genotypes than susceptible ones. In goats, genetic susceptibility to classical scrapie also presents a great genetic variability for the Prnp gene. In this line, recent studies highlighted that isoleucine (I) at codon 142 may increase susceptibility, and methionine (M) at the same codon prolongs the incubation period for classical scrapie in goats but does not confer resistance. Nevertheless, the role of I/M142 polymorphism has not yet been assessed in the resistance/susceptibility of goats to atypical scrapie infection. For this purpose, transgenic mice expressing the goat M142-PrPC variant (M142-Tg541 mice) were intracranial inoculated with a panel of scrapie and BSE isolates and their transmission features were compared to those of the goat I142-PrPC variant (WT-Tg501 mice). M142-Tg541 mice were fully susceptible to all BSE isolates regardless of the primary sequence of the isolate (cattle, sheep, or goat), as previously observed in WT-Tg501 mice. Susceptibility of M142-Tg541 mice was also identified in most of the tested classical scrapie isolates, albeit with significantly longer survival time than those observed in WT-Tg501 mice. Interestingly, M142-Tg541 mice were completely resistant to the primary transmission of the whole panel of atypical scrapie isolates. In fact, none of these mice showed any sign of neurological disease until the end of their lifespans and no PrPres was detected in their brains by western blot and immunohistochemical analysis. These results suggest that the I/M142 polymorphism might play a determinant role in susceptibility to atypical scrapie infection. To our knowledge, this is the first time that a goat PrPC variant has been associated with greater resistance to atypical scrapie, a finding that may have important implications in the control of this disease in goat herds.

Funding: - Project PID2019-105837RB-I00. - Project UE-FP6-2005-FOOD4B-036353

ORAL 2 - Innate immune status of glia and prion replication

Authors: Sang-Gyun Kang [1,2], Chiye Kim [1,3], Judd Aiken [*1,4] and Debbie McKenzie [*1,3]

1 Centre for Prions and Protein Folding Diseases, University of Alberta, Edmonton, AB Canada.
2 Department of Medicine
3 Department of Biological Sciences
4 Department of Agriculture, Food and Nutritional Sciences, University of Alberta, Edmonton, AB, Canada.

Corresponding author: sanggyun@ualberta.ca

The prominent pathological change in prion-affected brains is neuroinflammation. We investigated the impact of innate immune responses on prion replication in vitro. Glial cells from hamster cerebellum were susceptible to HY, but resistant to DY strain as determined by western blot analysis and animal bioassay. Glial cells from cerebral cortex were, however, refractory to both strains. Priming glial cells with lipopolysaccharide decreased prion replication, whereas pre-treatment with dexamethasone, inhibiting innate immunity, increased susceptibility to DY infection. Our results suggest that neuroinflammation resulting from prion infection might be an attempt to resolve prion propagation.

ORAL 3 - sPMCA conversion efficacies of Norwegian prions

Authors: Erez Harpaz[1], Tram Thu Vuong[2], Linh Tran[2], Michael Andreas Tranulis[3], Sylvie L. Benestad[2] and Cecilie Ersdal[1]

1 Department of Production Animal Clinical Sciences, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Svebastadveien 112, 4325 Sandnes, Norway
2 Norwegian Veterinary Institute, P.O. box 64, 1431 Ås, Norway
3 Department of Preclinical Sciences and Pathology, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Universitetstunet 3, 1433, Ås, Norway

Corresponding author: cecilie.ersdal@nmbu.no

We investigated prion conversion of Norwegian CWD isolates and classical scrapie by sPMCA. Brain homogenates from several cervids and small ruminants of different PRNP were used as substrates.

The reindeer CWD seed had the highest conversion efficacy (63%), and scrapie converted 60% of the substrates. Notably, the reindeer seed efficiently converted only the most susceptible AA-reindeer substrate and none of the other reindeer substrates from animals with less susceptible PRNP genotypes. The scrapie seed also had an early conversion of the reindeer AA-substrate. The moose and red deer seeds converted fewer substrates.

This investigation suggests that prions derived from reindeer and sheep have a potential for spillover. There is no reports of CWD in roe deer, but all seeds were amplified with roe deer brain.

ORAL 4 - Atypical scrapie evolution during propagation in different PrPC subtract both in vitro and in vivo.

Authors: Sara Canoyra[1], Alba Marín-Moreno[1], Juan Carlos Espinosa[1], Natalia Fernández-Borges+, Nuria Jerez-Garrido[1], Sylvie Benestad[2], Enric Vidal[3], Leonor Orge[4], Olivier Andreoletti[5] and Juan María Torres[1].

1 Centro de Investigación en Sanidad Animal, CISA-INIA-CSIC, Valdeolmos, Madrid, Spain.
2 Norwegian Veterinary Institute, N-0106 Oslo, Norway.
3 Centre de Recerca en Sanitat Animal, Universitat Autònoma de Barcelona (UAB)–Institut de Recerca i Tecnologia Agroalimentàries, Barcelona, Spain.
4 Laboratory of Pathology, National Institute for Agrarian and Veterinary Research, 2780-157 Oeiras, Portugal
5 UMR Institut National de la Recherche Agronomique (INRA)/École Nationale Vétérinaire de Toulouse (ENVT) 1225, Interactions Hôtes Agents Pathogènes, 31076 Toulouse, France

Corresponding author: jmtorres@inia.es (+34916202300)

Intra- or cross-species transmission of prions can lead to the emergence of new prion strains due to changes in their conformational characteristics. The process of prion strain emergence during species barrier propagation is explained by two non-mutually exclusive theories: the 'deformed templating' or mutation model, and the 'conformational selection' model. The conformational shift or mutation theory suggests that the new host's inability to replicate the prion forces a shift in the PrPSc conformation, resulting in the emergence of new prion strains de novo. However, the 'conformational selection' theory suggests that prion isolates consist of a conglomerate of PrPSc conformations, among which the energetically favourable conformation predominates. During cross-species transmission, the species barrier acts as a filter that selects other PrPSc conformers. Previous studies have shown that the transmission of atypical scrapie (AS) onto bovine PrP led to the emergence of the bovine spongiform encephalopathy agent (c-BSE). We analysed the differential strain characteristics of thermostability and in vitro prion amplification by PMCA in the AS transmission to examine the evolutionary dichotomy. Our findings suggest that conformational mutation is the primary mechanism responsible for BSE-C emergence. Moreover, we analysed the possible evolution of the AS prion without the species barrier pressure by modelling the transmission in a homologous ovine PrP context. Preliminary results in the ovine ARQ-PrP transgenic mice model the transmission of AS prion in vivo led to the emergence of 19K, 21K or atypical prions and mixtures of these agents.

Funding:
- Project PID2019-105837RB-I00 MCIN/ AEI /10.13039/501100011033
- Fundación La Marató de TV3 Enfermedades Infecciosas 201821-31

ORAL 5 - In vitro modelling of cervid prion strain emergence and evolution considering all the described polymorphic variants

Authors: Carlos M. Díaz-Domínguez[1,2], Hasier Eraña[1,3], Jorge M. Charco[1,3], Cristina Sampedro-Torres-Quevedo[1], Nuno Gonçalves-Anjo+, Josu Galarza-Ahumada[1], Maitena San-Juan-Ansoleaga[1], Eva Fernández-Muñoz[1], Nuria L. Lorenzo[5], Samanta Giler[6], Enric Vidal[6], Mariví Geijo[7], Glenn Telling[8], Jesús R. Requena[5], Joaquín Castilla[1,2,9]

1 Centre for Cooperative Research in Biosciences (CIC bioGUNE), Basque Research and Technology Alliance (BRTA), Bizkaia Technology Park, 48160, Derio, Spain.
2 Centro de Investigación Biomédica en Red de Enfermedades infecciosas, Carlos III National Health Institute, Madrid, Spain.
3 ATLAS Molecular Pharma S. L. Bizkaia Technology Park, 48160 Derio, Spain.
4 Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes and Alto Douro (UTAD), 5000-801 Vila Real, Portugal.
5 CIMUS Biomedical Research Institute & Department of Medicine, University of Santiago de Compostela-IDIS, 15782, Santiago de Compostela, Spain.
6 Centre de Recerca en Sanitat Animal (CReSA) – Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Campus de UAB, 08193 Bellaterra, Barcelona, Catalonia.
7 Animal Health Department, NEIKER-Basque Institute for Agricultural Research and Development, Basque Research and Technology Alliance (BRTA), Bizkaia Technology Park, 48160 Derio, Spain.
8 Prion Research Center (PRC), Colorado State University, Fort Collins, Colorado, USA.
9 IKERBASQUE, Basque Foundation for Science, Prion Research Lab, 48011 Bilbao, Spain.

Corresponding author: cdiaz@cicbiogune.es

The misfolding event by which PrPC turns into PrPSc can be spontaneous or induced by an exogenous agent. Regardless of the underlying cause, it is known that the presence of amino acid substitutions or polymorphisms in the PrP can affect the misfolding event. Moreover, numerous polymorphisms that promote spontaneous misfolding have been described and linked to the development of familial or hereditary forms of prion disease. As for the acquired prion diseases, the presence of amino acid variations between the PrPC from the host and the PrPSc from the donor can modify the prion strain characteristics upon transmission, including the host range. Thus, the possibility of emergence of new prion strains could be proportional to the amount of different polymorphic variants of a given PrP.

Chronic wasting disease, the prion disease affecting cervids is nowadays widely extended along the USA and Canada, with a few cases described in South Korea. Furthermore, it has also been recently detected in Norway, Finland, and Sweden. The characteristic high horizontal transmission capacity of chronic wasting disease, together with the numerous polymorphisms described in cervid PrP, could give rise to the emergence of new prion strains, as has been illustrated by those cases described in Northern Europe, different from the known American strains.

In this context and with the aim of analysing the effect of the distinct polymorphic PrP variants from cervids on the emergence of different strains, the PMSA (Protein Misfolding Shaking Amplification) technique was used to obtain distinct conformers. These conformers, which were obtained through spontaneous in vitro misfolding of the most frequent phenotypes of deer and elk PrP, were classified as different prion strains according to their biochemical properties. After proving that they showed all the features expected from bona fide prions, including the ability to induce a prion disease in animal models, we performed in vitro spontaneous misfolding experiments with every polymorphic variant of cervid PrP to evaluate their effect in the generation of different prion strains. The same in vitro prion propagation system was also used to assess the impact of residue 226 of cervid PrP in the evolution of the recombinant cervid prion strains generated previously, simulating their propagation to the most common genotypes in the wild. Thus, in the present study we propose a systematic procedure for

modelling the emergence and evolution of prion strains based on the in vitro misfolding and propagation of the polymorphic variants of cervid PrP, which could help to understand and predict the emergence of new cervid prion strains.

ORAL 6 - Vaccines for chronic wasting disease

Authors: Hermann M. Schatzl[1,2], Scott Napper[3,4], Sabine Gilch[1,2]

1 Calgary Prion Research Unit & Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada.
2 Hotchkiss Brain Institute & Snyder Institute for Chronic Diseases, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada.
3 Vaccine and Infectious Disease Organization (VIDO), University of Saskatchewan, Saskatoon, SK, Canada.
4 Department of Biochemistry, Microbiology and Immunology, University of Saskatchewan, Saskatoon, SK, Canada.

Corresponding author: hschaetz@ucalgary.ca

Prion diseases are strictly fatal infectious neurodegenerative disorders and prototypic conformational diseases, caused by the conformational conversion of the normal cellular prion protein (PrP^{C}) into the pathological PrP^{Sc} isoform. Examples are scrapie in sheep and goat, bovine spongiform encephalopathy (BSE) in cattle, chronic wasting disease (CWD) in cervids, and Creutzfeldt-Jacob disease (CJD) in humans. There are no therapies available, and animal prion diseases like BSE and CWD can negatively affect economy, ecology, animal health, and possibly human health. BSE is a confirmed threat to human health and mounting evidence supports the zoonotic potential of CWD. CWD is continuously expanding in North America in numbers and distribution, and was recently identified in Scandinavian countries. Incidence of CWD in Alberta is now reaching 15% in mule deer in hunting areas doing mandatory CWD testing, and 5% in white-tailed deer WTD). Caribou are still free of CWD, but transmission into caribou is likely to happen naturally under current conditions. CWD is the only prion disease occurring both in wild and farmed animals, which, together with extensive shedding of infectivity into the environment, impedes containment strategies. There is currently a strong push to develop vaccines against CWD, including ones that can be used in wildlife. The immune system does not develop a bona fide immune response against prion infection, as PrP^{C} and PrP^{Sc} share an identical protein primary structure, and prions seem not to represent a trigger for immune responses. This asks for alternative vaccine strategies, which focus on PrP^{C}-directed self-antibodies or exposure of disease-specific structures and epitopes.

Our groups have pioneered prion vaccines and established a proof-of-concept that vaccination is effective and can provide protection. Ongoing work, often with partners and within consortia, is testing vaccine efficacy (oral and parenteral) and effects on prion shedding in various animal models, including novel knock-in (KI) mice, reindeer and white-tailed deer.

The problem areas we currently address:

a) **Reduction of the negative impact of CWD on cervid populations by active vaccination and genetic selection,** creation of buffer zones to reduce CWD spread, and vaccination of caribou populations. This will positively impact cervid populations, ecosystem health, and First and Northern communities' food security.
b) **Reduction of CWD prion shedding into environment**, which reduces the infectious load in the environment, helps to slow the spread of CWD and the risk of cervids to get infected.
c) **Reduction of the risk for humans to get infected by zoonotic infection**. Even if the risk for humans might be minimal at the moment, CWD prions change and the more CWD prions have access to the human food chain, the higher this risk will be in the future. Caribou and other cervid species are essential components of human diet, and various parts of the Canadian population rely on them. CWD testing is problematic in many areas, so we have to make sure the risk to humans is minimal.

Main experimental platforms:

Vaccine formulation and delivery: Vaccines target either disease-specific epitopes or the normal isoform of the prion protein. Vaccines have complementary targets and can be used alone or in

combination. Adenovirus or vaccinia virus-based vectors and nanoparticles will be used for delivery to mice and cervids, delivery systems with the potential to work in wildlife.

CWD challenge and mechanisms of protection: Oral and parenteral vaccination will be compared, in mice and cervids challenged orally or peripherally with CWD prions. Mice and cervids will be monitored for side effects. As read-outs, we use incubation time to clinical disease and production of humoral immune responses. Antibodies will be tested for CWD neutralization capacity in cell culture and prion conversion assays. We have developed new knock-in mouse models that recapitulate accurately CWD pathogenesis as found in cervids, which is not the case with current transgenic mouse models. Since KI mice shed CWD prions into urine and feces, we can study in mice whether vaccination also reduces shedding of CWD infectivity. Vaccine efficacy is tested in reindeer and WTD. CWD infection of WTD is done by environmental exposition on CWD-contaminated pastures, with a controlled experimental indoor vaccination/challenge study done in parallel at the prion center in Fort Collins, CO, USA.

