Introducing SUPR-CM™ From Protein Stable

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

Protein Stable

Contents

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

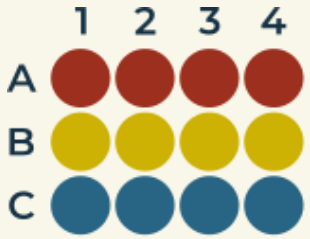
Protein Stable

Chemical Denaturation (Chemical Melt)

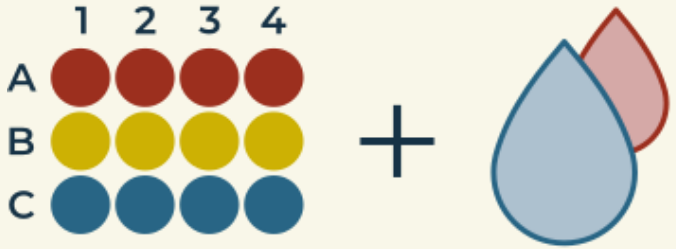
- Chemical denaturation (Chemical Melt) has been widely used in the protein research community
  - Reversible denaturation with urea or guanidine hydrochloride
  - Equilibrium measure of antibody stability
  - ΔG° energy of unfolding can be determined reliably at equilibrium
- Thermal denaturation (Thermal 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

Protein Stable

SUPR-CM Modular Approach



→ SUPR-CM Platereader



→ Plus Liquid Handling

→ Plus Automation

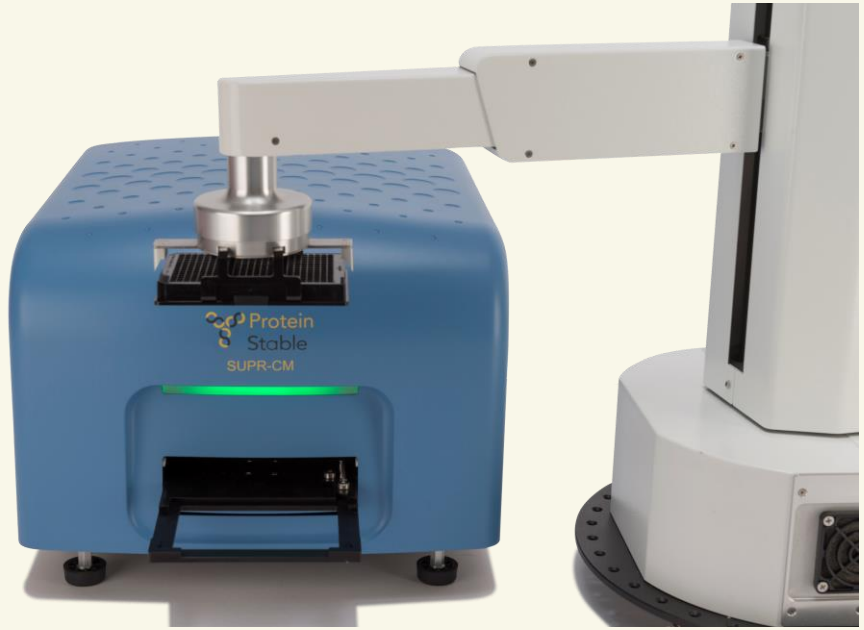
SUPR-CM Liquid Handling

- Modular and scalable approach
  - SUPR-CM uses industry standard 96/384-well microplates
    - Compatible with all liquid handling systems
    - Utilize the latest generation sub µL 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™

