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In devel.

Second-derivative UV Spectroscopy

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ABSTRACT

Second-derivative spectroscopy has proven to be an effective analytical tool because of its ability to resolve overlapping bands in the normal spectrum.

This protocol is used to determine the degree of tyrosine exposure in proteins using second-derivative UV spectroscopy.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Ragone, R, Colonna, G, Balestrieri, C, Servillo, L, and Irace, G
Biochemistry (1984) 23: 1871-75

TyrExposure_2ndDerUV.pdf

2ndDer_struct_Dynamics.pdf

Second-derivative_spectroscopy_of_protei.pdf

PROTOCOL STATUS

In development

We are still developing and optimizing this protocol

SAFETY WARNINGS

Protein Concentrations

1 Determine protein concentrations either using absorption coefficients (Abs @ 280 nm) or using BCA, Bradford, etc. colorimetric assays.

Proteins should be used at concentration where A_{280} ~ 0.2

UV Scan

2 Scan from 270-300 nm

Obtain Second-derivative

3 Copy UV scan data from instrument into a Prism file with columns for wavelength and absorbance values.

Analyze
>XY Analysis
> Smooth, Differentiate, or Integrate Curve
>Create Second Derivative of Curve
>smooth or not?

Prism