Introduction
Chirascan spectrometers go beyond the traditional use of CD. High-quality data supported by stringent statistical analysis provide unique insights into the higher order structure (HOS) of complex molecules.

Effect of transient exposure to nanoparticles on secondary structure and stability of proteins

Sample preparation and CD analysis
Two globular enzymes, submitted for analysis by a leading European university, were incubated with aluminium oxide nanoparticles (NPs) for one hour, centrifuged to remove NPs, and the supernatant analyzed. Measurements were performed using a Chirascan V100: far-UV, 0.5 mm pathlength cuvette, at 20°C, sample concentration 0.3 mg/mL. Continuous, multiwavelength temperature ramps (thermal denaturation) 20° to 90°C (heating 1°C/min) yielded 71 spectra in 71 min.

Data analysis
Thermal denaturation data was analyzed using a global fit of multiwavelength data.

Results: Exposure to nanoparticles alters secondary structure of enzyme 2

Enzyme 1. Far-UV spectrum typical for a predominantly α-helical protein with some β-sheet

Enzyme 2. Far-UV spectrum atypical for a globular protein: structure dominated by β-sheet in a distorted conformation

Conclusion
Analysis using Chirascan V100 reveals changes to both secondary structure and protein stability.

Statistically-validated HOS comparisons of NIST mAb variants

Data analysis
Data were compared using the weighted spectral difference (WSD) method to generate a quality attribute for statistical analysis. This attribute was analyzed with a quality range approach with +/-2SD acceptance criteria as recommended for intermediate (tier 2) risk ranking.

Results: Statistical significance of minor differences in secondary and tertiary structure determined

Tier 2 quality range approach applied +/-2SD acceptance criteria. Differences in secondary structure not significant. Differences in tertiary structure significant using +/-2SD criteria.

Conclusion
HOS comparisons using Chirascan Q100 enable detection and objective statistical quantification of minor differences in secondary and tertiary structure of complex molecules.

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