CREUTZFELDT-JAKOB DISEASE FOUNDATION, INC.
Supporting Families Affected by Prion Disease

CJD Surveillance
National Prion Disease
Pathology Surveillance Center

2023 CJD Foundation Family Conference
July 14–17, 2023, Hilton Washington DC Capitol Hill

Blocking the Neurotoxic Activities of Mutant Prion Proteins by a Novel Class of Therapeutic Agents

Maria Letizia Barreca

I2D Lab (*In Silico* Drug Discovery Lab)
Department of Pharmaceutical Sciences
University of Perugia, Italy
maria.barreca@unipg.it

I2D Lab
In Silico Drug Discovery Laboratory

STUDIUM GENERALE CIVITATIS PERUSII • A.D. MCCCVIII •

A.D. 1308
unipg
DEPARTMENT
OF PHARMACEUTICAL SCIENCES

Many Thanks to the Donors!

The Robert Dodd Memorial Research Grant
Contributed by Kathleen Dodd and Family

The Chuck Fear Memorial Research Grant
Contributed by Pamela Fear and Family

The Fred Glavan/Lee Gallagher Family Memorial Grant
Contributed by the Glavan and Gallagher Families

The Linda L. Sullivan Memorial Grant
Contributed by the Sullivan Family

The Donna Tepe Memorial Grant
Contributed by Jeff Tepe and Family

CJD FOUNDATION

CREUTZFELDT-JAKOB DISEASE FOUNDATION, INC.

Supporting Families Affected by Prion Disease

Prion Disease Drug Discovery Team

Department of Pharmaceutical Sciences
Maria Letizia Barreca
Giuseppe Manfroni

Medicinal Chemistry

Department CIBIO
Emiliano Biasini

Biochemistry/Cell Biology

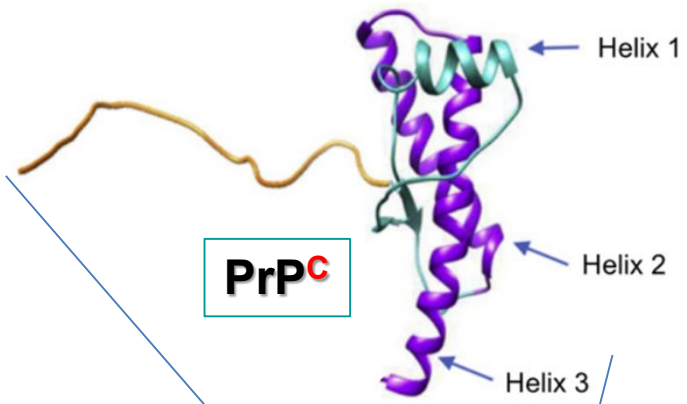
Department of Medicine and Surgery
Francesca Fallarino

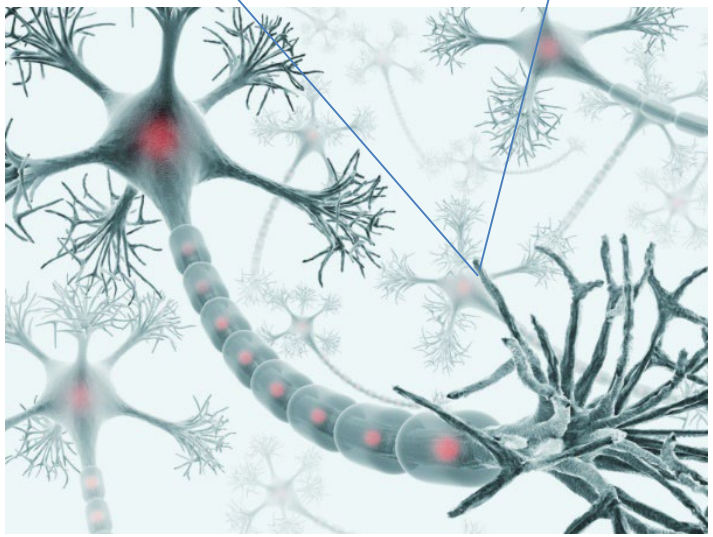
Pharmacology

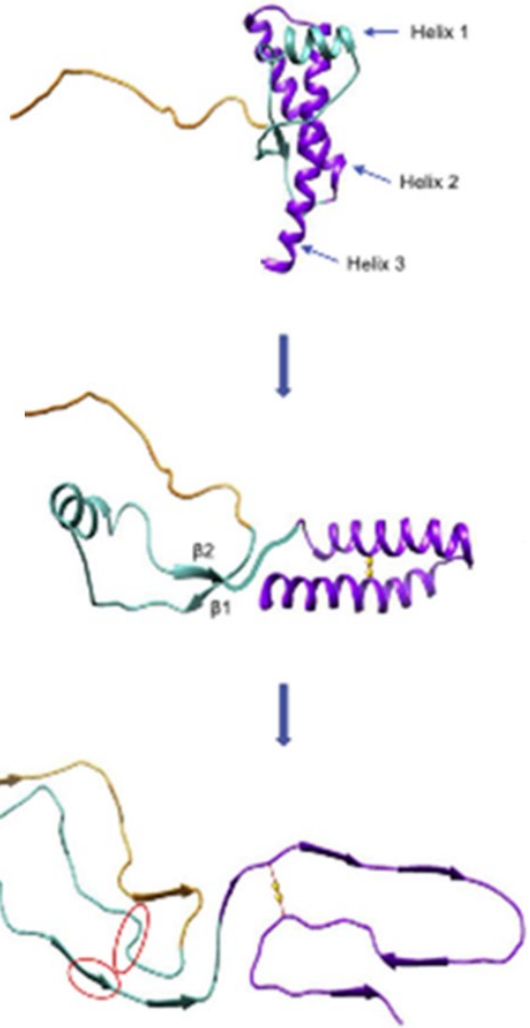
Department of Food Safety, Nutrition and Veterinary Public Health
Romolo Nonno

