

Introducing SUPR-CM™ From Protein Stable

High Throughput, Informative Chemical
Stability Studies For Protein Engineering
And Formulation In A Convenient
Format



Protein
Stable

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- Protein Stable
- SUPR-CM System
- Formulation Example
- Antibody Engineering Example
- Advantages Of SUPR-CM
- Summary



Protein Stable

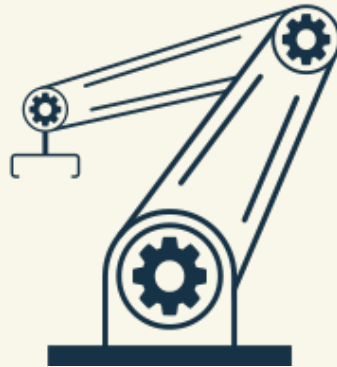
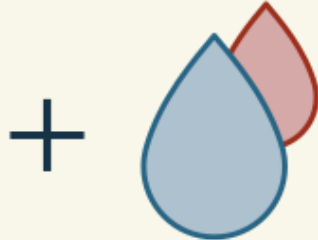
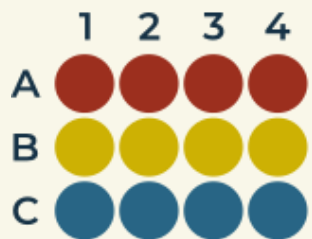
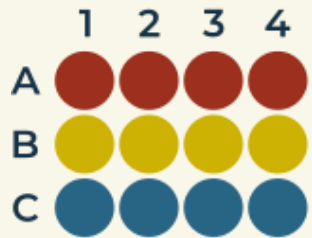
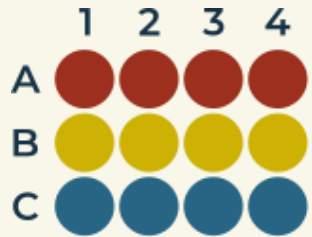
Introducing the SUPR-CM

- Intrinsic fluorescence measurements for native, label-free detection
- Standard microplate format for ease of sample handling
- High power 280 nm excitation with full emission spectrum acquisition for better data quality
- Scan a full 384-well plate in as little as 2.5 minutes for maximum productivity
- Operate with a few 100's of ng of antibody per well – preserve precious samples



- Chemical denaturation (Chemical Melt) has been widely used in the protein research community
 - Reversible denaturation with urea or guanidine hydrochloride
 - Equilibrium measure of antibody stability
 - ΔG° energy of unfolding can be determined reliably at equilibrium
- Thermal denaturation (Thermal Melt) is used extensively in biopharmaceutical development and formulation
 - Industry standard
 - Chemical melt is a different but truly complementary approach
- Combining both these complementary techniques gives you an enriched understanding of the stability of your antibody

SUPR-CM Modular Approach



→ SUPR-CM Platerreader

→ Plus Liquid Handling

→ Plus Automation

- Modular and scalable approach
 - SUPR-CM uses industry standard 96/384-well microplates
 - Compatible with all liquid handling systems
 - Utilize the latest generation sub μ L sample prep systems
 - Low volume
 - Low sample consumption
- Modular approach allows off instrument incubation
 - Antibodies can take over 24 hours to reach equilibrium
 - Off instrument incubation of samples in microplates ensure reliable results



