Constructing Melting Curves from TPS data

By examination of the temperature dependence of the spectra, thermal and thermodynamic information can be obtained about the macromolecule under observation.

Spectra collected as a function of temperature through the thermal melting transition are analyzed by singular value decomposition to extract basis spectra, linear combinations of which describe each of the spectra in the temperature profile. The matrix of spectra **A**, is decomposed into a matrix **U**, of orthogonal basis spectra, a matrix **S**, in which all of the elements are zero except for the singular values which lie on the diagonal, and a matrix **V** of coefficients which relate the basis vectors of **U** to the data matrix **A**; such that, $\mathbf{A} = \mathbf{USV}^T$.

Examination of the autocorrelation functions of the basis spectra (columns of **U**) and the coefficient vectors (columns of **V**) permits one to determine the minimum number of component spectra required to describe the data within the random noise in the spectra. This provides experimental confirmation of one of the primary assumptions of the van't Hoff model used for thermodynamic analysis of denaturation curves; i.e., there are no thermodynamically significant intermediate states. The coefficient vectors, which represent the temperature-induced variation in the absorbance signal integrated over the entire spectral range, can be used to construct melting curves. This provides a melting curve with enhanced signal to noise relative to single wavelength curves. Thus by using the multiplexed data as described, high quality denaturation data and the resulting thermodynamic analysis, can be obtained from TPS.

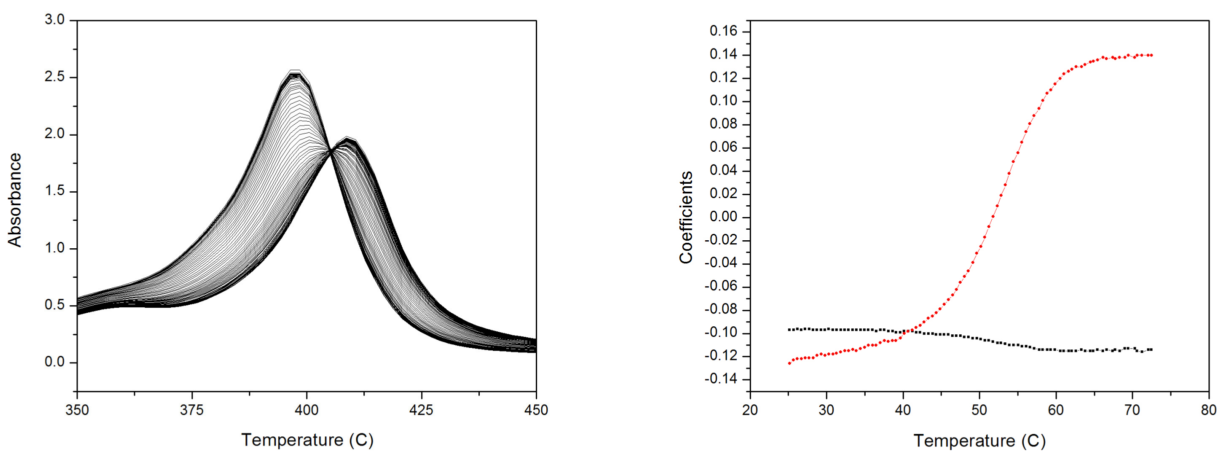

Figure 1 Singular Value Decomposition of Cytochrome c spectra

Left Panel: Spectra reconstructed using the two most significant basis spectra. **Right Panel:** denaturation curves comprising the two most significant coefficient vectors (**V**).

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