ESRF	**Experiment title:** Intermediate species along the self-assembly pathway of measles virus nucleocapsid-like particles	**Experiment number**: SC-4762
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Report:

Introduction and motivation. Measles virus (MeV) belongs to the *Paramyxoviridae* family. The viral genome in the form of single-stranded (ss)RNA is packaged by multiple copies of a nucleoprotein (N) forming a helical nucleocapsid (NC). Electron microscopy revealed that the diameter of NCs is about 20 nm and its length exceeds 100 nm at equilibrium. A recent study has shed light on the self-assembly mechanisms in the presence of short RNA segments [1]. A 50-aa peptide of the phosphoprotein (P) forming a stable complex with N was identified. Upon addition of this complex to six-nucleotide RNA segments, N self-assembled into tubular structures termed nucleocapsid-like particles (NCLPs) resembling the native NCs. At each capture of a six-nucleotide RNA segment by N, the C-terminal domain bound the preceding N while the N-terminal domain bound the following N in the helical structure of NCLPs (Fig. 1). The addition of one N entailed the release of one P. Kinetic measurements performed by nuclear magnetic resonance (NMR) revealed a time scale of about 45 minutes to complete the process in the high concentration conditions required by NMR. Interestingly, the use of 60-nucleotide RNA segments in similar conditions led to a slightly slower assembly. The same study has also demonstrated that the binding of RNA on N was sequence-specific. No assembly was detected in the presence of $polyU_6$ (UUUUUU) whereas the efficiency was increased with $polyA_6$ (AAAAAA) for instance.

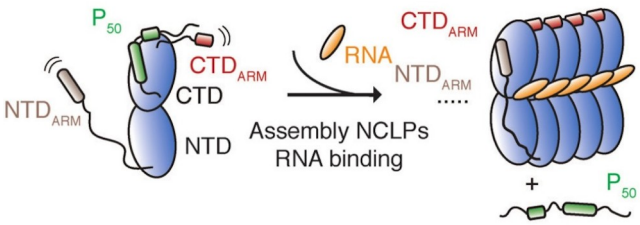

Figure 1 Principle of the assembly of a nucleocapsid-like particle. Adapted from [1].

Our goal was to elucidate the mechanisms regulating the formation of MeV NCLPs in the presence of short RNA segments with different sequences. The experiment aimed more specifically at identifying the structure and the composition of intermediate species possibly involved in the nucleation process.

Results and conclusions of the study. MeV N and P proteins were expressed in *E. coli* and purified by gel filtration. Six- and 60-nucleotide genomic RNA as well as $polyU_6$ and $polyA_6$ were purchased from Integrated DNA Technologies. The protein complex $P_{50}N_{405}$ and RNA were dispersed in 50 mM sodium acetate pH 6.0 and 150 mM NaCl. The SAXS pattern of $P_{50}N_{405}$ is shown on Fig. 2 and is consistent with a monomer. The radius of gyration was estimated to be 3.9 nm. Notice the raise of intensity at very low q-values, which is likely due to some problem in the definition of the mask but does not reflect the presence of aggregation whatsoever.

Various kinetics were performed by rapidly mixing $P_{50}N_{405}$ and RNA in different ratios with a stopped-flow device. Exposure time was set to 5 ms and the dead time between frames was geometrically increased up

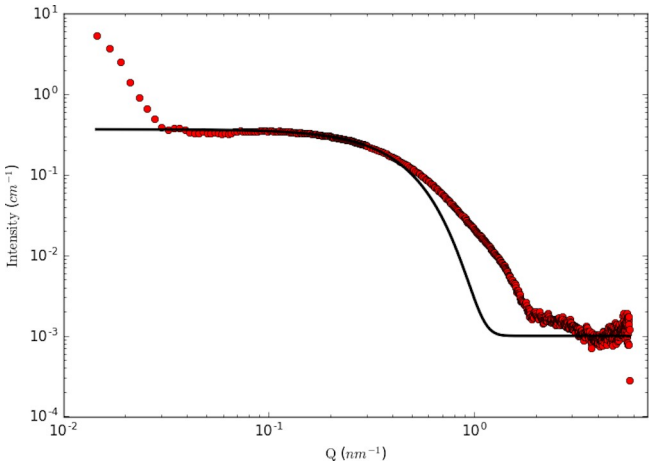

Figure 2 SAXS pattern of the $P_{50}N_{405}$ complex at 100 µM. The black line is a Guinier approximation.

to almost one hour. Since the self-assembly process turned out to be very long and because the hard-stop valve of the stopped-flow device could not stand long durations, we also followed the assembly with samples prepared in a test tube and regularly monitored by using the flow-through cell. We had thereby collected scattering patterns up to 16h after mixing. In the case of RNA_{60}, the final patterns (data not shown) suggested a majority of tubes having an inner diameter of 6.5 nm and an outer one of 20 nm, in good agreement with the structure of the native NC. The length could not be estimated because the smallest q-values were limited to 3×10^{-2} nm^{-1} but most likely exceeded 100 nm.

