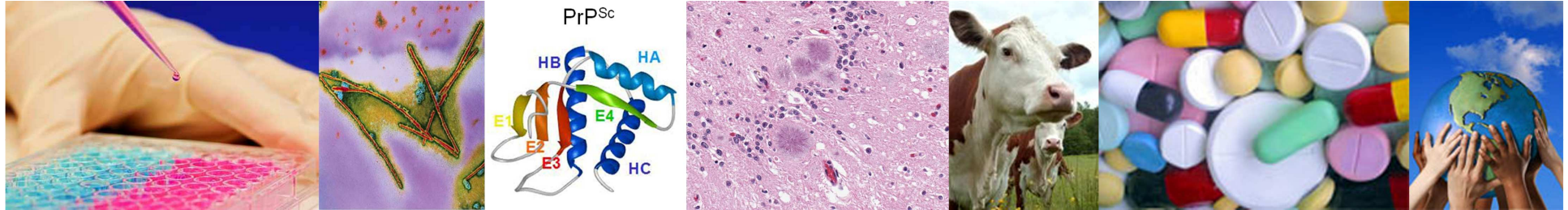


12 SAC Seminar Combating Global Infections



Recombinant prion proteins: conformation properties and application for diagnostic of transmissible spongiform encephalopathies.

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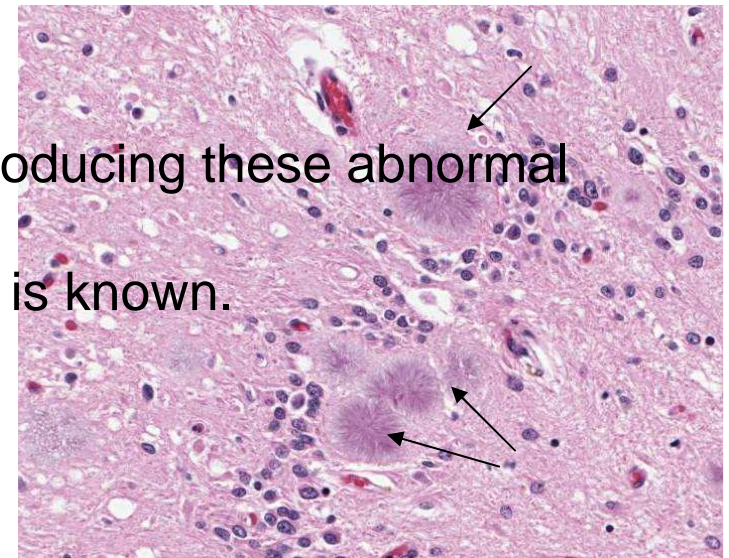
This material is based upon work supported by ISTC partner project #2777p and NARVAC R&D Co (Russia).

21-24 September, Irkutsk, Russia

Introduction

Amyloidosis is a pathological condition resulting from aggregation of extracellularly deposited abnormal proteins called amyloid fibrils that cause damage to organs and tissues.

- Proteins are deposited extracellularly
- Proteins aggregate and form fibrils called amyloid fibrils.
- Amyloid fibrils arise from misfolded proteins of host organism.
- Misfolded proteins may result from point mutations.
- Deposited as localized or systemic
 - i) localized: close to cells producing it;
 - ii) systemic: distant sites from these cells producing these abnormal proteins.
- In time more than 20 amyloidogenic protein is known.



Amyloidogenic proteins and the corresponding human diseases

Precursor protein	Fibril component	Clinical syndrome
APP	β -peptide 1-40 to 1-43	Alzheimer's disease
Immunoglobulin light chain	Intact light chain or fragments	Primary systemic amyloidosis
Serum amyloid A	Amyloid A (76 residue fragment)	Secondary systemic amyloidosis
Transthyretin	Over 45 transthyretin variants	Senile systemic amyloidosis Familial amyloid polyneuropathy I
Cystatin C	Cystatin C fragment	Hereditary cerebral amyloid angiopathy
β_2 -microglobulin	β_2 -microglobulin	Haemodialysis-related amyloidosis
Apolipoprotein A1	Fragments of ApoA1	Familial amyloid polyneuropathy III
Gelsolin	71 acid fragment of gelsolin	Finnish hereditary systemic amyloidosis
Islet amyloid polypeptide (IAPP)	Fragment of IAPP	Type II diabetes
Calcitonin	Fragments of calcitonin	Medullary carcinoma of the thyroid
Prion protein	Prion protein or fragments there of	Spongiform encephalopathies
Atrial natriuretic factor (ANF)	ANF	Atrial amyloidosis
Lysozyme	Lysozyme or fragments there of	Hereditary non-neuropathic systemic amyloidosis
Insulin	Insulin	Injection-localized amyloidosis
Fibrinogen	Fibrinogen fragments	Hereditary renal amyloidosis

Prion protein (PrP)



Misfolding



Transmissible Spongiform Encephalopathies (TSE)

Bovine Spongiform Encephalopathy (BSE)

Scrapie

Transmissible mink encephalopathy (TME)

Chronic Wasting Disease (CWD)

Feline spongiform encephalopathy (FSE)

Creutzfeldt-Jacob Disease (CJD)

Variant Creutzfeldt-Jacob Disease (vCJD)

Gerstmann-Straussler-Scheinker Disease (GSSD)

Fatal insomnia

**Prion diseases
TSE's**

!!! proved ability to inter- and intraspecies transmission !!!



Why we investigate this subject ?

1. New type of infection agent – only protein.
2. Transmission prion disease from species to species, including human.
3. Absence of any reliable means for TSE diagnostics in Russia



Aims of our investigation

1. Development test-system for detection abnormal isoform of PrP in human and animals tissues.
2. Research of recombinant prion proteins properties to form amyloid-like structures in vitro.
3. Produce model simulate interspecies transmission of TSE.



Production of recombinant prion proteins (rPrP's)

Full and truncated (with eliminate signal sequences and octarepeat region) PrP

PrP

Human (*Homo sapience*)
Bovine (*Bos taurus*)
Deer (*Cervus elaphus* and *C..nippon*)

Escherichia coli

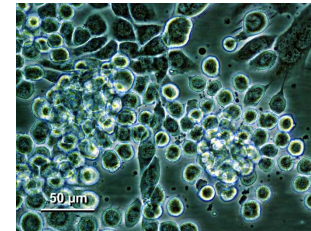
pET vectors



Only a.a. chain correspond to any PrP

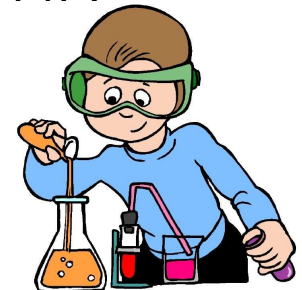
Sf21
(*Spodoptera frugiperda*)

pFastBac HT vectors



Eukaryotic folding molecule
with typical glycosilation of PrP.