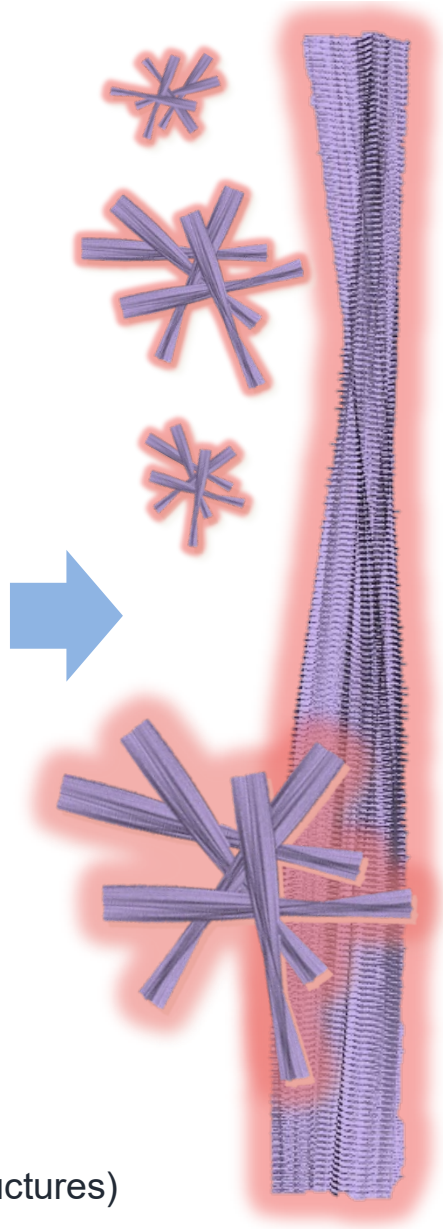
Veterinary Medicine

One Sequence, Two Proteins: PrP^{C} & PrP^{Sc}







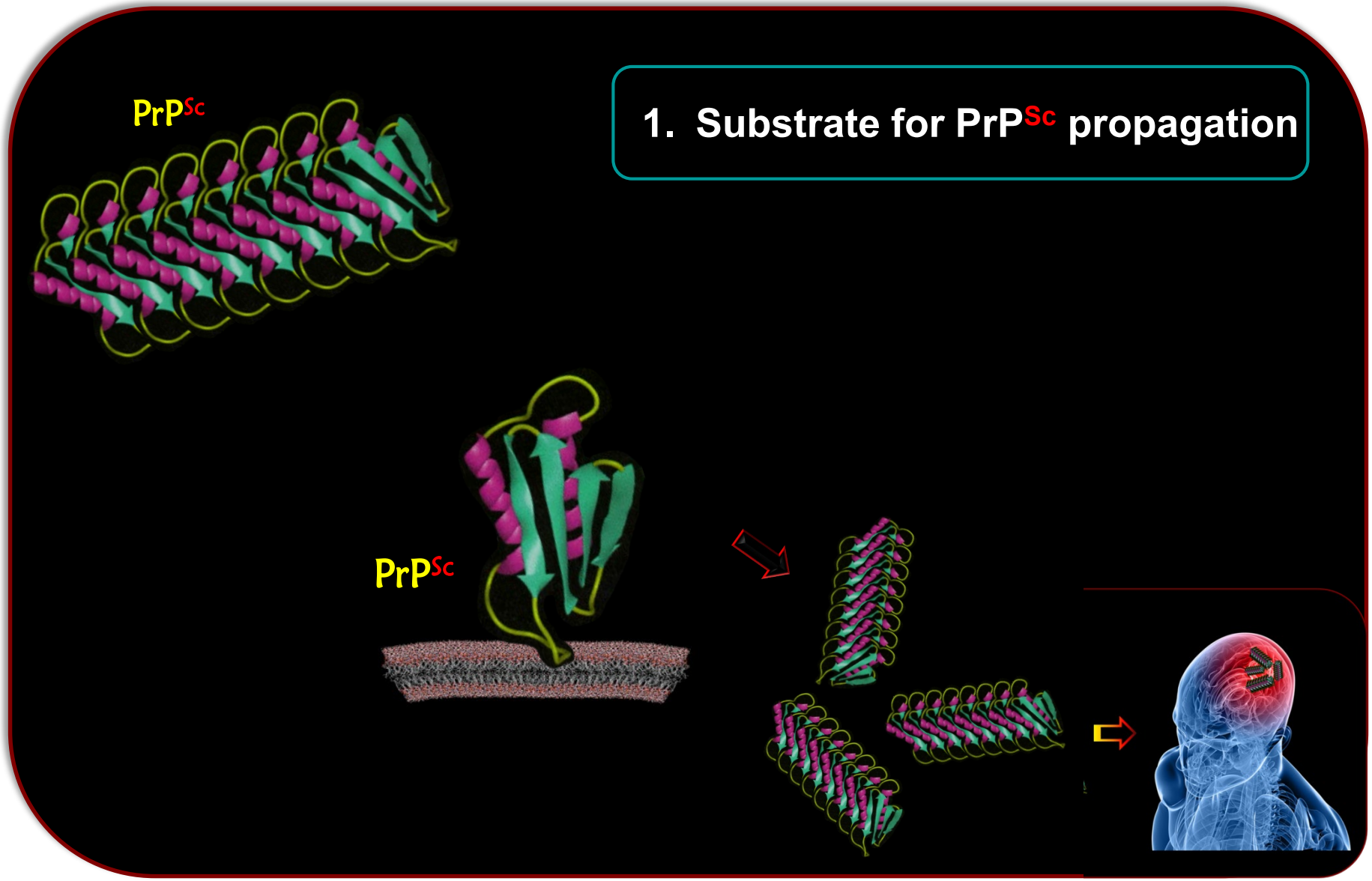


PrP^{Sc}

- self-propagates
- Aggregates (forms fibrils and amyloid structures)
- Accumulates in the brain
- induce toxic signals

Modified from Kraus A. et al. Mol Cell. 2021, 81, 4540-4551

A Dual Role for PrP^C in Prion Diseases

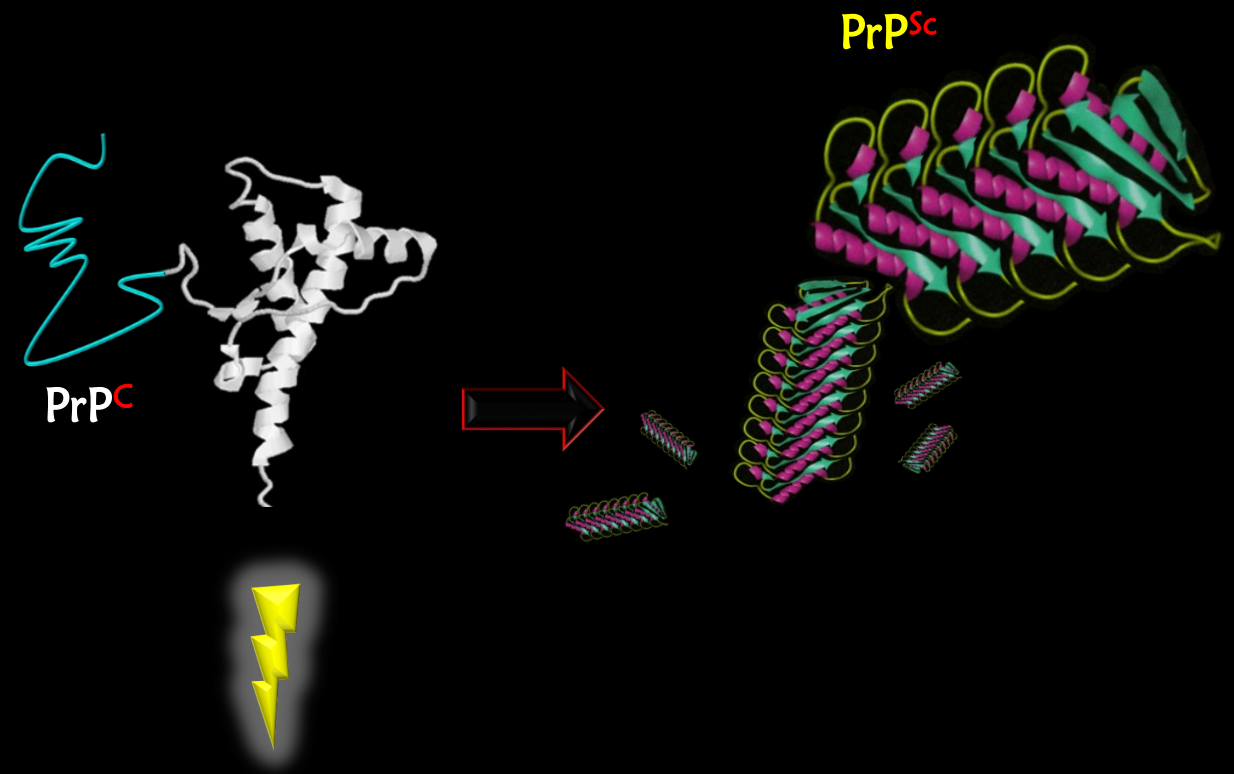


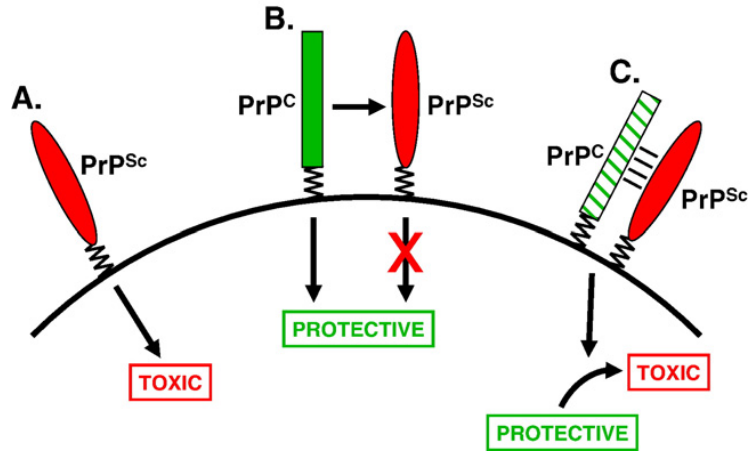
PrP^{Sc} is an **infectious** protein (PRION), capable of multiplying by directly getting in contact with PrP^C and imposing its conformational rearrangement into new PrP^{Sc} molecules

Biasini E. et al. Trends Neurosci, 2012, 35, 92-103
Prusiner S. B. PNAS USA, 1998, 95, 13363–13383

A Dual Role for PrP^C in Prion Diseases

2. Mediator of toxic signals



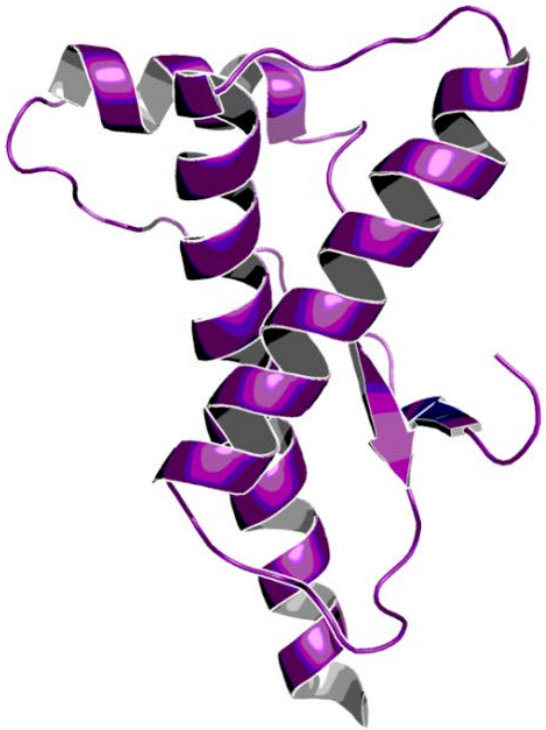


A. gain of function
B. loss of function
C. subversion of normal signaling function

Modified from Westergard L. et al. Biochim Biophys Acta. 2007, 1772, 629-644

Mercer R. CC et al. Cell. Tissue Res., 2023, 392, 81-96
Senesi M. et al. Ageing Res. Rev. 2017, 36, 156-154
Saà P. et al. Exp. Rev. Mol. Med. 2016, 18, e5, 1-18
Biasini E. et al. Trends Neurosci, 2012, 35, 92-103
Brandner S. et al. Nature, 1996, 379, 339–343

