BASIC INFORMATION ON SUB-PROJECT

NAME OF PROGRAMME/FUND	Scholarship Fund - Sciex NMS^{ch}
RESEARCH FIELD AND OTHER RESEARCH FIELDS INVOLVED (if applicable)	Physics, Basic Biological Research
TITLE OF THE SUB-PROJECT	Atomic structure of biologically important DNA fragments (DNAstruct)
REGION OF THE CZECH REPUBLIC (according to the location of the home institution)	South Moravian Region
GRANT AMOUNT SPENT	48 869,70
INTERMEDIATE BODY	Swissuniversities
HOME INSTITUTION	Academy of Sciences of the Czech Republic, v.v.i. Institute of Biophysics
HOST INSTITUTION	University of Zürich Institute of Inorganic Chemistry
NAME OF THE FELLOW	Daniel Renčiuk

ABSTRACT OF THE SUB-PROJECT	The project is aimed for the studies of the unusual DNA secondary structures important from the biological or medical point of view. It takes advantage of two complementary methodological approaches: CD spectroscopy based studies of conformational transitions and the high-resolution structural studies based on X-ray diffraction. It comprises two relatively independent problems: The 5-hydroxymethyl cytosine (hmC), hot topic of present epigenetics, was described in the past few years as a “sixth base” of the genome and is also considered as a potential intermediate in demethylation of cytosine. We are interested if and how the 5-hydroxymethyl cytosine for cytosine substitution influences the structure of a model B-DNA oligonucleotide, the Dickerson dodecamer CGCGAATTCGCG. We will determine by X-ray diffraction the high-resolution structure of Dickerson dodecamer containing hmC and compare it to the non-modified sequence. Fragile X syndrome type A (FRAXA) is a human neurodegenerative disorder characterised by multiple expansion of the (CGG) triplet in 5`UTR region of the FMR1 gene. (CGG)n forms unusual structures, which may be responsible for the disease: B-like homoduplex, quadruplex or Z-DNA. We wish to determine the atomic structures of both the unusual homoduplex and of Z-DNA formed either by non-modified or 5-methyl cytosine or 5-hydroxymethyl cytosine containing oligonucleotide. The project will be started at the home institution where preliminary CD and UV melting experiments will be done and then the high-resolution structures of the conformations selected in this part will be solved by X-ray crystallography at the host institution.

MAIN RESULTS	We crystallized, solved and published four structures of Drew-Dickerson DNA dodecamer with either 5-hydroxymethyl cytosine or 5-methyl cytosine modifications at two different positions. Using UV absorption melting experiment we determined the effects of cytosine modifications on the thermal stability of the DNA double helix. Results of this part of the project were published in prestigious peer-reviewed international journal Nucleic Acids Research (doi: 10.1093/nar/gkt738). The results were also actively presented on an international symposium (B. Spingler, 2013, Gordon Conferences, New England, USA). Using CD spectroscopy we found that Drew-Dickerson dodecamer does not form Z-DNA. We thus skipped this part of the project. During the project period we were not able to obtain any measurable crystals from FRAXA related (CGG)n sequences, even after intensive manual optimization of conditions. Thus there are no quantifiable results from this part of the project. According to this we started another project, not mentioned in the project proposal, which was focused on studies of the structure of the guanine quadruplex formed by thrombin-binding aptamer sequence. We started automated screening of crystallization conditions on three different DNA sequences. Until now we have not obtained measurable crystals.
DATE OF REALISATION OF THE FELLOWSHIP	7.5.2012 - 6.11.2012
MORE INFORMATION ON THE PROGRAMME	**www.sciex.ch**