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The nuclear export protein (NEP) of influenza virus is a crucial component for export of viral RNP through bilayer lipid membrane of the infected cell nucleus in complex with viral matrix protein (M1)/Exportin1/Nucleoporin. So, in this process NEP may be involved in some interactions as a constituent of supramolecular lipid-protein complexes. Earlier attempts to crystallize the full length NEP were unsuccessful: only the C-terminal domain (amino acids 64-121) was accessible for X-ray analysis. Insolubility of the protein at physiological conditions hampers its structural investigations. So we analyze the overall structure of NEP by synchrotron SAXS. The on-line size-exclusion chromatography (SEC-SAXS) experiments demonstrated a presence of two fractions of the protein in solution. The first fraction consisted of large aggregates, while the second one revealed formation of the NEP clusters, whose characteristics were determined by *ab initio* shape reconstruction method. It was shown that the clusters demonstrated ordered helix-like particles with the length of about 20 nm and the cross-section (diameter of the helix) ~ 5 nm. The helix-like structure consisted of small uniform particles with size of about 2 nm. These particles could be considered as individual NEP macromolecules which impossible to separate even by size-exclusion chromatography. They formed stable clusters, which are typical for the majority of viral proteins, especially for the membrane interacting proteins. To obtain additional information on the peculiar self-association properties of NEP we carried out searching for the  $\alpha$ -helices in NEP structure, and used the concept of intrinsically disordered/unstructured proteins, and predicted the SSE's with the Psipred software. The prediction of NEP N- domain 3D structure by Scratch Protein Predict program (SPP) and by Modeller 9.8 software resulted in contradictory information. Nevertheless, only the latter software (server RaptorX) led to almost an exact convergence program predicts of ordered (helix) and disordered fragments of the polypeptide chain.

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#### P10-4

##### **Potyvirus Potato Virus A coat protein posses unusual properties and forms short virus-like particles**

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Potyviruses are the largest and most economically important group of plant viruses. Earlier we have reported that virions of Potyvirus Potato Virus A (PVA) to have a peculiar combination of physicochemical properties and its coat protein (CP) contained unstructured domains [1]. Structural analysis of the PVA CP in solution was performed by small angle X-ray scattering (SAXS), fluorescence spectroscopy and electron microscopy. Overall structural characteristics of PVA CP obtained by SAXS (radii of gyration, Porod volumes, molecular masses) clearly point to the formation of large particles containing dozens (~ 60) of the individual PVA CP macromolecules. Porod asymptotic and Kratky plot indicate the existence of rather compact dense particles at pH 7.8, and the presence of partly disordered bodies without clearly defined borders at pH 10.5. *Ab initio* shape reconstruction of the CP associates at pH 7.8 demonstrates cylindrical particles similar to the shape of Potato Virus A. These particles undergo dramatic changes with increasing pH to 10.5: they lose their compactness and split into poorly connected spherical fragments.

The results of the electron microscopy analysis show that PVA CP dialyzed at pH 7.8 is assembled into short cylindrical virus-like particles, while the loose structures obtained at pH 10.5 form spherical shapes with the size of about 20-30 nm. Fluorescence spectra of both intact PVA virion and isolated PVA CP at pH 7.8 have two maxima at 314 and ~330 nm, *i.e.* these two species have similar tertiary



structure. Fluorescence spectrum of the isolated PVA CP in buffer with pH 10.5 has the only maximum -at 343 nm. This shift of the maximum position from 330 to 343 nm reflects the loss of the tertiary structure. Generally, the results of the present communication show that self-assembly of the isolated PVA CP in buffer with pH7.8 is the intrinsic biological property of the PVA coat protein needed for the formation of an viral envelope to protect genetic material of the virus.

**Reference:** [1] Ksenofontov, AL, Paalme, V., Arutyunyan, AM. et al.(2013) PLoS ONE, 8, e67830.

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## P10-5

### **Cryo-EM structure of a new internal membrane ss-DNA-bacteriophage found in a boreal lake**

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In this study we present the Cryo-EM structure of a new ssDNA-bacteriophage called FliP (*Flavobacterium* infecting, lipid-containing Phage). This structure allows the characterization at atomic level of the FliP capsid, which is found to be icosahedral as for other members of the *Microviridae* family but the its size greatly exceeds that of the known ssDNA-phages. Moreover, the presence of internal membrane makes FliP unique within the known ssDNA-phages.

Our Cryo-EM reconstruction shows that the major capsid proteins form the outer protein shell following T=21 icosahedral organization and pentameric spikes (13nm tall) protrude from twelve icosahedral vertices. Each capsid protein has two-barrel jelly rolls disposed perpendicularly to the surface of the capsid forming a trimer, which represents the assembly unit. The shape of the capsid is highly faceted, with facet-to-facet distance of 52 nm and edge-to-edge and vertex-to-vertex distances of 55 nm and 62 nm, respectively. The outer protein shell covers a 5-nm thick lipid bilayer membrane.

Although FliP capsid protein does not share significant sequence similarities with homologues from other viruses, the overall structure of the capsid virus suggests a relationship with bacteriophage PM2 and other members of the PRD1-adenovirus lineage despite the different genome types and lack of sequence similarity.

Furthermore, the host range experiments show that FliP is able to infect three different *Flavobacterium* strains, and currently there are no other reports of *Flavobacterium* as a host species for *Microviridae*. According to these results FliP has clear similarities to the *Microviridae*-family. Nevertheless, our results suggest that this bacteriophage belongs to a new *Microviridae*-subfamily, or possibly even to a completely new viral family.

## P11-2

### **Predication of sofosbuvir response using a single nucleotide polymorphism of interferon lambda-4 gene as a predictive factor**

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About 15% of the population in Egypt have chronic hepatitis C and over 90% out of them have HCV