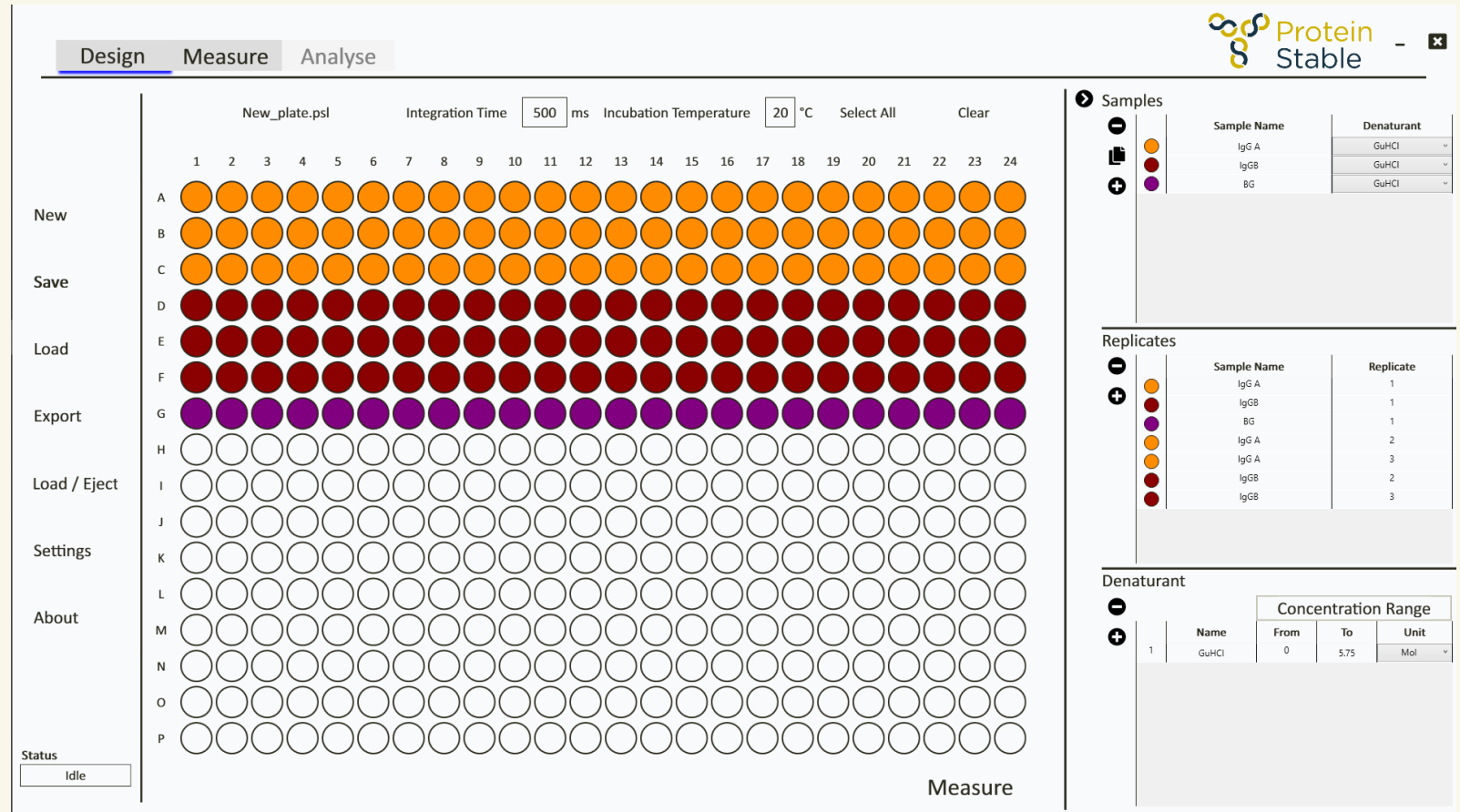
· Formulatrix Mantis™

- Modular and scalable approach
 - Automation compatible
 - Scalable from 10s to 1000s of samples with addition of microplate handling automation as needs expand
 - Improve throughput and walk-away time
 - Able to be implemented in already established analytical and sample prep workflows



- Intuitive software

- Application focused software simplifies analytical work-flow
- Easy to select system to identify sample groups and denaturant concentrations, or directly import

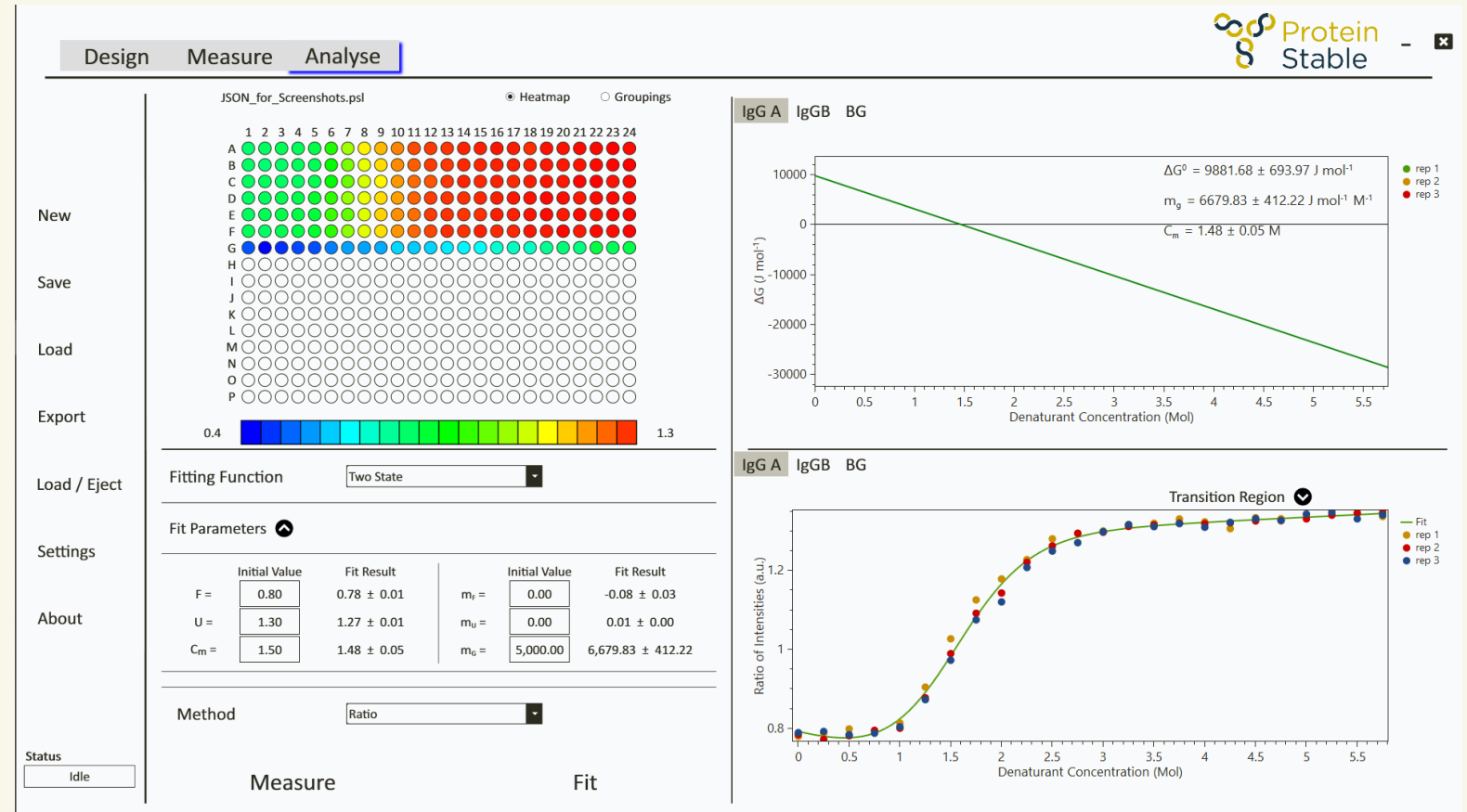


The screenshot displays the SUPR-CM software interface, which is divided into three main sections: Design, Measure, and Analyse. The Design section is currently active and shows a 24-well plate layout with columns numbered 1 to 24 and rows lettered A to P. The plate is populated with colored circles representing different sample groups: rows A, B, and C are orange; rows D, E, and F are dark red; row G is purple; and rows H through P are empty (white). Above the plate, settings for 'Integration Time' (500 ms) and 'Incubation Temperature' (20 °C) are visible. A sidebar on the left contains navigation options: New, Save, Load, Export, Load / Eject, Settings, About, and Status (Idle). On the right, there are three configuration panels: 'Samples' with a table of sample names and denaturants; 'Replicates' with a table of sample names and replicate counts; and 'Denaturant' with a table for concentration ranges. The 'Denaturant' table is as follows:

Name	Concentration Range		Unit
	From	To	
GuHCl	0	5.75	Mol

- Intuitive software

- Flows through to an inbuilt analytical suite streamlining data fitting and interpretation



The screenshot displays the SUPR-CM software interface, which is divided into three main sections: Design, Measure, and Analyse. The Design section shows a heatmap for a protein with 24 columns (residues) and 16 rows (conditions A-P). The Measure section shows a color scale from 0.4 to 1.3. The Analyse section is active and displays fitting parameters for a Two State model.