PrP^C-directed Drug Discovery Strategies in Prion Diseases

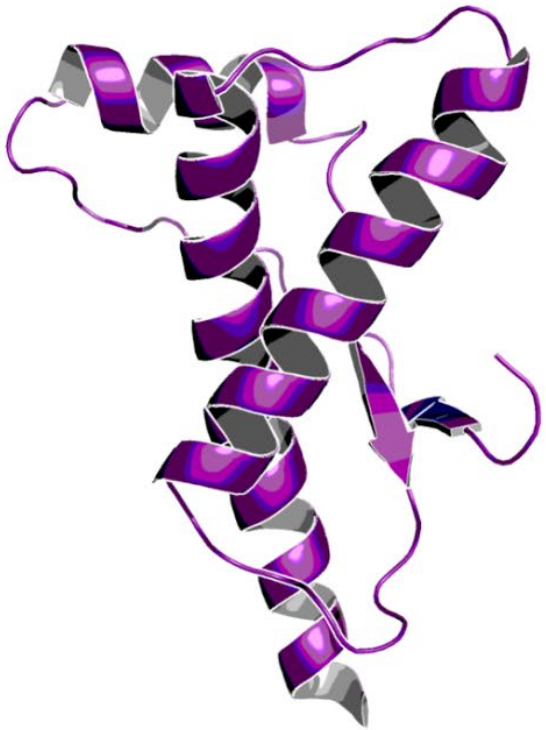


Prion protein

- Lowering PrP gene expression
- Promoting PrP degradation
- Relocalization of PrP^C
- Binding and stabilization of PrP^C
- Blocking PrP^C-mediated neurotoxic pathway

Modified from Astolfi A et al. ACS Med. Chem. Lett. 2020, 11, 2063-2067

PrP^C-directed Drug Discovery Strategies in Prion Diseases



Prion protein

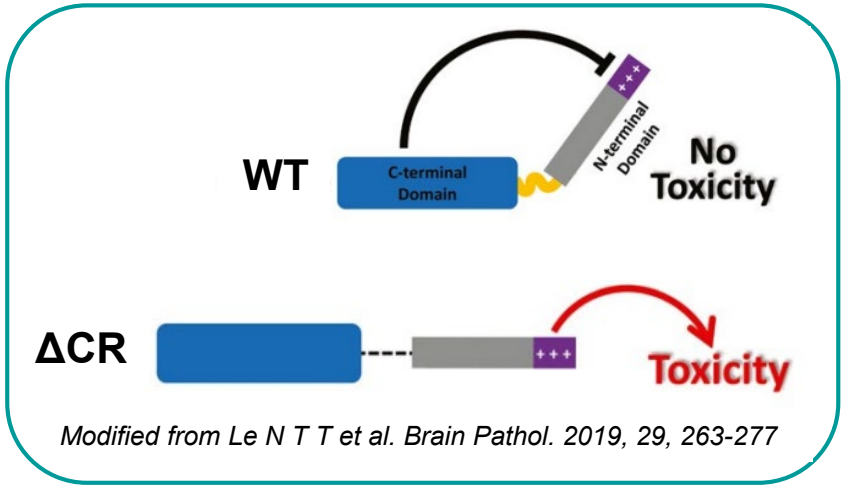
- Lowering PrP gene expression
- Promoting PrP degradation
- Relocalization of PrP^C
- Binding and stabilization of PrP^C
- Blocking PrP^C-mediated neurotoxic pathway

Modified from Astolfi A et al. ACS Med. Chem. Lett. 2020, 11, 2063-2067

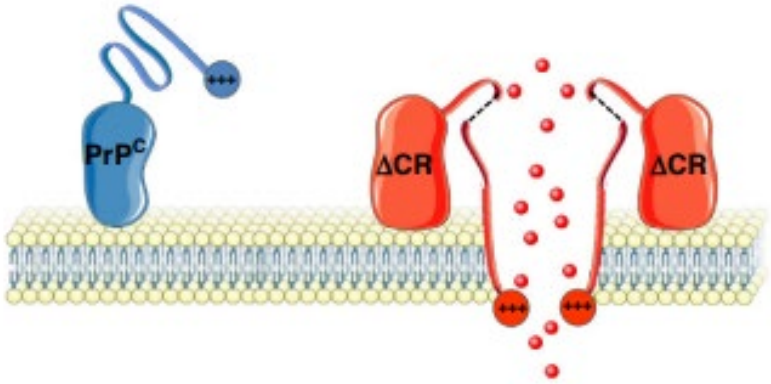
Drug-Based Cell Assay (DBCA)

Quick cellular assay to identify small molecules able to suppress mutant PrP^C-related toxicity

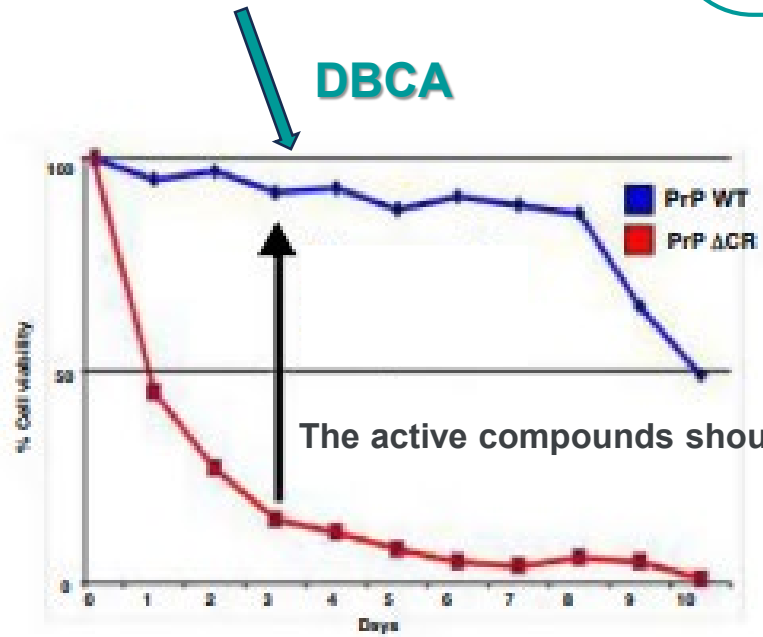
- Transgenic mice expressing PrP with a deletion of residues 105–125 in the central region of the protein (referred to as **ΔCR PrP**) developed dramatic neurodegeneration
- The DBCA can be used to evaluate the anti-**ΔCR PrP** effects of compounds
- When expressed in cells, **ΔCR PrP** induces 1) spontaneous large ionic currents and 2) <u>hypersensitivity to the toxic effects of two cationic antibiotics</u>



Modified from Le N T T et al. Brain Pathol. 2019, 29, 263-277



Spontaneous ionic currents



The active compounds should be able to restore cell viability

Modified from Mercer R CC et al. Curr. Opin. Pharmacol. 2019, 44, 20-27

Massignan, T. et al. J. Biol. Chem. 2010, 285, 7752-7765
Massignan, T. et al. Methods 2011, 53, 214-219

DBCA-based Drug Discovery

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 291, NO. 50, pp. 26164–26176, December 9, 2016

Identification of Anti-prion Compounds using a Novel Cellular Assay*[S]

Received for publication, July 1, 2016, and in revised form, October 19, 2016 Published, JBC Papers in Press, November 1, 2016, DOI 10.1074/jbc.M116.745612

Thibaut Imberdis[‡], James T. Heeres[‡], Han Yueh[§], Cheng Fang[‡], Jessie Zhen[§], Celeste B. Rich[‡], Marcie Glicksman[¶1], Aaron B. Beeler[§], and David A. Harris[‡2]

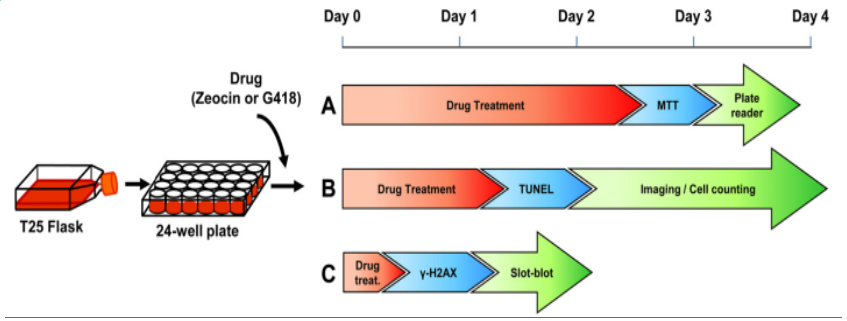
From the [‡]Department of Biochemistry, Boston University School of Medicine, Boston, Massachusetts 02118, the [§]Department of Chemistry, Boston University, Boston, Massachusetts 02115, and the [¶]Laboratory for Drug Discovery in Neurodegeneration, Brigham and Women's Hospital, Harvard Medical School, Cambridge, Massachusetts 02139

ChemPubSoc Europe DOI: 10.1002/cmdc.201700302 CHEMMEDCHEM Communications

SPECIAL ISSUE

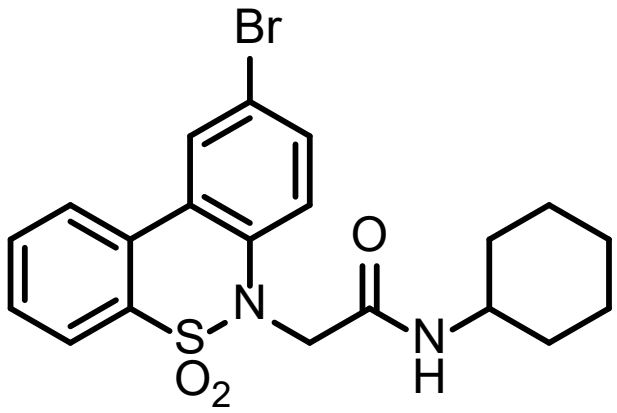
A Small-Molecule Inhibitor of Prion Replication and Mutant Prion Protein Toxicity

