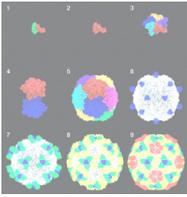


# Dynamic Light Scattering: Low Polydispersity related to Crystallization Success of Rice Virus



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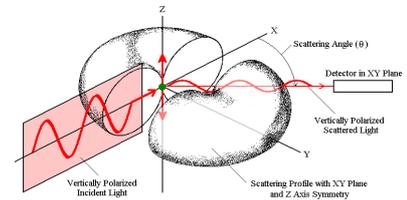
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## Abstract

Dynamic light scattering (DLS) is a technique to measure molecular size and size distributions in solution. In DLS the intensity fluctuations of scattered light are correlated in order to obtain the diffusion coefficient and equivalent hydrodynamic size of molecules. The technique can be used for dilute solutions from ~1 nm to 1 µm, corresponding to typical molar masses of a few kilo to beyond Mega Daltons. Here, we investigate a virus two different forms: One strain crystallized, the other did not. Significant differences in size and size distribution are observed.



## Experimental

Rice Dwarf Virus (RDV) was cultivated, purified and prepared in water at various concentrations (Nakagawa et al.). We looked at two different strains, RDV<sup>+</sup> (which crystallized) and RDV<sup>-</sup> (which failed to form crystals). Samples were prepared in a series of concentrations, appear clear and were free from precipitation. All preparations were analyzed on the Zetasizer Nano in a 12 µL cuvette.

	RDV+		RDV-	
conc.[g/mL]	Z-Ave [nm]	Pdl	Z-Ave [nm]	Pdl
0.500	75.2	0.004	119.2	0.25
0.250	76.3	0.037	122.4	0.24
0.125	76.9	0.053	117.0	0.19
0.063	75.9	0.011	120.8	0.29
0.031	77.7	0.050	121.5	0.28
0.016	79.1	0.009	119.4	0.26
Average:	76.9	0.027	120.1	0.25

## Hydrodynamic Size (Cumulants)

Dynamic light scattering (DLS) correlation functions were first analyzed with the (single exponential) cumulant method according to ISO13321. All data were repeatable over the concentration range and consistent for the two strains. Results are shown in Figure 2.

RDV<sup>+</sup> showed an average cumulant diameter of 77nm and a low polydispersity index of less than 0.03 - indicating that a monodisperse distribution of mostly a single species of scatterers are present in solution.

RDV<sup>-</sup> showed an average cumulant diameter of 120nm and a high polydispersity index of 0.25. This indicates that a broad, polydisperse distribution of several species of scatterers are present in solution.

The observed behaviour correlates well with prior studies showing a link between low polydispersity and higher crystallization success rates (D'Arcy).

## Hydrodynamic Size (Distribution)

A distribution analysis of the correlation (multiple exponential) regularization fit calculates the most likely range of different sizes present in the sample in solution.

The intensity distribution of RDV<sup>+</sup> in Figure 3 shows a single narrow peak near 75nm diameter for all concentrations. This is close to the size expected from transmission electron microscopy (TEM) data which suggest a diameter of about 70nm.

RDV<sup>-</sup> contains much larger components, it appears that the virus is present here as a mix of fragments and oligomers. There is large variation between the different concentration samples (Figure 4).

Figure 2: Cumulants results from dynamic light scattering of two different virus strains, RDV<sup>+</sup> is monodisperse and of smaller size, and RDV<sup>-</sup> is larger and polydisperse

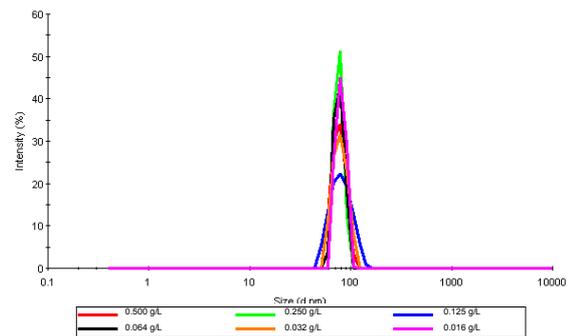


Figure 3: Size distribution analysis of DLS data for RDV<sup>+</sup>

## References

- D'Arcy, A. "Crystallizing proteins - a rational approach", Acta Cryst. D50 (1994), pp. 467-471.
- A. Nakagawa, N. Miyazaki, J. Taka, H. Naitow, A. Ogawa, Z. Fujimoto, H. Mizuno, T. Higashi, Y. Watanabe and T. Omura et al., The atomic structure of rice dwarf virus reveals the self-assembly mechanism of component proteins, Structure 11 (2003), pp. 1227-1238.

## Summary

Rice Dwarf Virus (RDV) is a double-shelled virus with a molecular mass of approximately 75 million Dalton. Virus samples from two different preparations were analysed with dynamic light scattering. One strain, RDV<sup>+</sup>, crystallized, the other RDV<sup>-</sup>, did not. The DLS size distributions show marked differences between the two strains: RDV<sup>+</sup> exhibited a single narrow peak with an average diameter of 77nm, RDV<sup>-</sup> shows a broad distribution, with a higher average size close to 120nm.

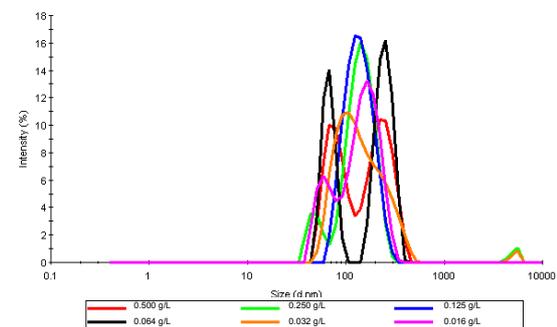


Figure 4: Size distribution analysis of DLS data for RDV<sup>-</sup>



The Zetasizer Nano from Malvern Instruments is the only commercial instrument to combine the measurement of size, molecular weight, and zeta potential of a wide range of samples in pharmaceutical and biomolecular applications.

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