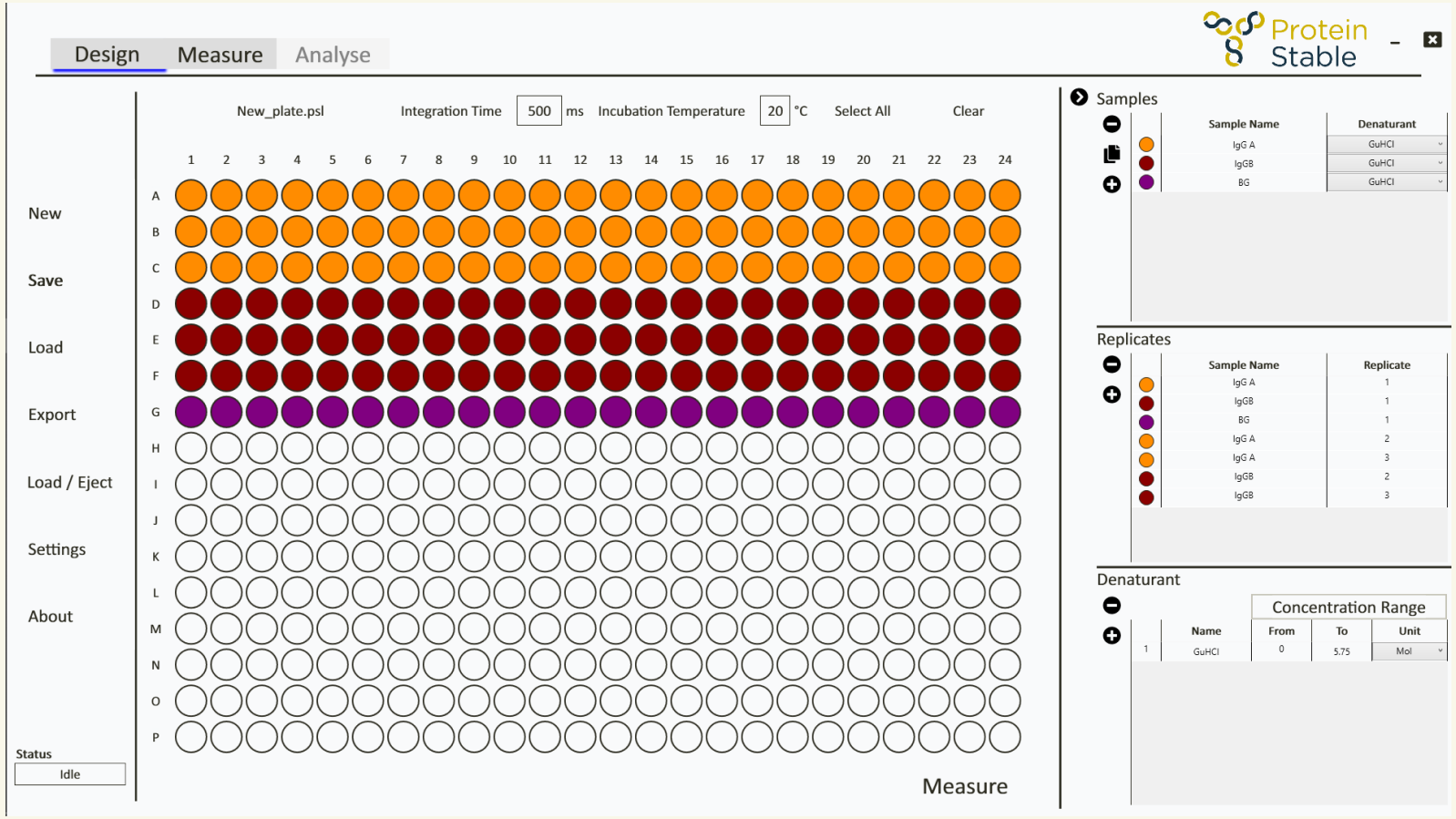
SUPR-CM Modular Approach

- 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



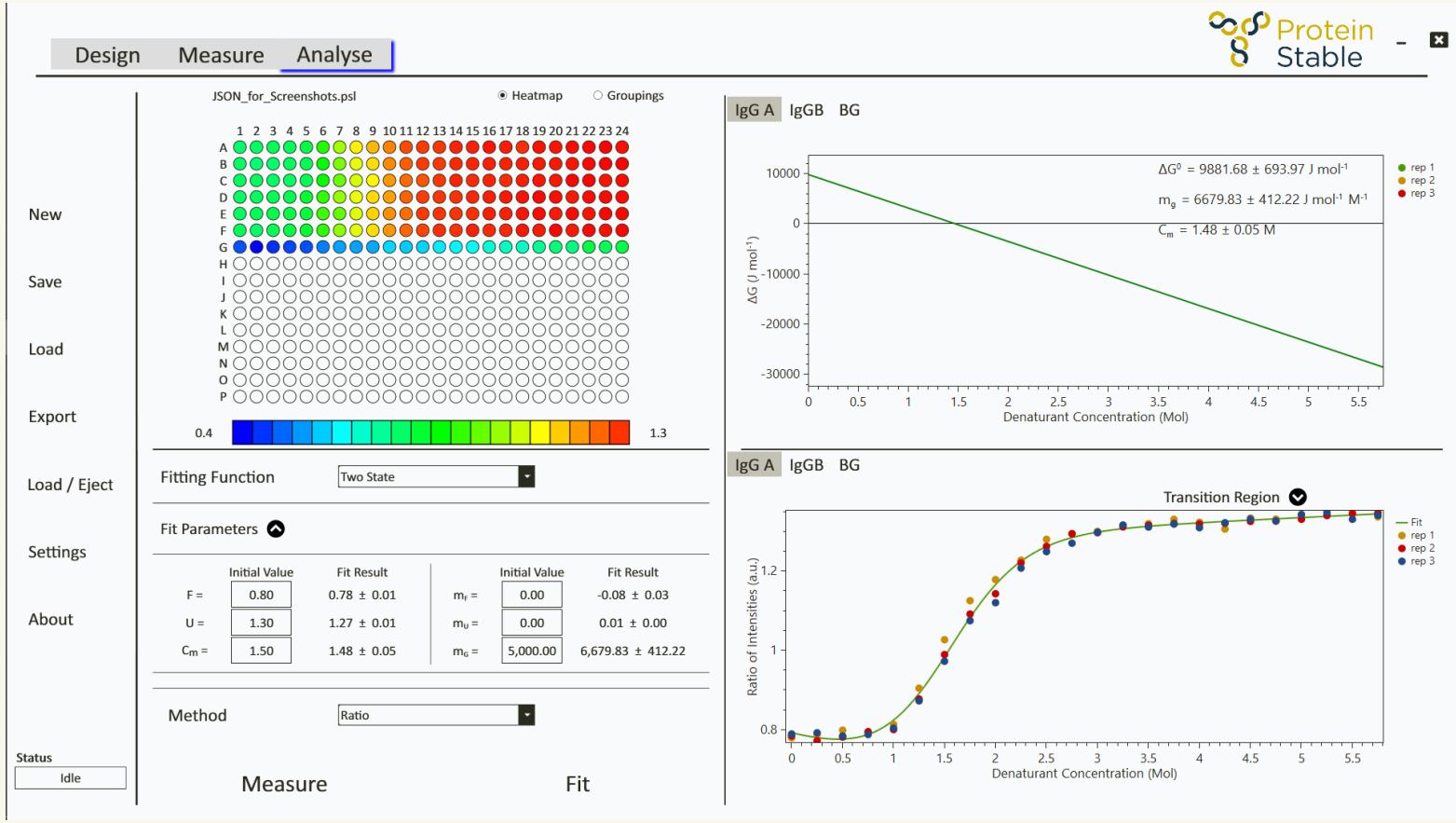
SUPR-CM Simplifies Chemical Melts

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