Easy, low-cost and fast purification with Ni-NTA agarose



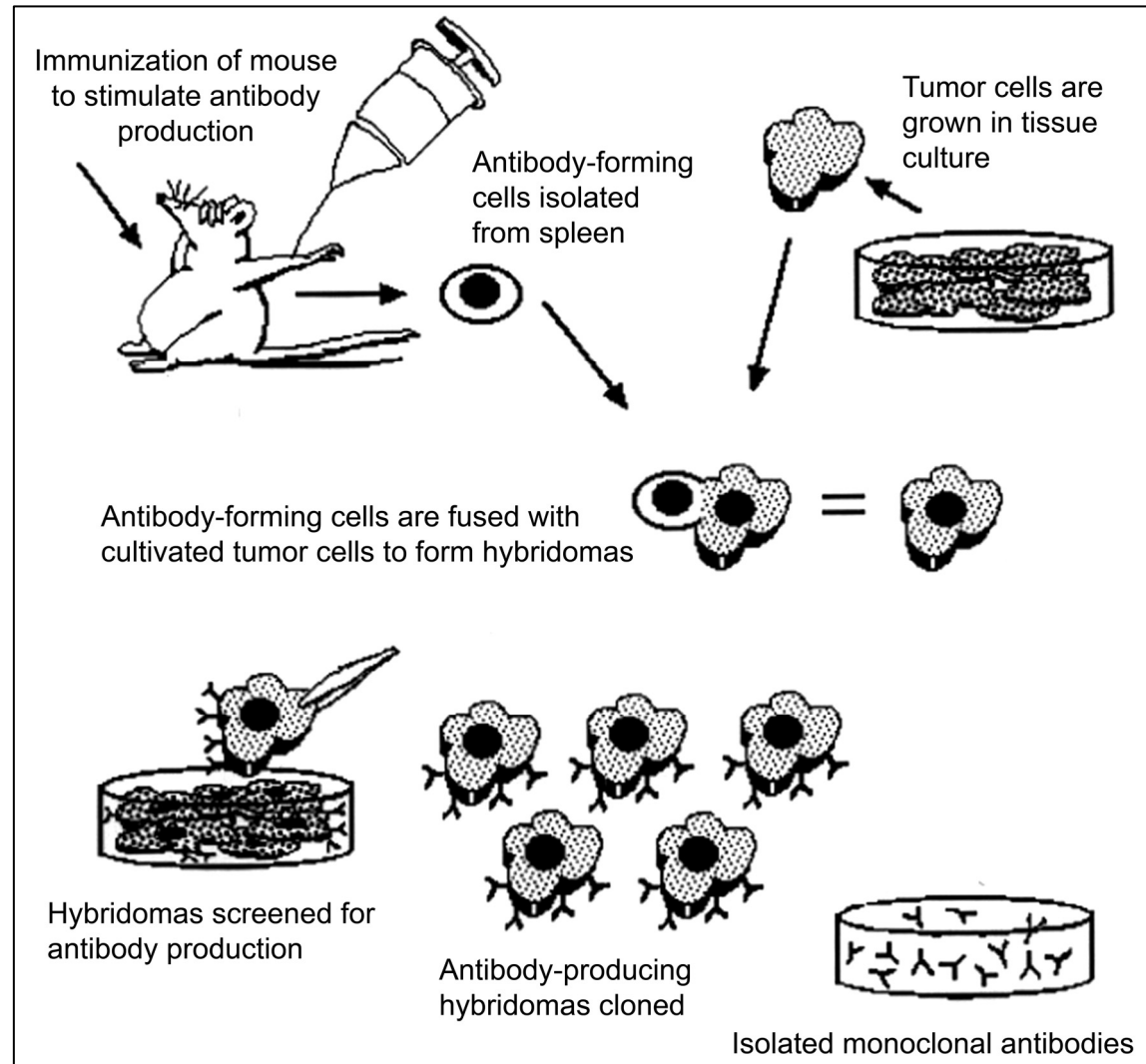
Production of monoclonal antibodies to prion protein

MAbs were prepared by conventional hybridoma technology

Mouse: BALB/c

Antigen:
recombinant fragment
of bovine PrP (102-240 a.a.),
expressed in E.Coli

Myeloma cell line: SP2/0-Ag14,

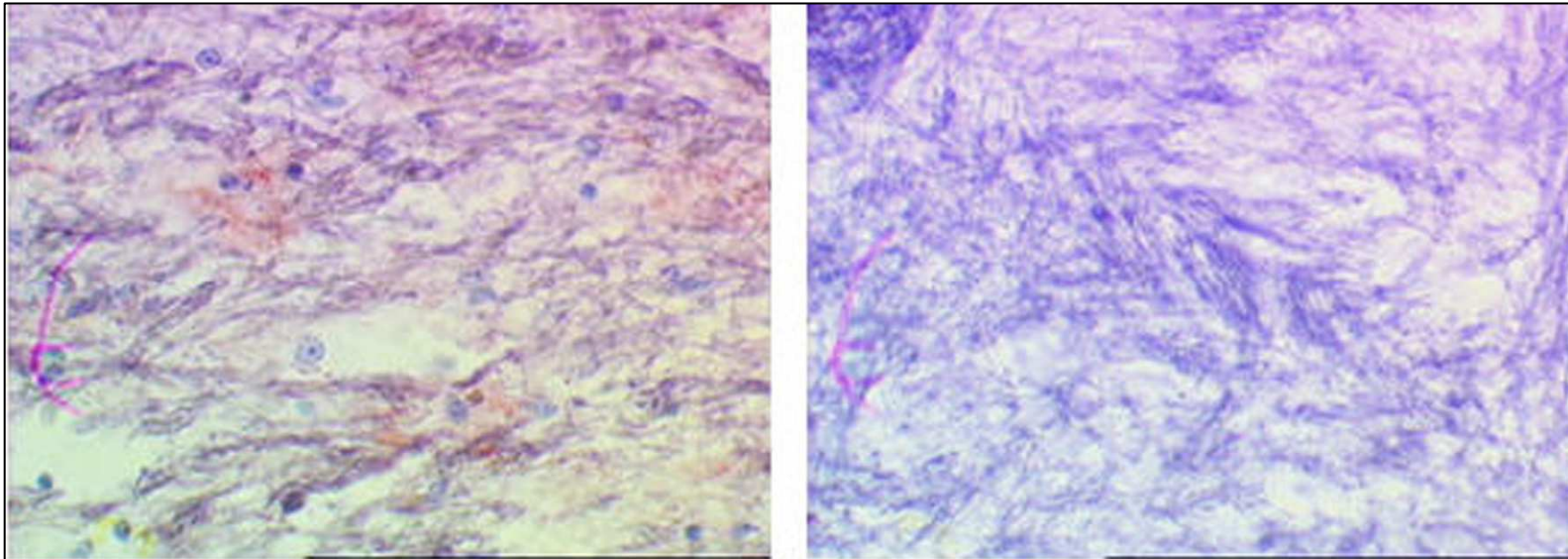


Application of produced Mab to detect
protease resistant isoform PrP.

Immunohistochemistry:

New generated Mab allow to reveal accumulation of PrP^d by IHC in brain organism with TSE

Bovine (*Bos taurus*):



BSE positive

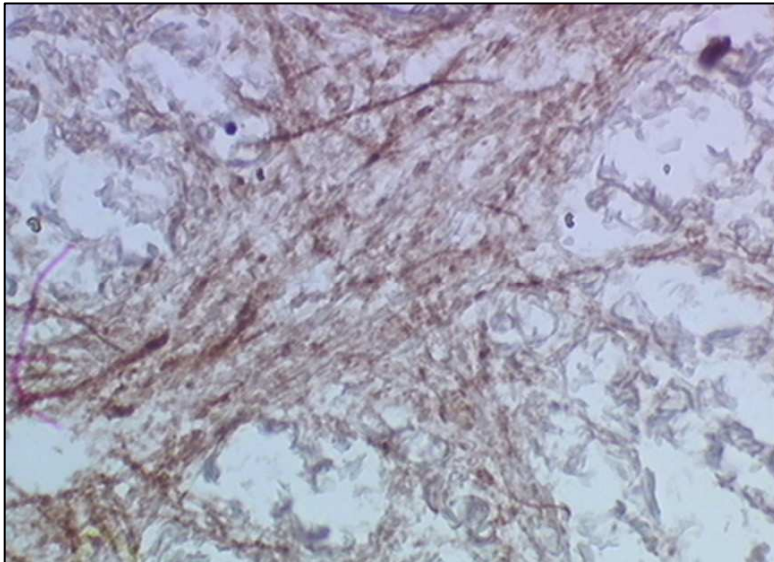
BSE negative

Application of produced Mab to detect
protease resistant isoform PrP.

