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EVROPSKÁ UNIE
MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY
OP Vzdělávání pro konkurenceschopnost
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Název: Electrochemical determination of PrP and its interractions with metals and metallothionein

Školitel: Alžběta Cardová

Datum: 6. 2. 2014

Reg.č.projektu: CZ.1.07/2.4.00/31.0023

Název projektu: Partnerská síť centra excelentního bionanotechnologického výzkumu

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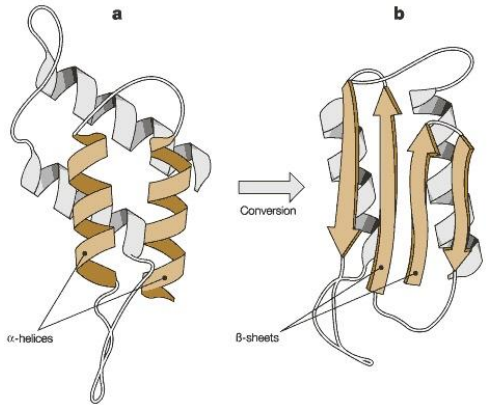
- PrP^{C} is glycosylphosphatidylinositol-anchored host glycoprotein normally present in brain (Dormont, 2002)
- This protein with predominance of α-helix structure can be converted to an abnormal protease resistant isoform with increased ratio of β-sheet structure called prion (PrP^{Sc}) (Kong et al, 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



Source: *Prion Biology and Diseases*, Cold Spring Harbor Laboratory Press, New York, 1999

Dormont D (2002) Prion diseases: pathogenesis and public health concerns. FEBS Lett ***529:*** *17-21*
Kong QZ, Mills JL, Kundu B, Li XY, Qing LT, Surewicz K, Cali I, Huang SH, Zheng MJ, Swietnicki W, Sonnichsen FD, Gambetti P, Surewicz WK (2013) Thermodynamic Stabilization of the Folded Domain of Prion Protein Inhibits Prion Infection in Vivo. *Cell Rep* **4:** 248-254
Tiraboschi P, Tagliavini F (2013) Prion disease: a promising rating scale for prion disease clinical research. *Nat Rev Neurol* **9:** 366-367

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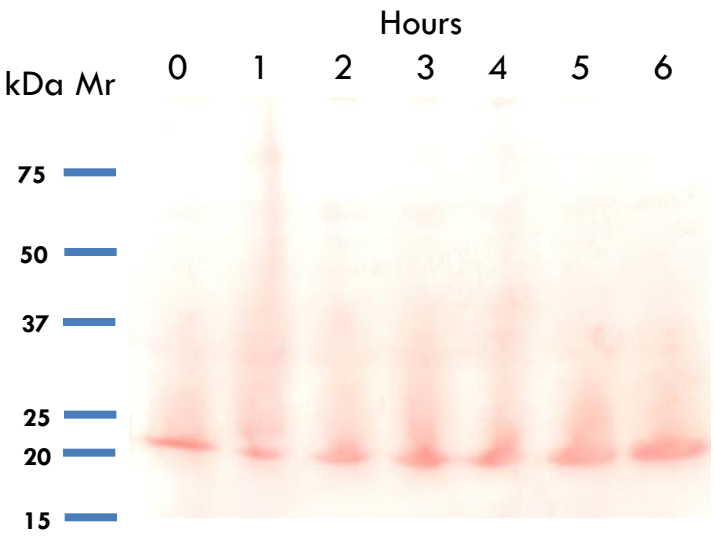
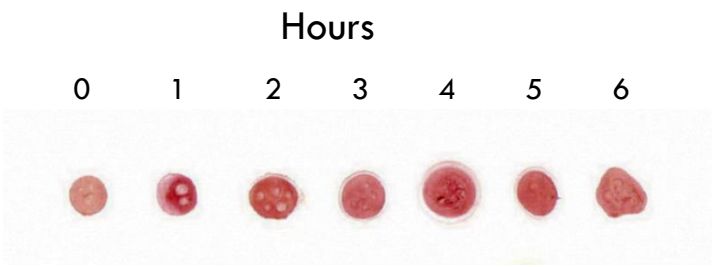
Bacterial production of recombinant PrP^C

