RTX toxins (Repeat in ToXin) are proteins bearing so called RTX domain characterized by nonapeptide glycine-rich repetitions. They are produced by Gram negative bacteria often representing their virulence factors. FrpC, representative of RTX group, which is studied in this work, is produced by Neisseria meningitidis during meningitis, the invasive form of meningococcal infection often leading to death. Although FrpC protein is highly immunogenic, its importance for disease etiology and pathogenesis is still unknown. The biological activity of FrpC protein consists in autocatalytic cleavage of the peptide bond between residues Asp414 and Pro415 outside its RTX domain and is followed by formation of isopeptide bond between Asp414 and ε-amino group of Lys. These activities depend strongly on presence of calcium ions.
The aim of this work was to describe one of several calcium binding sites in FrpC protein outside RTX domain. For this purpose FrpC without RTX domain (FrpC□RTX) and its mutant form FrpC□RTX-D521K were used with point substitution in possible calcium binding site which disabled FrpC cleaving activity. The area around position 521 of FrpC shares sequence homology with EF-hand, eucaryotic calcium-binding protein motives. Terbium, calcium ion luminiscent analogue, was used to characterize calcium binding site(s) in FrpC□RTX protein. Fluorescence anisotropy measurements with intrinsic Trp and ANS-bound FrpC were used to observe conformational changes of FrpC□RTX protein induced by calcium. Molecular 3D structure modelling with Rosetta software was used to see the effect of D521K mutation on the putative calcium binding site. Finally, confocal studies of interaction of FrpC with J774 mouse macrophages showed the possible endocytic pathway of FrpC.