Figure 3 depicts three kinetics performed with RNA_{60} and $polyA_6$. The SAXS patterns on Fig. 3a were collected over about one hour. The forward scattering intensity increased steadily before dropping abruptly around 20 min. This happened regularly due to the hard-stop valve that opened unexpectedly letting the sample flow in the capillary and subsequently becoming diluted. The radius of gyration was unreliable in this case. A quite interesting feature was the presence of isosbestic points on the scattering patterns. They suggest that solely two species were exchanging matter over the course of the assembly. It was confirmed by singular value decomposition, which revealed that two components were sufficient to reconstruct the full set of data. We can therefore assume that during this phase, a mixture of $P_{50}N_{405}$ and RNA_{60} in the one hand, and assembled NCLPs in the other hand were coexisting, and that no other intermediate species contributed significantly to the scattering intensity. Upon decrease of the concentrations (Fig. 3b), we observed that the growth rate was roughly the same but the final fraction of NCLPs was of course smaller than previously. Measurements performed with $polyA_6$ (Fig. 3c) at yet higher protein concentrations revealed a slow assembly process and the formation of less regular objects, probably comprising some defects. It was likely due to the presence of short segments of $polyA_6$, much more difficult to capture by the growing NCLPs than RNA_{60}. Intriguingly, $polyU_6$ and RNA_6 did not yield any detectable NCLPs, pointing out a certain sequence specificity of $P_{50}N_{405}$.

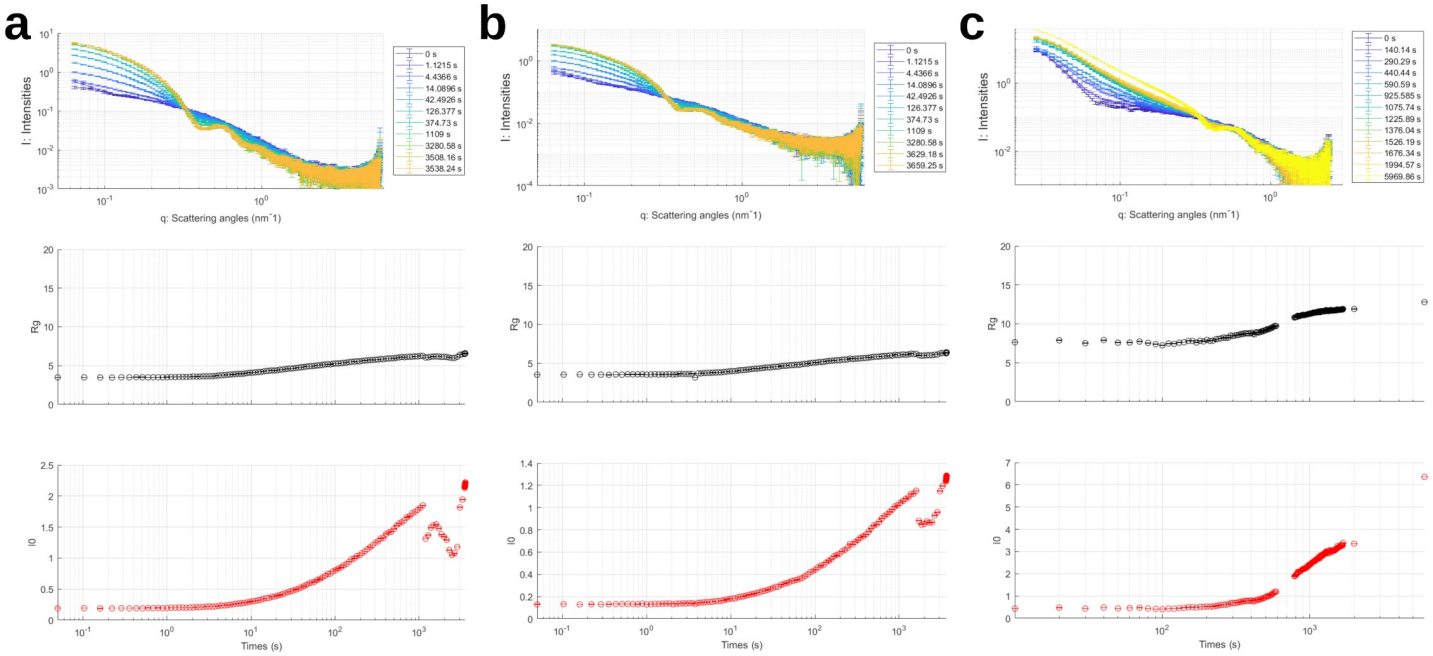

Figure 3 TR-SAXS patterns, radius of gyration R_g, and forward scattering intensity I_0 for the self-assembly of MeV NCLPs in various conditions: (a) 66 µM $P_{50}N_{405}$ with 33 µM RNA_{60}; (b) 33 µM $P_{50}N_{405}$ with 33 µM RNA_{60}; and (c) 100 µM $P_{50}N_{405}$ with 50 µM $polyA_6$.

[1] S. Milles *et al.* (2016). "Self-assembly of measles virus nucleocapsid-like particles: Kinetics and RNA sequence dependence". *Angew. Chem.* 128 9502.