- 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/cm^2 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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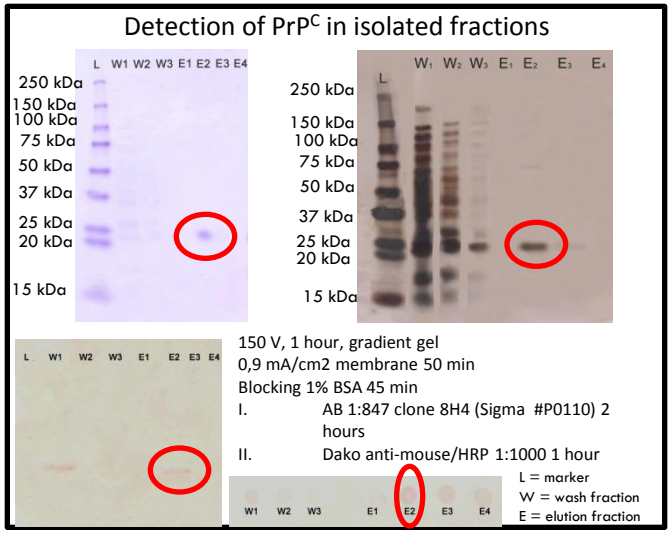
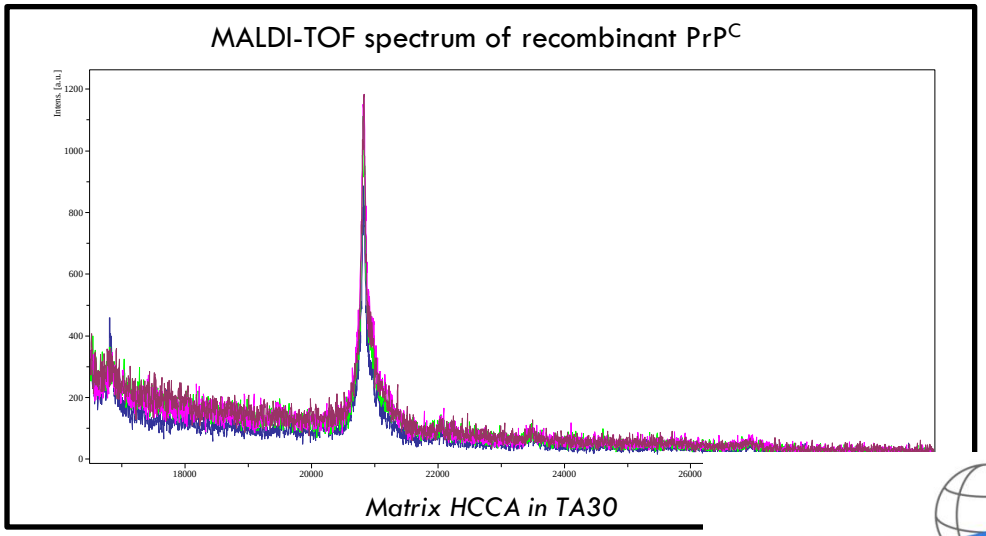
Recombinant PrP^C isolation and purification

- 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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Prions, metals and metallothionein

- 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) (Kozlowski 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 D, Stack MJ, Baron T, Balachandran A, Simmons M (2005) Diagnosis of transmissible spongiform encephalopathies in animals: a review. *J Vet Diagn Invest* **17:** 509-527

Kozlowski H, Luczkowski M, Remelli M, Valensin D (2012) Copper, zinc and iron in neurodegenerative diseases (Alzheimer's, Parkinson's and prion diseases). *Coordin Chem Rev* **256:** 2129-2141

Younan ND, Klewpatinond M, Davies P, Ruban AV, Brown DR, Viles JH (2011) Copper(II)-Induced Secondary Structure Changes and Reduced Folding Stability of the Prion Protein. *J Mol Biol* **410:** 369-382

Pedersen JT, Hureau C, Hemmingsen L, Heegaard NHH, Ostergaard J, Vasak M, Faller P (2012) Rapid Exchange of Metal between Zn-7-Metallothionein-3 and Amyloid-beta Peptide Promotes Amyloid-Related Structural Changes. *Biochemistry* **51:** 1697-1706

