Cu(II) and Fe(II) binding, DNA cleavage and radicals production by outer-membrane protein fragments from *F. nucleatum*

Kamila Stokowa-Sołtys,[a*] Karolina Kierpiec,[a] Valentyn Dzyhovskyi,[a] Robert Wieczorek [a]

[a] *Faculty of Chemistry, University of Wroclaw, F. Joliot-Curie 14, 50-383, Wroclaw, Poland*
kamila.stokowa-soltys@chem.uni.wroc.pl

Cancer is now one of the leading causes of morbidity and mortality worldwide. In many cases, human microbiota is associated with cancer progression, e.g. *F. nucleatum* promotes colon cancer growth and even metastasis[1]. *Fusobacterium* is one of the most abundant Gram-negative anaerobic bacteria, part of the gut and oral commensal flora. However, this bacterium can enter the blood circulation as a result of periodontal infection and cause numerous diseases, including cancers[2]. What is noteworthy, numerous of *F. nucleatum* outer-membrane proteins take part in cancerogenesis. Therefore, it is very interesting to study their interactions with metal ions and their ability to produce reactive oxygen species, which may be involved in cancer progression.

We have shown that outer-membrane proteins are able to coordinate copper(II) ions tightly[3,4,5]. The full characteristic of the coordination process was possible through combined experimental and computational methods. All studied complexes have a square-pyramidal geometry and build rich networks of hydrogen bonds that additionally stabilize the complex structure. Moreover, the DNA-cleaving activity of studied peptides was discovered. Even total DNA degradation was observed at higher ligands concentrations which induces double-stranded DNA cuts *via* the nucleolytic mechanism[5]. Also, the ability of the cupric complexes to degrade plasmid DNA in the presence of hydrogen peroxide or ascorbic acid was shown. In this case, DNA cleavage is accompanied by reactive oxygen species production and occurs *via* a radical mechanism[3,4,5,]. We have also shown that these peptides bind iron(II) ions and form thermodynamically stable complexes. Based on the potentiometric titrations performed in anaerobic conditions and in the presence of one-electron reductant (ascorbic acid) we have shown that Fe(II) ions form complexes with studied peptides. Obtained results indicate also that ascorbic acid does not affect the overall stability constants of the Fe(II) complexes with the peptides.

[1] S. Bullman, C.S. Pedamallu, E. Sicinska, T. Clancy, S. Ogino, J. Tabernero, C. Fuchs, W.C. Hahn, P. Nuciforo, M. Meyerson, *Cancer Res*, **2018**, *78*, 5129.
[2] K. Stokowa-Sołtys Kamila, K. Wojtkowiak, K. Jagiełło, *J Inorg Biochem*, **2021**, *224*, 111586/1-111586/10
[3] K. Stokowa-Sołtys, V. Dzyhovskyi, R. Wieczorek, M. Jeżowska-Bojczuk, *J Inorg Biochem*, **2021**, *215*, 111332/1-111332/10
[4] K. Stokowa-Sołtys, K. Wojtkowiak, V. Dzyhovskyi, R. Wieczorek, *Int J Mol Sci*, **2021**, *22*, 12541/1-12541/20
[5] K. Stokowa-Sołtys, K. Kierpiec, R. Wieczorek, *Dalton Trans*, **2022**, *51*, 1-13