Taken together, our work will result in measures that help to protect cervid populations against CWD infection. It also will reduce the risk of zoonotic transmission to humans in the future and preserve food security and food safety for Northern and Indigenous populations.

KEYNOTE 2

Thirty years of cohabitation with prion diseases: my personal experience

Author: Martí Pumarola[1]

1 Professor of Pathology. Department of Animal Medicine and Surgery. Universitat Autònoma de Barcelona
Corresponding author: marti.pumarola@uab.cat

In my talk I'll summarize my personal point of view about the main events that I've been in contact with along the last thirty years related to prion diseases. In 1991 I made contact for the first time with prion diseases during my stage at the Neurology Institute of the University of Bern (Switzerland), where I was directly involved in the diagnose of Bovine Spongiform Encephalopathy (BSE) cases. Back in Barcelona and supported by the Public Health Department of Catalan government, we started a local surveillance programme (1996-2000) with the collaboration and supervision of the University of Zaragoza (Dr J. Badiola). In 2000, when the first two cases of BSE were detected in Spain, I was commissioned to participate in the TSE Surveillance Programme in Catalonia by the Catalan Government, in close collaboration with the human counterpart (Dr I. Ferrer). Therefore, we created PRIOCAT, the prion lab for rapid testing and confirmatory immunohistochemical techniques. This laboratory was a member of the Spanish network of official prion laboratories. In 2005, PRIOCAT was transferred to the new building of the Centre de Recerca en Sanitat Animal (CReSA), whose high biocontainment facilities (BSL3) allowed us to work properly with such dangerous material. Using samples collected from local cases, we established the Bank of Animal Tissues of Catalonia (BTAC, 2002), an open facility where samples were stored and made available for scientific research.

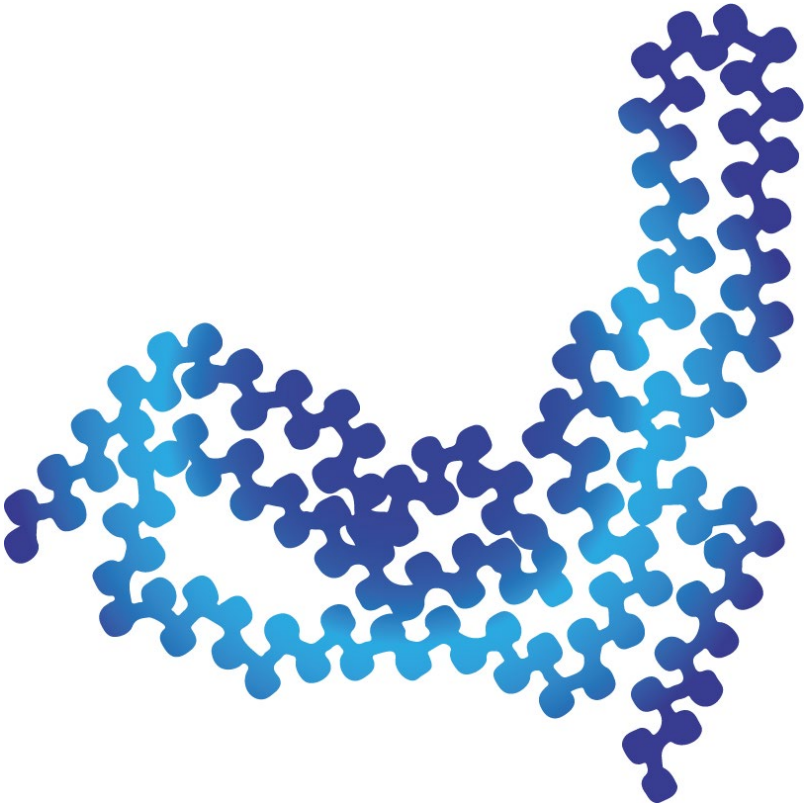
The research activities developed in the aforementioned structures, financed by Spanish and European research projects, have generated multiple doctoral thesis, scientific papers, book chapters, oral communications, and posters at many national and international meetings. In parallel, we have organized multiple seminars and workshops to provide up to date information and training to our social promoters, health authorities and veterinarians, from students to health professionals, from farmers to consumers, etc.

We have obtained all these results thanks to the human ingredient, especially veterinarians and biologists, pre- and post- doctorate scientists, technicians, undergraduate students, etc, that have been working and collaborating in our lab along these years. All of them have become essential and fundamental to do it.

From 2010 our work has moved on to other neuropathological fields in veterinary medicine, but we have continued participating and collaborating with the responsible of PRIOCAT (Dr E. Vidal) in the research activities.

A long, challenging but absolutely useful experience that has trained me personally, in collaboration with multidisciplinary specialized groups, in all professional fields, from research, to science teaching and communication activities in different environments.

PRION AND PRION-LIKE DISEASES IN HUMANS



KEYNOTE 3

Strain-specific lesioning patterns in sporadic CJD - a neuropathological perspective

Authors: Ellen Gelpi[1]

1 Division of Neuropathology and Neurochemistry, Department of Neurology, Medical University of Vienna, and Austrian Reference Center for Human Prion diseases

Corresponding author: ellen.gelpi@meduniwien.ac.at

The clinical and neuropathological phenotype of human prion diseases, particularly of sporadic CJD, is modulated and defined by the methionine/valine polymorphism at PRNP codon 129 and the glycosylation pattern of PrPres. The different strain-related phenotypes (M1, V1, M2, V2 in homo- or heterozygosis) will be presented from a neuropathological perspective with particular emphasis on the lesion profile and PrP deposition patterns across different neuroanatomical regions, responsible of clinical manifestations.

ORAL 7 - Human prion strain discrimination by RT-QuIC

Authors: Alba Marín-Moreno[1], Fabienne Reine[1], Laetitia Herzog[1], Human Rezai[1], Vincent Beringue[1], Davy Martin[1]

1 Université Paris-Saclay, INRAE, UVSQ, VIM, 78 350 Jouy-en-Josas, France

Corresponding author: alba.marin-moreno@inrae.fr

The most common human prion disease is sporadic Creutzfeldt-Jakob (sCJD). Depending on Prion protein polymorphisms or neuropathological features for instance, experimental transmission of sCJD cases to animal models suggests the existence of different sCJD strains (>10 molecular subtypes).

RT-QuIC measures the capacity of PrPSc seeds to convert recombinant PrP substrate into thioflavin-T fluorescently positive amyloids in vitro.

Here, we report using a panel of recombinant PrP substrates that RT-QuIC discriminates human prion strains, even some barely differentiable by bioassay. Thus RT-QuIC may be a promising tool to study sCJD strain diversity and to rapidly identify strains in patients.

ORAL 8 - Gene therapy for sCJD in a humanized mouse model

Authors: Jun Zhang[1], Manuel Camacho[1], Cameron Choi[1], Victoria Warady[1], Qingzhong Kong[1,2,3]

1 Department of Pathology, Case Western Reserve University, Cleveland, Ohio, USA
2 Department of Neurology, Case Western Reserve University, Cleveland, Ohio, USA
3 National Prion Disease Pathology Surveillance Center, Case Western Reserve University, Cleveland, Ohio, USA

Corresponding author: qxk2@case.edu

Creutzfeldt-Jakob disease (CJD) is the most common prion disease in humans, which progresses rapidly, is always fatal, and has no treatments. Effective and long-lasting therapeutics against CJD and other human prions is urgently needed.

The cellular prion protein (PrP) is essential for both prion replication and prion pathogenesis but not required for life or cell survival, making it an attractive target for prion therapeutics development. Knocking out or knocking down the PrP gene expression with siRNA/shRNA or antisense oligonucleotides (ASOs) targeting the PrP gene has been shown to be safe and effective against rodent prions in mouse models. But the ASO and shRNA/siRNA approaches have not been tested against human prions, and there has been no report of rAAV-based shRNA/siRNA gene therapy for any prion disease.

We tested 4 shRNAs that each knocked down human PrP by ~90% in a modified M17 human neuroblastoma cell line, and one rAAV-shRNA administered retro-orbitally at near clinical onset knocked down human PrP by ~26% in the brain and led to a significant extension of survival in sCJDMM1-inoculated Tg40h mice overexpressing human PrP-129M. Our data indicate that rAAV-based gene therapy via systematic shRNA/siRNA delivery is a promising and realistic approach for development of effective CJD treatment.

ORAL 9 - A robust method for evaluation of prionicide treatments

Authors: Laetitia Herzog[1], Fabienne Reine[1], Mohammed Moudjou[1], Roger Bonnet[2], Human Rezaei[1], Vincent Béringue[1] and Angélique Igel[1,2]

1 Université Paris Saclay/INRAE/UVSQ, VIM, Jouy-en-Josas, France
2 FB.Product, Torcé viviers en charnie, France

Corresponding author: angelique.igel@inrae.fr

Prions are a class of infectious agents that challenge sterilization processes with no prophylactic or curative treatment. Recent data and new guidelines imposed by various government agencies have highlighted the urgent need for a prionicide method efficient to treat prion-contaminated medical equipment/devices. In this work, we aimed to compare two humanized bioassays with their PMCA counterparts to propose a robust method for evaluating decontamination process.

To assess effectiveness of decontamination procedures, stainless steel wires were contaminated with three different prion strains : variant CJD subtype (vCJD), sporadic CJD subtype VV2 (sCJD-VV2) and the conventional reference 263K. After treatment with various prionicide protocols, residual prion seeding-activity present in the surface of the wire and in waste-water were quantified using bioassay and/or ultra-sensitive PMCA.

We demonstrated that the sCJD-VV2 prion strain appears more resistant to inactivation process than vCJD prion and we validated here a first prionicide procedures efficient to inactivate a panel of human prion strains. The use of this formulation, called TFD Premium, in presoaking or in washing machine made this process accessible to all sterile processing departments and laboratories.

ORAL 10 - Neuronal networks in absence of the prion protein

Authors: Anna Burato[1], Lorenca Sadiraj[2], Matteo Manzati[2], Beatrice Pastore[1,2], Marco Zattoni[1], Giuseppe Legname[1*], Michele Giugliano[2*]

1Laboratory of Prion Biology, Department of Neuroscience, SISSA, Via Bonomea 265, 34136 Trieste, Italy
2 Neuronal Dynamics Laboratory, Department of Neuroscience, SISSA, Via Bonomea 265, 34136 Trieste, Italy
*Author to whom correspondence should be addressed

Corresponding author: aburato@sissa.it

The cellular form of the prion protein (PrPC) is a ubiquitously expressed protein that, besides its role in prion diseases, exerts a plethora of physiological functions. We hypothesize that manipulating the protein function could modulate both neuronal excitability and synaptic transmission, thus unveiling the role of PrPC at microcircuit and network levels: the aim is to observe and quantify electrical activity alterations (spontaneous and evoked) in cultured neuronal networks developing ex vivo as a result of PrPC knock out. We employed substrate-integrated MicroElectrode Arrays (MEAs) to detect non-invasively the extracellular electrical activity of large networks of cultured primary neurons. Preliminary evidence revealed some differences between PrP+/+ and PrP-/- mice, both in terms of duration and occurrence rate of episodic spontaneous synchronous electrical events.

ORAL 11 - The small aromatic compound SynuClean-D inhibits the aggregation and seeded polymerization of multiple α-synuclein strains

Authors: Samuel Peña-Díaz[1,2,‡], Jordi Pujols[1,2,‡], Eftychia Vasili[3,4], Francisca Pinheiro[1,2], Jaime Santos[1,2], Zoe Manglano-Artuñedo[1,2], Tiago F. Outeiro[3,4,5,6], and Salvador Ventura[1,2,7,*]

1 Institut de Biotecnologia i Biomedicina. Universitat Autonoma de Barcelona, Bellaterra, Spain;
2 Departament de Bioquimica i Biologia Molecular. Universitat Autonoma de Barcelona, Bellaterra, Spain;
3 Department of Experimental Neurodegeneration, Center for Biostructural Imaging of Neurodegeneration, University Medical Center Göttingen, Göttingen, Germany;
4 Max Planck Institute for Experimental Medicine, Göttingen, Germany
5 Translational and Clinical Research Institute, Faculty of Medical Sciences, Newcastle University, Framlington Place, Newcastle Upon Tyne, Newcastle, United Kingdom
6 Scientific Employee With a Honorary Contract at Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE), Göttingen, Germany
7 ICREA, Passeig Lluis Companys 23, Barcelona, Spain
‡ These authors contributed equally to this work.

Corresponding author: salvador.ventura@uab.es

Parkinson's disease is a neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra, as well as the accumulation of intraneuronal proteinaceous inclusions known as Lewy bodies and Lewy neurites. The major protein component of Lewy inclusions is the intrinsically disordered protein α-synuclein (α-Syn), which can adopt diverse amyloid structures. Different conformational strains of α-Syn have been proposed to be related to the onset of distinct synucleinopathies; however, how specific amyloid fibrils cause distinctive pathological traits is not clear. Here, we generated three different α-Syn amyloid conformations at different pH and salt concentrations and analyzed the activity of SynuClean-D (SC-D), a small aromatic molecule, on these strains. We show that incubation of α-Syn with SC-D reduced the formation of aggregates and the seeded polymerization of α-Syn in all cases. Moreover, we found that SC-D exhibited a general fibril disaggregation activity. Finally, we demonstrate that treatment with SC-D also reduced strain-specific intracellular accumulation of phosphorylated α-Syn inclusions. Taken together, we conclude that SC-D may be a promising hit compound to inhibit polymorphic α-Syn aggregation.

