ESRF	**Experiment title:** Cryo-EM structural studies of a potential bioscavenger and detoxification biocatalysts - human butyrylcholinesterase in its native tetrameric form	**Experiment number**: MX 2007
Beamline: CM01	**Date of experiment**: from: 23/04/2018 to: 27/04/2018	**Date of report**: 20/07/2018
Shifts: 6	**Local contact(s)**: Michael Hons	*Received at ESRF:*

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Report:

Human butyrylcholinesterase (BChE) is a potential bioscavenger of toxic organophosphates. It can be used as an antidote to protect acetylcholinesterase and is a protein of choice for development of detoxification biocatalysts for clinical applications. To date, all the attempts to obtain the atomic structure of the full-length glycosylated tetrameric BChE were unsuccessful. The aim of the project was to use Cryo-EM technique to reveal the structure of the native tetrameric BChE and to clarify the details of its tetrameric and dimeric interfaces. Based on our preliminary experiments we estimated the optimal concentration of the sample for the experiment, prepared the grids and conducted Cryo-EM experiments at the ESRF instrument equipped with K2 detector.

Table 1. CryoEM data collection and processing statistics

Data collection	
Accelerating voltage, kV	300
Nominal / Calibrated magnification	130 000 / 46860
Pixel size[1], Å	1.067
Total exposure time, sec	7
Number of stacks	1500
Total dose per stack, $e^-/Å^2$	42
Number of frames per stack	28
Defocus range, μm	[-3.0; -1.0]
Defocus step, μm	0.4
Data processing	
Total extracted particles	170963
Refined particles	49261
Final particles used	6712

Symmetry	C2
Global resolution[2], Å	
$FSC_{0.5}$ (unmasked / masked)	13.0 / 9.4
$FSC_{0.143}$ (unmasked / masked)	9.1 / 7.6
Local resolution range[3], Å	[6.2; 8.7]
EMDB ID	EMD-0256

[1]Calibrated pixel size at the detector
[2]According to RELION 2.1
[3]According to ResMap 1.1.4

Examples of obtained images are shown on Figure 1. Noteworthy, that side view on BChE tetramers can also be projection of BChE dimers. Obtained images were processed as described below (Table 1).

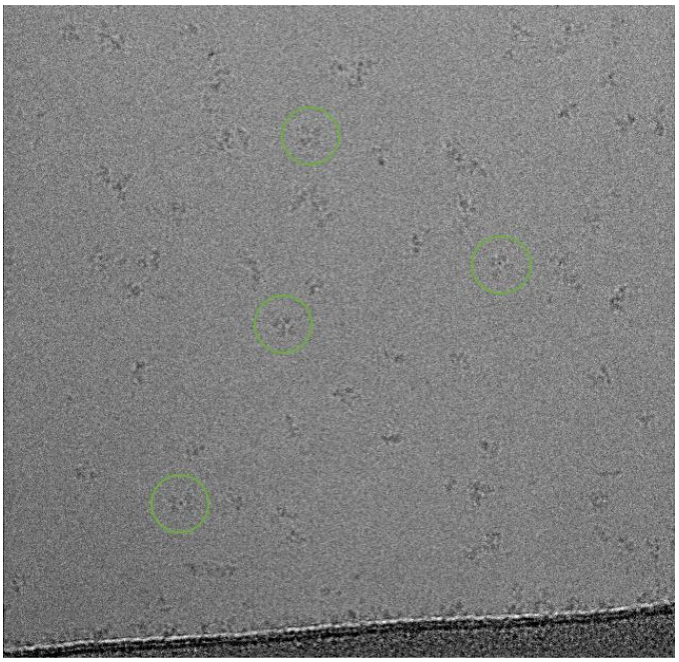

Fig. 1a: Top view on BChE tetramers are highlighted by green circle.