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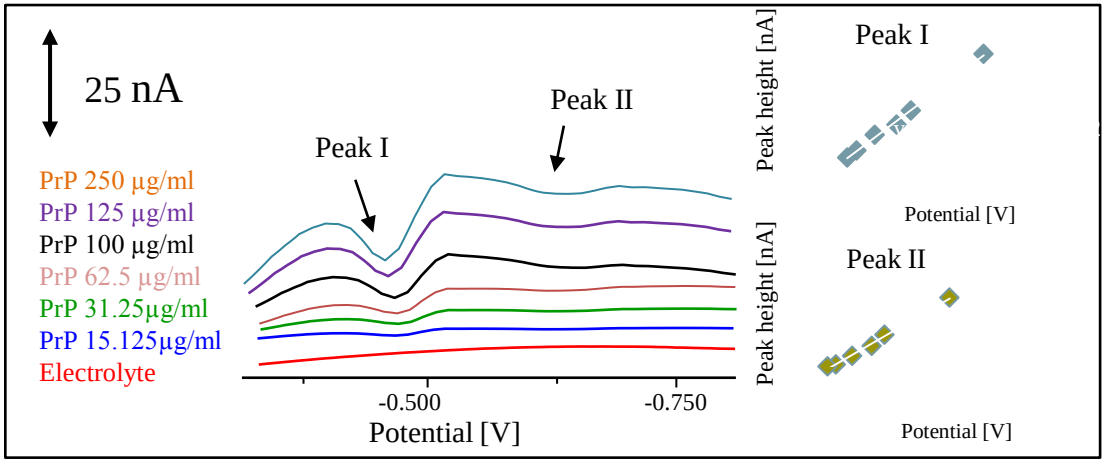
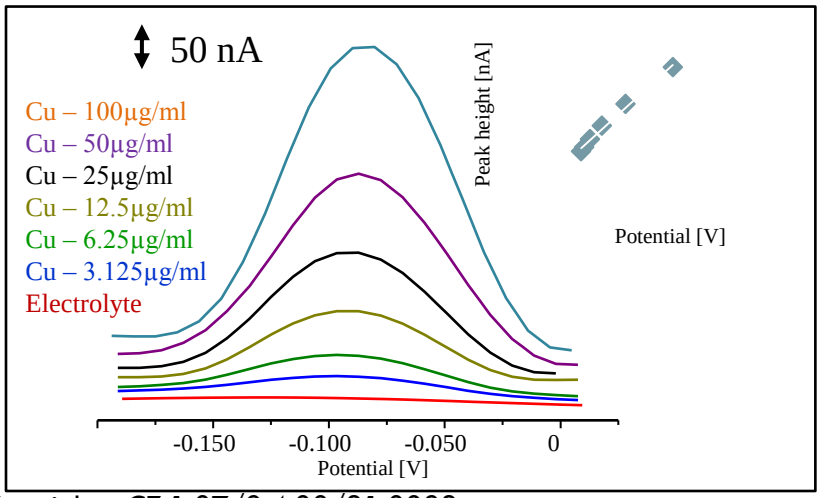
Electrochemical determination of PrP^C, zinc and copper (differential pulse voltammetry)

PrP^C

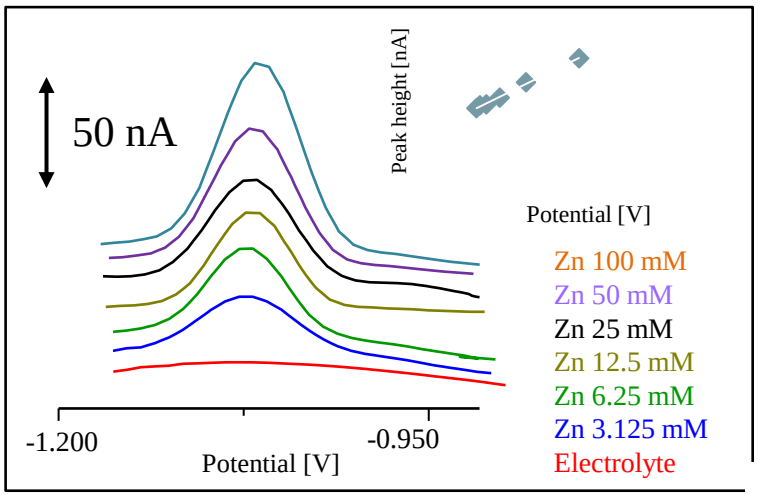


- *measured in acetate buffer pH 5*
- *Electrochemical signal corresponds to the concentration.*
- *Dependence of all peaks is linear (R^2 higher than 0.9)*