ORAL 12 - The structural architecture of an α-synuclein toxic oligomer

Authors: Jaime Santos[1], Jorge Cuellar[2†], Irantzu Pallarès[1†], Emily J Byrd[3], Alons Lends[4], Fernando Moro[5], Muhammed Bilal Abdul-Shukkoor[4], Jordi Pujols[1], Lorea Velasco-Carneros[5], Frank Sobott[3], Daniel E Otzen[6], Antonio N Calabrese[3], Arturo Muga[5], Jan Skov Pedersen[7], Antoine Loquet[4], Jose María Valpuesta[2], Sheena E Radford[3] and Salvador Ventura[1*]

1 Institut de Biotecnologia i Biomedicina and Departament de Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona; Bellaterra, Barcelona, Spain
2 Department of Macromolecular Structures, Centro Nacional de Biotecnología (CNB-CSIC); Madrid, Spain
3 Astbury Centre for Structural Molecular Biology, School of Molecular and Cellular Biology, Faculty of Biological Sciences, University of Leeds; Leeds, LS2 9JT, UK
4 Univ. Bordeaux, CNRS, Bordeaux INP, CBMN, UMR 5248, IECB; Pessac, France
5 Instituto Biofisika (UPV/EHU, CSIC) y Dpto. de Bioquímica y Biología Molecular, Facultad de Ciencia y Tecnología, Universidad del País Vasco; Barrio Sarriena S/N, 48940 Leioa, Spain
6 Interdisciplinary Nanoscience Center (iNANO) and Department of Molecular Biology and Genetics, Aarhus University; Gustav Wieds Vej 14, 8000 Aarhus C, Denmark
7 Interdisciplinary Nanoscience Center (iNANO) and Department of Chemistry, Aarhus University; Gustav Wieds Vej 14, 8000 Aarhus C, Denmark

Corresponding author: samuel.pdiaz@gmail.com

Oligomeric species populated during α-synuclein aggregation are considered key drivers of neurodegeneration in Parkinson's disease. However, their structure and the molecular determinants driving their conversion to fibrils remain elusive. In this work, we determined the symmetry and architecture of α-synuclein oligomers, dissecting the conformational properties of individual chains within these toxic assemblies. We demonstrate that the NAC domain is insufficient to promote oligomer to fibril conversion; instead, this transition is controlled by a short α-synuclein N-terminal motif. A missense mutation causing early-onset Parkinson's disease remodels this N-terminal region conformation, which results in a population of long-lived oligomers less susceptible to disaggregation by the human Hsp70 machinery. Our results provide a structural understanding of oligomer to amyloid conversion and identify targets for therapeutic intervention.

KEYNOTE 4

Interventional clinical trials in human prion diseases: is now the time?

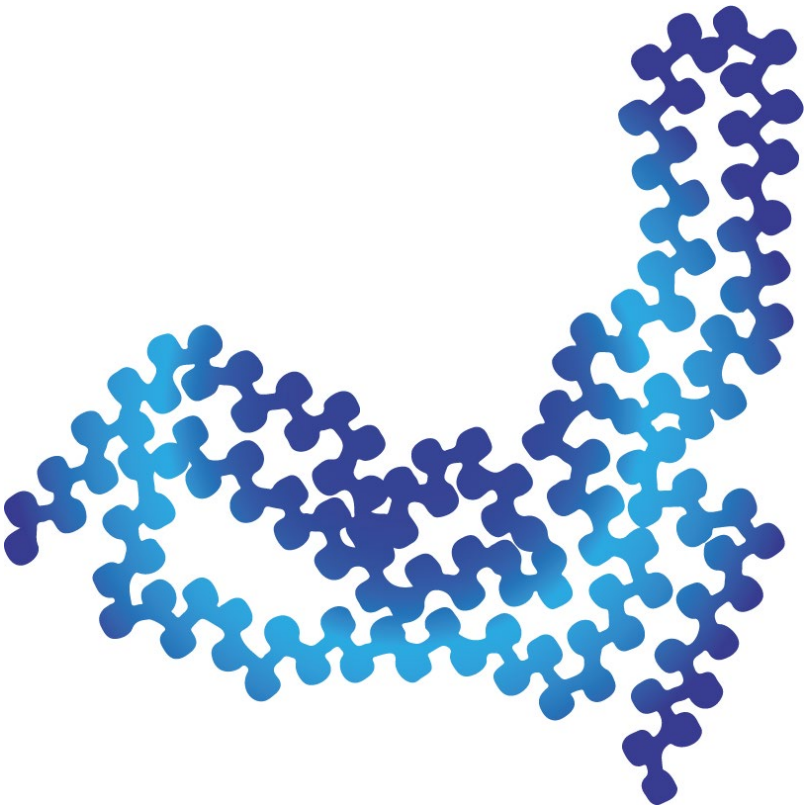
Authors: Raquel Sánchez-Valle[1]

1 Alzheimer's Disease and Other Cognitive Disorders Unit, Department of Neurology, Hospital Clínic, Institut d'Investigació. Biomèdica August Pi i Sunyer, University of Barcelona, Barcelona, Spain

Corresponding author: RSANCHEZ@clinic.cat

In this talk, we will summarize and discuss the advances, opportunities and challenges in clinical trials in human prion diseases in the context of the new era of clinical trials in other neurodegenerative diseases.

PRION STRUCTURE AND BIOLOGY



KEYNOTE 5

Interventional clinical trials in human prion diseases: is now the time?

Authors: Emiliano Biasini[1]

1 Department CIBIO, University of Trento, Via Sommarive 9, 38121 Trento (TN), Italy

Corresponding author: emiliano.biasini@unitn.it

Protein expression and function in eukaryotic cells are tightly harmonized processes modulated by the combination of different layers of regulation, including transcription, processing, stability, and translation of messanger RNA, as well as assembly, maturation, sorting, recycling, and degradation of polypeptides. The integration between all these pathways and the protein quality control machinery, deputed to avoid the production and accumulation of aberrantly folded proteins, determines protein homeostasis. Recent computational advancements in the simulation of biochemical processes open the possibility of investigating biological mechanisms with physics-based models. One of these methods, called Bias Functional, allows the reconstruction of protein folding and misfolding pathways at an atomistic level of resolution.1,2 By coupling this innovative computational technology with rigorous experimental techniques, we discovered the existence of functional, nonnative metastable states (folding intermediates) transiently appearing along the folding process of several proteins. We collected evidence indicating that protein folding intermediates could play a role in disparate biological processes, including post-translational regulation3 and host-pathogen interactions. Inspired by such an unexpected biological paradigm, we designed a novel drug discovery approach to selectively suppress target proteins by impairing their folding process rather than targeting their native conformations (named Pharmacological Protein Inactivation by Folding Intermediate Targeting, PPI-FIT).4,5 PPI-FIT was employed for the first time to identify a pharmacological degrader of the cellular prion protein (PrP), a cell surface glycoprotein playing a central role in fatal and transmissible neurodegenerative pathologies known as prion diseases.5,6 Our data reveal a previously unappreciated role for folding intermediates in the regulation of protein homeostasis and directly support the concept of modulating the expression of virtually any protein by acting on folding pathways.

References:

1. Beccara, S. A., Fant, L. & Faccioli, P. Variational scheme to compute protein reaction pathways using atomistic force fields with explicit solvent. Phys. Rev. Lett. 114, 098103 (2015).
2. Spagnolli, G. et al. Full atomistic model of prion structure and conversion. PLoS Pathog. 15, e1007864 (2019).
3. Spagnolli, G., Massignan, T., Astolfi, A. et al. Pharmacological protein inactivation by targeting folding intermediates. BioRxiv doi: https://doi.org/10.1101/2020.03.31.018069
4. Spagnolli, G., Massignan, T., Astolfi, A. et al. Pharmacological inactivation of the prion protein by targeting a folding intermediate. Commun Biol 4, 62 2021).
5. Faccioli, P. and Biasini, E. Patent n. PCT/IB2019/056371, A method for identifying folding intermediates of proteins.
6. Biasini, E. Barreca, ML. Faccioli, P. and Mancini, I. Patent n. WO/2021/191883, Small molecules inducing the degradation of the cellular prion protein.

ORAL 13 - Antibody binding modulates PrPC dynamics

Authors: Iona Ilie[1], A. Caflisch[1]

1 Van't Hoff Institute for Molecular Sciences, University of Amsterdam, P.O. Box 94157, 1090 GD Amsterdam, The Netherlands

Corresponding author: i.m.ilie@uva.nl

We investigate the influence of a neurotoxic and a neuroprotective antibody with similar epitopes, on the flexibility and the interaction with the membrane of PrPC by molecular dynamics simulations. Results show that antibody binding limits the range of orientations of the GD with respect to the membrane and decreases the distance between PrPC and membrane. Furthermore, the GD flexibility (Ilie & Caflisch 2022) and the interactions of the flexible tail and the GD are modulated differently by the two antibodies (Ilie et al. 2022).

ORAL 14 - Prion protein-based phylogeny inferred from hundreds of species of the class Mammalia shows overall conservation with canonical mammalian phylogenetic tree

Authors: Cristina Sampedro-Torres-Quevedo[1], Hasier Eraña[1,2,3], Jorge M. Charco[1,2,3], Carlos M. Díaz-Domínguez[1,3], Maitena San Juan-Ansoleaga[1], Eva Fernández-Muñoz[1], Ana R. Cortazar[1], Roberto F. Nespolo[4,5,6], Julian F. Quintero-Galvis[4], Africa Manero-Azua[7], Diego Polanco-Alonso[7], Guiomar Perez de Nanclares[7], Ana M. Aransay[1] & Joaquín Castilla[1,3,8]

1 Center for Cooperative Research in Biosciences (CIC bioGUNE), Basque Research and Technology Alliance (BRTA), Bizkaia Technology Park, 48160, Derio, Spain.
2 ATLAS Molecular Pharma S. L. Bizkaia Technology Park, 800, 48160 Derio, Spain.
3 Centro de Investigación Biomédica en Red de Enfermedades Infecciosas (CIBERINFEC), Carlos III National Health Institute, Madrid, Spain.
4 Instituto de Ciencias Ambientales y Evolutivas, Universidad Austral de Chile, Valdivia. Chile.
5 Center of Applied Ecology and Sustainability (CAPES), Facultad de Ciencias Biológicas, Universidad Católica de Chile, Santiago 6513677, Chile.
6 Millenium Institute for Integrative Biology (iBio), Santiago, Chile.
7 Molecular (Epi)Genetics Laboratory, Bioaraba Health Research Institute, Araba University Hospital, Vitoria-Gasteiz, Araba, Spain.
8 IKERBASQUE, Basque Foundation for Science, Prion Research Lab, 48011 Bilbao, Spain.

Corresponding author: csampedro@cicbiogune.es

Despite the similarity between amino acid sequences of the prion protein (PrP) across mammalian species, some present much lower susceptibility to prion diseases than others do. The key to this behaviour likely lays on the enhanced resistance of these animal species' prion proteins to acquire a bona fide prion conformation. Therefore, it is conceivable that it is the tertiary structure and interspecific variations ultimately encoded in the primary structure, what determines the proneness of a PrP to misfolding. For this reason, we are interested in analysing the differences in PrP sequence between mammalian species.

Phylogeny has come a long way since the 19th Century when it was entirely based upon morphological features. Subsequently, the use of gene sequences to infer phylogenetic relationships was put forward and trees were generated using distinct proteins. A combination of mitochondrial DNA and nuclear genes has been used to establish the current phylogeny of species, which is nevertheless susceptible to change. We are interested in looking at the PRNP gene from a phylogenetic perspective, as we believe we could find evolutionary events of interest potentially related to prion diseases.

We thus generated a database of hundreds of mammalian PrP sequences and constructed phylogenetic trees based on amino acid sequence variations. To this aim, 922 protein sequences (from residue 90 approximately to the C-ter) from 655 different mammalian species were aligned using CLUSTALW. After the alignment, identical sequences were merged in order to reduce background noise, leaving 842 different sequences. Trees were generated using the Bayesian Evolutionary by Sampling Trees package (BEAST) using the best-fit model identified by MEGA X (the general time-reversible model (GTR), with a proportion of invariable sites (+I) and rate of variation across sites (+G)). Analysis were run for 10,000,000 generations, sampling every 5,000 chains under a relaxed log normal clock model and Yule model. Three independent runs were combined in LogCombiner. The tree was then analysed and branches that did not have a high bootstrap value were combined into phylogenetically supported degenerated consensus sequences, leaving us with 440 different sequences. A tree was then again generated as previously described and posterior result analysis was done grouping species by orders.

Our results show a strong tendency of the classical phylogenetic orders to maintain their clusterization when using the PRNP gene as a readout of phylogenetic similarity. Despite the fact that only one gene was used to generate the phylogenetic tree, leading to an increase in uncertainty of the analysis performed by the bioinformatics tools, the general topology of the phylogenetic tree was considerably well conserved compared to the canonical mammalian phylogenetic tree. It is possible this is due to the high number of sequences that have been included in the analysis to generate the alignment and subsequent phylogenetic tree.

This work shows the comparison of the aforementioned trees with the classical phylogenetic classification. The differences we find may shed some light on potential evolutionary constraints posed by prion disorders, or detect motifs associated to prion resistance/susceptibility, by combining the information obtained from the tree with information about the ability to misfold in vivo or in vitro of those species' PrP.

ORAL 15 - Atypical scrapie evolution during propagation in different PrPC subtract both in vitro and in vivo

Authors: Ilaria Vanni[1], Vineet Rathod[2], Laura Pirisinu[1], Stefano Marcon[1], Elena Esposito[1], Michele Angelo Di Bari[1], Geraldina Riccardi[1], Claudia D'Agostino[1], Matteo Giovannelli[1], Umberto Agrimi[1], Holger Wille[2], Romolo Nonno[1]

1 Istituto superiore di Sanità, Department of Food Safety, Nutrition and Veterinary Public Health, Rome (Italy)
2 Centre for Prions and Protein Folding Diseases, University of Alberta, Edmonton (Canada)

Corresponding author: ilaria.vanni@iss.it

It is known that classical scrapie (CS) purified prions contain PrPSc fibrils, which have been recently resolved at atomic resolution. As atypical scrapie (AS) differs from CS in terms of clinical, pathological, epidemiological and biochemical behaviors, we purified AS prions to evaluate if such differences could be associated with different structural assemblies. By EM, purified AS prions, sheep and tg338-derived, showed only small non-fibrillar PrPSc particles. Nevertheless, they were infectious and maintained the original strain features. Our data suggest that AS and CS prions have different structural arrangements, which might explain their different behaviors. Finally, these data concur with previous evidence from some human GSS cases, confirming that fibrils are dispensable for prion infectivity.