SUPR-CM Simplifies Chemical Melts

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



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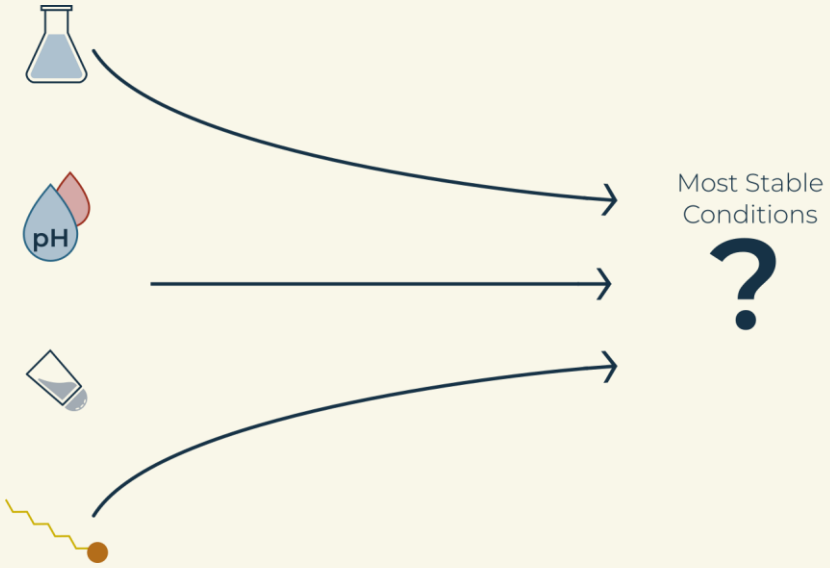
Antibody Stability Challenge