Copper



Zinc



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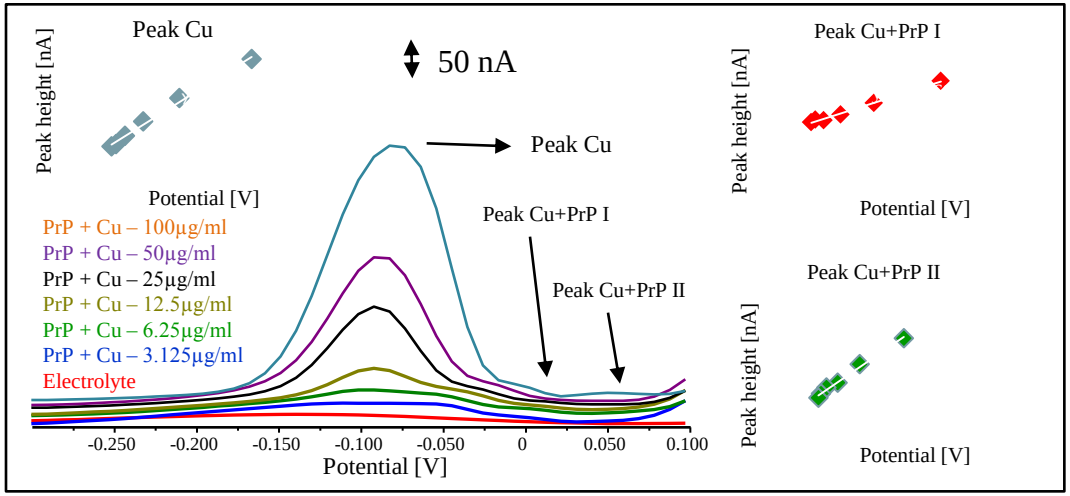
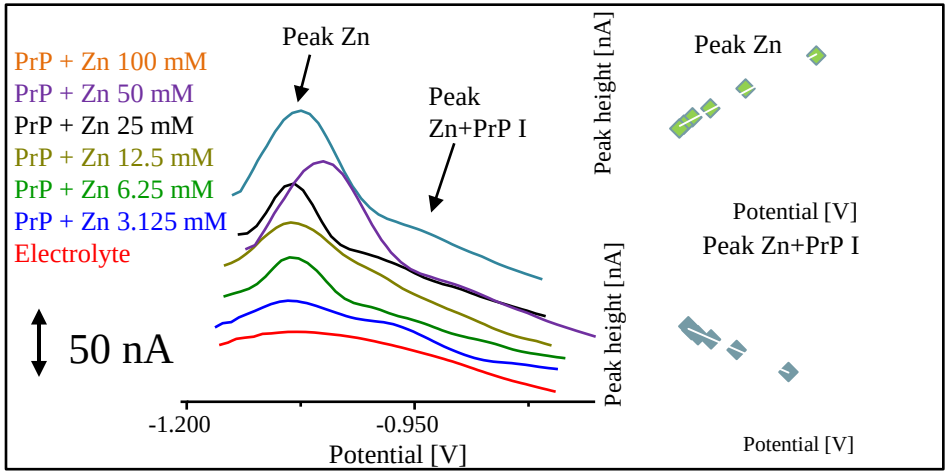
Electrochemical determination of PrP^C interactions with copper and zinc

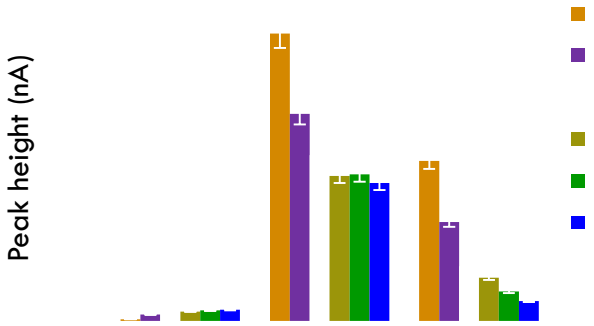
PrP^C + Cu



PrP^C + Zn



- Constant PrP^C concentration 100 µg/ml and various conc. of Cu and Zn (100, 50, 25, 12.5, 6.25, 3.125 µg/ml) measured in acetate buffer pH 5
- The diagram below shows ascending or descending trends of PrP and metals interactions