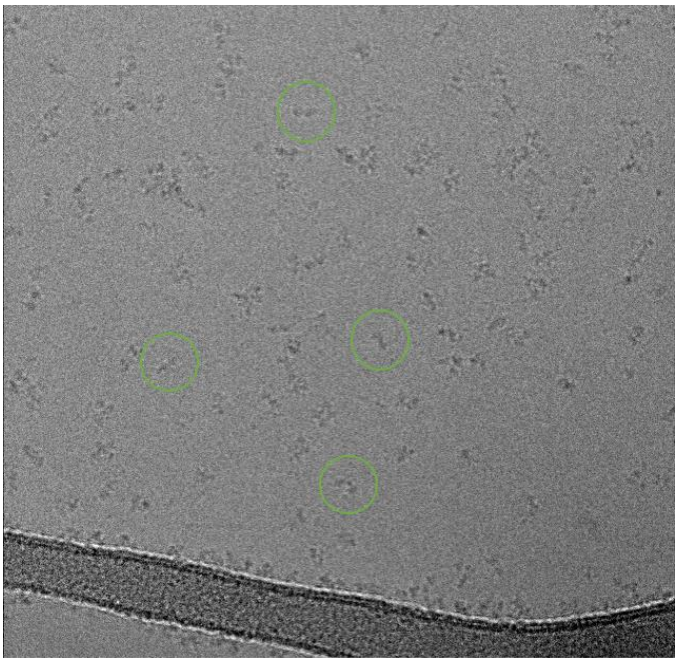

Fig. 1b: Side view on BChE tetramers / dimers are highlighted by green circle.

Data processing

Frame alignment and dose weighting were performed using UCSF Motioncor2. CTF estimation on aligned, unweighted, micrographs was performed with Gctf. All data processing steps except for the particle picking procedure were conducted in RELION 2.1. The BChE particles were picked automatically using Gautomatch software (https://www.mrc-lmb.cam.ac.uk/kzhang/Gautomatch/) with an automatically generated template. This resulted in 170,963 particles, which were extracted (256×256 pixels) and downsampled (128×128 and 64×64 pixels) for the iterative reference-free 2D classification. In total, 49,261 particles of 2D classes that possessed the quaternary structure features (Figure 2A) were subjected to 3D classification. A low-resolution initial tetramer model, required for the 3D classification stage, was built in EMAN2 using the corresponding class averages. The 3D classification resulted in five 3D classes, of which only one class had the quaternary structure features characteristic of tetramer. Subsequent 3D refinement of this tetrameric class containing 6712 particles was done without applying symmetry. This subset of particles was used for the final refinement (Figure 2B). The refinement was made using a soft mask encompassing the whole map and C2 symmetry based on preliminary knowledge of the tetramer structure and led to an improvement of the resolution. The 7.6 Å resolution of the final map was estimated by the 0.143 FSC criterion after a postprocessing procedure (Figure 2C). Estimation of the local resolution was done with ResMap with the soft mask used in postprocessing (Figure 2D). The final 3D map was deposited in the Electron Microscopy Data Bank (EMDB) under the accession code EMD-0256.

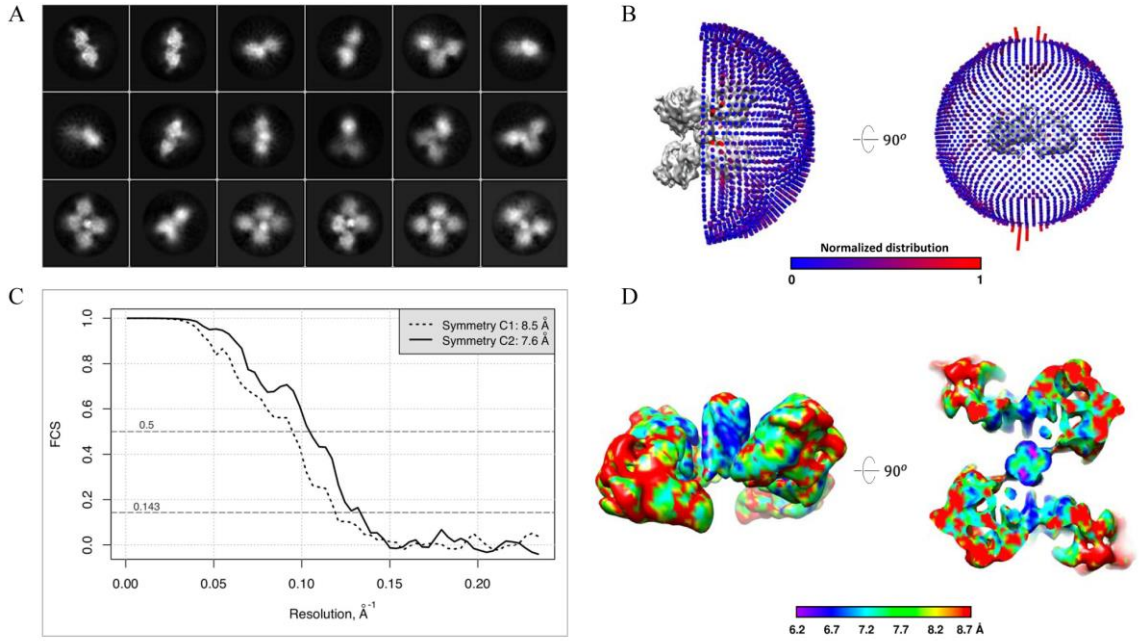

Figure 2. Quality of the cryoEM data. A – Class averages obtained after 2D classification, subjected to 3D classification. B - Distribution of Euler angles (generated by RELION). C - FSC curves of the masked density maps without imposing symmetry (dotted line) and with C2 symmetry applied (solid line) (according to RELION). D - Local resolution of the final density map estimated with MonoRes, shown on the complete volume (left) and on a coronal cross-section (right).

