Coupling of Stability and Self-Association of a Therapeutic Protein

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Construct 5o is a small 2-domain recombinant protein candidate drug for which limited conclusions could be drawn based in traditional optical methods in size exclusion chromatography (SEC) elution was dependent on the column load, indicating self-association. Additional studies using differential scanning calorimetry (DSC) and isothermal titration calorimetry (ITC) were initiated to better understand the behavior and stability of this molecule.

DSC revealed two distinct thermal transitions, Tm1 ~27-37 °C and Tm2 ~5-70 °C. Parameters varied with ionic strength of the buffer. The first transition was strongly dependent on protein concentration, while the second showed less response to concentration. The first transition could be coupled to self-association of the protein, which is favored at low temperatures and low ionic strength. Twelve other genetic variants were studied with constructs varying at one or more amino acid positions and yielded significantly different DSC thermograms. For all variants, two transitions were identified, and most variability was observed for the first transition. In ITC experiments concentrated protein was diluted in a stepwise manner into corresponding buffer. The data confirmed temperature and buffer dependent oligomerization of the protein, correlating with SEC results.

The results of these experiments indicated variants with maximum structure linked oligomerisation, enabling improved candidate selection and guidance for formulation development. ITC data also provided measure of equilibrium constants for oligomerisation, providing assessment of drug behavior and state in solution.