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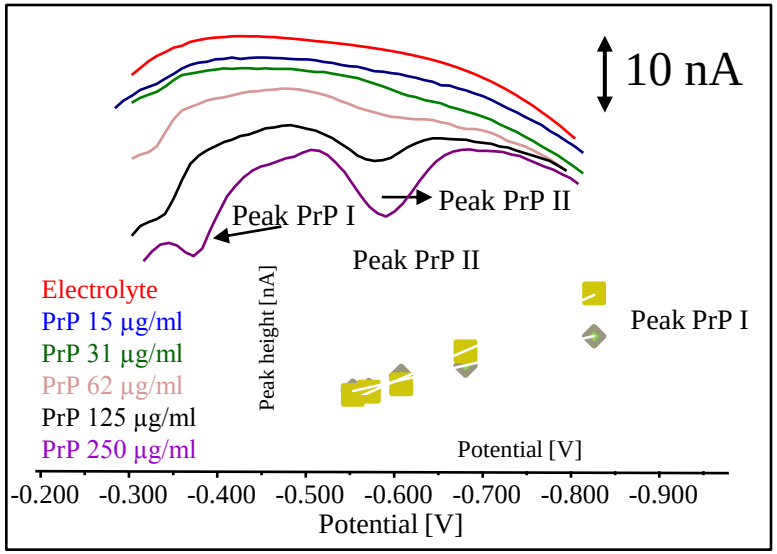
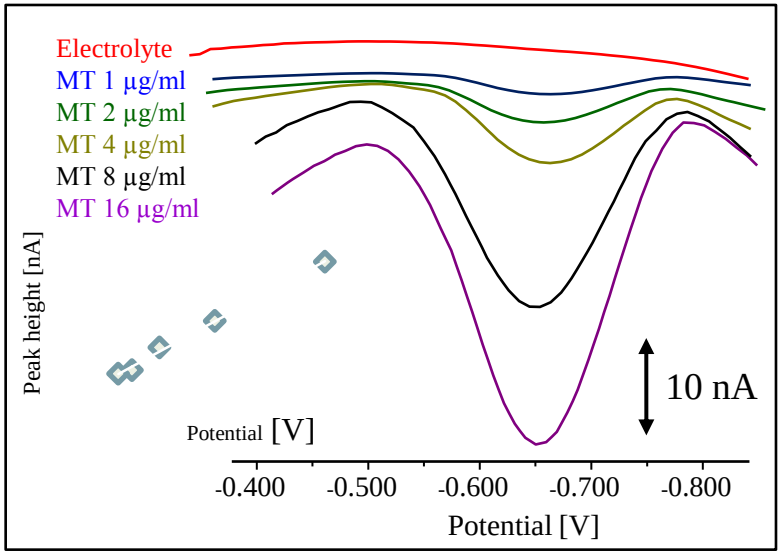
Electrochemical determination of PrP^C and MT (DPV coupled with adsorptive transfer stripping technique)

PrP^C



MT



- *measured in sodium phosphate buffer pH 7*
- *AdTS – accumulation time = 120s*
- *Electrochemical signal corresponds to the concentration.*
- *Dependence of all peaks is linear (R^2 higher than 0.9)*