ORAL 16 - A porphyrin with dual anti-prion activity

Authors: Antonio Masone[1], Chiara Zucchelli[2], Enrico Caruso[3], Giada Lavigna1, Hasier Eraña[4], Gabriele Giachin[5], Ilaria Raimondi[1], Laura Tapella[1], Liliana Comerio[1], Elena Restelli[1], Saioa R. Elezgarai[1], Federica De Leo[2], Giacomo Quilici[2], Lorenzo Taiarol[1], Marvin Oldrati[1], Nuria López-Lorenzo[6], Sandra García-Martínez[4], Alfredo Cagnotto[7], Jacopo Lucchetti[8], Marco Gobbi[8], Ilaria Vanni[9], Romolo Nonno[9], Michele A. Di Bari[9], Mark D. Tully[10], Valentina Cecatiello[11], Giuseppe Ciossani[11], Sebastiano Pasqualato[11], Eelco Van Anken[12], Mario Salmona[7], Joaquín Castilla[4,13], Jesús R. Requena[6], Stefano Banfi[3], Giovanna Musco[2], Roberto Chiesa[1]

1 Laboratory of Prion Neurobiology, Department of Neuroscience, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy.
2 Biomolecular NMR Unit, Division of Genetics and Cell Biology, IRCCS Ospedale San Raffaele, Milano, Italy.
3 Department of Biotechnology and Life Sciences, University of Insubria, Varese, Italy.
4 CICbioGUNE, Basque Research and Technology Alliance (BRTA), Bizkaia Technology Park, Derio, Spain.
5 Department of Chemical Sciences (DiSC), University of Padua, Italy.
6 CIMUS Biomedical Research Institute and Department of Medical Sciences, University of Santiago de Compostela-IDIS, Santiago de Compostela, Spain.
7 Laboratory of Biochemistry and Protein Chemistry, Department of Molecular Biochemistry and Pharmacology, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy.
8 Laboratory of Pharmacodynamics and Pharmacokinetics, Department of Molecular Biochemistry and Pharmacology, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy.
9 Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy.
10 Structural Biology Group, European Synchrotron Radiation Facility (ESRF), Grenoble, France.
11 Department of Experimental Oncology, European Institute of Oncology IRCCS, Milan, Italy.
12 Protein Transport and Secretion Unit, Division of Genetics and Cell Biology, IRCCS Ospedale San Raffaele, Milano, Italy.
13 Centro de Investigación Biomédica en Red de Enfermedades Infecciosas (CIBERINFEC), Carlos III National Health Institute, Madrid, Spain.

Corresponding author: roberto.chiesa@marionegri.it

We report the identification of Zn(II)-BnPyP, a tetracationic porphyrin that binds to distinct domains of native PrPC, eliciting a dual anti-prion effect. Zn(II)-BnPyP binding to a C-terminal pocket destabilizes the native PrPC fold, hindering conversion to PrPSc; Zn(II)-BnPyP binding to the flexible N-terminal tail disrupts N- to C-terminal interactions, triggering PrPC endocytosis and lysosomal degradation, thus reducing the substrate for PrPSc generation. With its bimodal action on the prion precursor, Zn(II)-BnPyP efficiently inhibits propagation of different prion strains in vitro, in neuronal cells and organotypic brain cultures, curing prion infection. These results identify a PrPC-targeting compound with an unprecedented dual mechanism of action which may be exploited to attain anti-prion effects without engendering drug resistance.

ORAL 17 - Spectroscopic heterogeneity in recombinant PrPSc prion strains

Authors: Sanaz Sabhezei[1], Hasier Eraña[2,3], Yaiza B. Codeseira[1], Nuria L. Lorenzo[1], Víctor Sánchez-Pedregal[4], Maite Freire-Delgado[1,5], Sonia Veiga[1], Jorge Moreno-Charco[2,3], Susana Bravo[6], Manuel Martín-Pastor[7], Joaquín Castilla[2,8,9], Jesús R. Requena[1].

1 CIMUS Biomedical Research Institute, University of Santiago de Compostela-IDIS
2 CIC-BIOGUNE
3 Atlas Molecular Pharma
4 Department of Organic Chemistry, University of Santiago de Compostela
5 Present address: Institut Pasteur
6 Proteomics & Mass Spectrometry Lab, IDIS
7 NMR Core facility, University of Santiago de Compostela
8 Ikerbasque
9 Centro de Investigación Biomédica en Red de Enfermedades infecciosas (CIBERINFEC).

Corresponding author: sanaz.sabzehei@rai.usc.es

Protein misfolding shaking amplification (PMSA) allows generation of unlimited quantities of distinct recombinant (rec) PrPSc preparations whose biochemical properties, v.g., pattern of proteinase K (PK)-resistant fragments, remain stable over many propagation cycles in vitro (Eraña et al., PLoS Pathog. 2019, 15(10):e1008117; Fernández-Borges et al., Acta Neuropathol. 2018, 135(2):179-199). They can therefore be considered stable recPrPSc strains. We subjected two of these recPrPSc prions to solid state NMR analysis. One of them is Sst01, a bank vole (BV) PrPSc(109I) strain whose biochemical and biological characteristics have been extensively described (Eraña et al., op. cit.). The other one is an ovine (Ov) PrPSc (VRQ) that we term UstSh01, and whose interesting biological properties will be reported elsewhere.

Biochemical analysis of UstSh01, revealed a pattern of proteinase K resistant fragments with nicks at positions 99/100 and 155, equivalent to those seen in Sst01, but also prevalent nicks at positions 135/136 and 164. This is suggestive of a similar general architecture of the two recombinant prions featuring, nevertheless, some differences. Negative stain transmission electron microscopy (EM) images showed in both cases flat 10 nm fibers with very little, if any, evidence of twisting. Extensive lateral association of fibers was seen.

We prepared UstSh01 and Sst01 PrPSc with uniformly (13C,15N) labelled Phe residues and subjected them to C-C solid state NMR. DARR (100 ms) spectra were acquired at 278 K with magic angle spinning at 20 kHz. Unexpectedly, spectra showed, for both recombinant prions, the presence of six Phe resonances in the CO-CA region,, instead of the expected three, one for each one of the three Phe residues in the PrP sequence. Chemical shifts, characteristic in all cases of a β-strand secondary structure environment, were different for the two prions, allowing a "fingerprint" differentiation of both analytes.

The unexpected spectroscopic heterogeneity can have several explanations. On the one hand, it might reflect the lateral association of PrPSc fibers, which might place specific Phe residues in a different chemical environment depending on whether the fiber in which they are contained is isolated or laterally paired. However, this is unlikely given that DARR reflects very short range interactions. A more likely and parsimonious explanation is the existence of a mixture of conformers, akin to the quasispecies that have been invoked to make up brain PrPSc prions and explain adaptation phenomena. However, these would need to propagate in a stable way in vitro. Other methodological explanations cannot be ruled out.

Further biochemical and structural studies will be needed to resolve this intriguing result whose understanding will be of obvious interest for the general understanding of recombinant prions.

ORAL 18 - Spectroscopic heterogeneity in recombinant PrPSc prion strains

Authors: A Amayra Hernández-Vega[1], Marcus Braun[2], Valerie Siahaan[2], Jochen Krattenmacher[2], Zdenek Lansky[2], Lara Scharrel[3], Marcus Jahnel[4], Susanne Wegmann[5] , Bradley T Hyman [5], Simon Alberti [6] , Stefan Diez[3,7], Anthony A Hyman[7].

1 Institute for BioEngineering of Catalonia (IBEC), Barcelona 08028, Spain. CIBERNED (Network Centre of Biomedical Research of Neurodegenerative Diseases), Spain.
2 Institute of Biotechnology CAS, BIOCEV, Vestec 25250, Czech Republic.
3 B CUBE Center for Molecular Bioengineering and Cluster of Excellence Physics of Life, Technische Universität Dresden, Dresden 01307, Germany.
4 Cluster of Excellence Physics of Life and the Biotechnology Center (BIOTEC), Center for Molecular and Cellular Bioengineering (CMCB), Technische Universität Dresden, Dresden 01307, Germany.
5 Department Neurology, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA 02129, USA.
6 Biotechnology Center (BIOTEC), Center for Molecular and Cellular Bioengineering (CMCB), Technische Universität Dresden, Dresden 01307, Germany.
7 Max Planck Institute of Molecular Cell Biology and Genetics, Dresden 01307, Germany.

Corresponding author: ahernandez@ibecbarcelona.eu

Tau is a highly disordered protein, with multiple binding sites for tubulin dimers, and a stereotyped blocky distribution of charges along its length. These characteristics made us wondered if Tau could form molecular condensates and, if so, if this was relevant for its biological function or pathological solid transition. Using recombinant Tau, we found that under a crowded environment Tau is able to undergo Liquid-liquid Phase separation. We showed that Tau drops are sensitive to the ionic strength suggesting that electrostatic interactions are involved in their formation. In line with this, we found that both RNA and tubulin enhance their formation. **Tau condensates concentrate tubulin and locally nucleate microtubule (MT) bundles**. We showed that this process is reversible and, by outcompeting MT binding with heparin, we could reverse Tau into drop-like configuration and debundled MTs. Further, while we were investigating conditions that will trigger Tau phase separation in the absence of molecular crowders, we found that **MTs could serve as a platform to nucleate Tau dynamic cooperative assemblies, Tau island**. Like condensates, these structures are in equilibrium with Tau in solution: shrinking or growing depending on the concentration of Tau in the surrounding media. Unlike condensates, these structures are composed by a single layer of Tau, suggesting that in-cis interactions between Tau molecules are favoured in this configuration. We showed that these structures **shield MTs from** katanin 2 **severing** function and **interfere with motor displacement**. In parallel, we have investigated **conditions/co-factors that will enhance Tau solid transitions in vitro.** Similar to what has been recently reported by Sjors Scheres and Michel Goedert groups, we found that shaking was essential to generate Tau fibers in vitro, while heparin was dispensable for it. Unexpectedly, we also found that amyloid beta ($A\beta_{1-42}$) trigger Tau transition into solid structures. These aggregates resemble amyloid plaques rather than Tau fibers. By analysing these "co-aggregates", we found that ThT signal only responded to increase concentrations of Aβ. These and other results suggest that Tau decorates Aβ plaques rather than synergizing with it in these solid structures questioning its biological relevance. We are now applying the knowledge obtained from this work to investigate how Tau transition into solid structures in neurons, in neurodegenerative diseases.

KEYNOTE 6

Kinetic stabilization of biomolecular condensates by heterotypic interactions

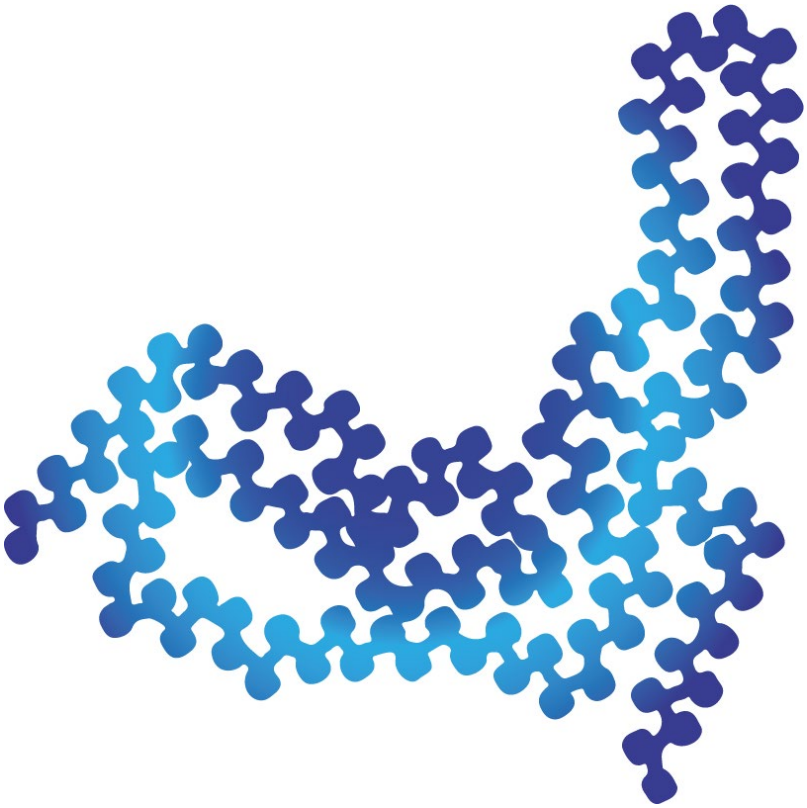
Authors: Xavier Salvatella[1]

1 Institute for Research in Biomedicine (IRB Barcelona), The Barcelona Institute of Science and Technology, Baldiri Reixac 10, 08028, Barcelona, Spain

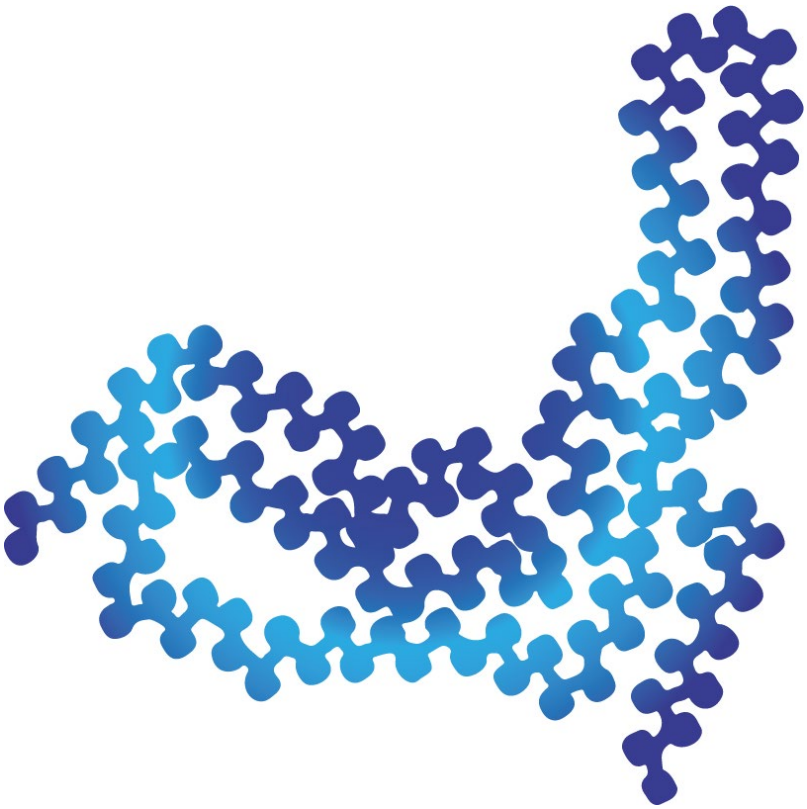
Corresponding author: xavier.salvatella@irbbarcelona.org

The inclusion of microexons by alternative splicing is frequent in neuronal proteins. The roles of these sequences are in most cases unknown, but changes in their degree of inclusion are associated with neurodevelopmental diseases. We recently found that the decreased inclusion of a 24-nucleotide neuron-specific microexon in CPEB4, an RNA-binding protein that regulates translation through cytoplasmic changes in poly(A) tail length, is linked to idiopathic autism spectrum disorder (ASD). Why this microexon is required and how small changes in its degree of inclusion generate a dominant-negative effect on the expression of ASD-linked genes is not clear. Here we show that neuronal CPEB4 forms condensates that dissolve upon depolarization, a phase transition associated with a switch from translational repression to activation. Heterotypic intermolecular interactions between the microexon and a cluster of histidine residues kinetically stabilize the condensates by competing with homotypic interactions between clusters, that otherwise lead to the irreversible aggregation of CPEB4. We conclude that microexon 4 in neuronal CPEB4 is required to preserve the reversible regulation of CPEB4-mediated gene expression in response to neuronal stimulation.