Fit Parameters:

	Initial Value	Fit Result		Initial Value	Fit Result
F =	0.80	0.78 ± 0.01	m _y =	0.00	-0.08 ± 0.03
U =	1.30	1.27 ± 0.01	m _u =	0.00	0.01 ± 0.00
C _m =	1.50	1.48 ± 0.05	m ₀ =	5,000.00	6,679.83 ± 412.22

Method: Ratio

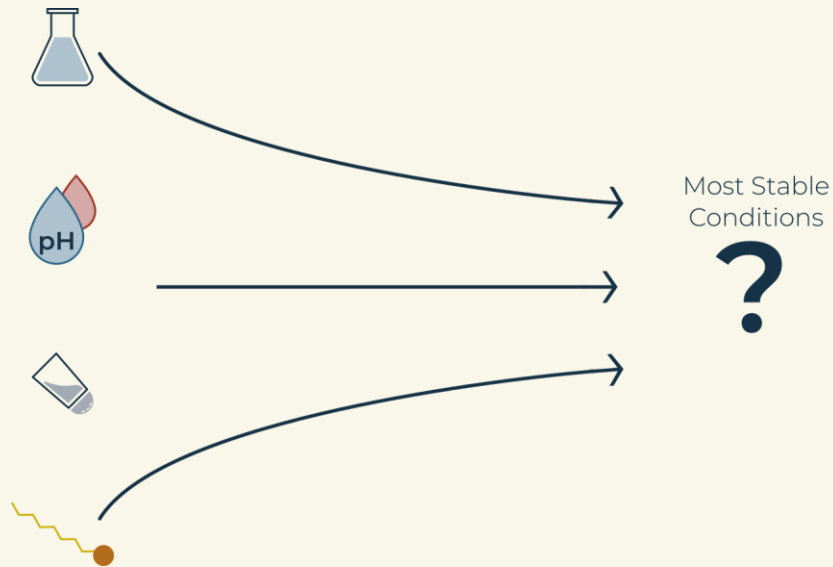
Measure Plot: ΔG (J mol⁻¹) vs Denaturant Concentration (Mol). The plot shows a linear decrease in ΔG with increasing denaturant concentration. The fit parameters are: ΔG° = 9881.68 ± 693.97 J mol⁻¹, m_y = 6679.83 ± 412.22 J mol⁻¹ M⁻¹, and C_m = 1.48 ± 0.05 M.

Fit Plot: Ratio of Intensities (a.u.) vs Denaturant Concentration (Mol). The plot shows a sigmoidal transition region. The fit parameters are: Fit, rep 1 (yellow), rep 2 (red), and rep 3 (blue).

Antibody Stability Challenge

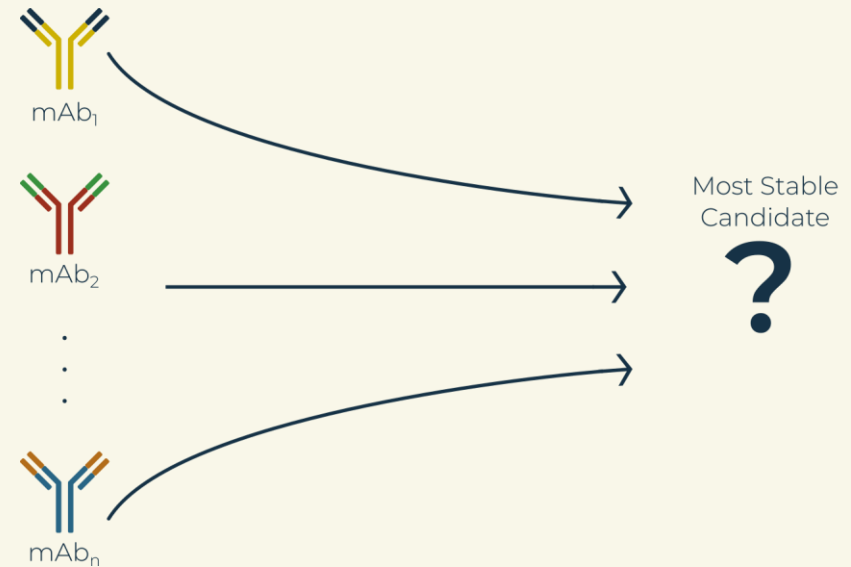
Formulation

- Large number of solution conditions to process
- Quickly identify optimal formulation

