


 INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ


Název: **Electrochemical determination of PrP and its interactions with metals and metallothionein**

Školitel: **Alžběta Cardová**

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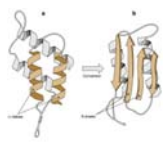
Reg.č.projektu: CZ.1.07/2.4.00/31.0023
 Název projektu: Partnerská síť centra excelentního bionanotechnologického výzkumu






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- PrP^C is glycosylphosphatidylinositol-anchored host glycoprotein normally present in brain ([Dormont, 2002](#))
- This protein with predominance of α -helix structure can be converted to an abnormal protease resistant isoform with increased ratio of β -sheet structure called prion (PrP^{Sc}) ([Kong et al., 2013](#))
- PrP^{Sc} isoform can cause a range of slow neurodegenerative disorders called transmissible spongiform encephalopathies ([Tiraboschi & Tagliavini, 2013](#)). The most famous prion caused disease is BSE



Dormont D (2002) Prion disease: pathogenesis and public health concerns. *FEBS Lett* 529: 17-21
 Kong QJ, Huh H, Kwon K, Li Y, Qian L, Saravanan K, Gill S, Hooper SL, Zhang M, Sontagkai W, Sorensen PD, Giombetti P, Surveika WC (2013) Thermodynamic Stabilization of the Folded Domain of Prion Protein Inhibits Prion Infection in *Yers. Cell Rep* 4: 248-254
 Tiraboschi F, Tagliavini F (2013) Prion disease: a promising target for prion disease clinical research. *Nat Rev Neurol* 9: 356-367

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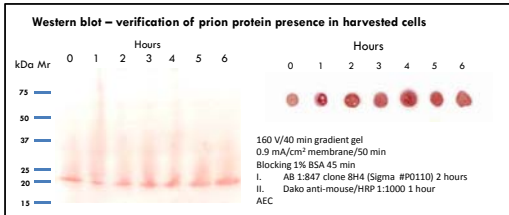




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- pRSET B cloning kit (Invitrogen, Germany) for high-level expression of recombinant proteins in *E. coli* was used. For subsequent *E. coli* cultivation we followed the manual by Invitrogen
- Expression of PrP^C in *E. coli* was verified by gel electrophoresis and western-blot

Western blot – verification of prion protein presence in harvested cells



160 V/40 min gradient gel
 0.9 mA/cm² membrane/50 min
 Blocking 1% BSA 45 min
 I. AB 1.847 clone 8H4 (Sigma #P0110) 2 hours
 II. Dako anti-mouse/HRP 1:1000 1 hour
 AEC

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• PrP^C was isolated on HisTrap excel 1 ml column (GE Healthcare, Uppsala, Sweden) using histidine protein anchors. We optimized the elutions according to the western-blot (picture below)

• Protein was purified by cut-off filtration (Amicon Ultra 3K-0.5 mL 3K Centrifugal Filters for Protein Purification and Concentration)

• Finally we verified our results by MALDI-TOF

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• PrP^C may play a role in cell signaling or in binding and transport of **Cu(II)** and **Zn(II)** ions (Gavier-Widen et al., 2005) (Kozłowski et al., 2012). Cu together with Zn ions are involved in the formation of amyloid plaques in case of neurodegenerative disorders (Pedersen et al., 2012).

• According to some authors Cu ions can destabilise the native fold of PrP^C and can facilitate the conversion to PrP^{Sc} isoform (Younan et al., 2011).

• **Metallothionein (MT)** fulfils multiple functions including the involvement in zinc and copper homeostasis and protection against heavy metal toxicity and oxidative damage. Due to its physiological role, zinc and copper belong to the most investigated metal ions connected to metallothionein.

• Brain specific subtype of MT is called **MT-III** and this protein is able to bind copper when Cu homeostasis is disrupted.

Gavier-Widen A, et al. (2005) PrP^C is a redox-active protein. *Journal of Neurochemistry*, 94, 117-121.
 Kozłowski M, et al. (2012) Copper(II)-induced Secondary Structure Changes and Reduced Folding Stability of the Prion Protein. *J Mol Biol* 419: 369-382.
 Pedersen C, et al. (2012) Rapid Exchange of metal between Zn7-Paradoxin2 and Amyloid-beta Peptide Promotes Amyloid-Related Structure Changes. *Biochemistry* 51: 1697-1704.
 Younan S, et al. (2011) Copper(II) Induced Secondary Structure Changes and Reduced Folding Stability of the Prion Protein. *J Mol Biol* 419: 369-382.