Tania Massignan[+,[a]] Valeria Sangiovanni[+,[a]] Silvia Biggi,[[a]] Claudia Stincardini,[[a]] Saioa R. Elezgarai,[[a, b]] Giulia Maietta,[[a]] Ivan A. Andreev,[[d]] Nina K. Ratmanova,[[d]] Dmitry S. Belov,[[d]] Evgeny R. Lukyanenko,[[d]] Grigory M. Belov,[[d, e]] Maria Letizia Barreca,[[c]] Andrea Altieri,*[[d, e]] Alexander V. Kurkin,[[d]] and Emiliano Biasini*[[a, b]]



Modified from Massignan T et al. Methods 2011, 53, 214-219

Selection of a DBCA-based Compound



LD24

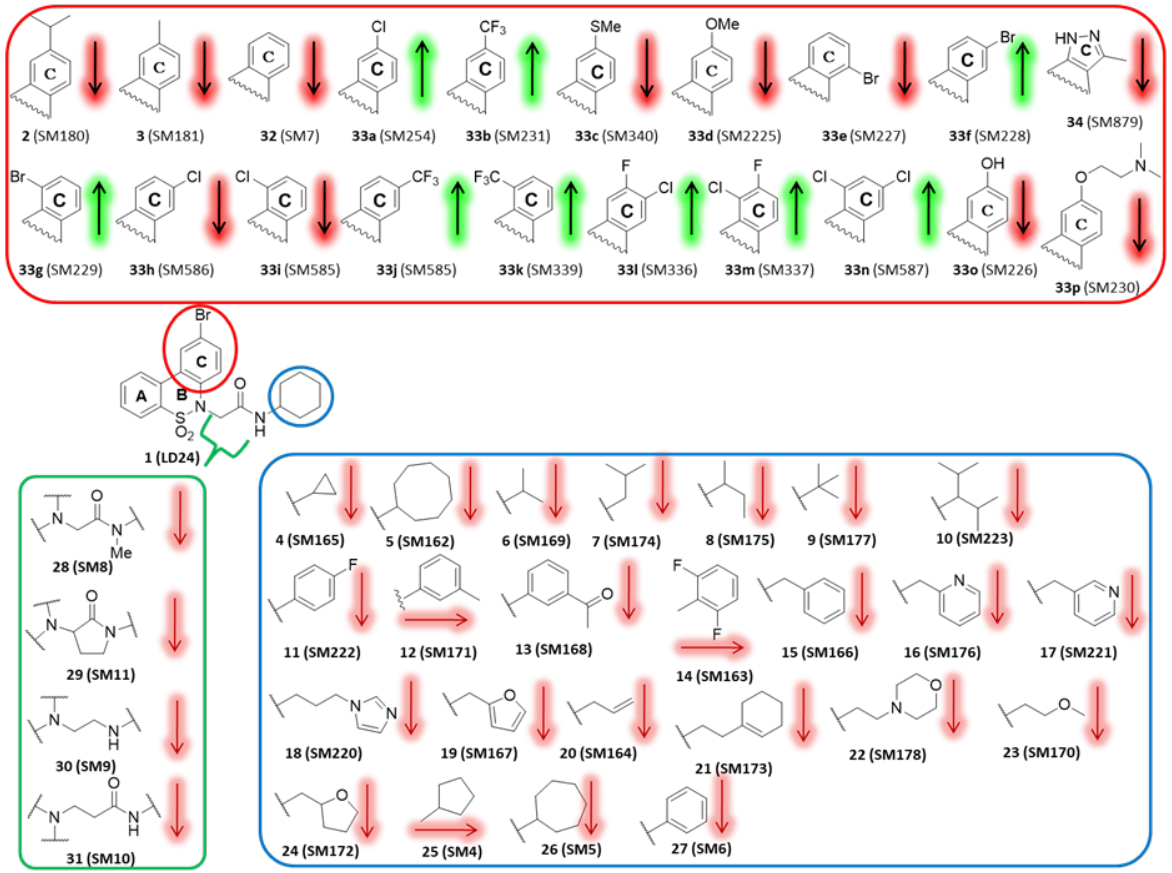
DBCA
RD_{50} = 0.4 µM
LD_{50} >100 µM

Prion infection
EC_{50} (22L and RML) ~50 µM

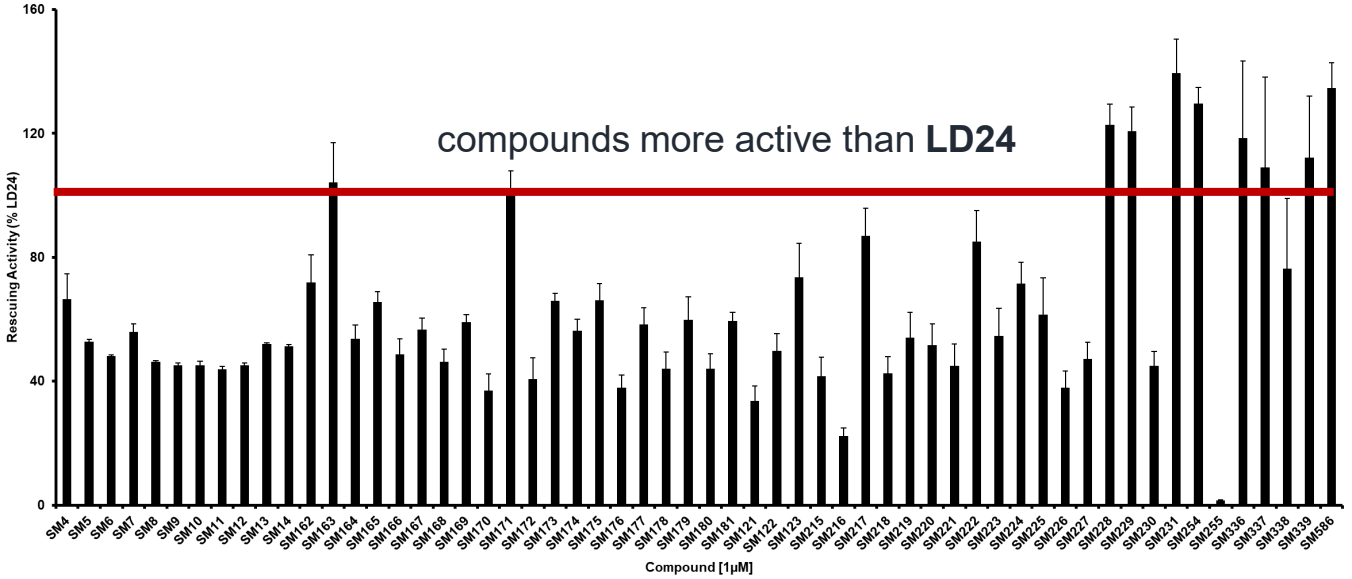
- Dibenzothiazine scaffold suitable for chemical modification and structure-activity relationship (SAR) studies.
- **Synthetic procedures developed in our laboratory**

Imberdis I H et al. J. Biol. Chem. 2016, 291, 26164-26176

First round of Chemical Optimization

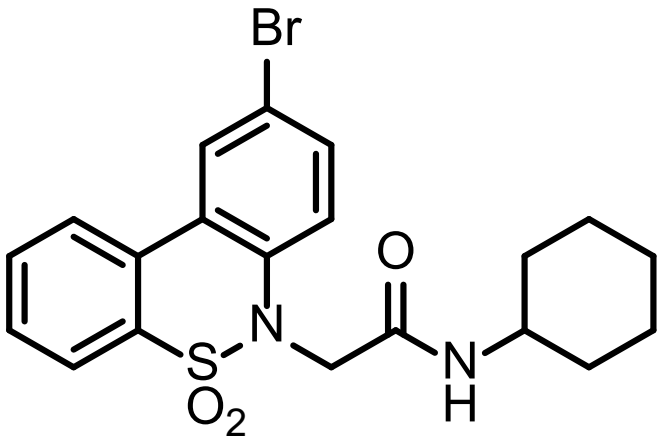


More than 80 compounds tested



First round of Chemical Optimization

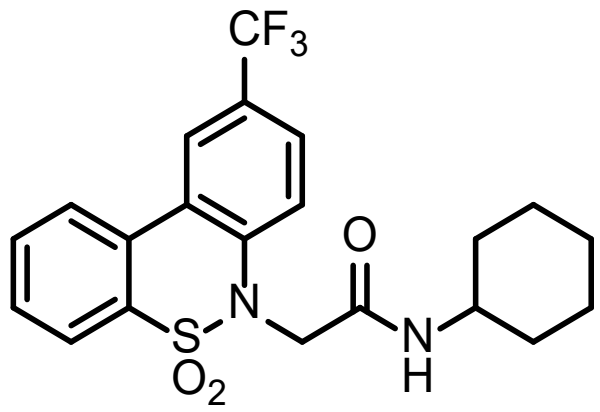
Potency improvement in the DBCA



LD24

RD_{50}= 1.1 µM
LD_{50} > 50 µM
SI > 48

>3-folds increase in the cell protection potency
Similar toxicity



SM231

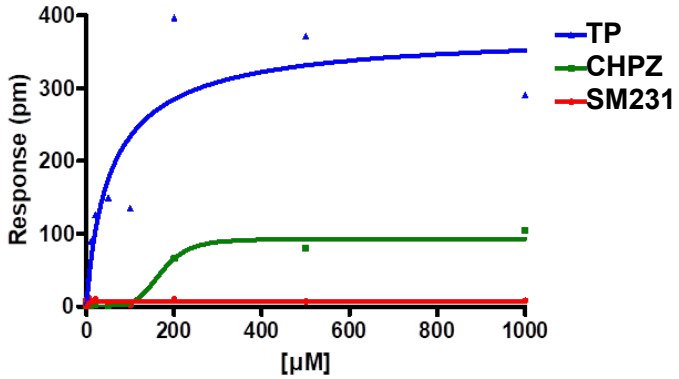
RD_{50}= 0.32 µM
LD_{50} = 22.4 µM
SI = 70

