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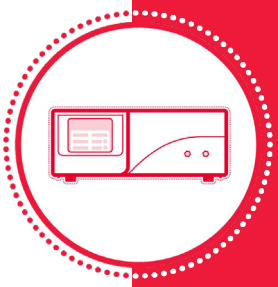


Trimeric SARS-CoV-2 spike protein analysis with SEC-MALS

Your Challenge

- You deal with complex proteins and protein assemblies used in biotherapeutics or diagnostics.
- You need to achieve advanced characterization of their critical quality attributes.

Our Solution

LenS3 MALS detector

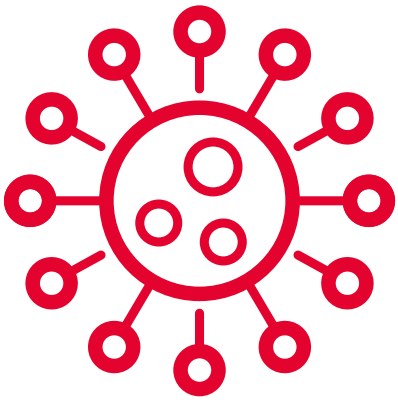
- A highly sensitive light scattering detector

What was done?

- Several variants and modified versions of trimeric Spike proteins were characterized by SEC-MALS.

What was the result?

- Significant structural differences were revealed from molecular weight and size determination



Analysis of complex proteins

The LenS3 MALS detector coupled with SEC allows a thorough characterization of complex proteins by providing their molecular weight and size profile with unmatched sensitivity.

Your Benefit

Characterize and differentiate critical quality attributes of complex proteins.

TOSOH BIOSCIENCE

https://www.separations.eu.tosohbioscience.com/products/chromatography-instruments-and-accessories/stand-alone-detectors

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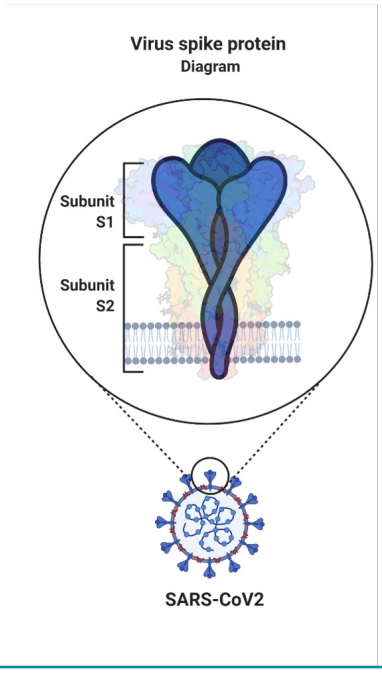
Application Note

Trimeric SARS-CoV-2 Spike Protein Analysis with Size Exclusion Chromatography Coupled with MALS

The scientific community recently focused on overcoming the coronavirus disease-19 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This RNA virus contains four structural proteins (spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins). The spike proteins are pointing outward, as depicted in *Figure 1*. These glycoproteins help the virus attach to various surfaces and mediate entry into host cells. Therefore, pure spike proteins are used as antigens for detecting SARS-CoV-2 antibodies to develop vaccines and other therapeutics and diagnostics applications.

Figure 1. Trimeric spike protein on the surface of SARS-CoV-2.



As the SARS-CoV-2 trimeric spike complex *(Figure 1)* is a major target of the immune system, stabilized trimeric spike proteins have been selected as antigens of choice for RNA and virus-vector-based vaccine candidates and provide the best sensitivity and specificity for the detection of SARS-CoV-2 antibodies in diagnostics applications. Excellgene SA produces soluble trimeric SARS-CoV-2 spike proteins in CHO cells[1].

Glycosylation has emerged as an important parameter in the activity of the spike proteins, and glycosylation of the spike proteins produced using different production platforms has been evaluated in a precedent work. Glycosylation patterns are conserved across the various platforms. This can be associated with site-specific stalling of glycan maturation that acts as a highly sensitive reporter of protein structure. Molecular dynamics simulations of a fully glycosylated spike support a model of steric restrictions that shape the enzymatic processing of the glycans[2]. However, the expression of spike proteins with the desired glycosylation pattern is challenging. A HexaPro modification locks the trimer in the prefusion state as found on the virus, which is relevant for vaccines and diagnostic tools. Additionally, the HexaPro variant leads to higher expression and improved stability[3].

Suitable analytical techniques are needed to verify the structure and ensure the quality of those spike proteins. Three critical quality parameters for oligomeric proteins are purity, molecular weight (MW), and size (radius of gyration, or Rg).