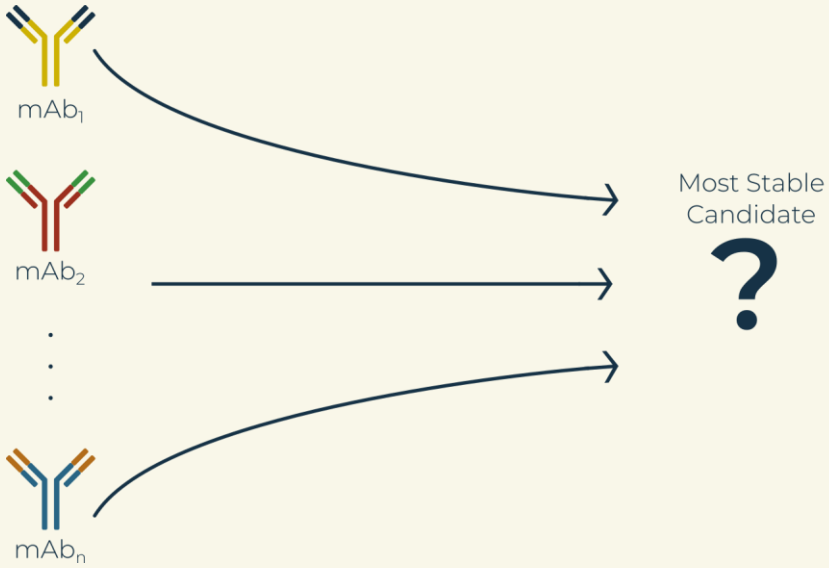
Formulation

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

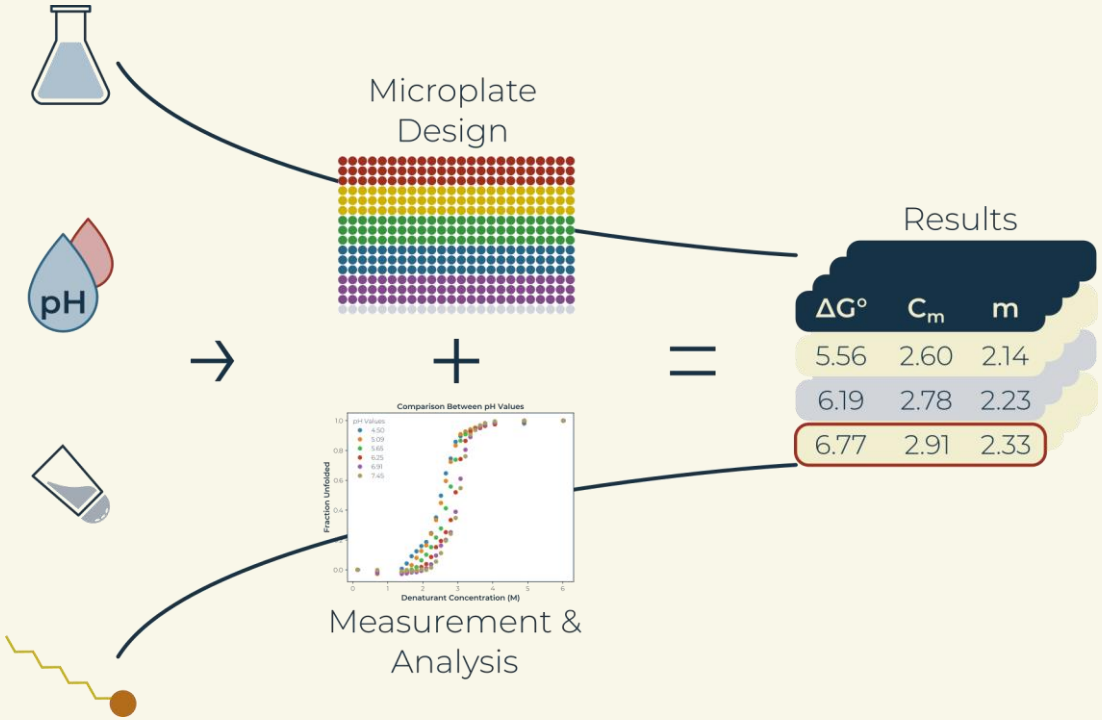


Antibody Engineering

- 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 $^{\mu g}/_{ml}$ 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