SM321 Does Not Target PrP^C Directly

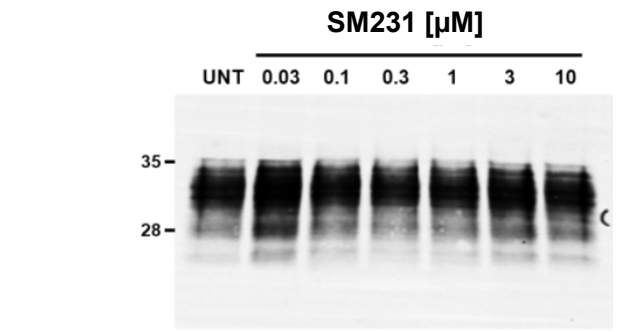
SM231 does not act by directly binding PrP^C, or by altering its expression or localization.

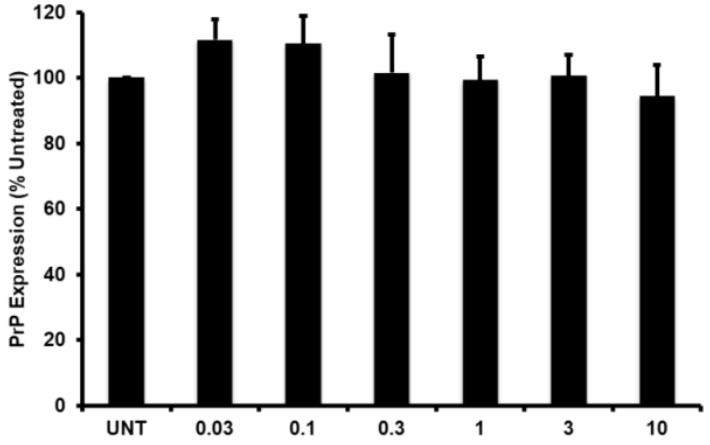
Dynamic Mass Redistribution (DMR)

Massignan et al. SciRep 2016

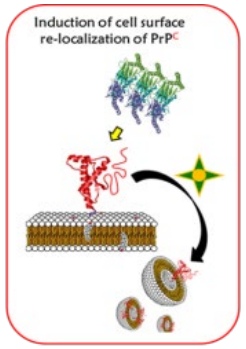


SM231 does not directly bind PrP^C

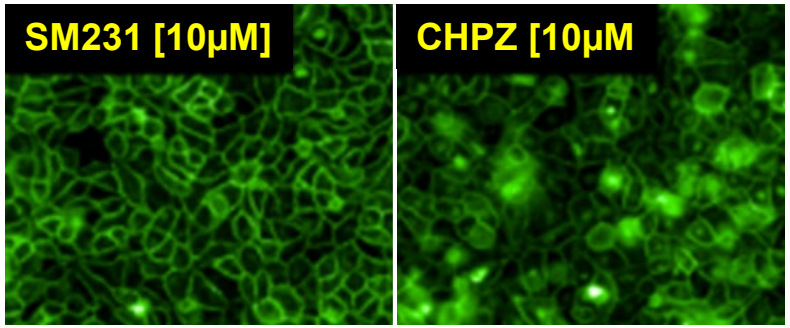




SM231 does not alter the expression of PrP^C



Stincardini et al. PLoSONE 2017

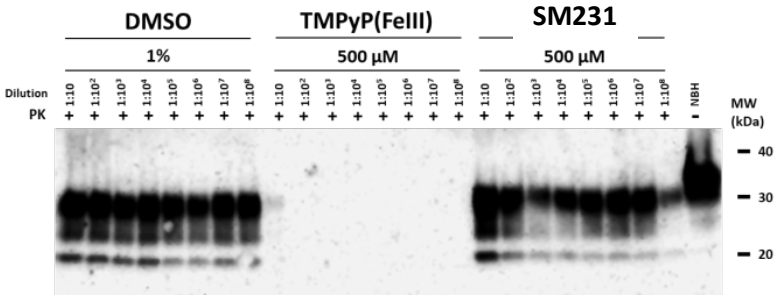


SM231 does not alter the cell-surface localization of PrP^C

SM231 is a Weak Inhibitor of Prion Replication

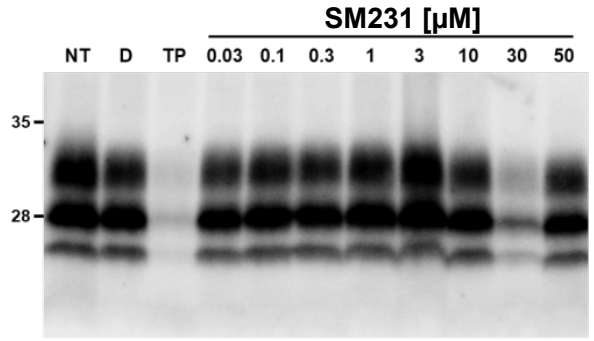
SM231 fails to suppress prion replication in vitro, in cells and in brain slices.

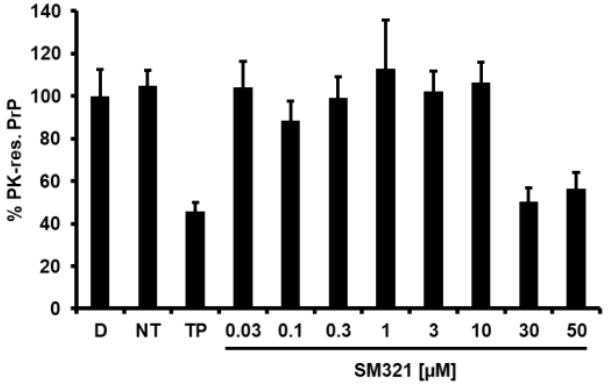
protein misfolding cyclic amplification (PMCA) reaction



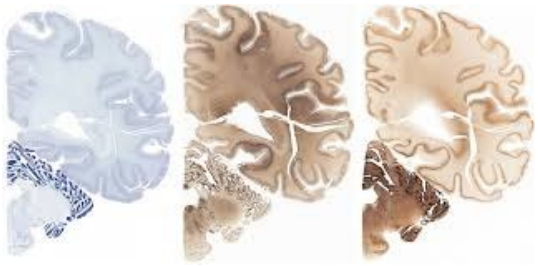
SM231 does not inhibit prion replication in a cell-free system

Prion-Infected Cells (RML and 22L)

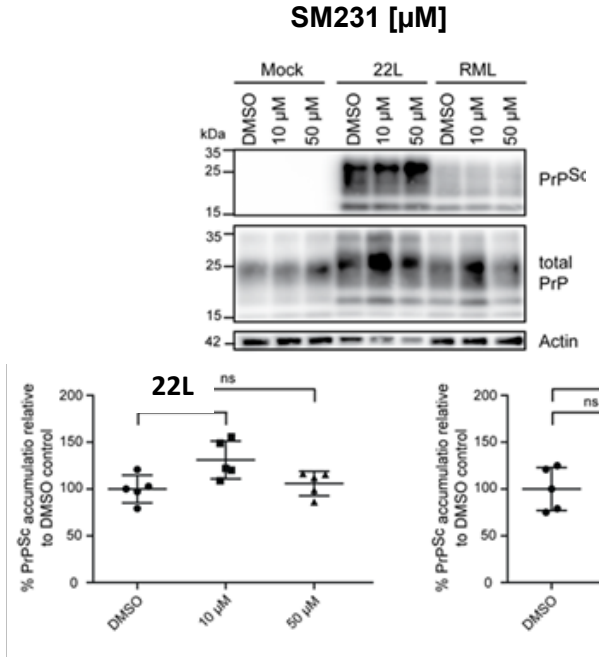




SM231 does not reduce PrP^{SC} formation in infected cells (reduction only at highest compound concentrations)