POSTERS



PRION DISEASES IN ANIMALS



POSTER A1 - Chronic Wasting Disease Surveillance in Italy

Authors: Daniela Loprevitte[1], Maria Concetta Cavarretta[1], Francesco Ingravalle[1], Daniela Gastaldi[1], Francesco Pennisi[1], Barbara Iulini[1], Valentina Campia[1], Tiziana Gasti[1], Claudia Cavallari[1], Serena Ravetta[1], Florio Caterina Lucia[1], Daniela Meloni[1], Maria Mazza[1], Elena Bozzetta[1]

1 Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Italy

Corresponding author: francesco.pennisi@izsto.it

Chronic Wasting Disease (CWD) is a prions-caused illness of increasing concern all over Europe. Following the identification in 2016 of the first cases of CWD in the reindeer and moose population of Nordic countries, in order to prevent its spread the EU established a surveillance system in six countires having such species of cervids. Although there are no reindeer or elk in its cervid population, our country established a surveillance program to monitor the occurrence of CWD in the Italian cervid population.

Specific guidelines (DGSAF 24007/2016 – DGSAF 3128/2017) have been issued by the Italian Ministry of Health. To enhance the chances of detecting the disease, the surveillance program focused only on fallen-stock, sick or symptomatic animals over 18 months old, excluding those hunted for human consumption.

POSTER A2 – CWD monitoring in Italy: neuropathological findings

Authors: B. Iulini[1], P. Gazzuola[1], L. Tripodi[1], G. Ru[1], D. Meloni[1], M. Mazza[1], E. Bozzetta[1], M.G. Perrotta[2], M. Caramelli[1], C. Casalone[1].

1 Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Italy
2 Ministero della Salute, Italy

Corresponding author: barbara.iulini@izsto.it

Chronic Wasting disease (CWD) is a prion disease that affects cervids. The Neuropathology laboratory of National Reference Centre for Animal Encephalopathies (CEA) carries out diagnostic services, research into neurological diseases in animals and monitoring of prion diseases. From 2017 to 2019 the CEA performed a histopathological survey of the brains of the wild ruminants sampled in the frame of Italian surveillance plan for CWD and resulted negative to rapid diagnostic screening. One hundred and thirteen brains were subjected to histopathological examination in order to investigate the main patterns of neuropathological lesions and possible correlated pathogens.

POSTER A3 – Chronic Wasting Disease Surveillance Plan in Italy

Authors: Daniela Loprevitte[1], Maria Concetta Cavarretta[1], Francesco Ingravalle[1], Daniela Gastaldi[1], Francesco Pennisi[1], Barbara Iulini[1], Valentina Campia[1], Tiziana Gasti[1], Claudia Cavallari[1], Serena Ravetta[1], Florio Caterina Lucia[1], Daniela Meloni[1], Maria Mazza[1], Elena Bozzetta[1]

1 Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Italy

Corresponding author: daniela.loprevite@gmail.com

Since Italy has established a surveillance plan to monitor the occurrence of CWD in the Italian cervid population, the aim of this study is to report the results for the period 2016–2022.

The program focused on fallen-stock and symptomatic animals older than 18 months, excluding those hunted for human consumption. The targets sampled for the diagnosis were the brain stem and medial retropharyngeal lymph nodes; whenever possible, the whole brain and the tonsils too. Samples from 3234 cervids were collected and tested by the rapid test. The cervid species analyzed were: roe deer (2475), red deer (532), fallow deer (201) and reindeer (26).

3063 samples resulted negative, 155 unsuitable due to advanced autolysis and 12 suspects, hereafter resulted negative by confirmatory tests.

No CWD cases were identified in all samples analyzed in Italy after six years of monitoring activity.

POSTER A4 – Chronic Wasting Disease Surveillance Plan in Italy

Authors: Maria Mazza[1,2,†], Linh Tran[3,†], Francesco Pennisi[1,2], Daniela Loprevite[1,2], Maria C. Cavarretta[1,2], Luana Dell'Atti[1,2], Daniela Meloni[1,2], Jørn Våge[3], Knut Madslien[3], Turid Vikøren[3], Tram Thu Vuong[3], Sylvie L. Benestad[3‡], Elena Bozzetta[1,2‡]

†, ‡*These Authors equally contributed to this study*
1 European Reference Laboratory for Transmissible Spongiform Encephalopathies - Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Italy
2 Italian Reference Laboratory for Transmissible Spongiform Encephalopathies - Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Italy
3 Norwegian Veterinary Institute, WOAH Reference Laboratory for Chronic Wasting Disease – Norway

Corresponding author: francesco.pennisi@izsto.it

The identification in 2016 of the first cases of CWD in Norwegian reindeer and moose raised great concern. To prevent the spread of CWD in Europe the EC established a surveillance system in six countries having reindeer and/or moose.

European CWD strains also appeared to be different from North American ones. To identify the different strains of CWD circulating in Europe, we evaluated on CWD Norwegian cases the diagnostic performance of three different rapid test approved for TSE diagnosis in cattle and small ruminants, two different confirmatory western blot and an amplification in vitro method as RT-QuIC.

All the diagnostic methods applied were able to detect the CWD positive samples and thus showing that they are reliable tools for the CWD surveillance in cervids population.

POSTER A5 – Extraction and decontamination of scrapie prions

Authors: Paula Ariadna Marco Lorente[1], Nerea Larrañaga[1], Fernando López[1], Marina Betancor[1], Sonia Pérez Lázaro[1], Rosa Bolea[1], Juan J. Badiola[1], Alicia Otero[1]

1 Centro de Encefalopatías y Enfermedades Transmisibles Emergentes, Facultad de Veterinaria, Universidad de Zaragoza — IA2, Zaragoza, España

Corresponding author: 716602@unizar.es

There is a shortage of effective techniques for the extraction and detection of prions present in the herd environment and there is no fully effective decontamination method available. Therefore, two protocols for the extraction and amplification by protein misfolding cyclic amplification of classical scrapie prions adsorbed on different materials (soil, paint, straw, and alfalfa) were developed. One was successful for all types of materials analyzed, while the other only allowed the extraction of prions adsorbed to soil samples. In addition, an autoclaving decontamination protocol was developed. Quite favorable decontamination levels began to be observed after 2 hours at 130ºC.

POSTER A6 – Delayed Wisc-1 CWD lymphotropic replication may explain differences in neuroinvasion between host expressing G96 or S96 cellular prion protein polymorphisms.

Authors: Camilo Duque Velásquez[1,3]; Chiye Kim[1,3]; Judd Aiken[2,3] and Debbie McKenzie[1,3]

1 Department of Biological Science, University of Alberta, Edmonton, AB T6G 2E9, Canada
2 Department of Agriculture, Food & Nutritional Science, University of Alberta, Edmonton, AB T6G 2P5, Canada
3 Centre for Prions and Protein Folding Diseases, University of Alberta, Edmonton, AB T6G 2M8, Canada

Corresponding author: duquevel@ualberta.ca

The main host factor affecting the likelihood of cervids of becoming infected and developing chronic wasting disease (CWD) is the primary structure of the cellular prion protein (PrPC). Single amino acid polymorphism in PrPC can impair continuous generation of PrPCWD. For example, the serine polymorphism at amino acid 96 (S96) of PrPC slows progression of CWD in white-tailed deer. In tg60 mice, S96-PrPC protects against prion disease following oral, intraperitoneal and intracranial exposure with Wisc-1 CWD strain. Interestingly, H95/S96 CWD (containing the H95+ strain), which causes disease by the intracranial route, failed to produce full attack rate and phenotypically similar prion disease by the oral and intraperitoneal routes. In vitro PrPCWD amplification by PMCA shows H95+ strain can be perpetually propagated with S96-PrPC substrate and indicates Wisc-1 loses replicative capacity once encoded on S96-PrPCWD. Together, this data suggests impaired neuroinvasion in hosts expressing S96-PrPC.

To evaluate the impact of PrPC polymorphisms and route of exposure on strain-tissue tropism, PrPres glycotype and disease progression, we inoculated cohorts of tg33 and tg60 mice with Wisc-1 or H95+, by the intracerebral, intraperitoneal and oral routes. To compare the rate of lymphotropic accumulation in spleen, mesenteric lymph nodes and Peyer patches, samples were taken at specific time points for PrPres evaluation. Western blot analysis indicates Wisc-1 PrPCWD accumulation in the spleen of intraperitoneally inoculated tg33 mice (i.e., homologous transmission) as early as 1-month post-exposure. Comparable levels of PrPres accumulation in tg60 mice spleens by 6 months post exposure. These data suggest that Wisc-1 lymphoid replication is impaired in hosts expressing S96-PrPC compared to hosts expressing G96-PrPC.

POSTER A7 – Lymphoid tropism of prions in dromedary camels

Authors: Rosalia Bruno [1]*, Baaissa Babelhadj [2]*, Laura Pirisinu[1], Geraldina Riccardi [1], Romolo Nonno [1], Umberto Agrimi [1], Gabriele Vaccari [1] and Michele Angelo Di Bari [1]

1 Department of Food Safety, Nutrition and Veterinary Public Health; Italian National Institute of Health; Rome, Italy.
2 Ecole Normale Superieure Ouargla Laboratoire de Protection des Écosystèmes en Zones Arides et Semi Arides University Kasdi Merbah Ouargla, Algeria.

Corresponding author: rosalia.bruno@iss.it

Camel prion disease (CPrD) is an emerging disease of dromedary camels. We have previously shown PrPSc deposition in a lymph node of a CPrD-affected dromedary (Babelhadj et al. 2018). Here, we investigated the presence of PrPSc in lymph nodes, spleen, Peyer's patches and RAMALT in four symptomatic (CNS+) and one asymptomatic (CNS-) Algerian dromedaries. We detected PrPSc deposition in all lymphoid tissues analyzed, regardless of the clinical status. Our results confirm the lymphoid tropism of CPrD and suggest that lymphoid involvement precedes neuroinvasion in CPrD, similarly to contagious TSEs such as classical scrapie and CWD.

POSTER A8 – Proteomic scrapie biomarker analysis in the CNS

Authors: Sonia Pérez Lázaro[1], Susana B. Bravo[2], Neelam Younas[3], Tomás Barrio[4], Alicia Otero[1], Jesús R. Requena[5], Inmaculada Martín-Burriel[6], Inga Zerr[3], Juan J. Badiola[1], Rosa Bolea[1]

1 Centro de Investigación en Encefalopatías y Enfermedades Transmisibles Emergentes, Facultad de Veterinaria, Universidad de Zaragoza – IA2, Zaragoza, Spain.
2 Laboratorio de Proteómica, Instituto de Investigación Sanitaria de Santiago de Compostela (IDIS), Complejo Hospitalario Universitario de Santiago, Santiago de Compostela, Spain.
3 Department of Neurology, Clinical Dementia Center and DZNE, Georg-August University, University Medical Center Göttingen (UMG), Göttingen, Germany.
4 UMR INRAE-ENVT 1255 Interactions Hôtes-Agents Pathogènes (IHAP), École Nationale Vétérinaire de Toulouse, Toulouse, France.
5 CIMUS Instituto de Investigación Biomédica, Universidad de Santiago de Compostela-IDIS, Santiago de Compostela, Spain.
6 Laboratorio de Genética bioquímica (LAGENBIO), Facultad de Veterinaria, Universidad de Zaragoza – IA2, Zaragoza, Spain.

Corresponding author: soniaperez@unizar.es

Prion diseases are usually diagnosed in the clinical stage when neuronal damage throughout the central nervous system (CNS) has occurred. In scrapie, PrPSc accumulates in lymphoid tissue before spreading in the CNS and its detection in vivo provides us with a preclinical prion disease natural model.

In this study we analysed the cerebrospinal fluid proteome through mass spectrometry in healthy control sheep and preclinical and clinical sheep naturally infected with scrapie.

We have selected two significant proteins, validated them in CSF by ELISA and evaluated their distribution in the CNS through immunohistochemical analysis, to check protein expression and deposits in nervous system cells.

POSTER A9 – Endoplasmic reticulum stress and ubiquitin-proteasome system impairment in natural scrapie

Authors: Jenny Lozada[1], Marina Betancor[1], Sonia Pérez Lázaro[1], Rosa Bolea[1], Juan J. Badiola[1] and Alicia Otero[1]

1 Centro de Encefalopatías y Enfermedades Transmisibles Emergentes, Universidad de Zaragoza

Corresponding author: aliciaogar@unizar.es

The chronic accumulation of misfolded proteins such as PrP Sc can alter endoplasmic reticulum homeostasis triggering the unfolded protein response (UPR). In this pathogenic event, the molecular chaperones play an important role and are overexpressed in prion diseases. Several reports in humans and animals have suggested that neurodegeneration is related to endoplasmic reticulum stress in diseases caused by the accumulation of misfolded proteins. In this study, we investigated the expression of three endoplasmic reticulum stress markers: PERK (endoplasmic reticulum kinase), BiP (binding immunoglobulin protein) and PDI (Protein Disulfide Isomerase). In addition, we valuated the accumulation of ubiquitin as a marker for protein degradation mediated by the proteasome. These proteins were studied by immunohistochemistry and western blot in brain tissue of sheep affected by scrapie in clinical and preclinical stages of the disease. Protein accumulation was semiquantitatively evaluated.

Results were compared with those observed in healthy controls. Scrapie-infected sheep showed a significant higher accumulation of the PRK-like ER kinase (PERK) where the thalamus and hippocampus were the regions showing the most intense PERK accumulation in clinical and preclinical sheep. Also, the ER chaperone binding immnunoglobulin protein (BiP/Grp78) that is overexpressed in thalamus and frontal cortex region in clinical and preclinical sheep. Scrapie-infected sheep showed significantly higher levels of PDI than healthy animals in almost all evaluated brain areas being the most altered marker. Significantly increased intraneuronal and neuropil ubiquitinated deposits in the form of granules were observed in certain brain areas such as the medulla oblongata and hippocampus in scrapie-affected animals compared to controls. Our results suggest that the neuropathological and neuroinflammatory phenomena that develop in prion diseases cause endoplasmic reticulum stress in brain cells triggering the UPR. In addition, the significantly higher accumulation of ubiquitin aggregates in scrapie-affected animals suggests an impairment of the ubiquitin-proteasome system in natural scrapie, these proteins may contribute as biomarkers of ER stress in prion diseases.

