Author of PhD thesis: Joanna Panecka
Title: *Dynamical properties of the ribosomal decoding site and its complexes with antibiotics*
Abstract:
Translation process is one of the key processes of life and it occurs in every living cell. In this process amino acid chains of proteins are synthesized based on the matrix of messenger ribonucleic acid (mRNA) sequence with the participation of transfer RNA (tRNA). Translation is performed by ribosomes, which are large macromolecular assemblies of RNA and proteins. Importantly, this process must proceed with sufficient accuracy, which is ensured by the mRNA decoding site (A-site) on the ribosome. During elongation of peptide chain, the two flexible adenines in the A-site mediate interactions between mRNA and tRNA that carries the proper amino acid. A-site is also a target for aminoglycoside antibiotics, which perturb the mRNA decoding process in bacteria. This class of compounds is used in hospitals for severe infections. Unfortunately, aminoglycoside therapy may cause serious adverse effects. One of the reasons is insufficient specificity of aminoglycosides to their main target; they also bind to the A-site on the human ribosome. Another problem is that bacteria relatively quickly develop resistance to any antibacterials, including aminoglycosides. Thus, there is a need for research aimed at improving the properties of these antibiotics.

In this thesis we applied computational methods to study the A-site physico-chemical properties in the context of the mRNA decoding accuracy and aminoglycoside binding. Since the dynamical aspect is critically important for the mRNA decoding process, we used molecular dynamics simulation techniques as a main research method. First, we investigated the physico-chemical properties of the modified oligonucleotides (like peptide nucleic acid) that can be covalently attached to aminoglycoside. Such chemical compounds can increase selectivity of aminoglycosides by complementary binding to ribosomal RNA in the proximity of the A-site. We found different conformational preferences of the studied oligonucleotides, which could be useful for the oligonucleotide-based antibiotic design. Further, we compared physico-chemical properties of the aminoglycoside targets: the bacterial and human A-site variants, which differ in RNA sequences. We identified conformational factors explaining the differences in aminoglycoside binding affinities towards the A-site variants and the known different translation accuracies in different life domains (e.g., human and bacteria). Finally, we investigated resistance mutations in the ribosomal protein S12, which is located proximal to the A-site. The mutations are known to counteract the bactericidal mechanism of action of aminoglycosides. Based on our data we suggested the structural mechanisms of the antibiotic deactivation in the mutants.

In conclusion, this work extends the knowledge on the mRNA decoding in bacteria and human, and on the factors that may affect accuracy of this process. Results of this work also explain the differences in affinities and activities of aminoglycosides towards the human and bacterial A-site. In the future this knowledge may help in the design of more effective antibiotics. Finally, in this thesis we thoroughly discussed the choice of the applied simulation methods, which may serve as a guide in choosing the proper computational protocols for the studies of the RNA systems similar to the A-site.