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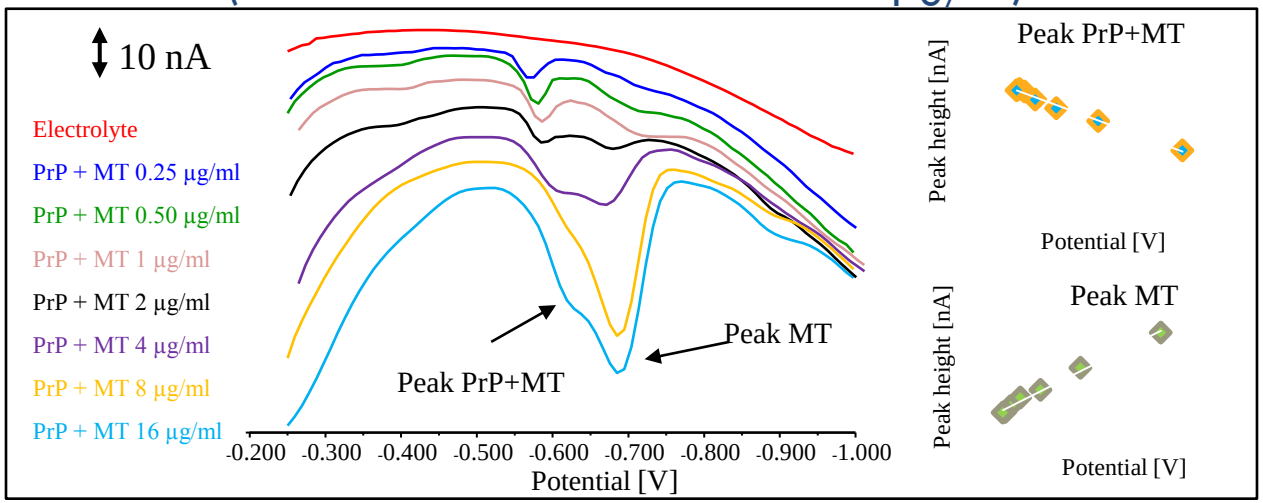
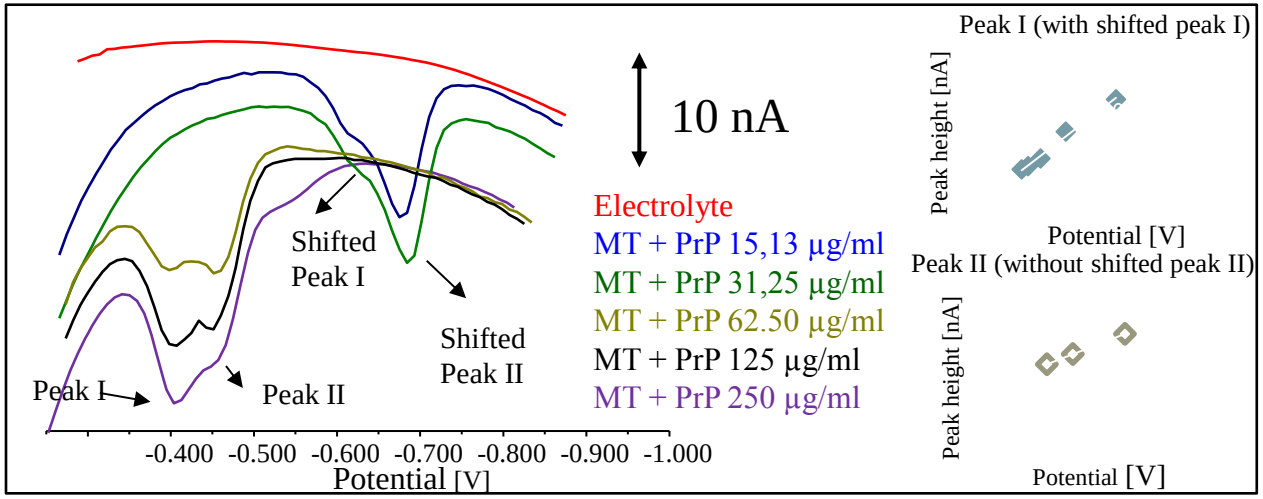
Electrochemical determination of PrP^C interaction with MT and vice versa

PrP^C + MT (constant PrP^C concentration = 100µg/ml)



