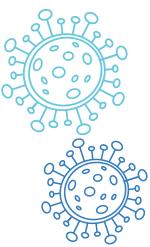


## **Additional Validation:**

## SARS-CoV-2 D614G variant spike protein

Daresbury Proteins reports additional validation data for its recombinant full-length trimeric SARS-CoV-2 D614G variant spike protein. In-depth analysis of the spike protein by Circular Dichroism (CD) spectroscopy confirms correct secondary structure and trimeric assembly. Two different batches of S protein were tested; the first batch in PBS buffer and the second batch in PBS buffer with 20% glycerol. The gel filtration profile demonstrates correct protein size and a lack of aggregation whether stabilised by the protectant glycerol or not. Differential scanning fluorimetry data of the two batches shows the protein is both stable and functional, with heparin binding activity.



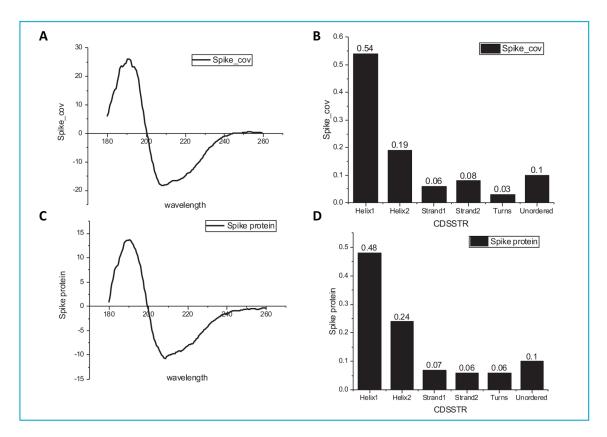


Figure 1. CD spectra of different cation forms of spike protein.

A and C. CD spectra were recorded on J-1100 spectrometer between 180 and 260

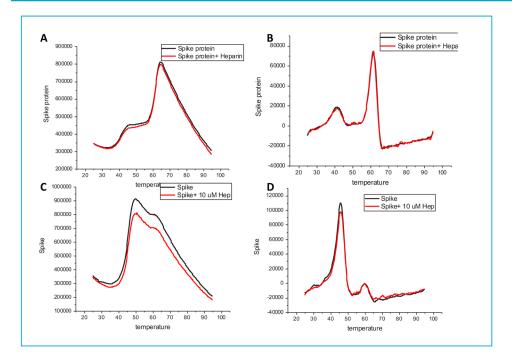
A and C, CD spectra were recorded on J-1100 spectrometer between 180 and 260 nm of spike protein (0.5 mg/mL). B and D, Secondary structure were analysed by program CDSSTR of (A/C). A and B is the first batch of spike protein (PBS buffer), C and D is the second batch of spike protein (PBS 20% Glycerol).

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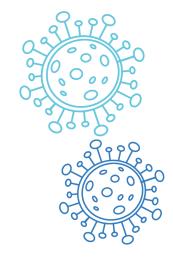
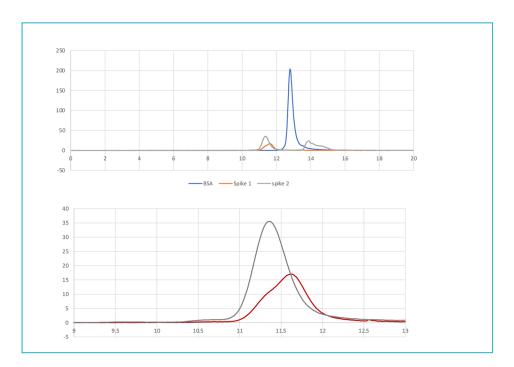


Figure 2. Differential scanning fluorimetry (DFS) analysis the stability and the heparin binding ability. DSF of proteins were performed in the absence or presence of heparin (10 $\mu$ M). A and B is the first batch of spike protein (PBS), C and D is the second batch of spike protein (PBS 20% Glycerol). A and C, Melting curve of spike protein. B and D, First derivative of the melting curves of spike to show its melting temperature as peak.



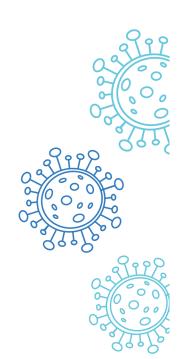


Figure 3. Gel filtration on BSA, Spike 1 (the first batch, in PBS buffer) and Spike 2 (the second batch, in PBS buffer and 20% Glycerol).

