ESRF	**Experiment title:** Study the Spatial Organization of Eukaryotic archtectural proteins from *D.melanogaster* and their complexes	**Experiment number**: MX-1862
Beamline: ID29, BM29	**Date of experiment**: from: 2016 to: 2017	**Date of report**:
Shifts: 10	**Local contact(s)**: Christoph Muller-Dieckmann	*Received at ESRF:*

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Report:

The spatial organization of the genome plays an important role in the regulation of all processes related to the translation of genetic information in any eukaryotes including human. Advances in genome-wide studies of chromatin architecture have shown that chromosomes consist of topologically associated domains (TAD), where the interactions between sequentially distal regulatory elements (enhancers, silencers and promoters) can occur due to their spatial proximity. The presence of specific although sequence-distal interactions between the boundaries of TADs and between regulatory elements within the TADs ensure the correct gene expression and subsequent organism development. Disruption of TAD boundaries may result in aberrant gene expression by exposing genes to inappropriate regulatory elements.

Spatial genome organization is coordinated by architectural proteins which bind to specific regulatory DNA sequences and establish long-range interactions, through a formation of various multi-protein complexes. Of special interest are those proteins, which are capable of, directly or through the recruitment of other protein partners, bring remotely separated regulatory elements of the genome in close spatial proximity or loop out chromatin domains. However, there is a severe lack of functional and especially structural data on these proteins as well as their complexes. The problem is in complex domain structure of archtiectural proteins which prevents their crystallization in full length.

In a frame of curret project we selected for futher structural studies a number functional domains of architrectural proteins including different Bric-a-brac/tramtrack and broad complex (BTB) domains as well as Zinc Finger Associated (ZAD) domains. These are widespread protein motifs involved in various protein–protein interactions.

To date we have obtained the following results:

1. **BTB domain of the CP190 protein from *D. melanogaster***. X-ray diffraction study at beamline ID29 allow to collect data at 1.5Å resolution (Figure 1), which is significantly higher compared to the structures available in protein data bank. Structure analysis is in progress now.

2. **BTB domain of the LOLA protein from *D. melanogaster***. It was supposed that BTB-domains of architectural proteins can form only dimers. SAXS data collected at BM29 beamline demonstrated that the BTB domain of the LOLA protein exists as an octamer (Table 1), which is in consistence with our solution studies. Unfortunately, crystallization of this domain lead to crystals diffracting to about 9Å.

Table 1. Calculated parameters of oligomers of the BTB domain of the LOLA protein in solution.

Protein concentration, mg/ml	Rg, nm	Dmax, nm	Vp, nm^3	Mol. weight, kDa	Oligomeric state
1.5	3.78	12.96	175.3	100-105	Octamer
6.0	4.55	16.16	208.8	120-125	Octamer

3. **ZAD-domain of Serendipity-d protein from *D. melanogaster***. X-ray diffraction study at beamline ID29 allow to collect data at 2.3 Å resolution. Structure was solved SAD technique based on the anomalous scattering of Zn atom. Despite the protein fold similarity to only known structure of ZAD-domain form Grauzone proteins, Zad-domain from Serendipity-d has some unique spatial features (Figure 1). Publication of this result is in progress.

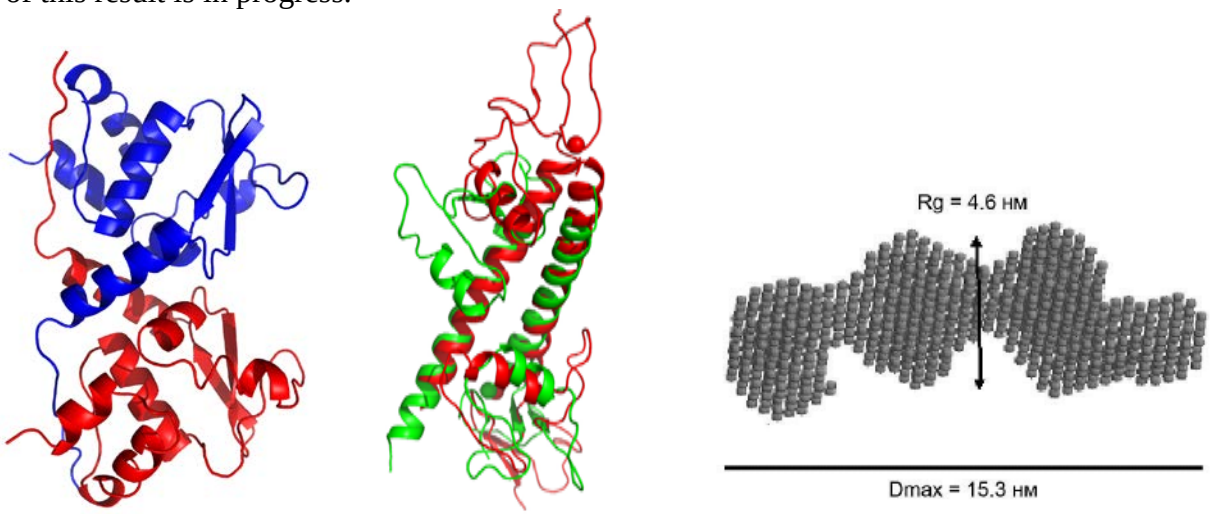

Figure 1. (Left) BTB-domain of CP190 protein in homodimeric state.(Middle) Comparison of ZAD-domains from Serendipity-d (red) and Grauzone (green). (Right) One of the possible low-resolution model of N-terminal domain of CTCF protein estimated by SAXS.

4. **N-terminal domain of CTCF protein**. This 163 a.a. long domain has no aminoacis homology to other known proteins. Despite the results of circular dichroism spectra which demonstrated almost complete lack of secondary structure elements, the SAXS data collected at BM29 beamline indicated that N-terminal CTCF domain possess a stable compact conformation in solution and seems to have a tetrameric form (Figure 1).

The preliminary results of the project have been presented on five international conferences and published in a following papers:

1. Bonchuk A.N., Kachalova G.S., Boyko K.M., Maksimenko O.G., Georgiev P.G. "N-terminal multimerization domain of *Drosophila melanogaster* CTCF protein has compact spatial organization but lacks secondary structure", Actual issues of biological physics and chemistry (RUS), 2016, 1, 243-245.
2. K. M. Boyko, A. Yu. Nikolaeva, G. S. Kachalova, A. N. Bonchuk, P. V. Dorovatovskii, and V. O. Popov "Preliminary Small-Angle X-Ray Scattering and X-Ray Diffraction Studies of the BTB Domain of Lola Protein from *Drosophila Melanogaster*", 2017, 62(6), 912-915.
3. K. M. Boyko, A. Yu. Nikolaeva, G. S. Kachalova, A. N. Bonchuk and V. O. Popov "Purification, Isolation, Crystallization, and Preliminary X-ray Diffraction Study of the BTB Domain of the Centrosomal Protein 190 from *Drosophila Melanogaster*", 2017, 62(6), 909-911.

Preparation of other manuscripts are in progress.