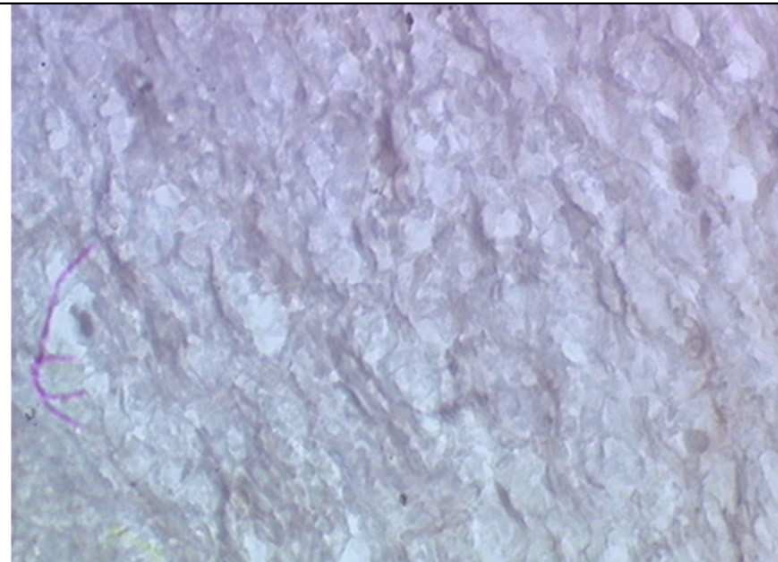
Immunohistochemistry:

New generated Mab allow to reveal accumulation of PrP^d by IHC in brain organism with TSE

Sheep (*Ovis aries*):



Scrapie positive



Scrapie negative

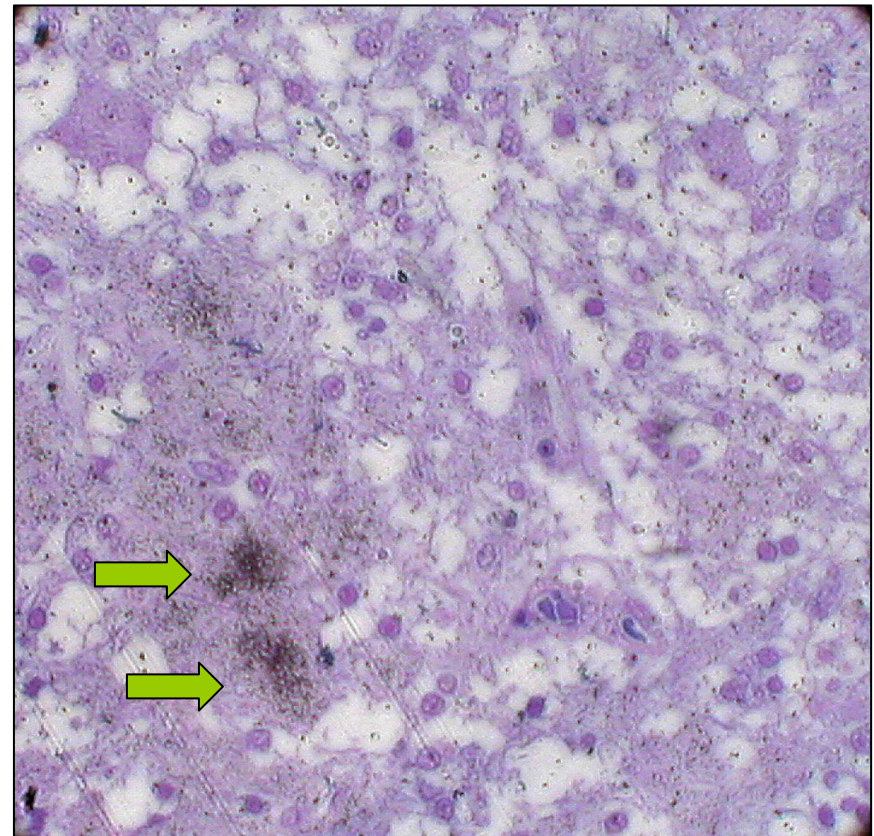
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Immunohistochemistry:

New generated Mab allow to reveal accumulation of PrP^d by IHC in brain organism with TSE

Human (*Homo sapiens*):

Amyloid plaques in brain of patient with vCJD
accumulation of PrP^d were detected by Mab 2C8.



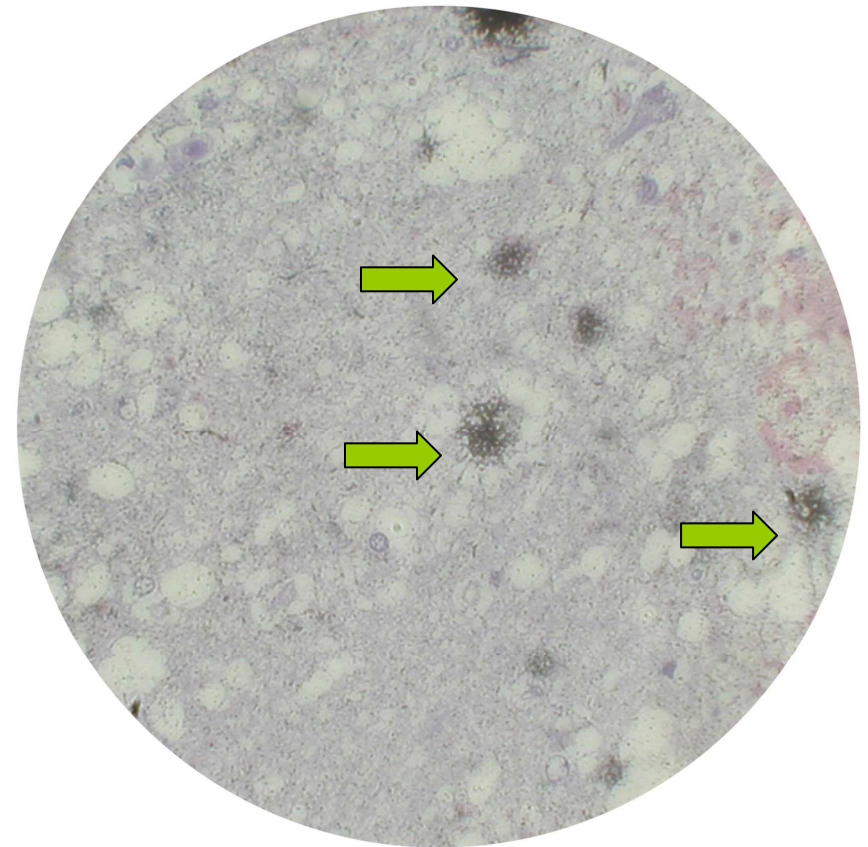
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Immunohistochemistry:

New generated Mab allow to reveal accumulation of PrP^d by IHC in brain organism with TSE

Human (*Homo sapience*):

Amyloid plaques in brain of patient with GSSS
accumulation of PrP^d were detected by Mab 2C8.



