Structural investigation of G-rich sequences in human promoter regions of proto-oncogenes

Guanine-rich DNA oligonucleotides can form higher-order structures called G-quadruplexes. Since G-quadruplexes have been associated with regulation of gene expression and DNA replication as well as linked to telomerase dysfunction and genetic instability they could potentially be targets for antitumor therapies. In this doctoral thesis we focused on structural characteristics of G-quadruplexes from the promoter regions of *c-KIT* and *EGFR* proto-oncogenes, both encode for tyrosine kinase receptors, important targets for anticancer treatment. Additionally, we studied interactions between a G-quadruplex and a fluorescence probe DAOTA-M2, which is used for detection of G-quadruplexes in live cells.

In the promoter of *c-KIT* proto-oncogene we focused on the kit* guanine-rich region, which has a key role in regulation of *c-KIT* transcription. Using NMR spectroscopy we revealed that kit* adopts a chair-type antiparallel G-quadruplex with two G-quartets in the presence of K^+ ions. The kinetics of kit* G-quadruplex formation is on the timescale of the transcriptional processes. In the promoter of *EGFR* proto-oncogene, we studied eight guanine rich regions, which represent a yet unexplored point of intervention to potentially silence this gene. We identified formation of G-quadruplexes with unique structural elements such as long loop arranged in a hairpin-like structure, adenine bulges and a A(GGGG)A hexade. DAOTA-M2 is a triangulenium optical probe that has significantly longer fluorescence lifetime upon binding to G-quadruplexes in comparison to double- and single-stranded nucleic acids. NMR structural study of interactions between DAOTA-M2 and a G-quadruplex revealed that they interact at 2:1 binding stoichiometry. Binding of DAOTA-M2 occurs mainly through π-π stacking interactions between the ligand and guanine residues of the outer G-quartets. Detailed insights in the structural characteristics of interactions offered an explanation for specific fluorescence features of DAOTA-M2 when bound to G-quadruplex structures.

Keywords: G-quadruplex, promoter, structure, NMR, *c-KIT*, *EGFR*, fluorescence probe DAOTA-M2