Multiplexing optical methods in Prometheus Panta for comprehensive protein characterization

INFORMATION	OPTICAL METHOD	PARAMETER	WHY IT MATTERS	SOFTWARE OUTPUT
Conformational Stability (thermal and chemical unfolding)	nanoDSF	**T_m** Melting temperature or point at which 50% of a protein or domain is unfolded.	Ranking candidates by melting temperature is a well-established method for identifying the most thermostable constructs/candidates/conditions, enabling better selections for further experimentation. How is the protein melting point (Tm) determined? →	**IP/T_m**
		T_{onset} Temperature at which thermal unfolding begins (onset of thermal unfolding).	More thermostable proteins have higher onset of unfolding; furthermore, proteins with T_{onset} values close to their T_ms unfold more cooperatively. Onset parameters →	**ON**
		Ea Activation energy of unfolding.	The higher the activation energy, the slower the unfolding rate. This is relevant for accelerated stability studies and shelf-life predictions. Activation energy of unfolding →	**N/A**
		Initial ratio Value of the 350nm/330nm ratio at the starting temperature of the unfolding measurement.	The starting value or initial ratio is strongly dependent on quantity and position of Tryptophan residues. This value can be used to determine the percentage of folded protein in a sample. What does the starting value of the 350 nm/330 nm ratio mean? →	**Initial value**
		C_m or C_{50} Concentration of a denaturant at which 50% of a protein or domain is unfolded.	A higher C_m value indicates greater protein stability. Chemical protein unfolding →	**IP/C_m**
		ΔG Gibbs free energy of protein unfolding, a thermodynamic measure of stability based on the relative population of folded and unfolded molecules when at equilibrium.	Proteins with a more negative ΔG are more likely to remain folded. Changes in Gibbs free energy are measured using ΔΔG, which indicates the relative stability of a protein at various concentrations or conditions. How is ΔG calculated by the PR.ChemControl software? →	**N/A**
Purity	DLS	**PDI** Polydispersity Index, derived from fitting the autocorrelation function.	The polydispersity index (PDI) indicates the heterogeneity of particle sizes in a sample. Low PDI values indicate that the sample preparation is free of large aggregates or multiple protein populations. How is the polydispersity index (PDI) calculated? →	**Cumulant PDI** **PDI1, PDI2, …**
Sizing	DLS	**rH** Hydrodynamic radius, derived from the diffusion coefficient.	The rH is calculated by fitting scattering data with the autocorrelation function. The result is the cumulant radius for all particles in the solution or a distribution of radii. It establishes a baseline sizing parameter for a protein or particle of interest. The hydrodynamic radius (rH) →	**Cumulant Radius** **Peak 1, Peak 2, …**
		T_{size} Temperature at which average particle size (cumulant radius) begins to increase (onset of size change).	Size increase indicates a major conformational or structural change to the particles in solution, which translates to several possible events on a molecular level: cooperative unfolding, oligomerization or formation of the first aggregates. Onset parameters →	**ON (Radius)**
	SLS	**MW** Average molecular weight of all particles in solution.	This average measure of molar mass can indicate the presence of oligomers or aggregates, if different than the nominal molar mass based on the amino acid sequence. Like rH, this value can also be used to evaluate changes resulting from modifications to buffer or production processes. How to perform B22 and molecular weight measurements? →	**MW** 💡
Aggregation and Self-Association	Backreflection	**$T_{turbidity}$** Temperature at which turbidity begins to increase (onset of turbidity).	An increase in turbidity often indicates the formation of large, amorphous aggregates (> 25 nm in diameter). As aggregates are unwanted, high values or no change in $T_{turbidity}$ is desirable. Onset parameters →	**ON (Turbidity)**
	SLS	**$T_{scattering}$** Temperature at which scattering intensity begins to increase (onset of scattering).	$T_{scattering}$ can very precisely indicate the starting point of aggregation even in the presence of minute amounts of aggregates, or very small aggregates at low concentrations. A higher $T_{scattering}$ value indicates higher protein stability. Onset parameters →	**ON (Scattering)**
		B_{22} Self-interaction parameter that examines protein-protein interactions in solution.	Negative B_{22} values indicate propensity towards self-interaction, and therefore greater likelihood of aggregating at high concentrations required for clinical use. How to perform B22 and molecular weight measurements? →	**B_{22}** 💡
	DLS	**kD** Diffusion interaction parameter used to assess weak inter-particle interactions.	The diffusion interaction parameter is used to understand and predict colloidal protein stability and often required during clinical stages. Negative kD values indicate propensity towards self-interaction, and therefore greater likelihood of aggregating at high concentrations. How to perform kD measurements? →	**kD** 💡

💡 5 different concentrations of the sample are necessary to calculate this parameter.