Application of produced Mab to detect protease resistant isoform PrP.

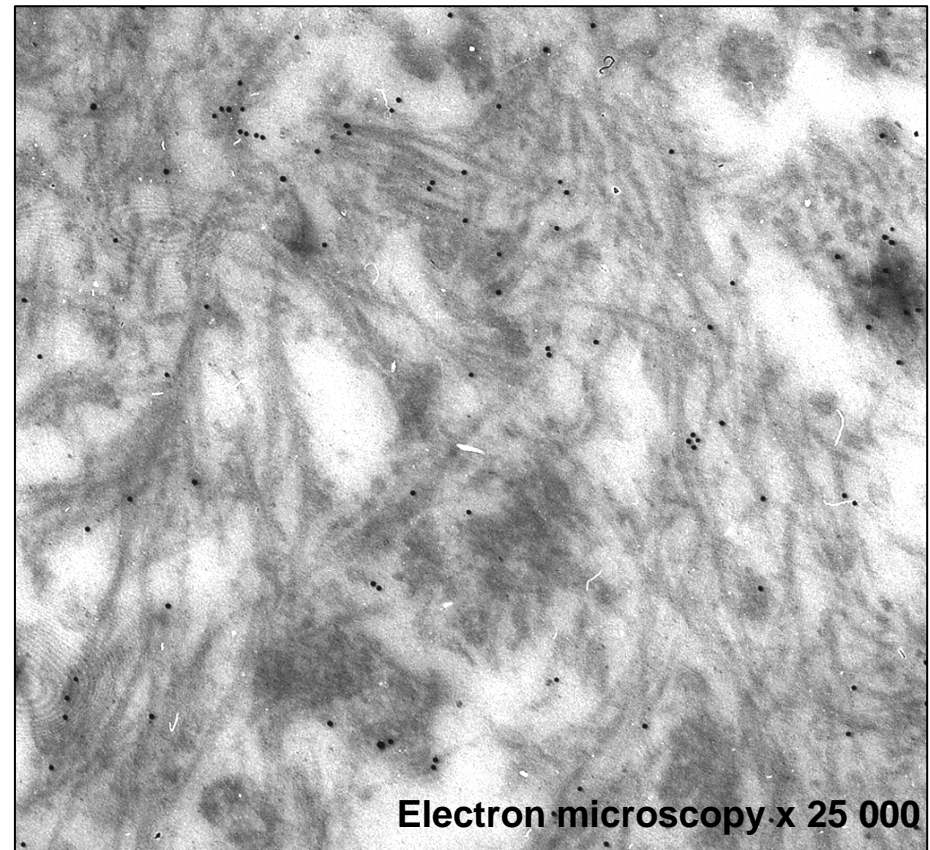
Immunohistochemistry:

New generated Mab allow to reveal accumulation of PrP^d by IHC in brain organism with TSE

Human (*Homo sapiens*):

Amyloid plaques in brain of patient with vCJD

accumulation of PrP^d were detected by Mab 2C8.
visualisation by anti-mouse Mab
conjugated with colloidal gold particles (d=20 nm)



Electron microscopy x 25 000

Application of produced Mab to detect protease resistant isoform PrP.

Western blotting:

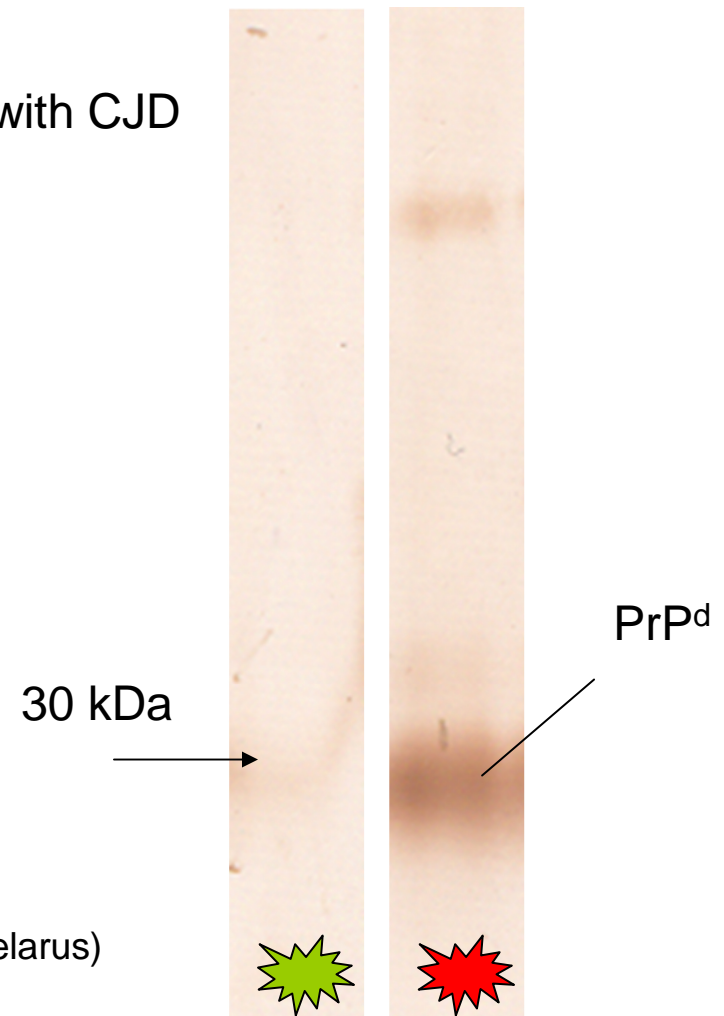
Detection of PrP^d in autopsy material from patient with CJD



- probe from healthy human



- probe from patient with CJD



This data was kindly provided
by D.Sc N.N. Poleshuk and Ph.D S.P. Kapitulets
(Research Institute for Epidemiology and Microbiology, Minsk, Belarus)

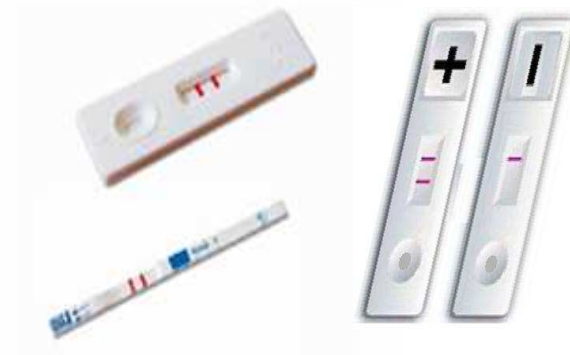
Application of produced Mab to detect protease resistant isoform PrP.

Fast immunoassay test:

Selected monoclonal antibodies

sandwich ELISA

Lateral flow immunoassay

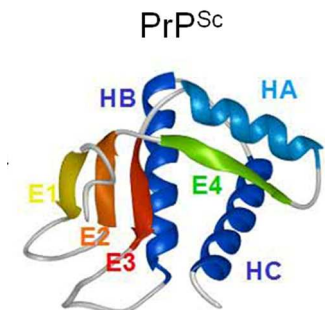
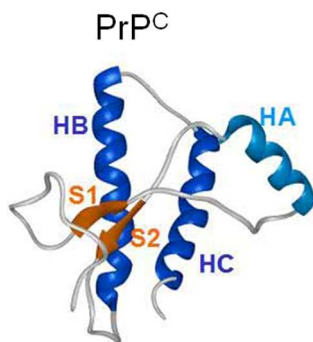


In work out

Properties of recombinant β -PrP

- Contains many β -sheet in its secondary structure
- High resistance to proteinase (proteinase K)
- Form fibrils similar to observed in amyloid plaques

By analogy with PrP^{disease}



Self-assembly formation of amyloid-like structures by rPrP

Stage I:

Formation of thin protofibrils approximately 8 nm in diameter and up to 5 μm in length, which represented helices. These protofibrils were arranged into bundles comprised of three to five flexible protofibrils, which began to twist with one another.