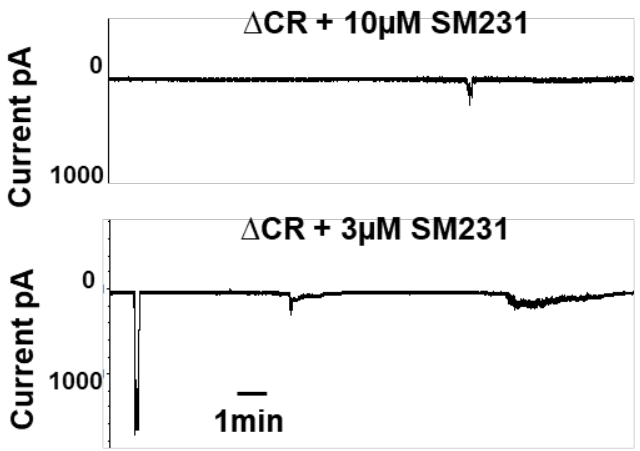
Prion-Infected Brain Slices /RML and 22L)

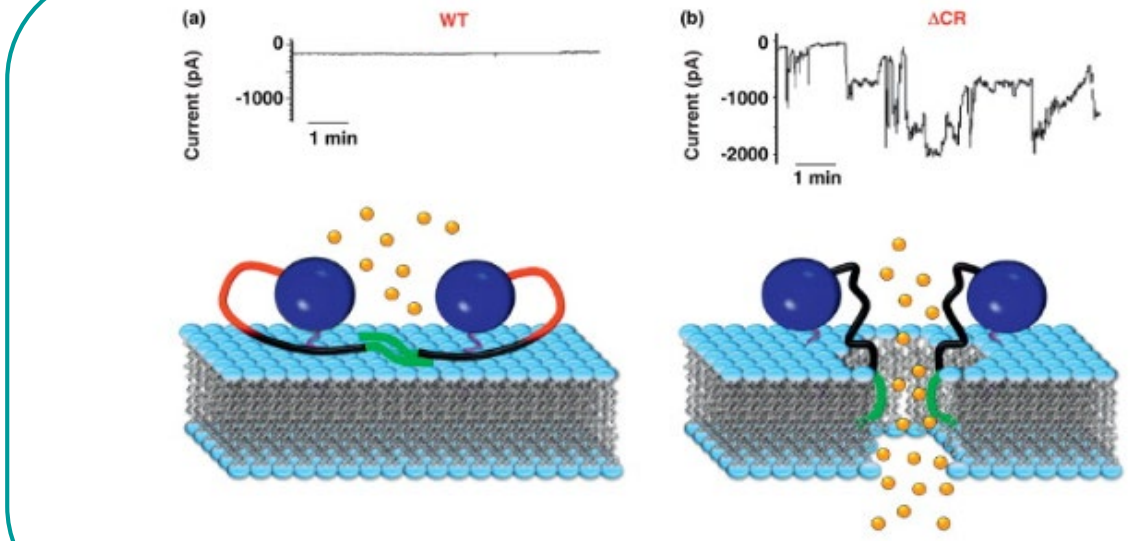


Slightly reduction of PrP^{SC} at 50 µM only with RML strain

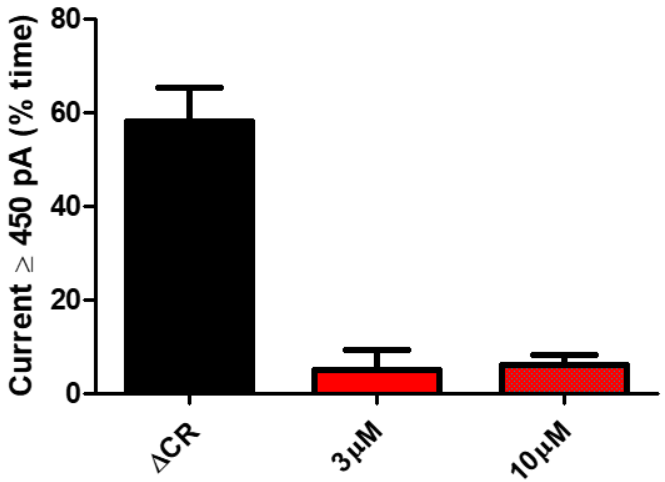
SM231 Suppresses the channel activity of Mutant PrP

SM231 abrogates inward ionic currents generated by *Δ*CR PrP





Modified by Biasini E et al. Trends in Neur, 2012, 35, 92-103



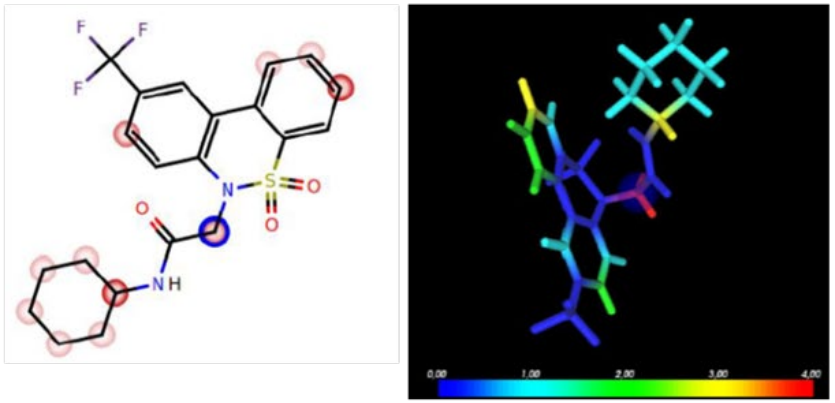
- **ΔCR PrP** induces spontaneous large ionic currents in a variety of cultured cells and neurons
- Several point mutations in PrP that cause genetic prion diseases also induce spontaneous currents
- The current-inducing activity of mutant PrPs observed *in vitro* is mechanistically related to their neurotoxicity *in vivo*

Solomon I.H. et al. J. Biol. Chem. 2010, 285, 26719-26726

Metabolic Studies on Derivative SM231

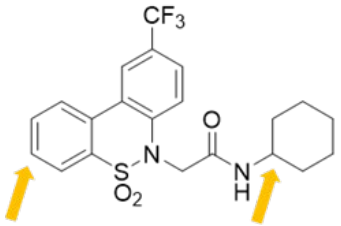
SM231 showed low metabolic stability and poor solubility in aqueous medium, two properties which hampers its use in pre-clinical settings.

A



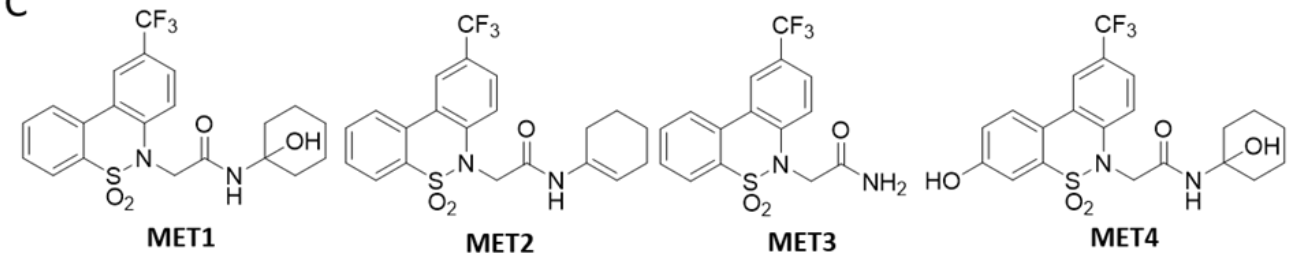
Computational predictions using MetaSite

B



Computational predictions based on docking experiments

C

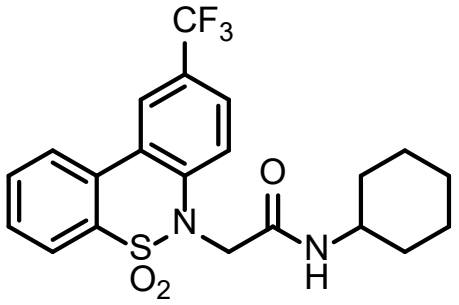


MET1 **MET2** **MET3** **MET4**

RLM/HPLC-MS

Second Round of Chemical Optimization

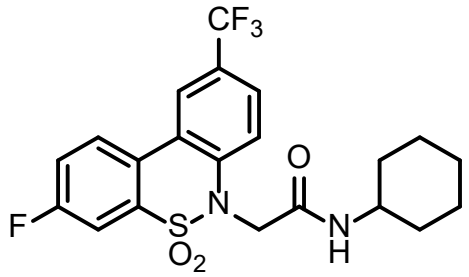
Improvement of potency and selectivity



SM231

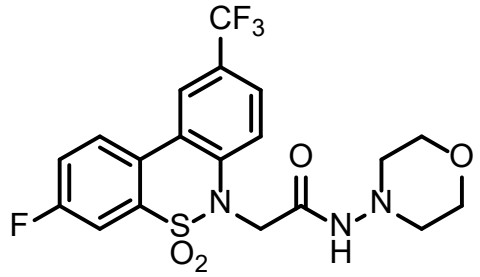
RD_{50}= 0.32 µM
LD_{50} = 22.4 µM
SI = 77

25-61-folds potency increase in cell protection
No toxicity



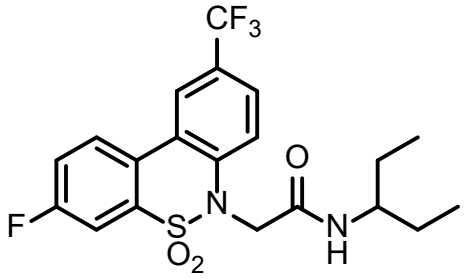
SM882

RD_{50}= 0.044 µM
LD_{50} > 100 µM
SI >2273



SM881

RD_{50}= 0.032 µM
LD_{50} > 100 µM
SI > 3333

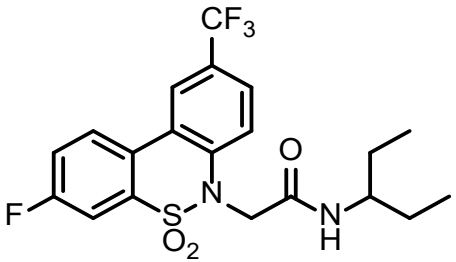


SM884

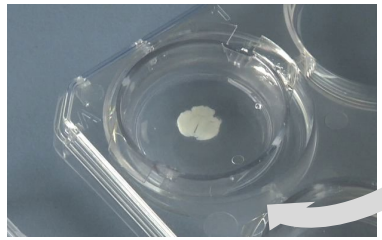
RD_{50}= 0.018 µM
LD_{50} > 100 µM
SI > 5555

SM884 rescues the synaptotoxic effects of prions in mouse brain slices.