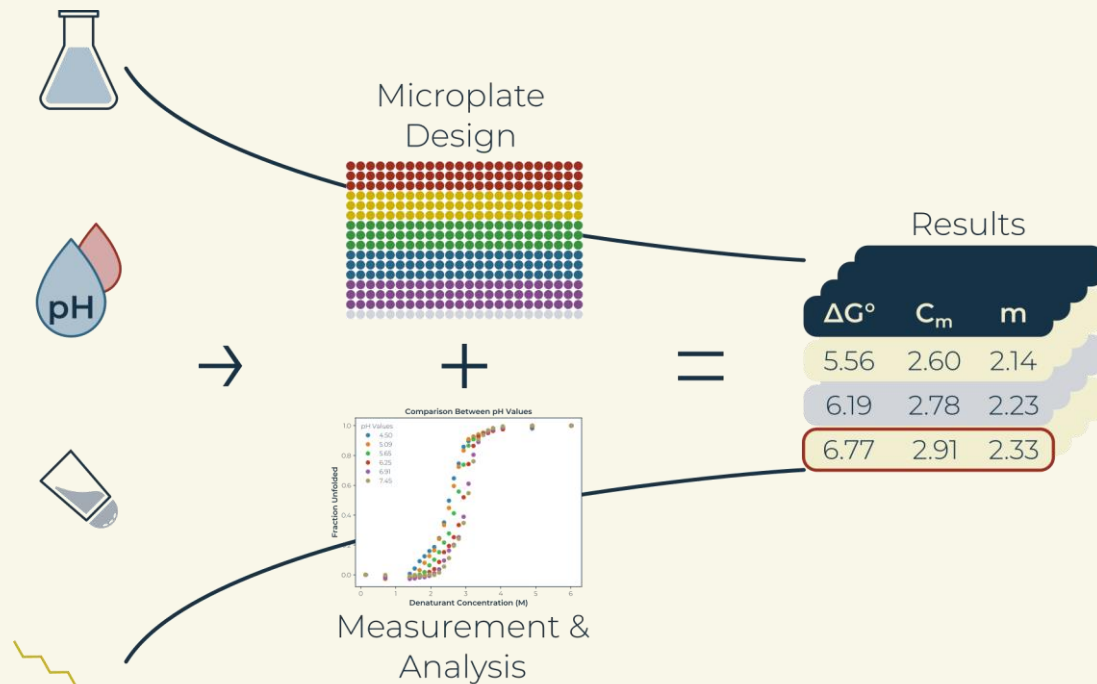


Antibody Engineering

- Sample limited while needing precision
- Rapid turn around time between batches



Formulation Example



Reagents:

- IgG1 monoclonal antibody (mAb)
- Guanidine Hydrochloride (GuHCl)
- Citrate-Phosphate Buffer at 7 different pH values

384 Microwell plate Set-Up:

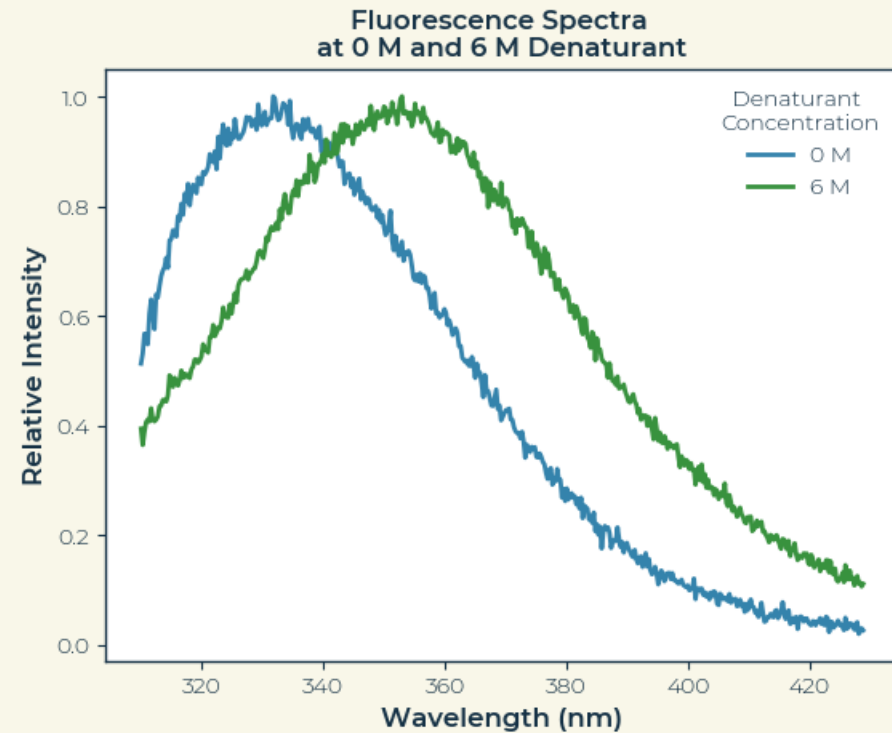
- 50 $\mu\text{g}/\text{ml}$ mAb concentration
- 24 GuHCl concentrations (0 M – 6 M)
- 3 replicates
- 50 μl total well volume

Measurement/Analysis:

- 2.5 mins scan time per plate
- Ratio of intensities (355 nm & 330 nm) calculated
- 3-state function fitted

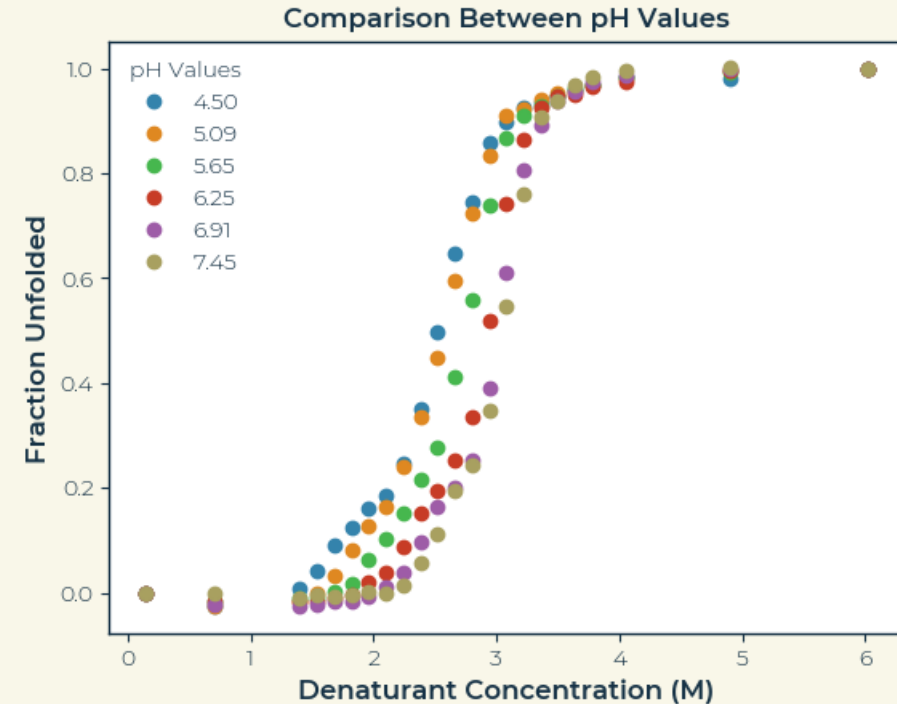
Formulation Example

- Denaturant unfolds the protein which causes a shift to a longer wavelength
- Full spectrum measurement allows for fast plate reading and highest quality data
- Not limited to 96 well microplates for quality denaturant experiments