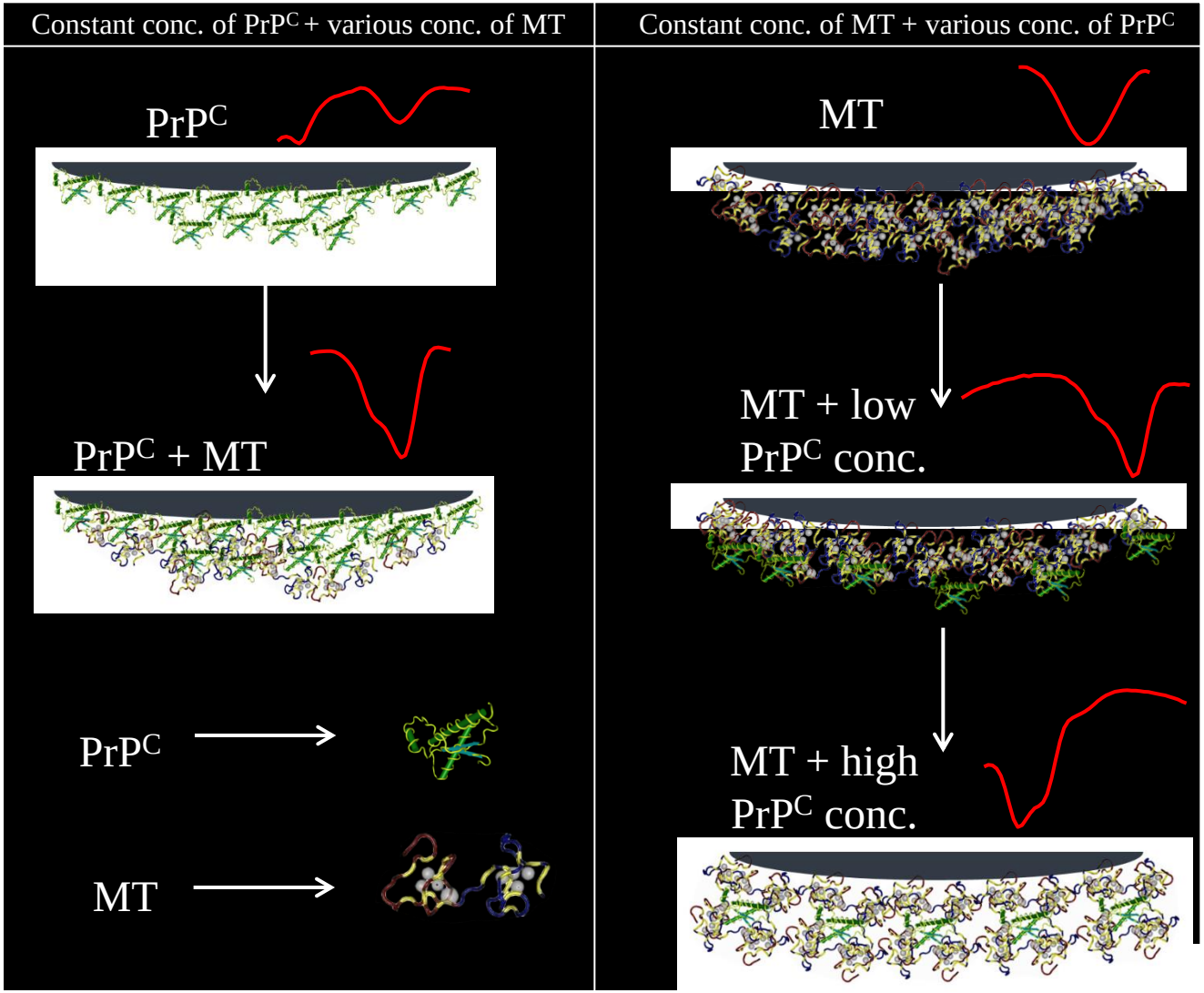
- In case of PrP^C and MT interaction there are two coalesced peaks
- Calibration curves of peaks are linear (R^2 higher than 0.9).

MT + PrP^C (constant MT concentration = 8µg/ml)



- In case of MT and PrP^C interaction there is a peak shift to the new position with increased conc. of PrP^C
- There is a massive change of structure

Diagram of PrP^C interaction with MT and vice versa



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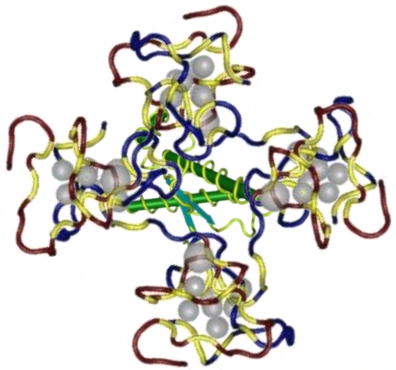
Conclusion

- 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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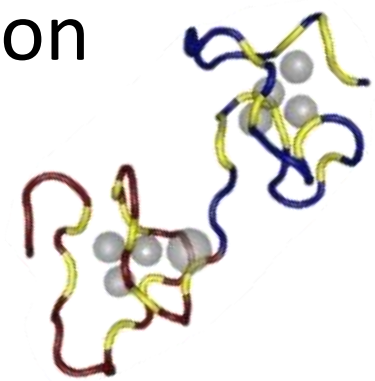
Thank you for your attention



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