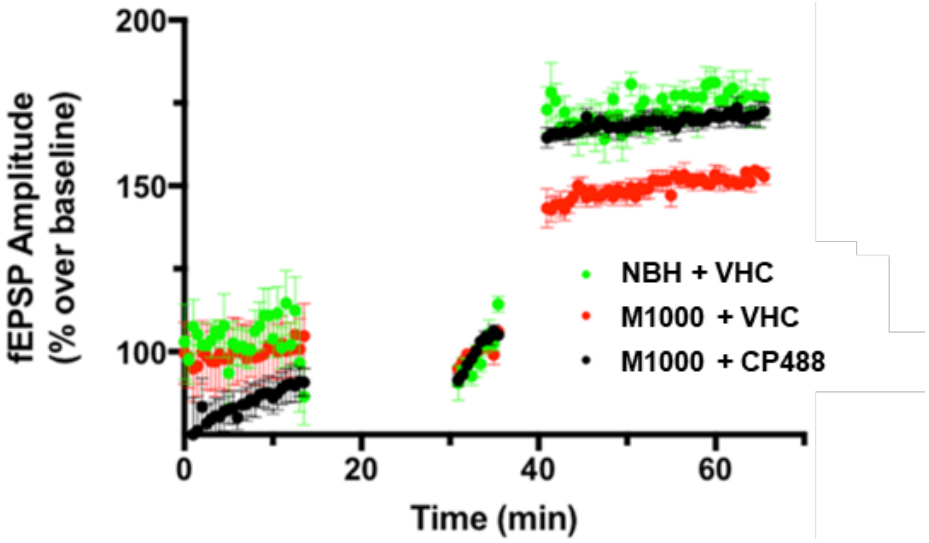
SM884 (at 0.03 μM concentration) restores normal synaptic plasticity and excitatory post-synaptic potential in *ex-vivo* model of prion toxicity



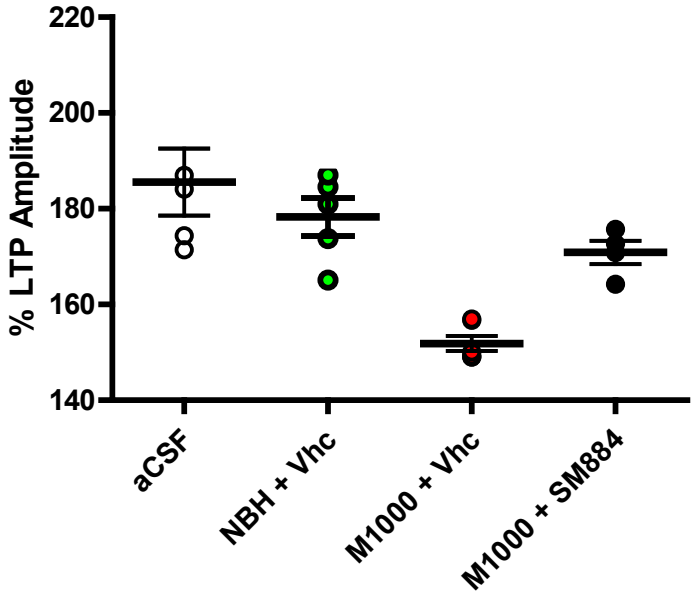
SM884

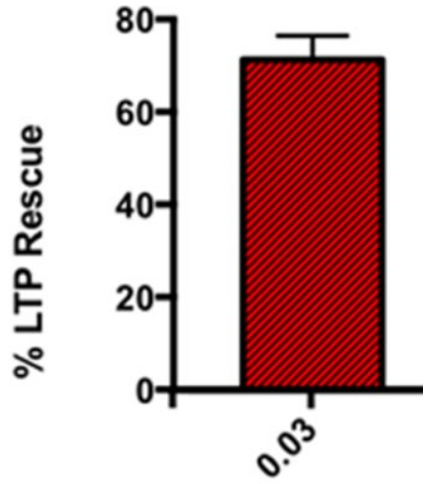


moRK13 cells infected with M1000 prions



Excitatory-Post-Synaptic Potential (EPSP) as an index of synaptic functionality

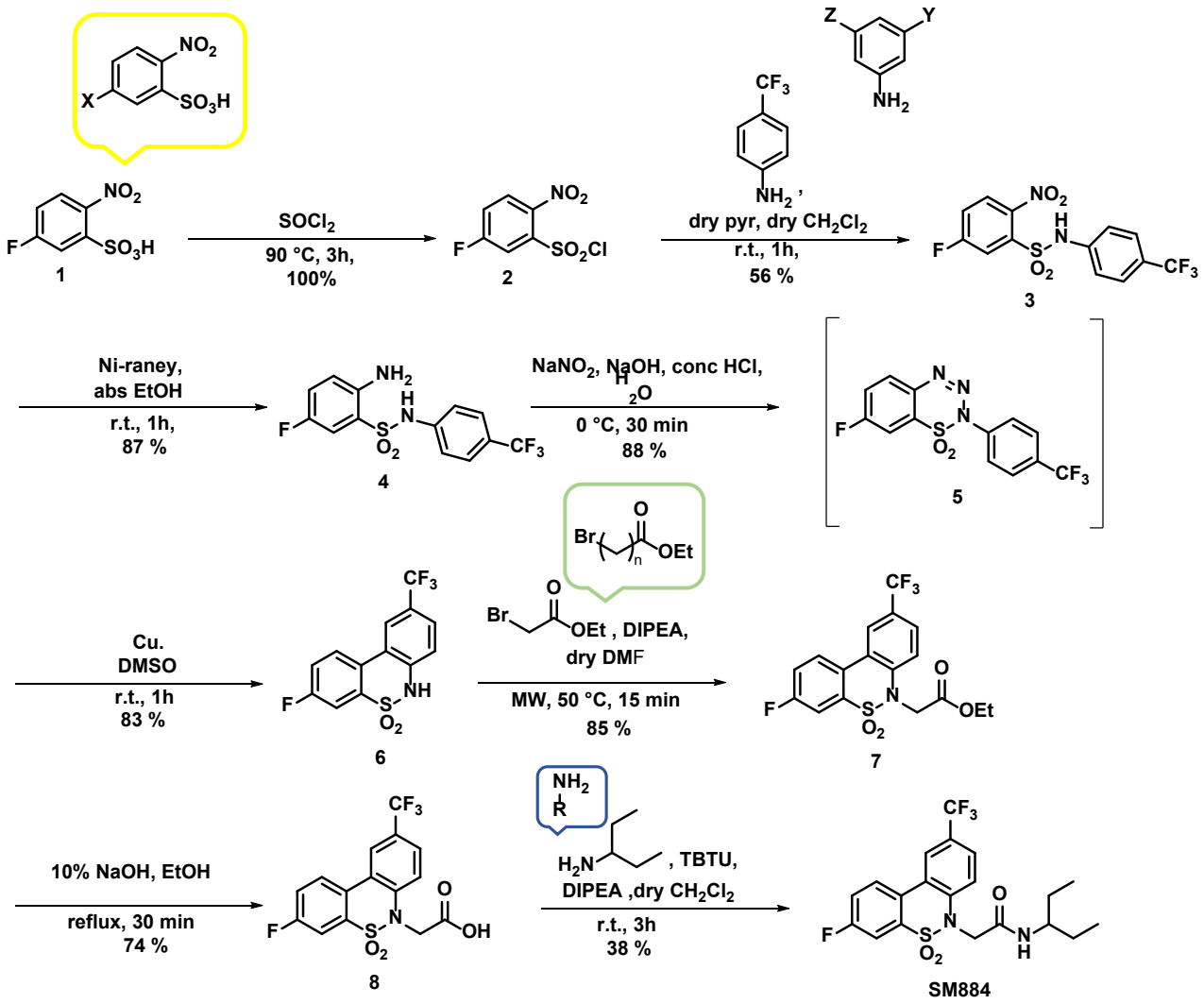


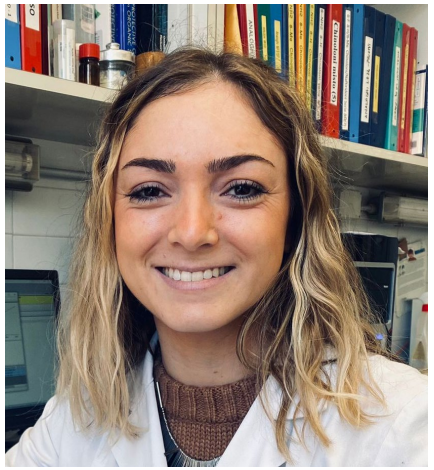


Long-Term Potentiation (LTP) as and index of synaptic plasticity

Foliaki S. T. et al. PLoS Pathog. 2018, 14(8): e1007214

Synthetic procedures developed in our laboratory at UNIPG





Maria Giulia Nizi

CJD Foundation bursary

- **First task**

Synthesis of the required amounts of compounds for the *in vitro* and *in vivo* experiments

CREUTZFELDT-JAKOB DISEASE FOUNDATION, INC.