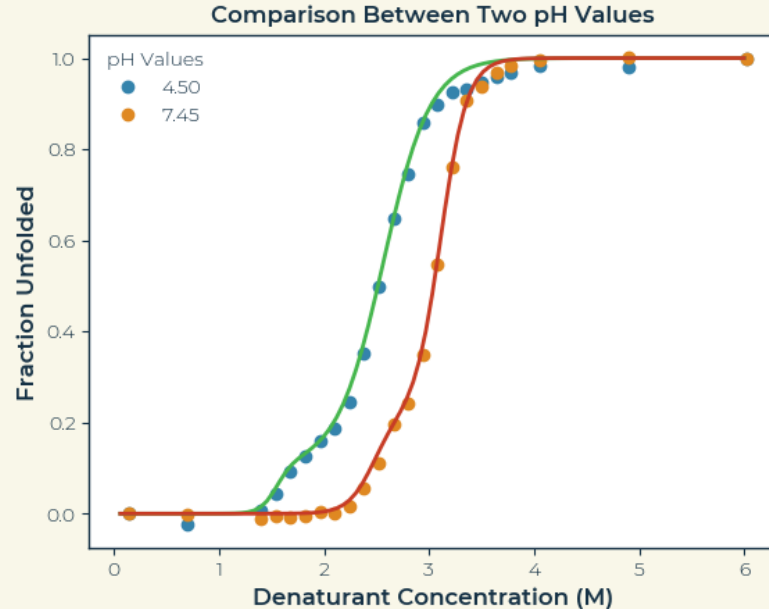
Formulation Example

- Fraction unfolded illustrates effect pH has on antibody stability
- Multiple sample conditions tested with only a couple of 384 microwell plates



Formulation Example

- Data fitted to a 3-state function and plotted to observe changes to protein stability



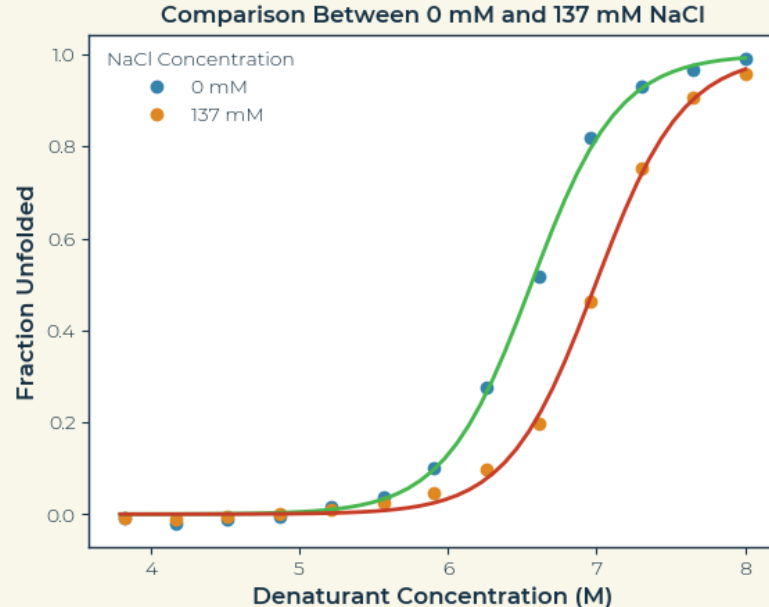
- The fitted parameters can be compared and demonstrate which formulation improved stability the most

Sample pH	ΔG°_1 (kJ mol ⁻¹)	C_{m1} (M)	ΔG°_2 (kJ mol ⁻¹)	C_{m2} (M)
4.50	48.17	1.54	28.19	2.57
5.09	45.13	1.73	28.86	2.60
5.65	37.33	2.01	39.89	2.79
6.25	41.88	2.24	56.37	2.98
6.91	55.28	2.32	55.99	3.06
7.45	49.47	2.46	60.51	3.11

Formulation Example

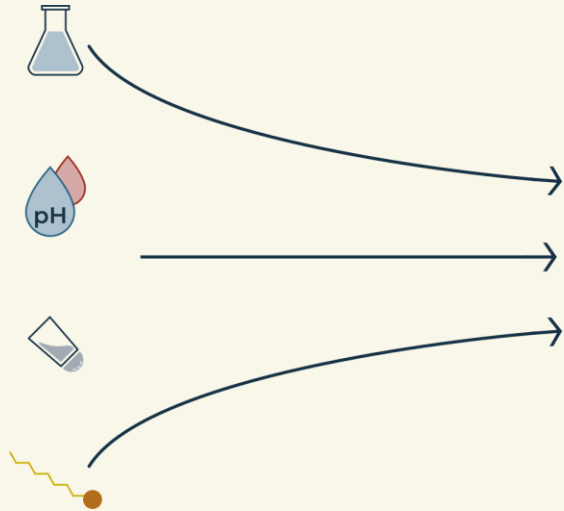
- Study extended to test NaCl effect on antibody stability

- SUPR-CM sensitive enough to detect small effect of NaCl on antibody

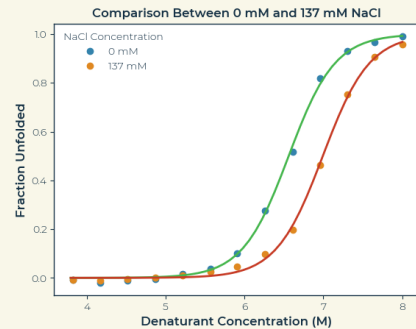
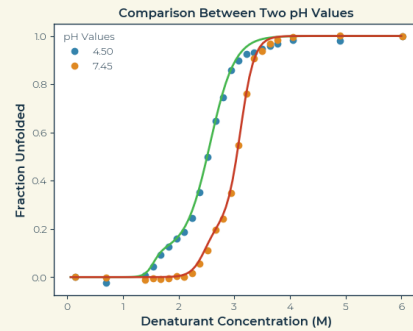