Protofibrils arrange into bundles comprised of three to five flexible protofibrils, which began to twist with one another.

Stage II:

Compact fibrils approximately 12 nm in diameter and up to 4 μm in length, in which the above periodicity was not observed.

Apparently, this type of fibrils formed as a result of tight twisting of protofibril bundles.

Stage III:

Short rigid fibrils 16–25 nm in diameter and 80–160 nm in length.

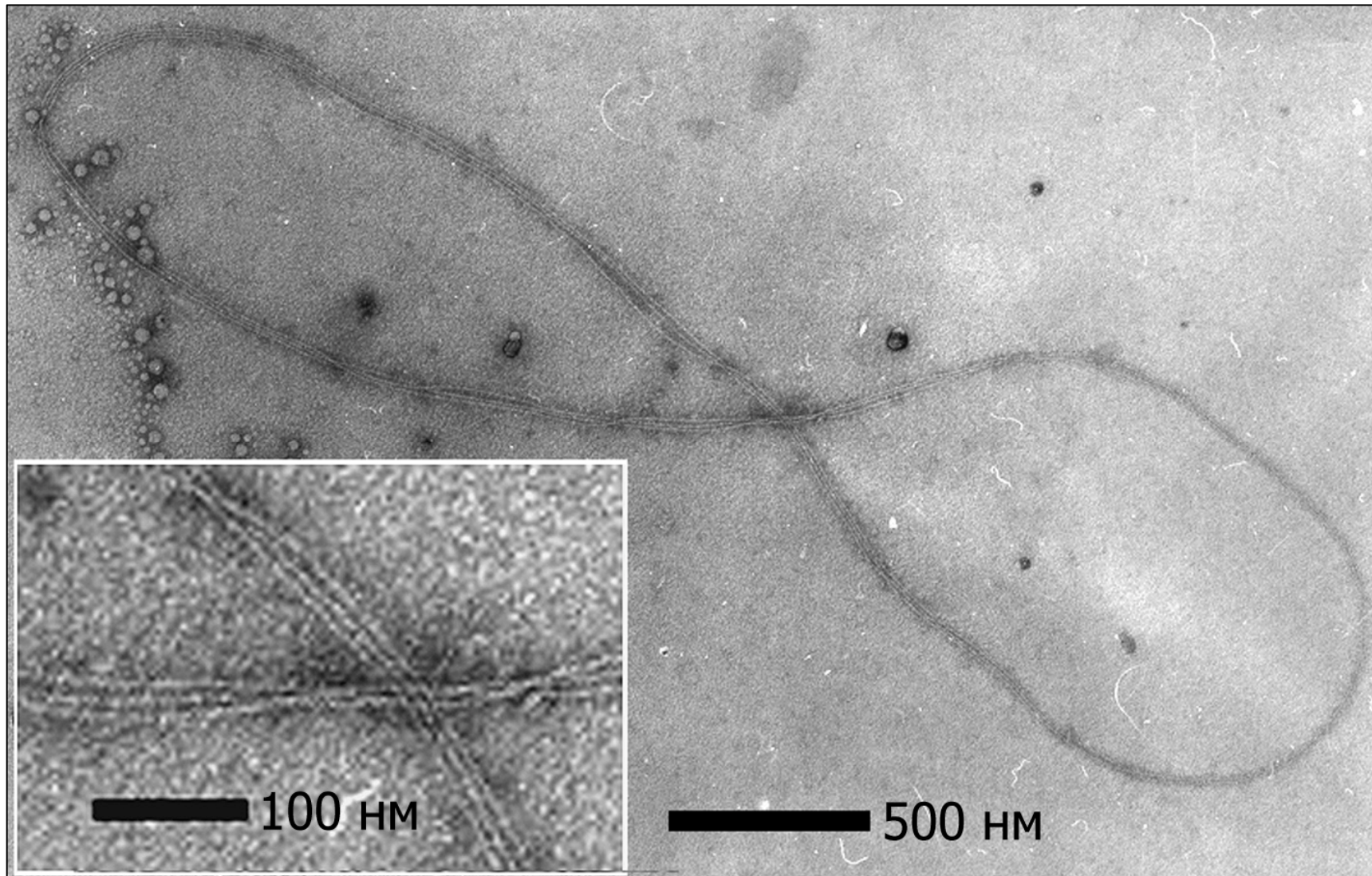
Apparently this type of fibrils formed as a result of fragmentation long fibrils observed on stage 2.

Stage IV:

Secondary aggregation of short rigid fibrils into big branch association

Self-assembly formation of amyloid-like structures by rPrP

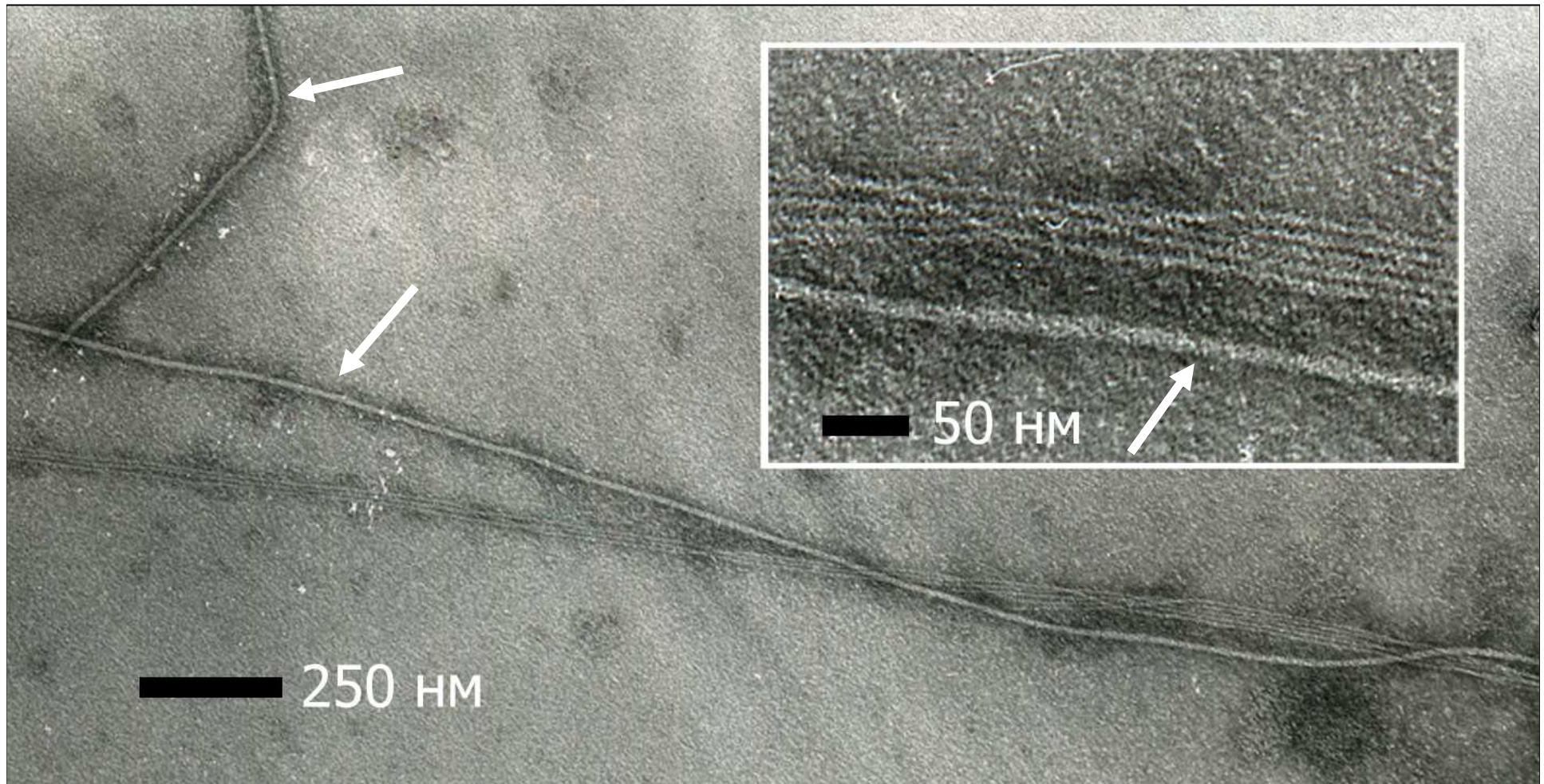
Stage I:



Electron microscopy, negative staining by uranyl acetate

Self-assembly formation of amyloid-like structures by rPrP

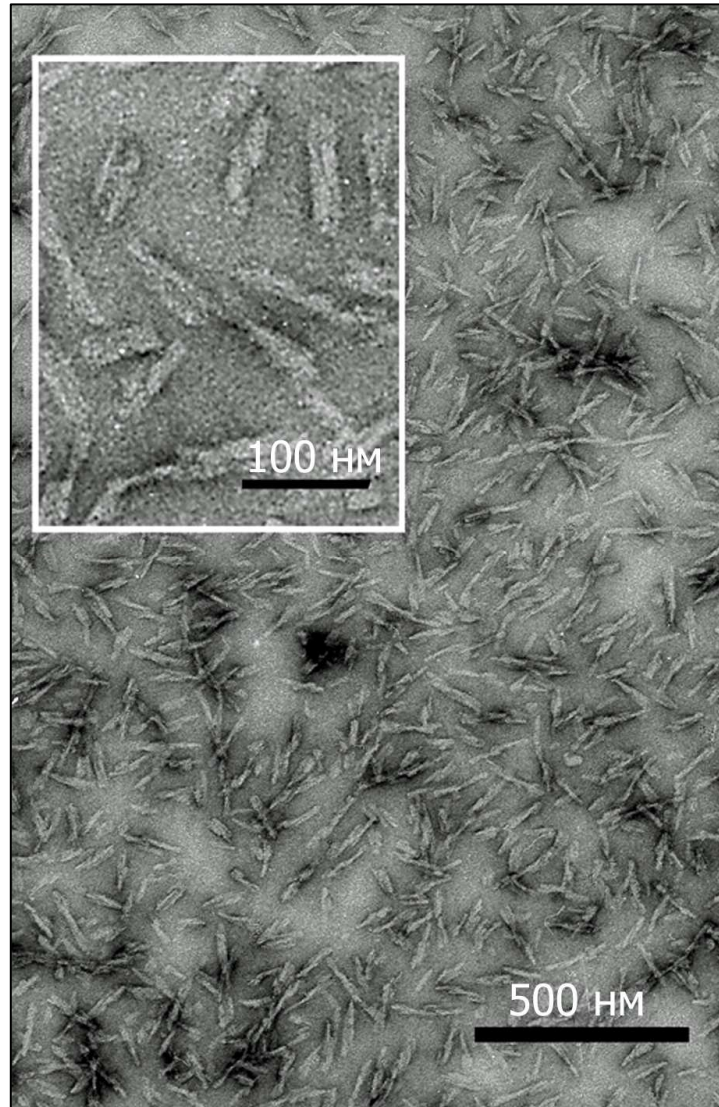
Stage II:



Electron microscopy, negative staining by uranyl acetate

Self-assembly formation of amyloid-like structures by rPrP

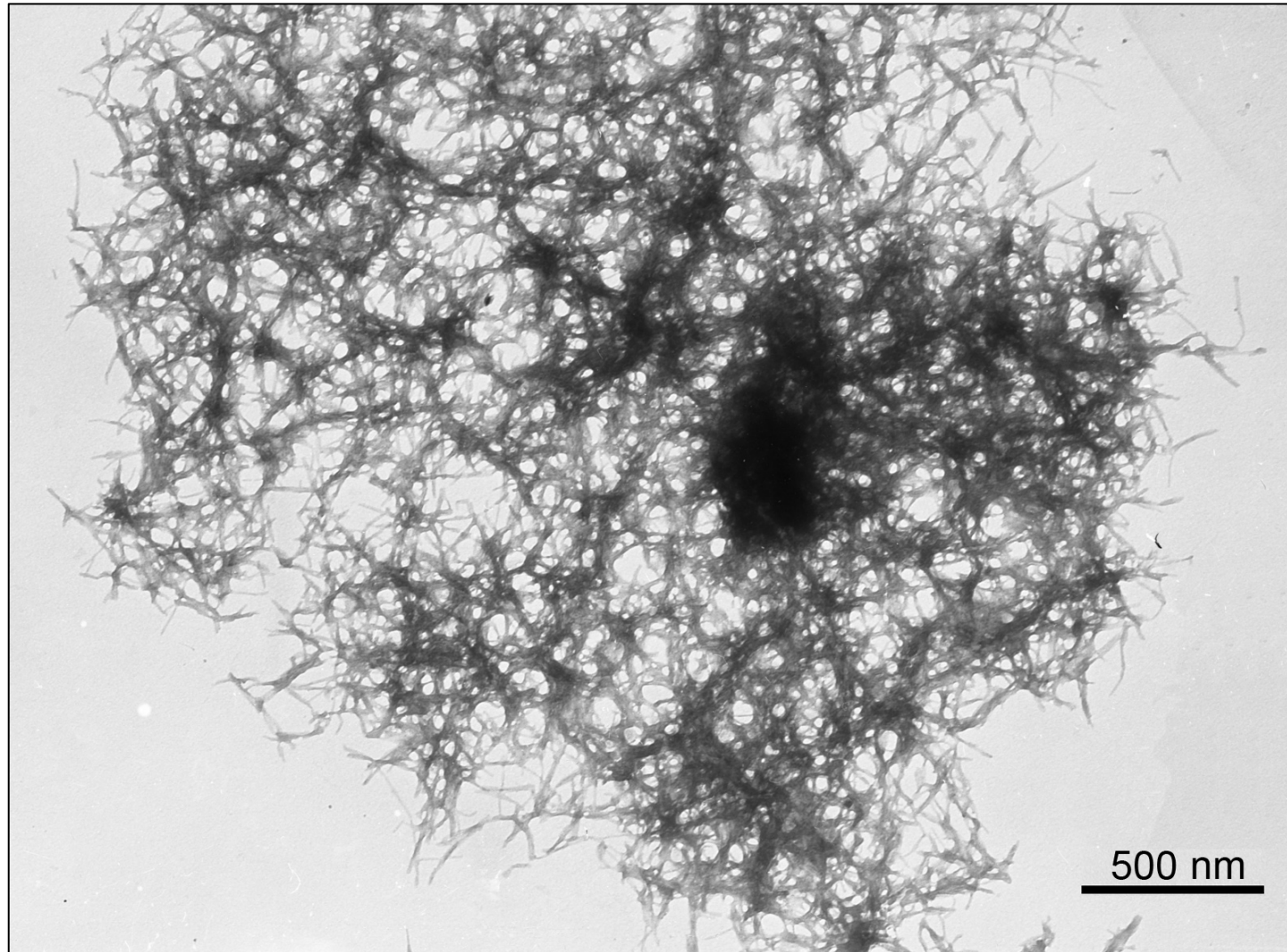
Stage III:



Electron microscopy, negative staining by uranyl acetate

Self-assembly formation of amyloid-like structures by rPrP

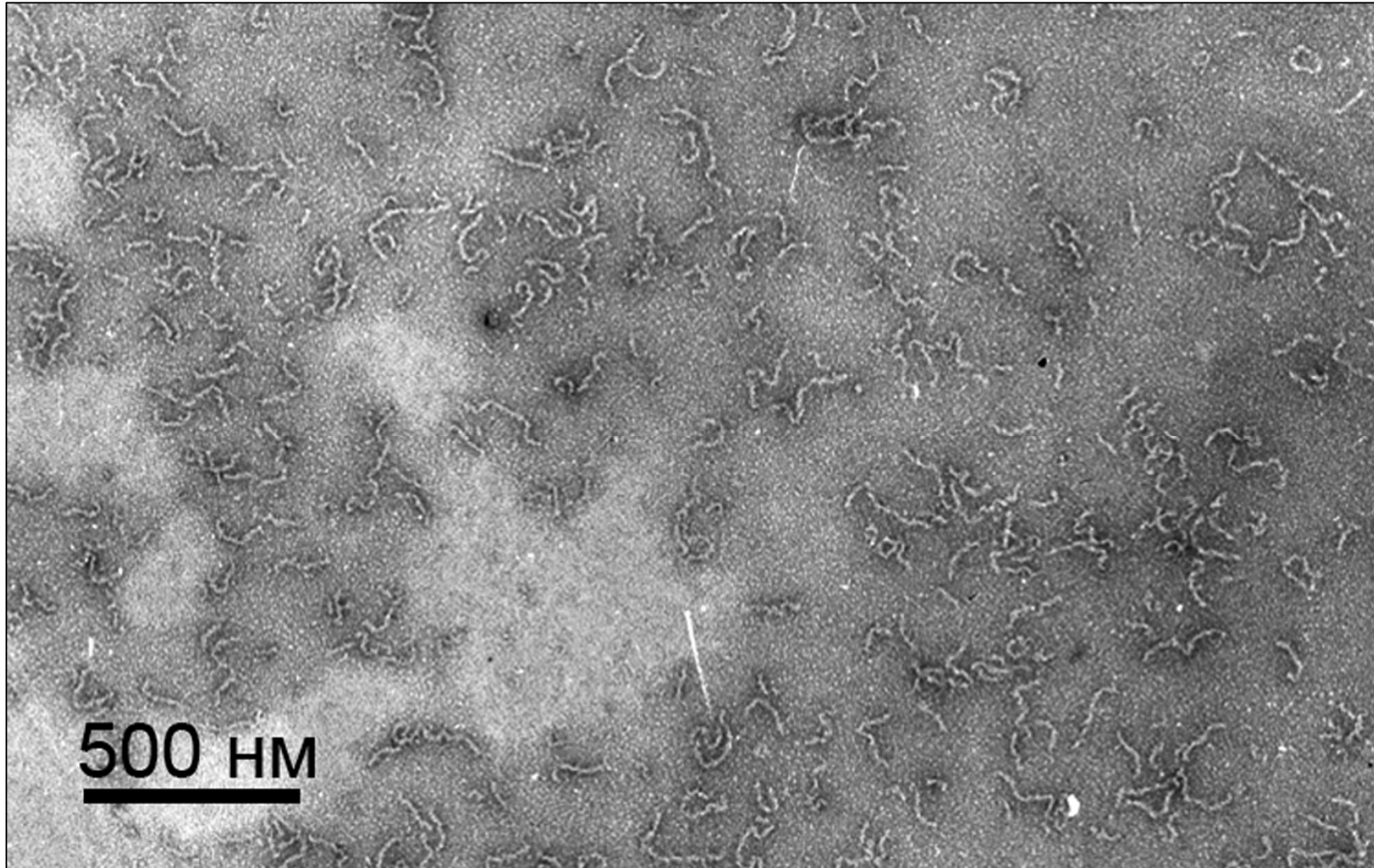
Stage IV:



Electron microscopy, negative staining by uranyl acetate

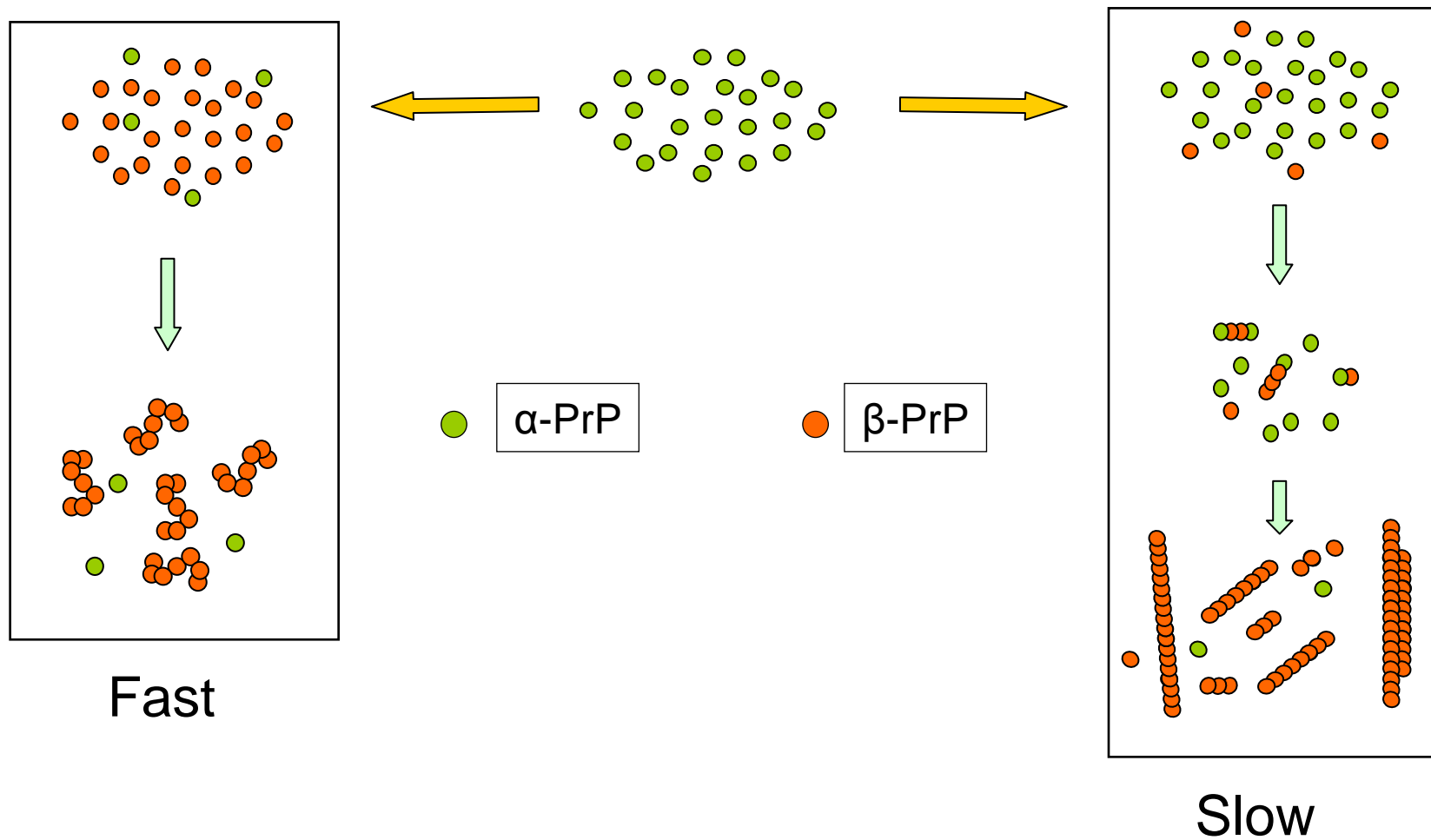
Another types of fibrils forming by recombinant PrP.

