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POSTER ABSTRACT PRESENTATIONS

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Abstract P-37: Phosphorous Mapping in Inactivated SARS-Cov-2 Particles by Electron Energy Loss Spectroscopy

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Background: The severe COVID-19 pandemic started in December 2019 is caused by the SARS-CoV-2 virus. The SARS-CoV-2 virion consists of a positive-sense single-stranded RNA (ssRNA), bound with the nucleocapsid N protein and surrounded by a lipid membrane with the embedded glycoprotein S and the transmembrane proteins M and E. The structure of inactivated SARS-CoV-2 virions is crucial for the development of vaccine-induced immunity. Here we characterized the nucleic acid distribution within β-propiolactone inactivated whole-virion SARS-CoV-2 vaccine CoviVac.
Methods: We used EELS to verify the presence of phosphorus (P) inside the β-propiolactone inactivated virions. Electron microscopy was performed with a JEM-2100 200kV LaB_6 transmission electron microscope (JEOL, Japan) equipped with a Gatan GIF Quantum ER energy filter (Gatan, USA) operating in spectrometer mode, along with a High-Angle Annular Dark-Field (HAADF) scanning transmission electron microscopy (STEM) detector. The cooling holder model 21090 (JEOL, Japan) was operated at -182 °C to reduce the contamination effects and to enhance the specimen's stability under the electron beam. We employ a negative stain with 2% $(NH_4)_2MoO_4$ rather than uranyl acetate since the Uranium $O_{4,5}$ peak (edge at 96 eV) is close to the P $L_{2,3}$ peak (edge at 132 eV) and interferes with the accurate background interpolation.
Results: The intensity under the P peak after the background subtraction was used for STEM-EELS mapping. We observed the characteristic P signal from

the inner part of the virion but not from the bare grid. The observed P signal could arise from either viral RNA or lipids of the virus membrane, and since the P signal is highly heterogeneous, it is more likely to originate from RNA.
Conclusion: So far, phosphorous mapping in individual virions using EELS was done only with samples prepared using highly specialized techniques, which minimized the sample thickness, including the substrate thickness. Here, we performed elemental mapping on ordinary samples of whole viruses. All investigated virions contained P signal, but its spatial distribution and intensity differed significantly. This clearly reflects the non-even distribution of the genomic RNA, which, apparently, accompanies their inner heterogeneity, previously observed by *in-situ* cryo-electron tomography.

Key Words: COVID-19 • β-propiolactone inactivated virus particles • EELS • nucleocapsid

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