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POSTER ABSTRACT PRESENTATIONS

SESSION TITLE: STRUCTURE OF VIRUSES AND CHAPERONINS

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Abstract P-29: Quality Control of Tick-Bone Encephalitis Virus Samples Using TEM and SAXS for XFEL Studies

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Background: Tick-borne encephalitis virus (TBEV) from flavivirus family is an important human pathogen causing a wide range of symptoms from uncomplicated fevers to encephalitis and meningoencephalitis. XFEL studies can provide new insight into TBEV structure to further improve the vaccine design. Flaviviruses are promising objects for XFEL/SPB (single particle biology) experiments for several reasons: 1) their structural investigations have clinical significance; 2) symmetry of particles is an advantage for XFEL data processing; 3) particles size (~50nm) is relatively large. XFEL is a cutting-edge method supposed to revolutionize structural biology by providing possibility to obtain 3D structures of biomolecules in their 'native state'. Although the determination of molecular structures at high resolution using the diffraction-before destruction approach with XFEL pulses is feasible in theory, there still remain many challenges for sample delivery, detector calibration and algorithm development for sample sorting. The method has special requirements regarding sample quantity and quality. Large volume, relatively high concentration, absence of aggregates and homogeneity of the sample are required. Aggregates and broken particles cause problems during sample delivery and data processing. Preparation of TBEV samples at high concentration (~10^{13}-10^{14}) without aggregates and with minimum amount of broken particles (which cannot be removed completely due to the TBEV structural heterogeneity) might be tricky and has to be controlled reliably. TEM negative staining technique is a common technique for characterizing viral suspensions, although it sometimes produces artifacts and has certain limitations.

Methods: Here we present an approach for the sample quality control which combines both negative staining TEM, cryoEM and small-angle X-ray scattering (SAXS).

Results: Negative staining TEM allows one to visualize TBEV particles while cryo-EM and SAXS are employed to check sample in case of "aggregate-like" clusters finding. SAXS additionally

gives an estimation of particles size volume distribution in at the same physical-chemical conditions as for XFEL experiment.

Conclusion: Combination of methods was significant for choice of sample preparation protocol. This hybrid approach makes an important contribution in successfulness of injection test at XFEL beamline. It can be useful for other virus samples evaluation for SPB experiment.

Key Words: flavivirus • cryo-EM • SAXS

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