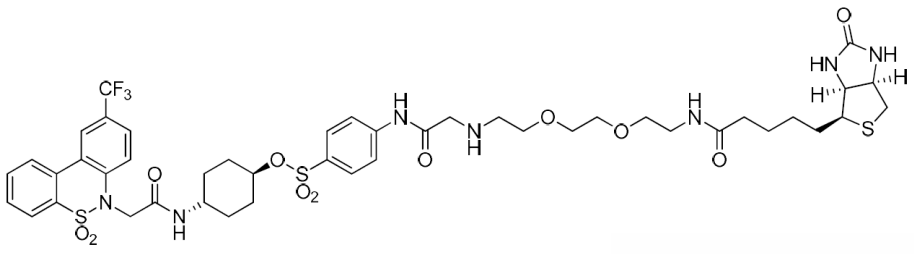
Supporting Families Affected by Prion Disease

Which is the molecular target of SM-derived compounds?

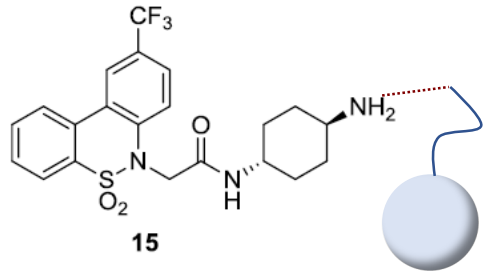
Output: short list of potential targets that we need to validate

- **Second task**

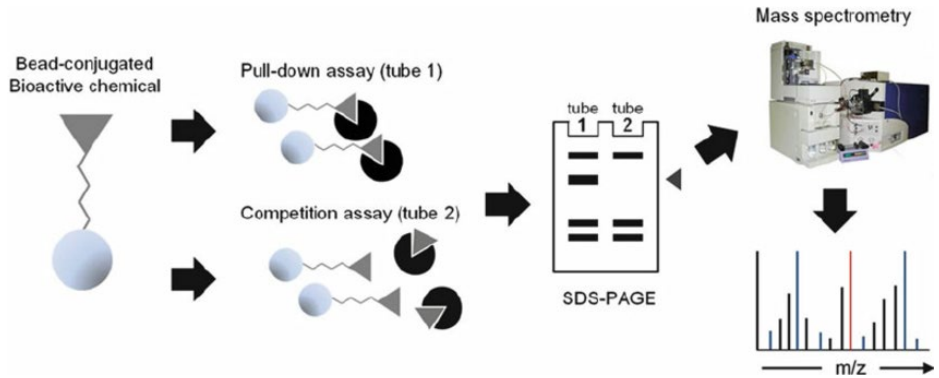
Identification of the possible molecular target(s) for our bioactive compounds, given that we are aware that these small molecules do not act on PrP^C directly



PrP15biot2



15



Transnational Call
CHEMOPROTEOMICS AND MASS SPECTROMETRY IMAGING 2021

CREUTZFELDT-JAKOB DISEASE FOUNDATION, INC.
Supporting Families Affected by Prion Disease

In vitro & *in vivo* Pharmacokinetic Profiling of SM881 and SM884

➢ **Third task**

Information obtained from *in vitro* and *in vivo* pharmacokinetic studies regarding how the body interacts with our compounds.

In vitro Adsorption, Distribution, Metabolism and Excretion (ADME)

- permeability in MDCKII-MDR1 cells (with and without inhibitor)
- binding (mouse plasma protein and brain tissue)
- metabolic stability in liver microsomes and hepatocytes.

In vivo Pharmacokinetic (PK)

Both compounds were administered via intravenous bolus administration in CD-1 mice at 2 mg/kg. Plasma and tissue (brain, kidney and liver) were collected by terminal sampling from 3 animals per time point up to 24 h.

CJD FOUNDATION
CREUTZFELDT-JAKOB DISEASE FOUNDATION, INC.
Supporting Families Affected by Prion Disease

In vitro & *in vivo* Pharmacokinetic Profiling of SM881 and SM884

➢ **Third task**

Information obtained from *in vitro* and *in vivo* pharmacokinetic studies regarding how the body interacts with our compounds.

In vitro Adsorption, Distribution, Metabolism and Excretion (ADME) studies in mouse

Test compound	Permeability	Plasma binding	Brain tissue homogenate binding	Metabolic stability in liver microsomes	Metabolic stability in hepatocytes
SM881	moderate P-gp substrate	Moderate (F_b = 94.1%)	Moderate (F_b = 95.1%)	Moderate predicted *in vivo* hepatic clearance	Moderate predicted *in vivo* hepatic clearance
SM884	high No P-gp substrate	very high (F_b = 99.8%)	high (F_b=98.6%)	High predicted *in vivo* hepatic clearance	High predicted *in vivo* hepatic clearance

CJD FOUNDATION
CREUTZFELDT-JAKOB DISEASE FOUNDATION, INC.
Supporting Families Affected by Prion Disease

Summary

- **Dibenzothiazine derivatives potently inhibit electrophysiological abnormalities induced by mutant PrP molecules**
- **The compounds do not directly target PrP^C and are weak inhibitors of prion propagation in cell cultures or brain slices, suggesting that their pharmacological target is mainly involved in prion toxicity but not replication**

Summary

- Dibenzothiazine derivatives potently inhibit electrophysiological abnormalities induced by mutant PrP molecules
- The compounds do not directly target PrP^C and are weak inhibitors of prion propagation in cell cultures or brain slices, suggesting that their pharmacological target is mainly involved in prion toxicity but not replication

Ongoing and Future Work

- **Additional experimental studies to validate the postulated molecular target(s)**

Summary

- Dibenzothiazine derivatives potently inhibit electrophysiological abnormalities induced by mutant PrP molecules
- The compounds do not directly target PrP^C and are weak inhibitors of prion propagation in cell cultures or brain slices, suggesting that their pharmacological target is mainly involved in prion toxicity but not replication

Ongoing and Future Work

- Additional experimental studies to validate the postulated molecular target(s)
- **Analysis of *in vivo* pharmacokinetic profiling results**

Summary

- Dibenzothiazine derivatives potently inhibit electrophysiological abnormalities induced by mutant PrP molecules
- The compounds do not directly target PrP^C and are weak inhibitors of prion propagation in cell cultures or brain slices, suggesting that their pharmacological target is mainly involved in prion toxicity but not replication

Ongoing and Future Work

- Additional experimental studies to validate the postulated molecular target(s)
- Analysis of *in vivo* pharmacokinetic profiling results
- **Testing *in vivo* the therapeutic potential of the most promising compound(s). The goal is to directly test the hypothesis that blocking PrP-mediated toxicity could provide therapeutic benefits in prion diseases**

Summary

- Dibenzothiazine derivatives potently inhibit electrophysiological abnormalities induced by mutant PrP molecules
- The compounds do not directly target PrP^C and are weak inhibitors of prion propagation in cell cultures or brain slices, suggesting that their pharmacological target is mainly involved in prion toxicity but not replication

Ongoing and Future Work

- Additional experimental studies to validate the postulated molecular target(s)
- Analysis of *in vivo* pharmacokinetic profiling results
- Testing *in vivo* the therapeutic potential of the most promising compound(s). The goal is to directly test the hypothesis that blocking PrP-mediated toxicity could provide therapeutic benefits in prion diseases
- **Our dibenzothiazine derivatives may serve as chemical biological tools to unravel poorly understood aspects of prion biology**

CREUTZFELDT-JAKOB DISEASE FOUNDATION, INC.

Supporting Families Affected by Prion Disease

CJD Surveillance
National Prion Disease
Pathology Surveillance Center

2023 CJD Foundation Family Conference

July 14–17, 2023, Hilton Washington DC Capitol Hill

Maria Letizia Barreca

Department of Pharmaceutical Sciences, University of Perugia, Italy

"Blocking the Neurotoxic Activities of Mutant Prion Proteins by a Novel Class of Therapeutic Agents"

SUMMARY

The following tasks have been achieved:

- Synthesis of the required amounts of compounds for the *in vitro* and *in vivo* experiments;
- Identification of the possible molecular target(s) for our bioactive compounds, given that we are aware that these small molecules do not bind the cellular prion protein;
- Information obtained from *in vitro* and *in vivo* pharmacokinetic studies regarding how the body interacts with our compounds.

ONGOING AND FUTURE WORK

- ✓ Additional experimental studies to definitively validate the postulated molecular target(s);
- ✓ Testing *in vivo* the therapeutic potential of the most promising compound by using mouse models of prion diseases. The goal is to directly test the hypothesis that blocking PrP-mediated toxicity could provide therapeutic benefits in prion diseases.