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Employing Multiple Spectroscopic Techniques Simultaneously to Observe Protein Unfolding MICHAEL CROWE, BEN KELTY, JUSTIN LINK, Xavier University — A protein's function is directly related to its native, folded structure. In order to study the structure of proteins, the unfolding process may be characterized. In our study, by using the spectroscopic techniques of circular dichroism (CD), absorption, and fluorescence simultaneously, we examined the unfolding of horse heart cytochrome c, a well-studied, model protein by gradually increasing the concentration of the chemical denaturant, guanidine hydrochloride. The signal changes from these modalities over the course of the unfolding reaction provides some of the thermodynamic properties like Gibbs free energy for insight into the stability of the protein. This allows us to compare the three techniques under the exact same conditions. The objective of this session is to present recent work in developing a protocol to observe the unfolding of cytochrome c using fluorescence, absorbance, and CD simultaneously.

Justin Link
Xavier University

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