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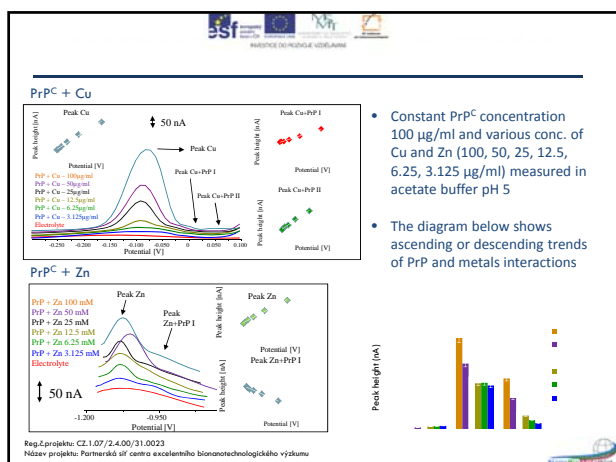
PrP^C

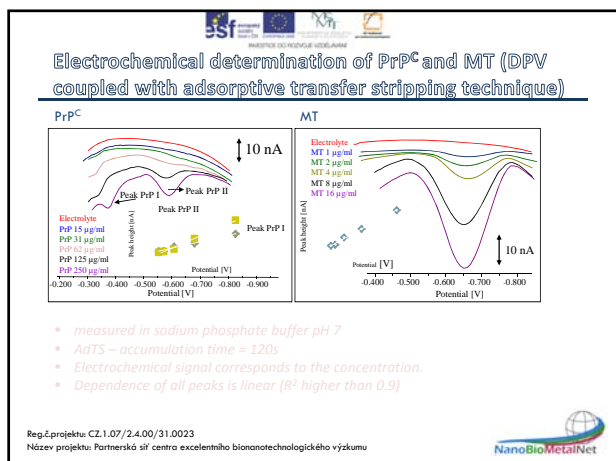
• measured in acetate buffer pH 5
 • Electrochemical signal corresponds to the concentration.
 • Dependence of all peaks is linear (R^2 higher than 0.9)

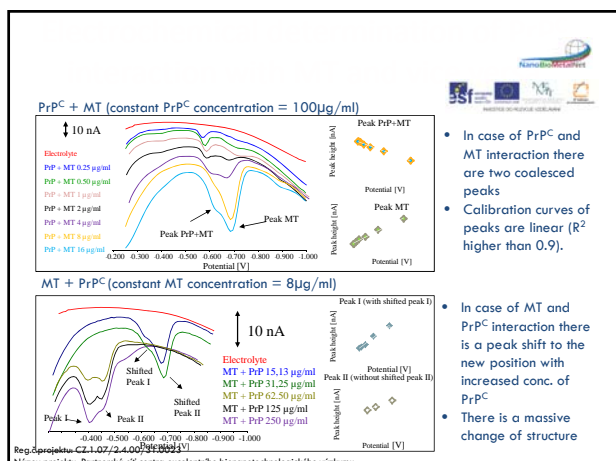
Copper

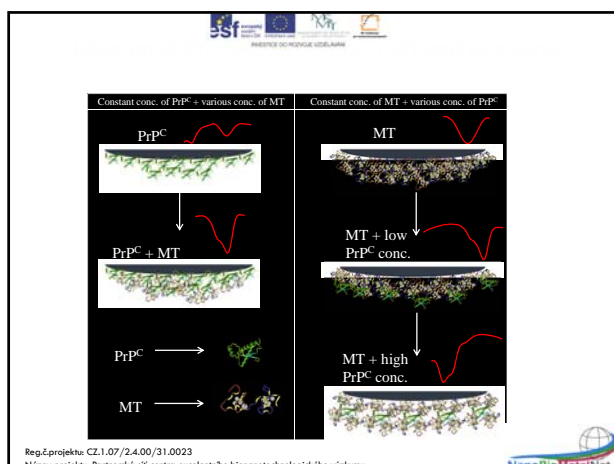
Zinc

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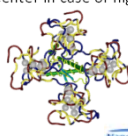








- Recombinant PrP^C was produced, isolated and purified
- PrP^C was used for electrochemical determination and for an investigation into its interactions with metals and metallothionein
- Massive interaction was discovered especially in case of MT and PrP^C interaction
- We presume that a change of the peak position is caused by the formation of MT tetramers enclosing the PrP^C molecule to the center in case of high PrP^C concentration



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- Doc. RNDr. Vojtech Adam, Ph.D.
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- Mgr. Ondřej Zítka, Ph.D.
- Mgr. Markéta Vaculovicová, Ph.D.

An entire Laboratory of Metallomics and Nanotechnologies...



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 Thank you for your attention 

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