Frequently Asked Questions

Chemical Denaturation Technique

Questions	Answers
Why is there a shift to a longer wavelength when protein unfolds?	The SUPR-CM measures the intrinsic fluorescence of the tryptophans, that are a part of the protein. The fluorescence response of tryptophan is affected by its local environment. When the protein unfolds, the tryptophans are exposed to a different local environment. Hence a change in the fluorescence spectrum is detected.
How sensitive is this technique?	Since the equilibrium constant is equivalent to exp(-$\Delta G°$/RT)) and RT ≈ 2.48 kJ mol^{-1} (assuming room temperature conditions), a change in Gibbs Free Energy of ≈ 2.48 kJ mol^{-1} would be significant.
Can chemical denaturation be applied to drug screening by detecting their stabilization on protein target?	Thermal denaturation is the basis of the ThermoFluor or Thermal Shift Assay and is widely used. The principle has been extended to chemical denaturation (see reference) and does provide similar information but hasn't been widely used yet. https://doi.org/10.1016/j.ab.2010.12.001
What is the sample volume for 96 and 384 well respectively?	The working volume of the 96-well microplate is 50 µl - 300 µl. The working volume for the 384-well microplate is 15 µl - 145 µl. By using PCR plates the 96-well working volume is reduced to <100 µl and the 384 PCR plate working volume is reduced to <30 µl.
Is there data to show how the improvement in chemical denaturation stability translates into long term storage stability in the optimized formulation or construction?	Just Biotherapeutics has a paper demonstrating that using isothermal chemical denaturation (with thermal stability) to select constructs leads to improved stability of constructs of an anti-HIV antibody IgG1, with subvisible particle formation being 9-fold lower under accelerated long term stability studies. https://doi.org/10.1016/j.xphs.2019.07.009
How do you account for the change in pH adding Guanidine hydrochloride has?	When preparing the stock solutions, the pH of the guanidine hydrochloride solution is adjusted to closely match the buffer pH.

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Question	Answer
Is the system 21 CFR compliant?	We plan to develop a full 21 CFR part 11 into our system software. The initial release will not be though.
Are you open to collaborations?	We are more than amenable to the possibility of collaboration. Contact us at sales@proteinstable.com with your proposal.
Are the measurements done in sequence for each well or simultaneously on all plates?	The SUPR-CM measures each well sequentially via an array detector, which captures full spectrum fluorescence intensity data. This approach records high quality information while minimizing the time needed to measure a full plate. The time that each well is measured for can be adjusted to better optimize your protocols.
How many data points from a spectrum are collected within the 2.5 minutes in 384-well microplate?	Fluorescence spectrum are measured from 310 nm to 430 nm with around 400 intensity values being measured. The SUPR-CM uses an array detector, so the entire fluorescence spectrum is captured simultaneously. The time taken to record a single well spectrum can be altered to accommodate the quantum yield of the sample. In the example presented each well was measured for 400 ms, and the SUPR-CM scans the plate sequentially.
What is the big advantage of the SUPR-CM over other conventional fluorescence plate readers?	The main limitation of the microplate readers on the market is from the general-purpose nature of their design. They use emission monochromators, which require scanning over multiple wavelengths to acquire the emission spectrum (a requirement for the technique). They also use broadband light sources like xenon flash lamps and monochromators for the excitation light, which are not as bright as our light source. When we have compared a prototype of the SUPR-CM to a standard plate reader, a 96 well plate was taking ~ 1 hour to scan with the plate reader and 1 minute to scan with the SUPR-CM. Also, the plate reader required more sample.
Can you do thermal denaturation studies?	The SUPR-CM is a dedicated chemical melt instrument that facilitate precise, high-throughput measurement of protein stability.
How do you account for aggregation and/or precipitation?	The wide range of protein concentrations the SUPR-CM can operate with means that aggregation effects can be negated by working with low amounts of protein. Additionally, it would be possible to measure protein stability at multiple protein concentrations in order to assess the aggregation propensity of the protein.

Software & Data Analysis

Question	Answer
Does the software facilitate analysis of the measured denaturation curves?	The SUPR-CM software is designed to be a complete package that makes the process of measurement and data analysis simple and intuitive. This includes fitting of 2-state/3-state models to the denaturation data as well as determining the errors in the values fitted.
Is the analysis software provided with the rest of the software?	Yes, data analysis is provided within the software. The SUPR-CM software is not split into separate applications. Instead, the process of designing the plate layout (i.e. telling the software which wells contain which samples), measuring the plate and analyzing the data can be done within the one application.
How are you evaluating the fluorescence signal?	The most common method for monitoring the unfolding process is the ratio of intensities at the wavelengths 350 nm and 330 nm (i.e. I_{350nm}/I_{330nm}) for each fluorescence spectrum. These wavelengths are typically the peak positions for the unfolded and folded spectra, respectfully. The software lets you select which wavelengths to use and the option to average the intensity values (for a particular bandpass value) to reduce the influence of signal-to-noise. The SUPR-CM also has the option the change the method of monitoring unfolding (ratio of intensities, Barycentric mean).