Size exclusion chromatography (SEC) is commonly used to provide information on the size and purity of proteins. When coupled with multi-angle light scattering (MALS), this advanced analytical method can determine MW and Rg simultaneously. The resulting true MW by light scattering reveals the aggregation state of proteins to elucidate their oligomeric structure. Until recently, the Rg of spike proteins was too small to be characterized by MALS, but recent development is now allowing the determination of Rg below 10 nm. The Rg calculated by MALS is compared with the results obtained from theoretical simulations or structural studies to gain comprehensive information on the possible conformation of proteins.

In this study, we characterized several variants and modified versions of trimeric spike proteins produced by Excellgene using the most recent development in SEC-MALS.

Experimental Conditions

Instrument:	UHPLC system
Detectors:	Refractive index detector Tosoh LenS3 MALS detector
Column:	1x TSKgel UP-SW Aggregate (0023524) 4.6 mm ID × 30 cm; 3 µm; 30 nm
Mobile Phase:	100 mM phosphate, 100 mM sodium sulfate, 150 mM sodium chloride, pH 6.7
Flow rate:	0.35 mL/min
Temperature:	Ambient
Injection vol.:	10 µL

Results and Discussion

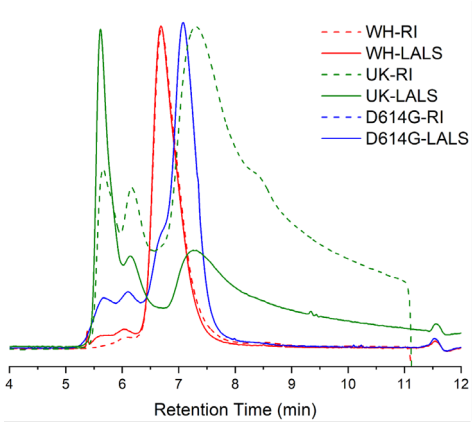
Comparison of low angle light scattering (LALS) and refractive index (RI) chromatograms of spike protein variants

When comparing RI and LALS chromatograms *(Figure 2)*, trace amounts of aggregates are significantly more visible by LALS due to the molecular weight-dependent response of light scattering. This higher sensitivity allows for a better characterization of the purity of the spike proteins.

Table 1. Sample information.

Sample ID	Concentration (mg/mL)
WH1	1.087
WH1	1.083
Beta	1.088
D614G-2Pro	1.135
D614G-6Pro	0.885

Figure 2. LALS (solid lines) and RI (dotted lines) elution profiles of SARS-CoV-2 spike proteins variants.



Molecular weight distributions of the trimeric spike protein variants determined by LALS

Regardless of the proportions of protein aggregates, trimeric proteins, and fragments/impurities, the well-overlaying MW distribution curves in *Figure 3* demonstrate the very close structural profiles of these variants. The average MW of ~500 kDa of the main peak *(Table 2)* is higher than the predicted 420 kDa value (as calculated from the amino acid sequence of the S protein in trimeric form without glycans) but agrees well with the SDS-PAGE result (150-170 kDa for the monomer). Similar findings have also been reported in the literature[4].

Size distribution of the trimeric spike protein variants

Although they have almost identical MW distribution profiles *(Figure 3)*, slightly different Rg values were observed *(Figure 4)*. The results suggest slightly dissimilar conformations of the trimeric spike protein variants. However, in their aggregated state, their sizes tend to converge. The obtained trimeric proteins' Rg values of ~6 and ~9 nm also agree with literature values[5, 6] (a length of 18-23 nm, not exceeding 30 nm, or a width of 5 nm. If analog to a cylinder shape: Rg = 6.3 to 9.4 nm).

Figure 3. Overlay of MW distributions and RI chromatograms of the three S protein variants.

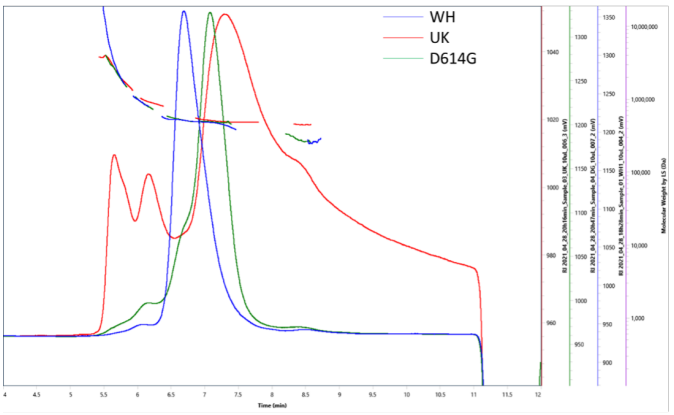
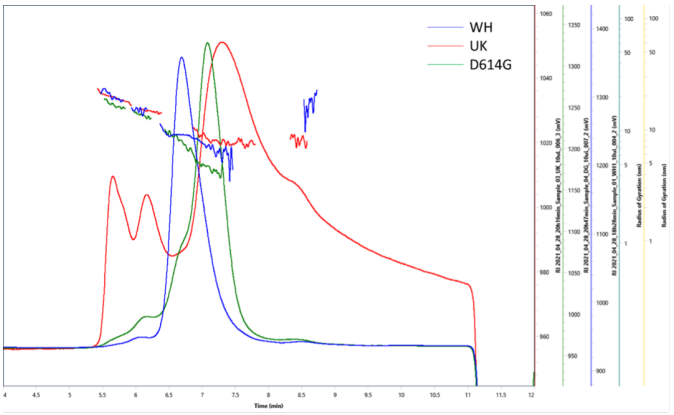