POSTER A10 – Study of the dynamics of 21kDa associated scrapie strains upon serial passage of scrapie isolates in ovine PrPC expressing mice

Authors: Marina Betancor[1], Tomás Barrio[2], Alicia Otero[1], Chloé Mesic[2], Séverine Lugan[2], Naïma Aron[2], Alvina Huor[2], Juan José Badiola[1], Rosa Bolea[1], Olivier Andréoletti[2].

1 Centro de Encefalopatías y Enfermedades Transmisibles Emergentes, Facultad de Veterinaria, Universidad de Zaragoza, Instituto Agroalimentario de Aragón - IA2, 50013, Zaragoza, Spain.
2 UMR École Nationale Vétérinaire de Toulouse (ENVT) - Institut National pour l'Agriculture, l'Alimentation et l'Environnement (INRAE) - 1225 Interactions Hôtes Agents Pathogènes (IHAP), 31300 Toulouse, France.

Corresponding author: mbetancorcaro@gmail.com

The transmission capacity of prions to different species is closely related to their strain characteristics. In scrapie, it has been demonstrated by bioassay that a single isolate might contain several strains, so it is considered that in certain cases different strains coexist and copropagate from a single isolate.

Thus, the objective of this study is to evaluate, using the Protein Misfolding Cyclic Amplification (PMCA) technique, the presence of scrapie strains associated with a 21kDa profile in isolates of ovine origin and their evolution during their serial transmission in a bioassay in mice expressing ovine PrPC. For this purpose, brain samples from sheep and mice experimentally infected with prions were subjected to PMCA under different conditions to evaluate the amount and proportion of subcomponents contained in them.

Results show that PMCA can be used under certain conditions in which the 19kDa phenotype, associated with a scrapie strain, is not amplifiable. Under these same conditions, PMCA is able to amplify the 21kDa subcomponent of scrapie, associated with several different strains of this disease. Using the PMCA method established in this study, the 21kDa component was also detected in ovine scrapie isolates that initially showed a 19kDa glycosylation profile. PMCA was also able to spot the tissue-dependent differential selection occurring in bioassays. Therefore, these results demonstrate, on the one hand, that PMCA can be used to detect 21kDa strains in homogenates that show a 19kDa signature and that it is capable of detecting the tissue-dependent differential selection that occurs in bioassays. On the other hand, and more importantly, the results suggest that some of the natural ovine scrapie isolates used in the study contained mixtures of strains and that co-infection with various strains may occur frequently in nature.

POSTER A11 – Neuropathological characterization of camel prion disease

Authors: Michele Angelo Di Bari[1], Baaissa Babelhadj[2], Geraldina Riccardi[1], Rosalia Bruno[1], Romolo Nonno[1], Umberto Agrimi[1], Gabriele Vaccari[1] and Laura Pirisinu[1]

1 Istituto Superiore di Sanità Department of Food Safety, Nutrition and Veterinary Public Health, Rome, Italy
2 Ecole Normale Superieure Ouargla Laboratoire de Protection des Écosystèmes en Zones Arides et Semi Arides University Kasdi Merbah Ouargla, Ouargla, Algeria

Corresponding author: michele.dibari@iss.it

In 2018, we described and designed as Camel prion disease (CPrD), a novel prion disease in dromedary camel in Algeria. Herein, we present a detailed neuropathological description of the phenotype of CPrD, in terms of both spongiform change and PrPSc accumulation. The analysis of the brain of eleven CPrD cases from Algeria revealed widespread vacuolation and PrPSc deposition in subcortical areas, cerebellum and caudal brainstem, while cortices were variably affected. This study highlighted a homogeneous disease phenotype among the dromedary cases analyzed and allowed us to define the brain regions relevant for the neuropathological diagnosis of CPrD.

POSTER A12 – PRNP gene polymorphisms in 20 horse breeds

Authors: Diego Sola[1], Rody Artigas[2], Pilar Zaragoza[3], Inmaculada Martín-Burriel[1-3], Juan José Badiola[1] and Cristina Acín[1].

1 Centro de Encefalopatías y Enfermedades Transmisibles Emergentes, Universidad de Zaragoza, IA2, IIS Aragón, 50013, Zaragoza, Spain.
2 Unidad Académica de Genética y Mejora Animal, Facultad de Veterinaria, Universidad de la República, Ruta 8 Km18, 13000, Montevideo, Uruguay.
3 Laboratory of Biochemical Genetics (LAGENBIO), Faculty of Veterinary, Institute for Health Research Aragon (IIS Aragón), AgriFood Institute of Aragon (IA2), University of Zaragoza, Miguel Servet 177, 50013 Zaragoza, Spain.

Corresponding author: diegosola95@gmail.com

Prion diseases are fatal neurodegenerative disorders in which the main pathogenic event is the conversion of the cellular prion protein (PrP^{C}) into an abnormal and misfolded isoform known as PrP^{Sc}. Most prion diseases and thus their susceptibility and pathogenesis are modulated mainly by the *PRNP* gene. Mutations in the *PRNP* gene can occur in different parts of the PrP^{C} sequence, leading to a change in transmission efficiency depending on the place where it occurs. Horses are animals which are considered to be highly resistant to prions. Several studies have attempted to identify polymorphisms in the *PRNP* gene that demonstrate this high resistance.

In this study we have analyzed 207 horses from 20 different breeds, discovering 3 novel polymorphisms, one synonymous SNPs (237T>C) and two nonsynonymous (5T>G, 544G>A).

By using computer programs such as PolyPhen-2, PROVEAN and PANTHER we have determined the impact that these new polymorphisms would have on the horse prion protein. PolyPhen-2 predicted 544G>A and 5T>G as “Benign”. PROVEAN estimated both as “Neutral”. PANTHER dictates as “Possibly damaging” in the 544G>A polymorphism and dictates as “Possibly benign” the polymorphism 5T>G.

In addition, we measured the propensity for amyloid aggregation using AMYCO and analyzed the lack of hydrogen bridges that these changes would entail together with their electrostatic potentials using Swiss-PdbViewer software.

In conclusion, the horse *PRNP* gene presents a low level of polymorphisms in the coding regions. This is the first description of these 3 polymorphisms in the equine breed (237T>C, 5T>G and 544G>A). Furthermore, none of these changes imply an increased susceptibility to amyloid propensity or changes in electrostatic potentials.

POSTER A13 – Update on the transmission experiments of idiopathic human prion diseases to small ruminant mouse models (Tg338, Tg501): evidence of CJD MM1 transmission to Tg338

Authors: Enric Vidal[1,2], Samanta Giler[1,2], Montse Ordóñez[1,2], Hasier Eraña[3,4], Jorge M. Charco[3,4], Guillermo Cantero[1,2], Juan C. Espinosa[5], Juan M. Torres[5], Vincent Béringue[6], Martí Pumarola[7] and Joaquín Castilla[3,8,9]

1 Unitat mixta d'Investigació IRTA-UAB en Sanitat Animal. Centre de Recerca en Sanitat Animal (CReSA). Campus de la Universitat Autònoma de Barcelona (UAB), Bellaterra, Catalonia.
2 IRTA. Programa de Sanitat Animal. Centre de Recerca en Sanitat Animal (CReSA). Campus de la Universitat Autònoma de Barcelona (UAB), Bellaterra, Catalonia.
3 Center for Cooperative Research in Biosciences (CIC BioGUNE), Basque Research and Technology Alliance (BRTA), Prion Research Lab, Derio, Spain.
4 ATLAS Molecular Pharma S. L. Derio (Bizkaia), Spain.
5 Centro de Investigación en Sanidad Animal, CISA-INIA-CSIC, Valdeolmos, Madrid 28130, Spain.
6 Molecular Virology and Immunology, Institut National de la Recherche Agronomique (INRA), Université Paris-Saclay, Jouy-en-Josas, France
7 Unitat de Patologia Murina i Comparada, Departament de Medicina i Cirurgia Animals, Facultat de Veterinària, Campus de UAB, 08193 Bellaterra, Barcelona, Catalonia.
8 IKERBASQUE, Basque Foundation for Science, Bilbao 48013, Bizkaia, Spain.
9 Centro de Investigación Biomédica en Red de Enfermedades infecciosas (CIBERINFEC), Carlos III National Health Institute, Madrid, Spain.

Corresponding author: enric.vidal@irta.cat

About 90% of Creutzfeldt-Jakob disease cases are classified as sporadic (sCJD), that is, occur infrequently, randomly and without a known cause. It is a fatal neurodegenerative disease with an incidence of 1-1.5 cases per million per year, without treatment or prophylaxis at present. Epidemiological studies have been so far unable to establish a causal relationship between sCJD and prion diseases in animals.

The zoonotic potential of sheep scrapie was demonstrated in 2014 (Cassard et al., Nature Communications) through inoculation of transgenic mice overexpressing the human prion protein with scrapie isolates. The resulting prion disease was indistinguishable from that occurring after sCJD inoculation in the same model and, while these results do not demonstrate that sCJD is caused by scrapie prions, they do show that the transmission barrier between ovine and human prions is not absolute.

To further assess this zoonotic risk, we have prepared inocula from 3 sCJD cases (MM1, MV2 and VV2) and 2 VPSPr cases (MM and MV) to verify if it is possible to recover the scrapie phenotype. Additionally, two different inocula gCJD (E200K) and GSS (A117V) have been also included in the bioassays as controls for classical and atypical genetic human prions, respectively.

No evidence of transmission was found on a first passage in Tg338 nor Tg501 ovinized mouse bioassays, but on second passage, 4/10 Tg338 mice succumbed to CJDMM1 (40% attack rate after 645 dpi). The remaining 2nd passages are still ongoing. In this poster, the neuropathological features of the resulting strain are analyzed and discussed.

This study has been funded by MINECO research project references AGL2017-88535-P and by RedPRION (Interreg POCTEFA EFA148/16).

POSTER A14 – Variation at residue 226 in cervid PrPC and PrPSc of CWD affect prion strain propagation

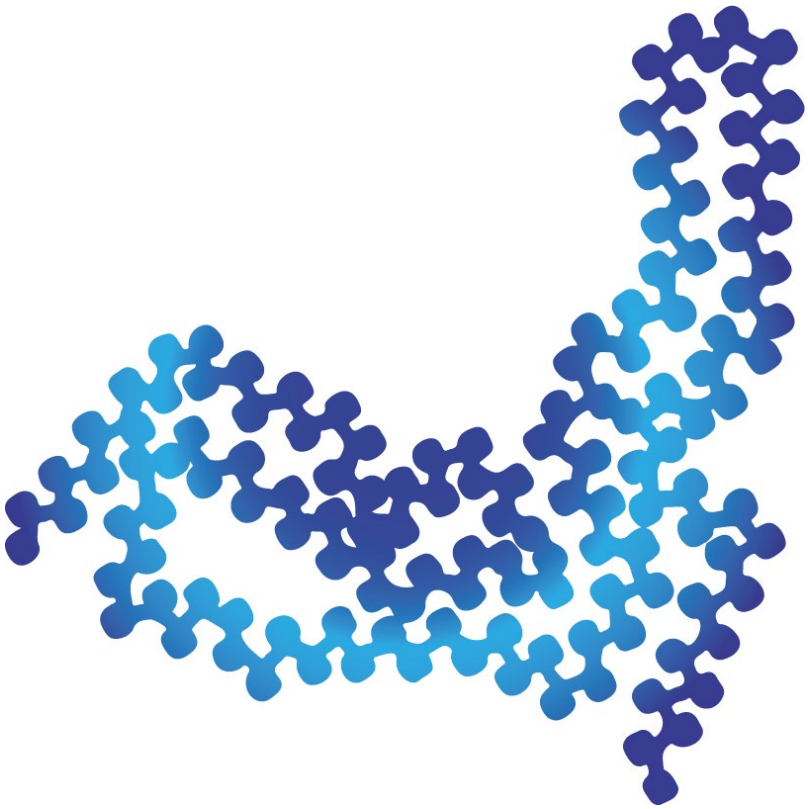
Authors: Tomas Barrio[1,2]*, Joseph DeFranco[1]*, Sehun Kim[1], Jifeng Bian[1,3], Jenna Crowell[1], Bailey Huser[1], Emma Kate Raisley[1], and Glenn C. Telling[1]

1 Prion Research Center (PRC), the Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, Colorado, United States of America, Program in Cell and Molecular Biology, Colorado State University, Fort Collins, Colorado, United States of America
2 Current address: UMR École Nationale Vétérinaire de Toulouse (ENVT) - Institut National pour l'Agriculture, l'Alimentation et l'Environnement (INRAE) - 1225 Interactions Hôtes- Agents Pathogènes (IHAP), 31300 Toulouse, France.
3 Current address: Virus and Prion Research Unit, USDA National Animal Disease Center, Ames, Iowa
*T.B. and J.D. contributed equally to this work

Corresponding author: tomas.barrio@envt.fr

Despite PrPSc (i.e., the pathogenic form of host-encoded PrPC) lacking informational nucleic acids, prions share strain diversity analogous to conventional pathogens. These strain properties affect prion infectivity and pathogenesis, PrPSc biochemical properties, and host-range dynamics. Chronic wasting disease (CWD) is a rapidly spreading, uncontrolled prion disease in wild and captive cervids in North America, Europe, and Asia. We have recently developed novel gene-targeted (Gt) models that express cervid prion protein under the wildtype murine genomic regulatory elements. Cervid PrP is polymorphic at residue 226, where elk and red deer express glutamic acid and deer, moose, and reindeer express glutamine. We have developed Gt models encoding cervid PrP with either glutamic acid (E) or glutamine (Q) expressed at residue 226, referred to as GtE and GtQ mice, respectively. Our previous findings demonstrated that variation at residue 226 in host-encoded PrPC influenced prion conversion and replication. We expanded on these results and analyzed the effect of inoculating CWD isolates sampled from animals with different genotypes (E226 and Q226 prions) when transmitted to different PrPC primary structures (GtE and GtQ mice). We found that, while there were no disparities in GtQ mice, there were pronounced differences in the GtE mice when inoculated with E226 and Q226 CWD PrPSc. These differences included varied disease kinetics, conformational properties, lesion profiles, and PrPSc deposition in the brain. These data indicate that GtE and GtQ models react differently to distinct sources of CWD prions, suggesting that E226 and Q226 PrPC may amplify different conformers present in the inocula, and reinforce the crucial role of the prnp genotype in the characterization of prion strains.