NaCl Conc. (mM)	ΔG° (kJ mol ⁻¹)	C_m (M)
0	54.48	6.56
137	57.69	6.99

SUPR-CM for Formulation

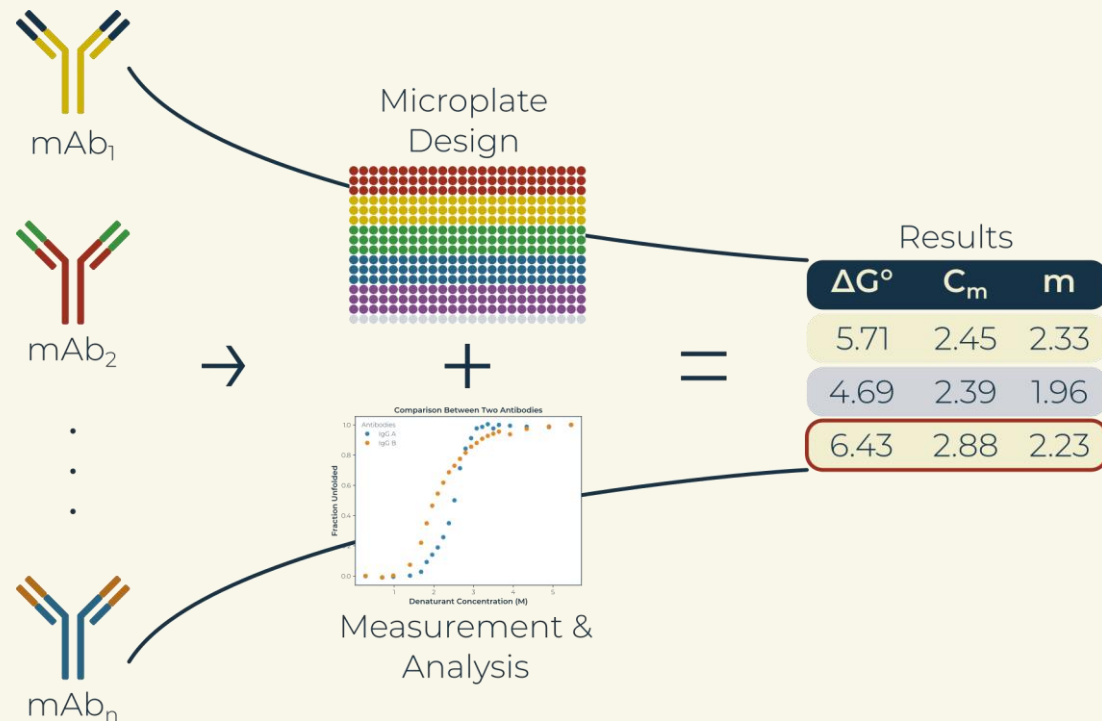


Most Stable Conditions
?



- Highest throughputs - wide range of solution conditions tested with ease
- Best data quality – confidence in decision making and regulatory submissions
- Microplates throughout simplify sample handling and minimise risk of errors

Antibody Engineering Example



- **Reagents:**

- 2 x IgG antibody (IgG A & IgG B)
- Guanidine Hydrochloride (GuHCl)
- Phosphate Buffer Solution (pH 7.2)

- **384 Microwell plate Set-Up:**

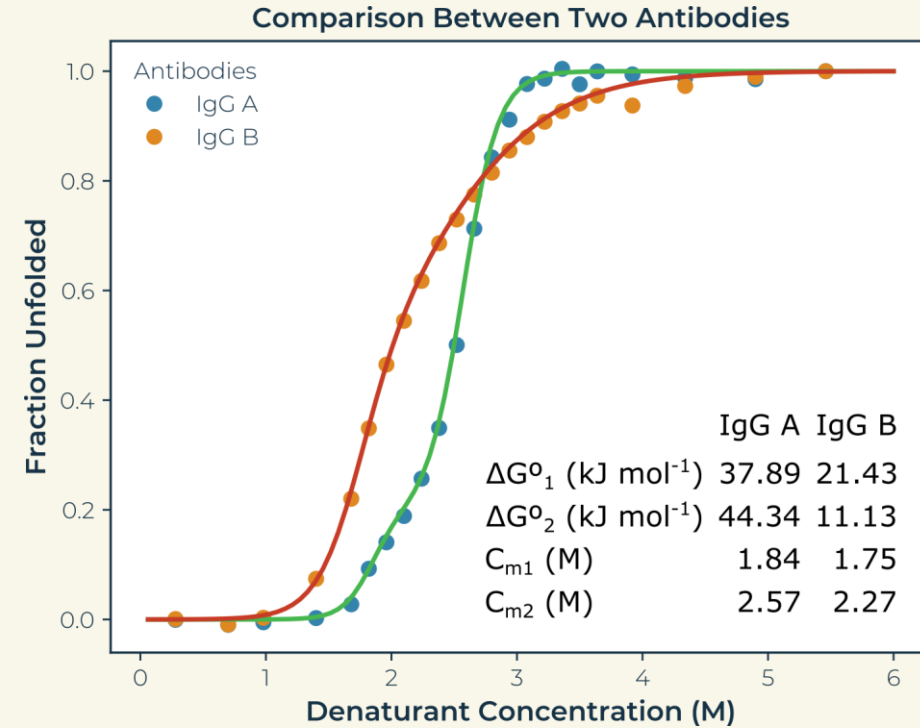
- 50 $\mu\text{g}/\text{ml}$ mAb concentration
- 24 GuHCl concentrations (0 M – 6 M)
- 3 replicates
- 50 μl total well volume

- **Measurement/Analysis:**

- 2.5 min scan time per plate
- Ratio of intensities (355 & 330nm) calculated
- 3-state function fitted

