

## Impact of Copper Oxide Nanoparticles on Human Cystatin C - Cell Viability and *In Vivo*-like Flow Studies

K. Rucińska<sup>1,2\*</sup>, J. Maksim<sup>1,2</sup>, Ł. Przysiecka<sup>3</sup>, A. Kertmen<sup>1</sup>, W. M. Kwiatek<sup>2</sup>, and M. Kozak<sup>1,4</sup>

<sup>1</sup>Adam Mickiewicz University in Poznań, Faculty of Physics, 61-614 Poznań, Poland

<sup>2</sup>Institute of Nuclear Physics Polish Academy of Sciences, 31-342 Kraków, Poland

<sup>3</sup>NanoBioMedical Centre AMU, 61-614 Poznań, Poland

<sup>4</sup>SOLARIS National Synchrotron Radiation Centre, 30-392 Kraków, Poland

\*e-mail: karolina.rucinska@amu.edu.pl

Well known fact is, that long-term exposure to air pollution is a risk factor for the development of serious diseases, including cancer or dementia. Among the harmful components of polluted air are heavy metals such as Cu and Zn and their compounds. These metals, especially in the form of micro- or nanoparticles, have been linked to toxic effects, posing significant health risks to humans and other organisms. Studies have shown that human SH-SY5Y cell lines exhibit concentration-dependent neurotoxic responses to copper oxide nanoparticles (CuO NPs), leading to apoptosis in neuronal cells and potentially contributing to Alzheimer's disease (AD)<sup>1</sup>.

Evidence suggests that metal ions can bind to amyloid beta (A $\beta$ ) and modulate its aggregation<sup>2</sup>. Additionally, copper cations have been found in high concentrations within senile plaques in the brains of Alzheimer's disease (AD) patients<sup>3</sup>. One of the co-depositors in these plaques is human cystatin C (HCC), which also exhibits amyloidogenic properties due to its flexible structural components. Although the mechanism of amyloidogenic aggregation of human cystatin C is not fully understood, one factor that can accelerate this process are shear forces, which in the model system can be induced by microfluidic *in vivo*-like flow.

Currently, no studies have investigated the amyloidogenic aggregation of human cystatin C both under flow conditions and in the presence of metallic nanoparticles. To address this gap, we applied a microfluidic system that mimics physiological flow conditions to examine the effects of flow dynamics and shear forces on protein aggregation in the presence of NPs.

In parallel, to gain deeper insights into the health implications and underlying mechanisms, we investigated also the interactions between human cystatin C and synthesized by us CuO nanoparticles using atomic force microscopy (AFM), thioflavin T fluorescence assay, circular dichroism (CD), and cell viability assays on SH-SY5Y cell lines. The nanoparticle samples were characterized at the BM29 (BioSAXS) beamline at ESRF (Grenoble, France), while protein aggregation in the presence of NPs under microfluidic flow was studied at the P12 (BioSAXS) beamline at DESY (Hamburg, Germany).

**Acknowledgements:** This project was financed by grant 2021/41/B/ST4/03807 from the National Science Centre (Poland).

### References

1. Y. Shi, A. R. Pillozzi, X. Huang, Int. J. Environ. Res. Public Health 17 (2020) 1005.
2. A. Abelein, Acc. Chem. Res. 56 (2023) 2653.
3. M. A. Lovell, J. D. Robertson, W. J. Teesdale, J. L. Campbell, W. R. Markesbery, J. Neurol. Sci. 158 (1998) 47.