PRION AND PRION-LIKE DISEASES IN HUMANS



POSTER H1 – Biobank of genetic CJD at Israel

Authors: Prof. Victor Novack[1], Alice Anane[2], Prof. Lior Rokach[3]

1 Head of The Research Authority and Clinical Research Center, Founder and the Head of the Negev BioBank (NBB) Chair, Department of Internal Medicine G, Soroka University Medical Center (SUMC) Vice Dean for Innovation, Faculty of Health Sciences, Ben-Gurion University. Alice Anane,
2 Founder and chair, CJD Foundation Israel.
3 Department of Software and Information Systems Engineering, Ben-Gurion University.

Corresponding author: alice@dido.co.il

A sharing longitudinal biobank of samples (including blood-plasma, serum, pbmcs) of E200K families and patients for founding a large database for AI research for biomarkers, early diagnosis, and therapeutic for gCJD.

I'm the founder of CJD Foundation Israel since 2008. My father died from gCJD, and I and my daughter and siblings were found as carriers. I unite the community of families. Our registry counts more than 400 members in Israel only (representatives of families).

Our partners: Prof. Victor Novack, Clinical Epidemiologist. MD, Ph.D. Head of the Research Authority, and Negev BioBank (NBB). AI research by Prof. Lior Rokach.

Our model: https://www.youtube.com/watch?v=8UQTpoBYvdU&t=10s

POSTER H2 – Microbiota prion-like proteins and Abeta

Authors: Jofre Seira Curto[1], Adan Dominguez Martinez[1], Maria Rosario Fernandez Gallegos[1], Natalia Sanchez de Groot[1]

1 Unitat de Biociències, Departament de Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona, 08193, Barcelona, Spain

Corresponding author: natalia.sanchez@uab.cat

Alterations in microbiota have been associated with the development of neurodegeneration and it has been proposed that amyloid structures formed by microorganisms may trigger host protein aggregation. We have computationally analysed the presence of prion-like sequences in the gut microbiota, detecting a diverse set of proteins. Based on their structure and function we selected 12 prion amyloid cores. All of them can aggregate into amyloid fibrils and can influence the aggregation of amyloid beta peptide, involved in Alzheimer's disease. Overall, our results indicate that the interaction between human and microbiota amyloid proteins could happen and could be more common than expected.

POSTER H3 – Aβ40 aggregation under changeable conditions

Authors: Jofre Seira Curto[1], Rosario Fernandez Gallegos[1], Josep Cladera Cerdà[2], Núria Benseny Cases[2],Natalia Sanchez de Groot[1]

1 Unitat de Biociències, Departament de Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona, Cerdanyola del Vallès
2 Unitat de Biofísica. Departament de Bioquímica i Biologia Molecular. Centre d'Estudis en Biofísica. Universitat Autònoma de Barcelona, Cerdanyola del Vallès

Corresponding author: jofre.seira@uab.cat

---

pH homeostasis is essential for cell well-being and its disturbance can lead to health disorders. In patients with Alzheimer's disease, the pH decreases in the brain, and it is altered inside the endosomes and the lysosomes. This pH variation is associated with the aggregation of the amyloid beta peptide. Here we analyze how the pH and ionic conditions alter the Aβ40 aggregation and its toxicity. When the pH decreases and gets close to the IP, the absence of charges promotes aggregation. Our results point to an interaction between different conformational species that can alter the aggregation process. Overall, we see that the pH can change the formation and stability of fibrils which in turn influences in their toxicity and seeding capabilities.

POSTER H4 – Sleep features in Gerstmann-Sträussler-Scheinker

Authors: Jordi Sarto[1], Laura Pérez-Carbonell[1], Carles Gaig[1], Amaia Muñoz-Lopetegi[1], Raquel Ruiz[2], Laura Naranjo[2], Josep María Augé[2], Andrés Perissinotti[3], Joan Santamaria[1], Alex Iranzo[1], Raquel Sánchez-Valle[1]

1 Neurology Department, Hospital Clínic de Barcelona;
2 Immunology Department, Hospital Clínic de Barcelona;
3 Nuclear Medicine Department, Hospital Clínic de Barcelona

Corresponding author: sarto@clinic.cat

Background. Gerstmann-Sträussler-Scheinker (GSS) is a rare prion disease with heterogeneous clinical presentation and challenging diagnosis. Although sleep-related abnormalities are prominent and well-known in other prion diseases such as in Creutzfeldt-Jakob disease and fatal familial insomnia, information on sleep is limited in GSS. We evaluate here the sleep features of three patients with the GSS phenotype.

Methods. This study is part of a cohort study in genetic prion diseases performed at the Hospital Clínic de Barcelona, Barcelona, Spain. Twenty-six patients (58% women) have been included, with a mean age (standard deviation, S.D.) of 43 (2.5) years. Fourteen participants were at-risk or suffered from fatal familial insomnia (FFI), 7 genetic Creutzfeldt-Jakob disease (gCJD) and 5 Gerstmann-Straüssler-Scheinker disease (GSS). Fifty-four percent of participants wanted to know their genetic status and 5 were already symptomatic at the time of the first visit. Subjects undergo a clinical and cognitive evaluation, MRI scan, blood and CSF biomarkers studies and a sleep evaluation that include sleep clinical history, sleep scales and video-polysomnography.

Results. We analysed the data on the 3 GSS patients evaluated so far. Two patients reported sleep maintenance insomnia attributed to leg stiffness and back pain while the remaining patient did not report sleep problems. Video-polysomnography showed normal sleep staging and architecture in all of them, with non-specific abnormalities such as reduced sleep efficiency, obstructive apneas, and periodic limb movements.

Conclusions. We found absence of critical or specific sleep alterations in GSS. The sleep variances across GSS and other prion diseases such as fatal familial insomnia may be due to dissimilar neuropathological involvement of the neuronal structures that modulate sleep.

POSTER H5 – Quantitative 14-3-3 protein and prion RT-QuIC concordance analysis of patients with suspected prion diseases in Spain

Authors: Laura Naranjo[1], Jordi Sarto[2], Natalia Egri[1], Maria Antonia Romera[1], María del Carmen Antón[1], Rocio Couso[1], Carlos Nos[3], Raquel Sánchez-Valle[2], Raquel Ruíz-García[1].

1 Immunology Department, Biomedical Diagnostic Center, Hospital Clínic de Barcelona, Barcelona, Spain.
2 Alzheimer's Disease and Other Cognitive Disorders Unit, Neurology Service, Hospital Clínic de Barcelona, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Universitat de Barcelona, Barcelona, Spain.
3 General Subdirectorate of Surveillance and Response to Emergencies in Public Health, Department of Public Health in Catalonia, Barcelona, Spain.

Corresponding author: lanaranjo@clinic.cat

Despite PrPSc (i.e., the pathogenic form of host-encoded PrPC) lacking informational nucleic acids, prions share strain diversity analogous to conventional pathogens. These strain properties affect prion infectivity and pathogenesis, PrPSc biochemical properties, and host-range dynamics. Chronic wasting disease (CWD) is a rapidly spreading, uncontrolled prion disease in wild and captive cervids in North America, Europe, and Asia. We have recently developed novel gene-targeted (Gt) models that express cervid prion protein under the wildtype murine genomic regulatory elements. Cervid PrP is polymorphic at residue 226, where elk and red deer express glutamic acid and deer, moose, and reindeer express glutamine. We have developed Gt models encoding cervid PrP with either glutamic acid (E) or glutamine (Q) expressed at residue 226, referred to as GtE and GtQ mice, respectively. Our previous findings demonstrated that variation at residue 226 in host-encoded PrPC influenced prion conversion and replication. We expanded on these results and analyzed the effect of inoculating CWD isolates sampled from animals with different genotypes (E226 and Q226 prions) when transmitted to different PrPC primary structures (GtE and GtQ mice). We found that, while there were no disparities in GtQ mice, there were pronounced differences in the GtE mice when inoculated with E226 and Q226 CWD PrPSc. These differences included varied disease kinetics, conformational properties, lesion profiles, and PrPSc deposition in the brain. These data indicate that GtE and GtQ models react differently to distinct sources of CWD prions, suggesting that E226 and Q226 PrPC may amplify different conformers present in the inocula, and reinforce the crucial role of the prnp genotype in the characterization of prion strains.

POSTER H6 – Improved Real-Time Quaking Induced Conversion for early diagnostics of Creutzfeldt-Jakob disease in Denmark

Authors: Remarh Bsoul[1], Eva Løbner Lund[1], Kimberley Burns[2], Mary Andrews[2], Neil McKenzie[2], Alison Green[2] and Ausrine Areskeviciute[1]

1 Danish Reference Center for Prion Diseases, Department of Pathology, Copenhagen University Hospital, 2100 Copenhagen, Denmark;
2 National CJD Research and Surveillance Unit, Centre for Clinical Brain Science, University of Edinburgh, Edinburgh EH4 2XU, UK;

Corresponding author: ausrine.areskeviciute@regionh.dk

Currently, cerebrospinal fluid-based Real-Time Quaking-Induced Conversion (CSF RT-QuIC) is the most prominent method for early detection of sporadic Creutzfeldt-Jakob disease (sCJD), the commonest prion disease. CSF RT-QuIC delivers high sensitivity (>90 %) and specificity (100 %), which has been demonstrated by large ring-trial studies testing probable and definitive sCJD cohorts. Following the CSF RT-QuIC inclusion in the revised European CJD Surveillance Network diagnostic criteria for sCJD, it has become a standard diagnostic procedure in many prion disease reference or surveillance centers around the world. Here, we present the implementation of the second-generation CSF RT-QuIC (commonly known as Improved QuIC or IQ) at the Danish Reference Center for Prion Diseases (DRCPD), that reduced assay time. The sensitivity and specificity were evaluated and validated by analyzing 63 CSF samples. These 63 samples were also analyzed at the National CJD Research and Surveillance Unit (NCJDRSU), University of Edinburgh, UK using first generation or previous CSF RT-QuIC method (PQ). The sensitivity and specificity of PQ at the NCJDRSU is 92% and 100%, respectively. Using these 63 CSF samples the agreement between the two RT-QuIC generations at DRCPD and NCJDRSU Prion laboratories was 100%.

POSTER H7 – Analysis of plasma neurofilament light chain in fatal familial insomnia

Authors: Ilaria Raimondi[1], Laura Pasetto[1], Ignazio Roiter[2], Valdimiro Artuso[3], Joaquín Castilla[4,5,6], Izaro Kortazar-Zubizarreta[7], Valentina Bonetto[1], Gianluigi Forloni[1], Roberto Chiesa[1]

1 Department of Neuroscience, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy.
2 ULSS 3 Serenissima, Venice, Italy.
3 ULSS 2 Marca Trevigiana, Ca' Foncello Hospital, Treviso, Italy.
4 Center for Cooperative Research in Biosciences (CIC bioGUNE), Basque Research and Technology Alliance (BRTA), Spain.
5 Centro de Investigación Biomédica en Red de Enfermedades infecciosas, Carlos III National Health Institute, Spain.
6IKERBASQUE, Basque Foundation for Science, Spain.
7 Department of Neurology, Bioaraba Health Research Institute, Araba University Hospital -Txagorritxu. Spain.

Corresponding author: ilaria.raimondi@marionegri.it

Fatal familial insomnia (FFI) is a genetic prion disease linked to the D178N-129M PRNP mutation. It has a long silent phase followed by a rapid neurological decline, which invariably leads to death. The lack of prodromal biomarkers leads to diagnosis when neurological damage has already occurred and the possibility of cure is remote. We measured neurofilament light chain (NfL) in longitudinal plasma samples of FFI carriers, including individuals who developed symptoms or are approaching the predicted age of onset, and non-carrier relatives. Results indicate a significant increase in NfL at onset or overt disease. NfL may be a diagnostic but not a prodromal FFI biomarker.

POSTER H8 – Exploring cryptic amyloidogenic regions in prion-like proteins from plants

Authors: Carlos Pintado-Grima[1], Jaime Santos[1], Valentín Iglesias[1], Zoe Manglano-Artuñedo[1], Irantzu Pallarès[1] and Salvador Ventura[1]

1 Institut de Biotecnologia i de Biomedicina and Departament de Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona, Barcelona, Spain.

Corresponding author: Carlos.Pintado@uab.cat

Prion-like domains (PrLDs) are intrinsically disordered regions (IDRs) of low sequence complexity with a similar composition to yeast prion domains. PrLDs-containing proteins have been involved in different organisms' regulatory processes. Regions of moderate amyloid propensity within IDRs have been shown to assemble autonomously into amyloid fibrils. These sequences tend to be rich in polar amino acids and often escape from the detection of classical bioinformatics screenings that look for highly aggregation-prone hydrophobic sequence stretches. We defined them as cryptic amyloidogenic regions (CARs) and recently developed an integrated database that collects thousands of predicted CARs in IDRs. CARs seem to be evolutionary conserved among disordered regions because of their potential to stablish functional contacts with other biomolecules. In this work we have focused on identifying and characterizing CARs in prion-like proteins (pCARs) from plants, a lineage that has been poorly studied in comparison with other prionomes. We confirmed the intrinsic amyloid potential for a selected pCAR from Arabidopsis thaliana and explored functional enrichments and compositional bias of pCARs in plant prion-like proteins.