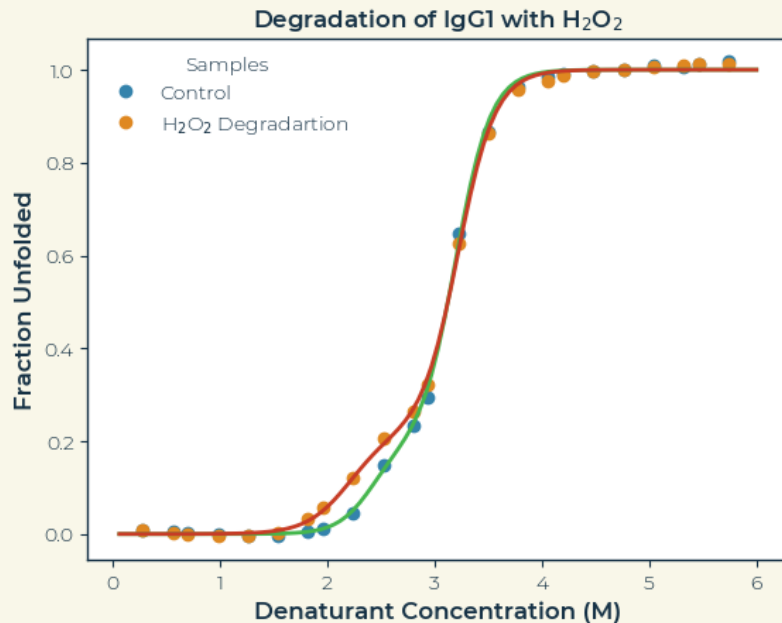
Antibody Engineering Example

- Quickly identify which antibody has the highest stability
- Easily expanded to test multiple antibodies

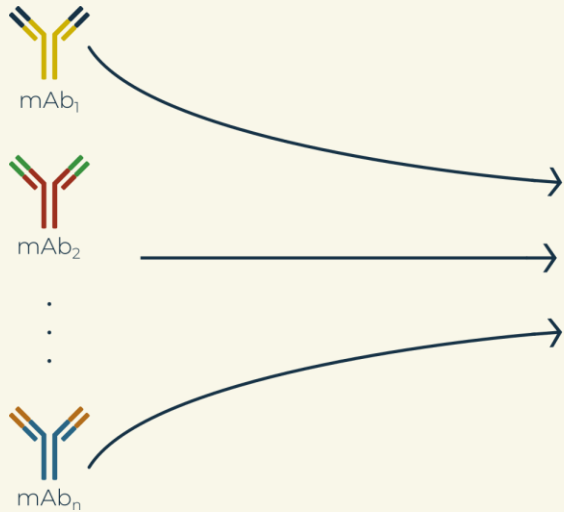


Antibody Engineering Example

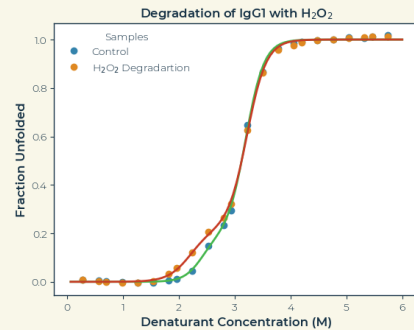
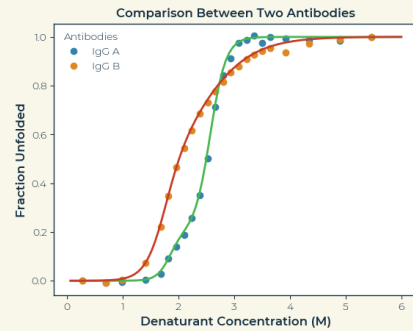
- Antibody stability tested by H_2O_2 degradation
- Precision to identify change in one domain but not the other



Samples	ΔG°_1 (kJ mol ⁻¹)	C_{m1} (M)	ΔG°_2 (kJ mol ⁻¹)	C_{m2} (M)
Control	36.30	2.40	47.45	3.20
H_2O_2	26.82	2.20	45.21	3.22



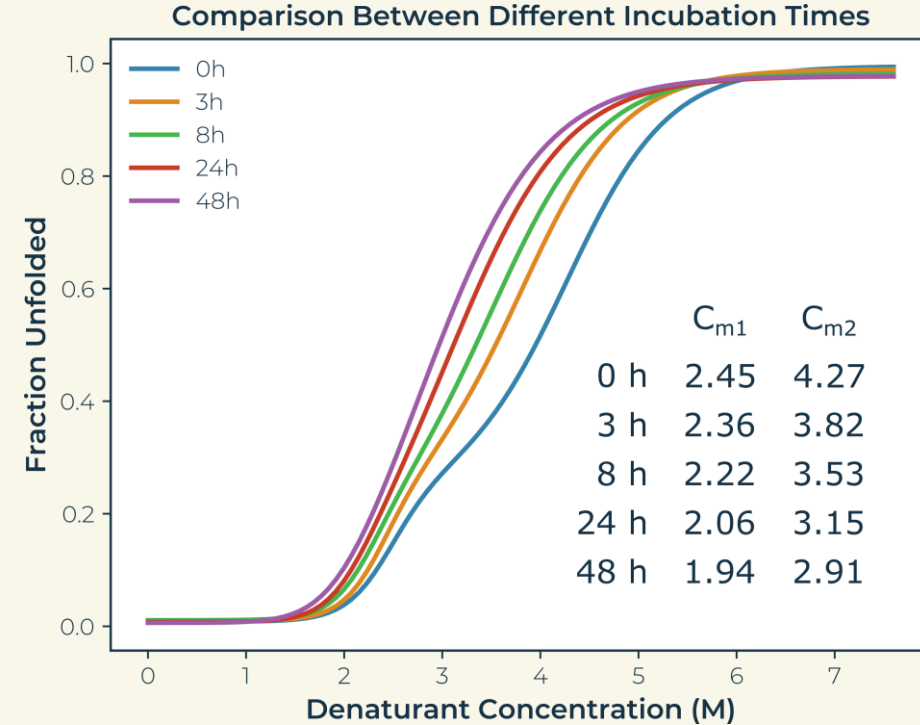
Most Stable
Candidate
?