Figure 4. Overlay of Rg distribution and RI chromatograms.

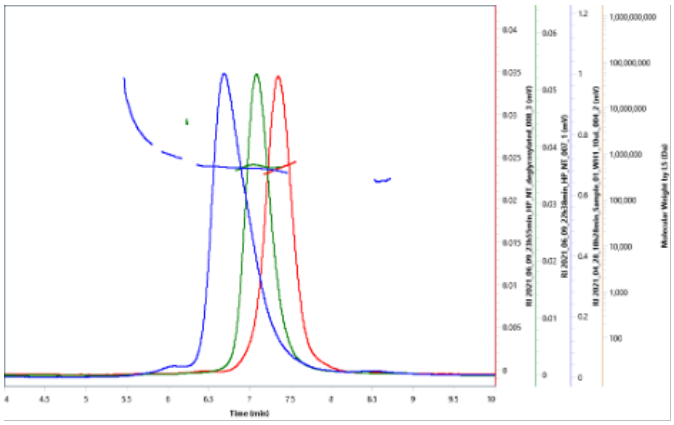


Effect of modifications of the spike protein on its molecular weight, size, and shape

Chromatograms and MW distributions were compared between the wildtype WH trimeric spike protein and two modified samples *(Figure 5)*. The HexaPro-modified trimeric spike protein shows fewer aggregates in the RI chromatograms.

The MALS calculation also indicates that the HexaPro-modified sample has a slightly higher MW (535.8 kDa vs. 498.9 kDa for WH) but a smaller size (later elution, Rg value of 7.7 nm vs. 8.6 nm for WH), which correlates well with the expected effect of the HexaPro modification. After deglycosylation of the HexaPro trimeric protein, the MW does not change. However, the apparent size is smaller (6.3 nm), possibly due to the protein's slight conformational adjustment after glycan removal.

Figure 5. Overlay of RI chromatograms and MW distributions of wildtype (blue) and two modified WH spike proteins (HexaPro, green, HexaPro-deglycosylated, red).



Conclusion

The molecular weight (MW) and size (Rg) of trimeric SARS-CoV-2 spike proteins from multiple variants and their HexaPro-stabilized and deglycosylated versions were determined by SEC-MALS. Slight MW and size changes were detected between different variants, modifications, or glycosylation profiles.

Such characterization using SEC-MALS is only possible using the LenS$_3$ MALS detector combined with the TSKgel UP-SW Aggregate column, as they show excellent separation performance and sensitivity, which allowed us to confirm the structure of the trimeric spike proteins and reveal significant structural differences.

As the LenS$_3$ MALS detector is the only MALS detector capable of measuring Rg below 10 nm, we could determine size variations between 6 nm and 9 nm in consistency with size information from the literature.

The LenS$_3$ MALS detector and the TSKgel UP-SW Aggregate column are the SEC-MALS technologies of choice to characterize spike proteins and other complex biomolecules in Research and Development and Quality Control.

References:

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Featured products.

P/N	Description	Dimension
0023524	TSKgel UP-SW Aggregate	4.6 mm ID x 30 cm L
0040000	LenS$_3$ Multi-Angle Light Scattering Detector	
0040001	UHPLC Conversion Service Kit for LenS$_3$ MALS Detector	

Table 2. Analysis of the trimeric spike protein samples.

Sample ID	Molecular weight (kDa)				Rg (nm)			
	Fraction	Main Peak	Aggregate 1	Aggregate 2	Fraction	Main Peak	Aggregate 1	Aggregate 2
WH	260.1	498.9	924.2	3,108.7		8.6	15.2	20.5
UK	446.7	494.6	899.8	2,370.8	8.4	8.3	15.2	20.0
D614G	286.8	493.9	857.6	2,247.4		6.2	14.0	17.2
HexaPro		535.8				7.7		
HexaPro deglyc.		497.4				6.3		