Incubation of rPrP with some conditions promote formation of “flexible” (worm-like) fibrils.

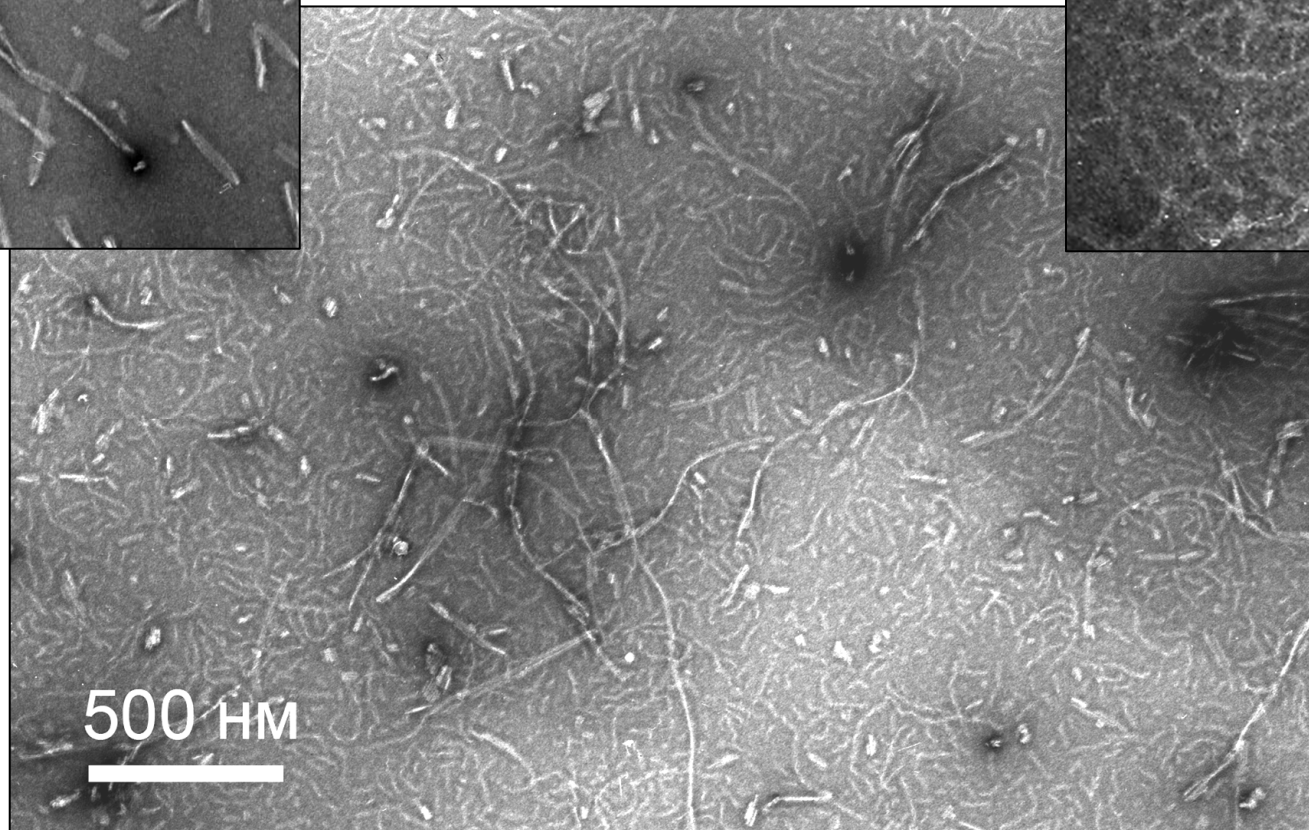
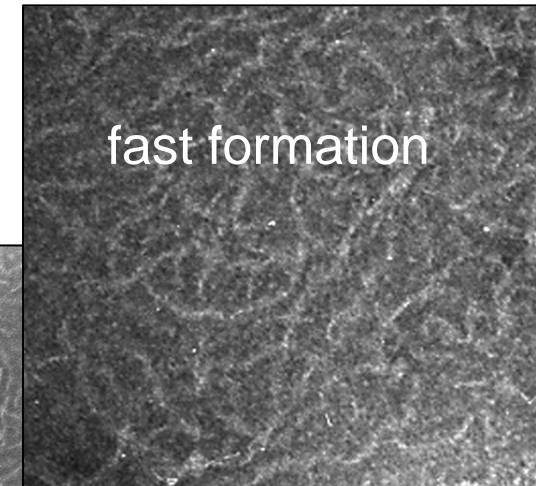
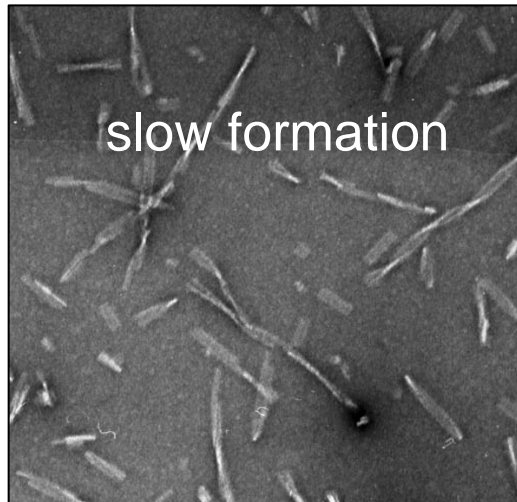


Electron microscopy, negative staining by uranyl acetate

Proposed scheme of rPrP fibrils formation

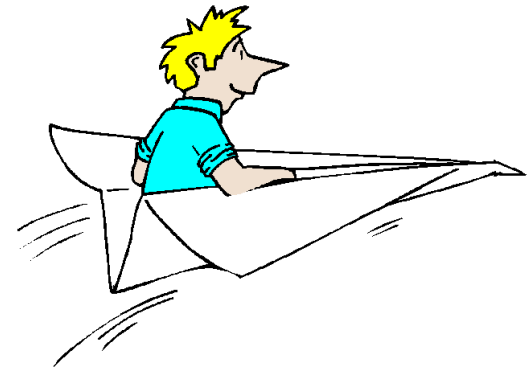


Simultaneous formation different types of fibrils by rBovinePrP



Conclusions and future prospects

1. Study molecular basis of prion infections and prion strain generation.
2. Develop new diagnostic systems for TSE's, including rapid tests, discriminating normal and infectious prion isoforms.
3. Select reagents for medical prevention and therapy of different amyloidosis.
4. Produce artificial prion fibrils for nanotechnology goals (fabrication of nano-wires, fibers, etc.)



Acknowledgments



Thank you for your attention !