- Which candidate to progress?
- Measure the stability of many antibodies in minutes
- Chemical denaturing able to capture complex antibody unfolding.
- Able to quantify subtle changes to the stability of antibodies

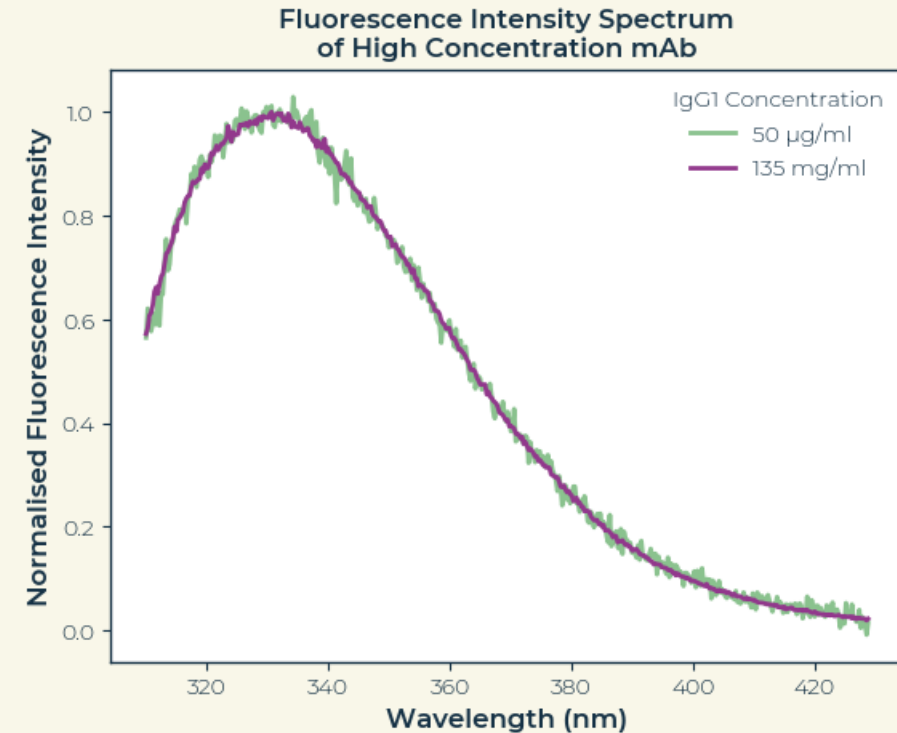
Advantages Of SUPR-CM

- Modular approach means no restrictions on incubation times
- Allow samples to reach equilibrium and improve quality of data



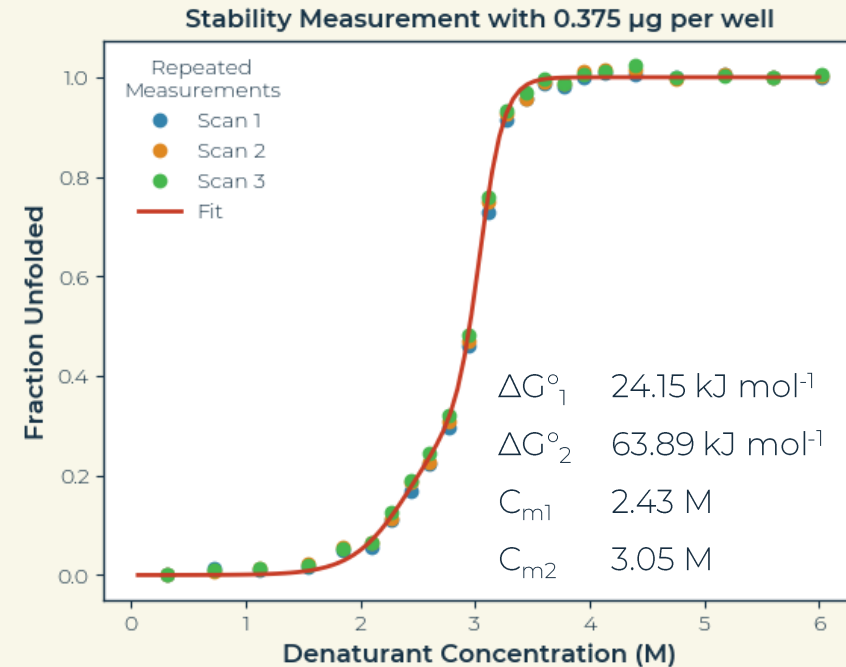
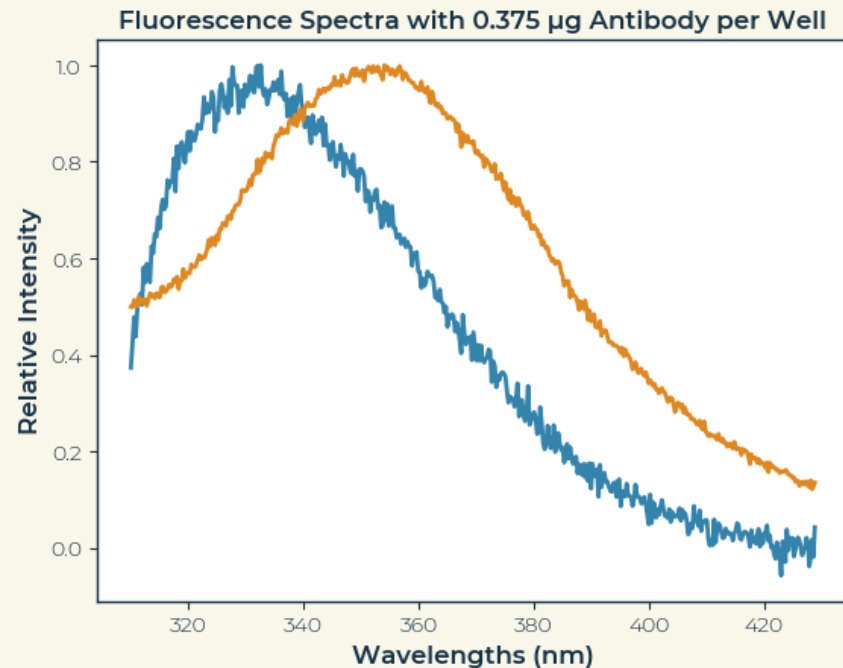
Advantages Of SUPR-CM

- Large range of antibody concentration can be used
- Can work at therapeutic concentration range (> 100 mg/ml)



Advantages Of The SUPR CM

- Can measure stability with as little as 0.375 μg of antibody per well
- No need to compromise when the amount of sample available is low



Summary



SUPR chemical denaturation

No compromise data quality for effective decision making in the development process



96 and 384-well microplates.

Convenient format for reduced errors, higher throughput and lower cost



Scan plate in 2.5 mins

Maximize productivity and make earlier decisions on optimal candidates



Wide range of protein concentrations.

Minimise precious samples or work at therapeutic concentration – your choice



Unrestricted incubation times

Make informed decisions using best quality data from samples at equilibrium



Modular approach.

Flexibility to meet your development needs now and in the future



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