CryoEM model of BChE tetramer

The dataset was collected using a K2 detector on a Titan Krios electron microscope. Raw particles showed a moderate distribution of the expected tetramers, and reference-free 2D averages (Figure 2A) clearly demonstrate the presence of the expected oligomeric state. An initial reconstruction without symmetry applied gave a 10.5 Å resolution. C2 symmetry application, together with mask-enforced reconstruction, provided a 9.1 Å resolution, which was finally increased to 7.6 Å after postprocessing (Figure 2C, Table 1). This confirmed that the tetramer is a dimer of dimers, as previously stated. The final cryoEM map exhibited characteristic features of the BChE tetramer: a four-monomer arrangement with well-resolved secondary structural elements, as well as a left-handed superhelical C-terminal tail which lies in the center of the molecule along the 2-fold axis of the tetramer, perpendicular to its "plane" (Figure 3A,B).

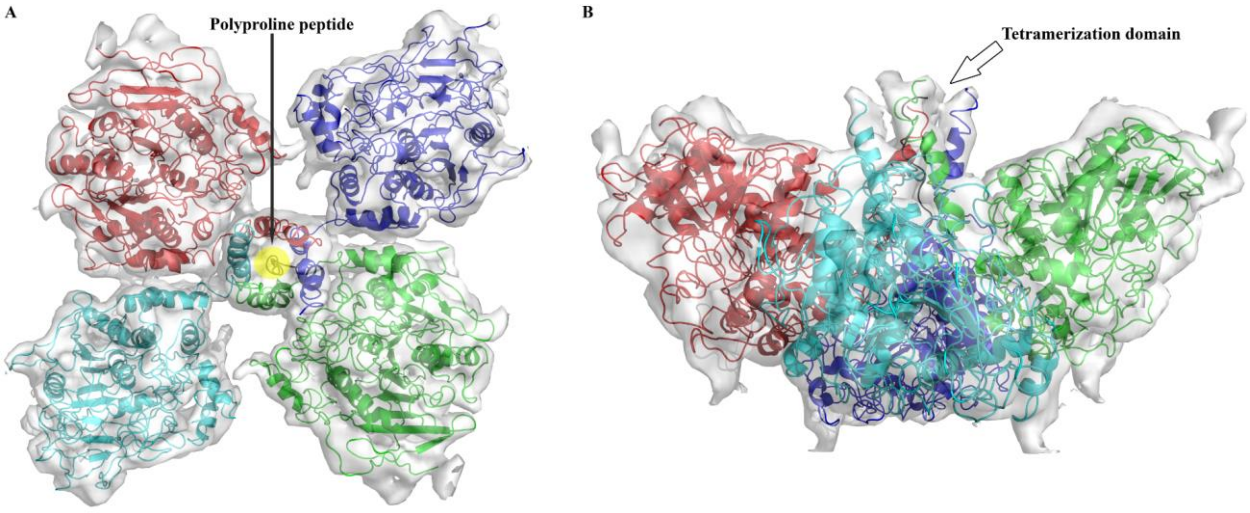

Figure 3. CryoEM map of the BChE tetramer. A- Top view of the map, where the 2-fold axis, which coincides with the C-terminal tail of BChE, is perpendicular to the plane of the screen. The map is depicted as a semitransparent gray surface. The BChE tetramer model fitted into the experimental density is shown as a cartoon model and colored by monomers. The polyproline peptide is colored in black and indicated with yellow circle. B - Side view of the map. The representation is the same as that in panel A.

To summarized, we obtained for the first time the natural tetrameric structure of human BChE at a 7.6 Å resolution. The structure differs significantly from the models proposed earlier at the level of the quaternary structure, including in the arrangement of monomers and orientation of the C-terminal tail. The data was published in the following article: *Konstantin M. Boyko, Timur N Baymukhametov, Yury M Chesnokov, Michael Hons, Sofya V Lushchekina, Petr V Konarev, Alexey V Lipkin, Alexandre L Vasiliev, Ph.D. Patrick Masson, Vladimir O Popov, Michail V Kovalchuk "3D structure of the natural tetrameric form of human butyrylcholinesterase as revealed by cryoEM, SAXS and MD" Biochimie, 2018, https://doi.org/10.1016/j.biochi.2018.10.017.*