EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

ESRF

Experiment Report

ESRF	**Experiment title:** The investigation of the RE31, 15TBA and 110 aptamers using the SAXS method	**Experiment number**: MX-2039
Beamline: BM29	**Date of experiment**: from: 17.06.2018 to: 18.06.2018	**Date of report**: *Received at ESRF:*
Shifts: 3	**Local contact(s)**: Bart Van Laer	

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Report:

Experiments were carried out on the BM29 beamline at ESRF from 17 to 18 June 2018. It was the SAXS measurements of the biological macromolecules in solution. The samples were photoproteins, DNA and RNA aptamers. Our work consists of the several directions. The first one is the thrombin binding aptamer RE31 (31 nucleotides), which was studied by SAXS. It is the derivation of the short DNA aptamer TBA (15 nucleotides). The second one is the new DNA aptamer 110 to the interleukin-6. The third one is the photoproteins berovin apo and luciferases from *Metridia longa* and *Gaussia princeps*. The fourth direction is the number of the DNA aptamers to the glioblastoma (Gli-225 and Gli-233), Ehrlich ascite adenocarcinoma (AS-14t) and lung cancer cells (LC-18t, LC-224t and LC2108t). And our last sample is the RNA aptamer to the human hemoglobin.

All the samples were prepared with different concentrations in solution of the correspondent buffer. Before experiments samples were heated to 90°C and then cooled to 4°C. The temperature in the sample changer was the same, 4°C. The measured solutions, its buffer, concentrations temperature and calculated gyration radius and maximal dimensions are listed below:

Biomolecule	Sequence	Buffer	Volume, μl	Conc, mg/ml	T,° C	Rg,nm	Dmax, nm
15TBA	GGTTGGTGTGGTTGG	Tris	40	0.04	4	Very low conc.	
110	GCTCGGGGGGGAGGAGAATGATGCTGGGTTA	Tris	60	0.03-11.5	4	3.44	12
110	GCTCGGGGGGGAGGAGAATGATGCTGGGTTA	Tris	60	0.5-3.9	4	3.44	12

BAwt	MTERLNEQNNESYRYLRSVGNQWQFNVEDLHPKMLSRLYKRF DTFDLDSDGKMEMDEVLYWPDRMRQLVNATDEQVEKMRDAV RVFFLHKGVEPVNGLLREDWVEANRVFAEAERERERRGEPSLIA LLSNSYYDVLDDDGDGTVDVDELKTMMKAFDVPQEAAYTFFE KADTDKSGKLERTELVHLFRKFWMEPYDPQWDGVYAYKY	Tris	60	1-20	4	2.1	8.4
C12	GKMPGKKLPLEVLIEMEANAFKAGCTRGCLICLSKIKCTAKMK QYIPGRCHDYGGDKKTGQAGIVGAIVDIPEISGFKEMEPMEQFIA QVDLCADCTTGCLKGLANVKCSELLKKWLPDRCASFADKIQK	Tris	60	1.7-5	4	1.97	6.7
28G	GKMPGKKLPLEVLIEMEANAFKAGCTRGCLICLSKIKCTAKMK QYIPGRCHDYGGDKKTGQAGIVGAIVDIPEISGFKEMEPMEQFIA QVDLCADCTTGCLKGLANVKCSELLKKWLPDRCASFADKIQKE AHNIKGLAGDR	Tris	60	2.4-4	4	1.93	7.0
GpL	KPTENNEDFNIVAVASNFATTDLDADRGKLPGKKLPLEVLKEM EANARKAGCTRGCLICLSHIKCTPKMKKFIPGRCHTYEGDKESA QGGIGEAIVDIPEIPGFKDLEPMEQFIAQVDLCVDCTTGCLKGLA NVQCSDLLKKWLPQRCATFASKIQGQVDKIKGAGGD	Tris	60	1.2-4.5	4	02.09	7.5
GpL	KPTENNEDFNIVAVASNFATTDLDADRGKLPGKKLPLEVLKEM EANARKAGCTRGCLICLSHIKCTPKMKKFIPGRCHTYEGDKESA QGGIGEAIVDIPEIPGFKDLEPMEQFIAQVDLCVDCTTGCLKGLA NVQCSDLLKKWLPQRCATFASKIQGQVDKIKGAGGD	Tris	60	1-4.5	4	02.12	7.7
Gli-225	AAACACTGGTCATAATCATGGTGGC	PBS	40	0.04	4	Not yet processed	
Gli-233	ACTATTCCACTGCAACAACTGAACGGACTGGAA	PBS	60	0.8-8.2	4	1.84	7.0
Gli-55t	GTCCG GTTCA CCTCT AGCAT TCCTG GCGTT ATTAA CGGAG CAGTC CTGTG GAGTG GGTGA	PBS	60	0.4-4.4	4	Not yet processed	
AS-14t	CTCCTCTGACTGTAACCACGAAGGTGTCGGCCTTAGTA AGGCTACAGCCAAGGGAACGT	PBS	60	0.6-9.7	4	Not yet processed	
LC-18t	CGAAC GCGAG TTGAG TTCCG AGAGC TCCGA CTTCT	PBS	60	1.3-10.2	4	Not yet processed	
LC-224t	TGTAA CCACG CCGGT AAATT CTCCT GACGC CGGGG TAAGT	PBS	60	0.6-8.1	4	Not yet processed	
LC2108t	CTGTA ACCAC GCCCA GAGTC AGTGC GGCCC TTCCT TACAG T	PBS	60	0.7-7.5	4	Not yet processed	
H5t21	5'-C(F)GC(F) U(F)C(F)A AC(F)C(F) GAG GGA GGG GGG GAG GAU(F) GGC(F) U(F)GU(F) GC(F)G-3' (CGCUC AACCG AGGGA GGGGG GGAGG AUGGC UGUGC G)	PBS	60	0.8-3.2	4	3.4	11.3

We have obtained good SAXS results and the most of data are now in processing and interpretation. There are difficulties in the biomolecule transportation, we will need in the future take into account the tendency of proteins to aggregate. We suppose that obtained data from photoproteins are related with the dimerisation of the molecules.

Data from BM29 beamline has better resolution and signal-to-noise quality in comparison with the DIKSI station at the Kurchatov institute in Moscow. The publication with obtained at ESRF SAXS data will be published in the near future.

Thank you to Bart Van Laer for the introduction into the work on the beamline and for the help with the new buffer preparation, it was very useful.