More than just α-helix and β-sheet: expanding the role of circular dichroism

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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.

HOS information	Secondary structure	Tertiary structure
Wavelength range	Far-UV (<250 nm)	Near-UV (>250 nm)
Chromophores	Peptide bonds and S-S bonds	Aromatic amino acids and S-S bonds
Information	Overall conformation, α-helix, β-sheet, turns etc.	Local conformation and side chain environment

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

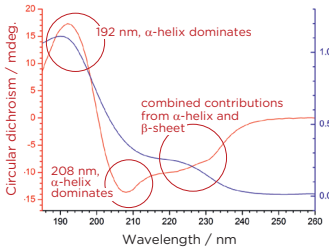
Sample preparation and CD analysis

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.

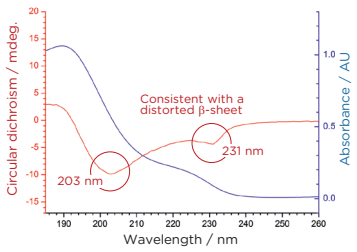
Data analysis

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

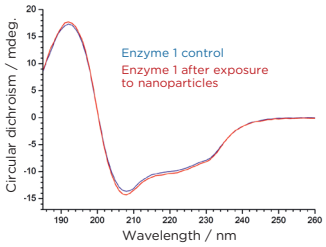
Results: Exposure to nanoparticles alters secondary structure of enzyme 2



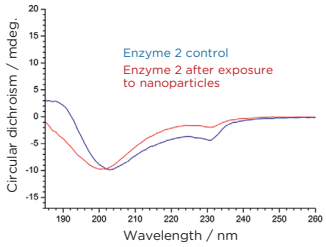
Enzyme 1. Far-UV spectrum typical for a predominantly α-helical protein with some β-sheet



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

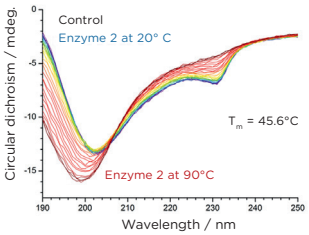


Enzyme 1, effect of NPs. Minor change in far-UV indicates minimal effect on secondary structure. Spectra normalized by absorbance.

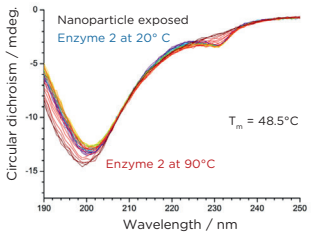


Enzyme 2, effect of NPs. Significant change in far-UV indicates perturbation of secondary structure. Spectra normalized by absorbance.

Results: Exposure to nanoparticles alters protein stability of enzyme 2



Enzyme 2 control. Signal at 231 nm reduced upon unfolding, increase and shift of signal at 203 nm.



Enzyme 2 after NP exposure. Modified unfolding profile, increased melting temperature.

Conclusion

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

Statistically-validated HOS comparisons of NIST mAb variants

Sample preparation and CD analysis

A primary monoclonal antibody standard (PS 8670) and reference material with a lower monomeric purity (RM 8671), supplied by the National Institute for Standards and Technology (NIST), were dialyzed against PBS and the dialysate used to establish a baseline spectrum.

Isothermal CD measurements were performed using a fully integrated Chirascan Q100 for HOS analysis: far-UV, six independent replicates, 0.1 mm pathlength flow cell; near-UV, five replicates, 10 mm pathlength flow cell.

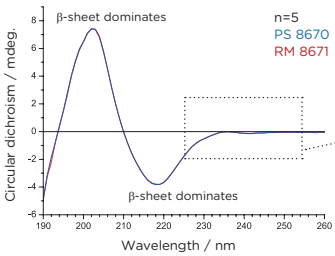
Data analysis

Data were compared using the weighted spectral difference (WSD) method to generate a quality attribute for statistical analysis[1]. This attribute was analyzed with a quality range approach with +/-2SD acceptance criteria as recommended for intermediate (tier 2) risk ranking[2].

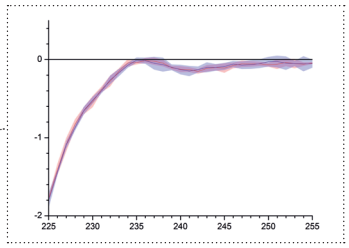
$$WSD = \sqrt{\sum_{i=1}^{n}\left[\frac{1}{n}\left(\frac{|x_i|}{|x_i|_{ave}}\right)(x_i - y_i)^2\right]}$$

[1] *Dinh et al., Anal. Biochem. 464 (2014):60-62*
[2] *Statistical approaches to evaluate analytical similarities; Guidance for Industry; CDER/CBER/FDA*

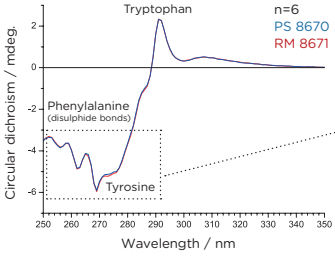
Results: Minor differences detected in far- and near-UV



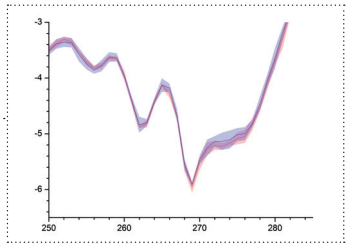
mAbs far-UV spectra are typical for antibodies.



Shaded area is the 2xSD envelope.

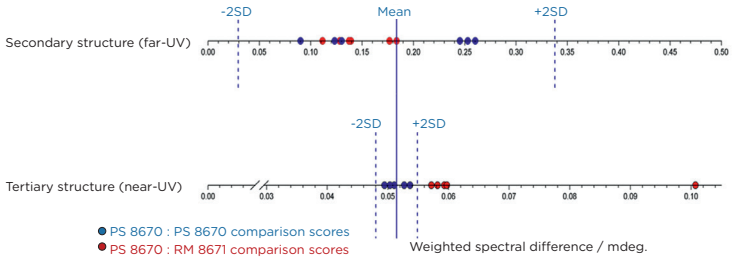


mAbs near-UV spectra. Aromatic side chains contribute with their own characteristic wavelength profile.



Shaded area is the 2xSD envelope.

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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