POSTER H9 – TAC-1: Advancing towards the development of small molecules to treat tauopathies

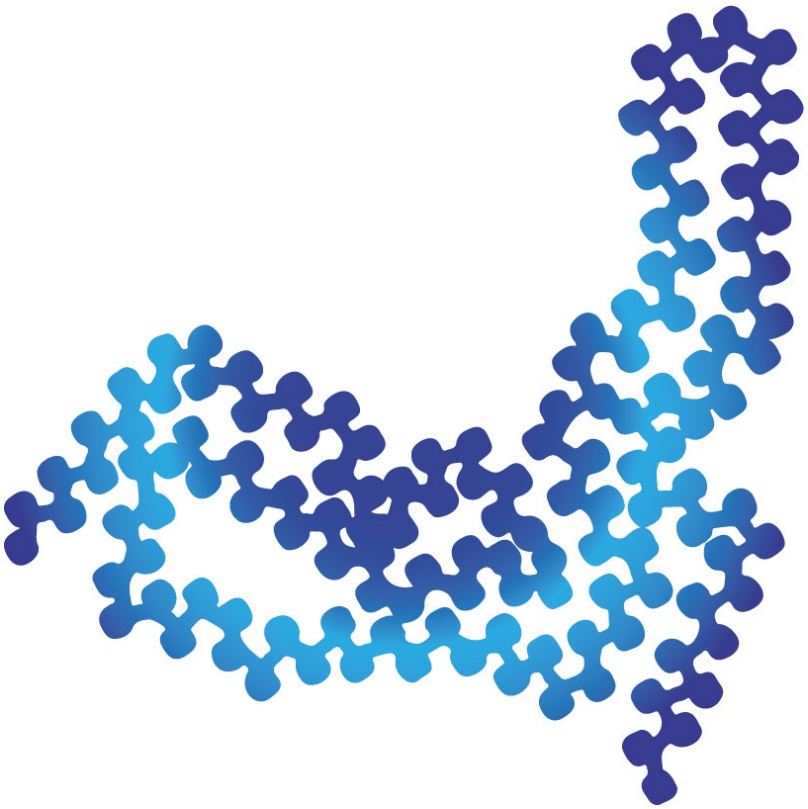
Authors: Zoe Manglano-Artuñedo[1], Samuel Peña-Díaz[1], Jaime Santos[1], Helena Østergaard Rasmussen[2,3], Jan Skov Pedersen[2,3], Daniel E Otzen[2,4], Irantzu Pallarès[1] and Salvador Ventura[1]

1 Institut de Biotecnologia i Biomedicina and Departament de Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona, Bellaterra, Spain.
2 Interdisciplinary Nanoscience Center (iNANO), Aarhus University, Aarhus, Denmark.
3 Department of Chemistry, Aarhus University, Aarhus, Denmark.
4 Department of Molecular Biology and Genetics, Aarhus University, Aarhus, Denmark.

Corresponding author: zoe.manglano@uab.cat

Tauopathies are a set of neurodegenerative diseases characterized by the amyloid aggregation of an intrinsically disordered protein named tau. The most common tauopathy is Alzheimer's disease, which is the main cause of dementia worldwide and leads to a high social and economic burden on our society. Nowadays, the current therapies are only addressed to treat the symptomatology, but not the onset nor progression of these disorders. Here, we developed a novel high-throughput screening methodology to find new tau aggregation inhibitors based on the implementation and optimisation of seeded tauK18 aggregation kinetics. We obtained 97 compounds with anti-aggregating properties that subsequently were tested on aggregation kinetics with full-length tau (Tau2N4R). This two-step screening process (TauK18 and Tau2N4R) allowed us to identify TAC-1, a promising compound that inhibits the amyloid aggregation of the two variants as reported by thioflavin-T and light scattering. We found that TAC-1 is an inhibitor with concentration-dependent activity that may impact aggregate structures. Flow Induced Dispersion Analysis and Small Angle X-Ray Scattering results suggest that TAC-1 targets Th-T binding species altering the structure of tau aggregates. Taken together, we conclude that TAC-1 may constitute a promising hit compound to inhibit and modulate tau amyloid aggregation.

PRION STRUCTURE AND BIOLOGY



POSTER S1 – PrPSc induce the oligomerization of Aβ peptide

Authors: Raimon Sabate[1,2], Alba Espargaró[1,2], Irene Álvarez-Berbel[1], Yixia Jin[1], Gabrielė Putriūtė[1], Maria Antònia Busquets[1,2]

1 Secció de Fisicoquímica. Departament de Farmàcia i Tecnologia Farmacèutica, i Fisicoquímica, Facultat de Farmàcia i Ciències de l'Alimentació. Universitat de Barcelona. Av. Joan XXIII, 27-31. 08028 Barcelona.
2 Institut de Nanociència i Nanotecnologia. IN2UB. Diagonal 645. 08028 Barcelona.

Corresponding author: rsabate@ub.edu

In the last few years several evidences suggest the existence of potential interactions between PrP and amyloid-beta peptide. In these lights, we have tested the effect of in-vitro preformed PrPSc fibrils in the aggregation of Aβ peptide, the main hallmark of Alzheimer's disease. Our assays show that the presence of PrPSc fibrils increase the lag time and nucleation constant of Aβ aggregation and reduce the fibril grown ratio. The obtained data suggest a potential direct binding between PrPSc seeds and Aβ monomers entailing the formation of larger aggregates in concomitance with the reduction in the concentration of mature fibers. Interestingly, we observe an increment of oligomeric species of potential toxicity and spreading capacity.

POSTER S2 – PrPSc aggregates modify the aggregation of α-syn

Authors: Alba Espargaró[1,2], Irene Álvarez-Berbel[1], Yixia Jin[1], Gabrielė Putriūtė[1], Raimon Sabate[1,2], Maria Antònia Busquets[1,2]

1 Secció de Fisicoquímica. Departament de Farmàcia i Tecnologia Farmacèutica, i Fisicoquímica, Facultat de Farmàcia i Ciències de l'Alimentació. Universitat de Barcelona. Av. Joan XXIII, 27-31. 08028 Barcelona.
2 Institut de Nanociència i Nanotecnologia. IN2UB. Diagonal 645. 08028 Barcelona.

Corresponding author: aespargaro@ub.edu

Recent research has demonstrated the co-occurrence of α-syn and tau aggregation in Parkinson disease as well as the co-occurrence of the aggregation of Aβ peptide, tau and proteins such as TDP-43 in Alzheimer disease. Since the aggregation of different amyloid proteins could be a characteristic feature in conformational diseases, we tested the effect of PrPSc in-vitro formed aggregates in the aggregation of α-synuclein protein, linked to Parkinson disease. Interestingly, our results show that in-vitro α-syn aggregates alter α-syn aggregation pattern. Thus, whereas nucleation constant is practically unaltered in presence of PrPSc seed, the elongation constant is drastically reduced, lowering concomitantly the concentration of α-syn mature fibers.

POSTER S3 – Lipid environment role in α-synuclein aggregation

Authors: Irene Álvarez-Berbel[1], Yixia Jin[1], Gabrielė Putriūtė[1], Raimon Sabate[1,2], Alba Espargaró[1,2], Maria Antònia Busquets[1,2]

1 Secció de Fisicoquímica. Departament de Farmàcia i Tecnologia Farmacèutica, i Fisicoquímica, Facultat de Farmàcia i Ciències de l'Alimentació. Universitat de Barcelona. Av. Joan XXIII, 27-31. 08028 Barcelona.
2 Institut de Nanociència i Nanotecnologia. IN2UB. Diagonal 645. 08028 Barcelona.

Corresponding author: mabusquetsvinas@ub.edu

α-synuclein is the main hallmark in Parkinson disease. It is known that the presence of lipids, thus cell membranes, could represent a key factor in the aggregation of PrPSc and other prion-like proteins. Liposomes (bicelles) represent the simplest model to mimic cell membranes. With the goal to decrypt the effect of lipid presence in the amyloid aggregation of α-synuclein, we assayed the effect of liposomes with different lipid composition in the aggregation of α-synuclein under physiological conditions. Remarkably, we have observed that the presence of liposomes could modify the aggregation pathways of α-synuclein involving the formation of different α-synuclein amyloid species displaying differential structure, size, stability, seeding capacity and potential toxicity.

POSTER S4 – Characterization of a PrP-HaloTag chimera

Authors: Antonio Masone[1], Lorenzo Taiarol[1], Marvin Oldrati[1], Ilaria Ortenzi[1], Eleonora Busani[1], Roberto Chiesa[1]

1 Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy

Corresponding author: antonio.masone@marionegri.it

We generated a PrP-HaloTag chimera that can be labelled with cell permeable and impermeant fluorescent ligands and detected by imaging and biochemical approaches. PrP-Halo is correctly expressed on the plasma membrane of HEK293 cells, it is glycosylated and subjected to physiological alpha-cleavage. When cells were stimulated with a PrPC-degrading compound, cell surface PrP-Halo was internalized by clathrin-coated pits and degraded through the lysosomal pathway. The possibility of specifically labeling different subsets of PrPC molecules will allow to precisely define the cellular trafficking and metabolism of PrP in normal and pathological conditions.

POSTER S5 – The Impact of Solvent Ions on Amyloid Aggregation of hnRNPDL-2

Authors: Andrea Bartolomé-Nafría[1]; F. Emil Thomasen[2]; Kresten Lindorff-Larsen[2]; Salvador Ventura[1]; Javier Garcia-Pardo[1].

1 Institut de Biotecnologia i Biomedicina (IBB), Universitat Autònoma de Barcelona (UAB), Bellaterra-Cerdanyola del Vallès, Spain.
2 Københavns Universitet, Copenhagen, Denmark.

Corresponding author: andrea.bartolome@uab.cat

Protein aggregation is dependent on two main factors: the primary sequence of the protein and the environmental conditions. Therefore, for the same protein sequence, changes in pH, temperature or solvent composition have an impact on the aggregation kinetics. In a similar manner, the presence of metal ions is thought to play a crucial role in many biological functions, including protein folding, stability and amyloid aggregation. In this context, the study of the environmental effects on amyloid aggregation can help understanding the cause of differences in disease progression and the in vitro assembly of amyloids for technological purposes.

hnRNPDL is a ribonucleoprotein (RNP) involved in transcription and RNA processing that hosts missense mutations causing limb-girdle muscular dystrophy D3 (LGMD D3). Mammalian-specific alternative splicing (AS) renders three natural isoforms, hnRNPDL-2 being predominant in humans and the only isoform capable of forming amyloid fibrils. The architecture and activity of the fibrils are reminiscent of functional amyloids, suggesting that LGMD D3 might be a loss-of-function disease associated with impaired fibrillation.

In the present work we have investigated the impact of solvent ions on amyloid aggregation of hnRNPDL-2. By using SAXS and computational simulations, we have investigated the changes in the structure hnRNPDL-2 induced by the presence of different metal ions and salt concentrations. In vitro aggregation experiments and structural data support our findings, suggesting that the environmental salt concentrations are a critical factor that affects the formation of hnRNPDL-2 amyloid complexes. Ultimately, we believe our findings can help understanding disease cause and progression associated to this protein and other functional amyloids involved in human diseases.

POSTER S6 – Protein-only Nanoparticles for T-cell Activation

Authors: Marc Fornt-Suñé[1,2], Gonzalo Lázaro Bermejo[1,3], Marcos Gil-Garcia[1,2], Andrea Aran[1,3], Javier Garcia-Pardo[1,2], Mercè Martí[1,3] and Salvador Ventura[1,2]

1 Institut de Biotecnologia i de Biomedicina (IBB), Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain.
2 Departament de Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain.
3 Departament de Biologia Cel·lular, Fisiologia i Immunologia, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain.

Corresponding author: marc.fornt@uab.cat

Adoptive T-cell immunotherapy (ACT) utilizing nanosized artificial antigen presenting cells (aAPCs) has emerged as a promising approach for cancer treatment. Although diverse nanomaterials have been explored as aAPC scaffolds, protein-only nanoparticles have been largely overlooked, despite their high designability and biocompatibility. In this study, we present a novel plug-and-play approach for the development of protein-only nanoparticles as aAPCs, using the self-assembling properties of ZapB coiled-coil and the Z-domain antibody-capturing ability. The resulting coiled-coil-based nanoparticles (ccNPs) can be easily and rapidly functionalized with a tailored combination of antibodies, making them a versatile platform for T-cell expansion. Our results demonstrate that ccNPs decorated with anti-CD3 and anti-CD28 antibodies induce T-cell proliferation and activation at a level comparable to commercial magnetic beads, while sustaining cytokine production for an extended period. The biocompatibility, modularity, and chemistry-free surface modification of this protein-only platform offers a valuable tool for developing personalized ACT for cancer treatment.

POSTER S7 – AmyloGraph: A comprehensive database of amyloid-amyloid interactions

Authors: Burdukiewicz M[1], Rafacz D[2], Barbach A[3], Hubicka K[3], Bąkała L[2], Lassota A[4], Stecko J[5], Szymańska N[5], Wojciechowski JW[3], Kozakiewicz D[6], Szulc N[3], Chilimoniuk J[7], Jęśkowiak I[8], Gąsior-Głogowska M[3], Kotulska M[3]

1 Institute of Biotechnology and Biomedicine, Autonomous University of Barcelona, Campus Universitat Autònoma de Barcelona Plaça Cívica Bellaterra, s/n, 08193 Cerdanyola del Vallès, Barcelona, Spain.
2 Faculty of Mathematics and Information Science, Warsaw University of Technology, Koszykowa 75, 00-662 Warsaw, Poland.
3 Department of Biomedical Engineering, Faculty of Fundamental Problems of Technology, Wrocław University of Science and Technology, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland.
4 School of Biosciences, College of Life and Environmental Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, United Kingdom.
5 Faculty of Medicine, Wrocław Medical University, Ludwika Pasteura 1, 50-367 Wrocław, Poland.
6 Laboratory of Microbiome Immunobiology, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Weigla 12, 53-114 Wrocław, Poland.
7 Department of Genomics, Faculty of Biotechnology, University of Wrocław, Fryderyka Joliot-Curie 14a, 50-383 Wrocław, Poland.
8 Department of Pharmacology, Wroclaw Medical University, Mikulicza-Radeckiego 2, 50-345 Wrocław, Poland.

Corresponding author: michalburdukiewicz@gmail.com

"Amyloid proteins and prions have a unique ability to assemble into filamentous aggregates characterized by cross-beta sheets. This process can be accelerated or slowed by already-formed amyloid fibrils constituting the same or other proteins.

However, it is very challenging to elucidate the sequence determinants that allow such interactions. Moreover, no databases are consolidating the results of numerous experiments studying interactions of amyloids and prions.

We decided to develop AmyloGraph, the first unified database of amyloid-amyloid interactions. As the AmyloGraph aims to standardize information, we describe each interaction using three descriptors: I) impact on the speed of fibrillization, II) physical contact during the interaction and III) development of the heterogeneous fibers. We also preserve the information on the amino acid sequence of amyloids participating in the interaction.

One of the strengths of AmyloGraph is rigorous data curation and validation. Each manuscript was revised independently by two curators. Moreover, curators used designed forms to ensure the best data quality.

The AmyloGraph database presents the interaction data as a fully interactive graph, where nodes represent amyloids and edges represent their interactions. The data is also available in the tabular format. Three describing properties can filter all interactions. The database can be accessed at AmyloGraph.com.

Burdukiewicz, M., Rafacz, D., Barbach, A., Hubicka, K., Bąkała, L., Lassota, A., Stecko, J., Szymańska, N., Wojciechowski, J.W., Kozakiewicz, D., Szulc, N., Chilimoniuk, J., Jęśkowiak, I., Gąsior-Głogowska, M., Kotulska, M., 2022. AmyloGraph: a comprehensive database of amyloid–amyloid interactions. Nucleic Acids Research gkac882. https://doi.org/10.1093/nar/gkac882"