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## Preliminary spectroscopic and thermodynamic studies of binding Zn<sup>2+</sup> ions to human PrP<sup>C</sup>

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Keywords: conformational stability of proteins, circular dichroism

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Human prion protein (PrP) is a neuronal membrane protein exposed into synaptic cleft [1]. Mature form of PrP is 208 amino acids long and can exist in two different structural conformations: normal (PrP<sup>C</sup>), and pathogenic (PrP<sup>Sc</sup>). PrP<sup>C</sup> consists of two domains: unstructured and flexible N-terminal domain containing four tandem octarepeats and structured C-terminal domain with three  $\alpha$ -helices and two anti-parallel  $\beta$ -sheets. Pathogenic PrP<sup>Sc</sup> contains predominantly  $\beta$ -sheets [2] and is involved in development of nine human [3] and seven animal neurodegenerative diseases [4] called transmissible spongiform encephalopathies (TSE).

Zinc is the second most common metal in living organisms. The highest concentration of zinc is observed in the nervous system [5]. During synapse excitation, concentration of zinc in synaptic cleft can reach even mM concentration. PrP can bind zinc with binding constant  $3 \times 10^4 \text{ M}^{-1}$  and is considered to be responsible for the zinc uptake [6]. What is also important from TSE point of view conversion of PrP<sup>C</sup> to PrP<sup>Sc</sup> decreases concentration of zinc in neural system up to 70%. PrP binds zinc ions by an octarepeat region [7].

Main goal of our studies was to measure conformational stability of PrP<sup>C</sup>, with and without zinc

ions. In order to measure conformational stability of human PrP<sup>C</sup> far UV circular dichroism spectroscopy (CD) was used to detect changes in secondary structure. Spectrum of PrP had double minima at 219 nm and 209 nm and a single maximum at 191 nm (Fig. 1). The biggest changes between spectra were 219 nm therefore this wavelength was used to perform thermal melting using CD spectroscopy.

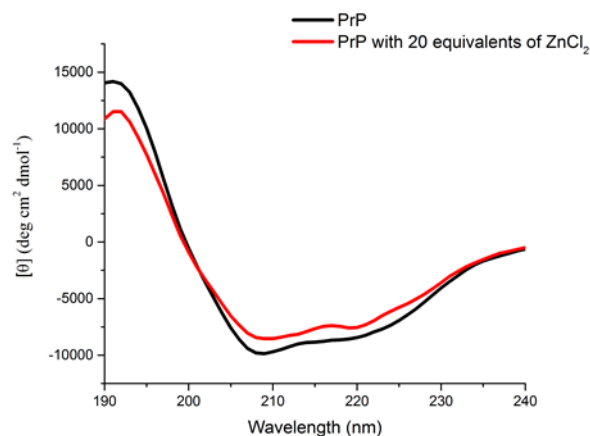


Figure 1. Far UV CD spectra of 20  $\mu\text{M}$  wild-type human PrP with and without 400  $\mu\text{M}$  of  $\text{ZnCl}_2$ .

**Acknowledgments:** This work was supported by the funds from the National Science Centre (Poland) granted on the basis of decision no. No. 2014/15/B/ST4/04839.

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