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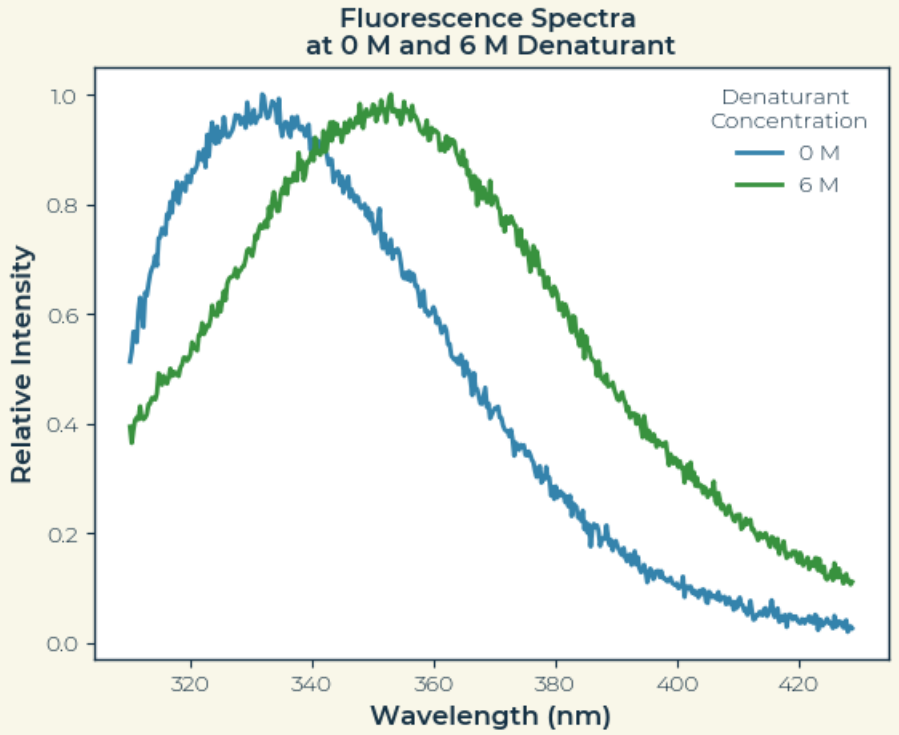
Formulation Example

- 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



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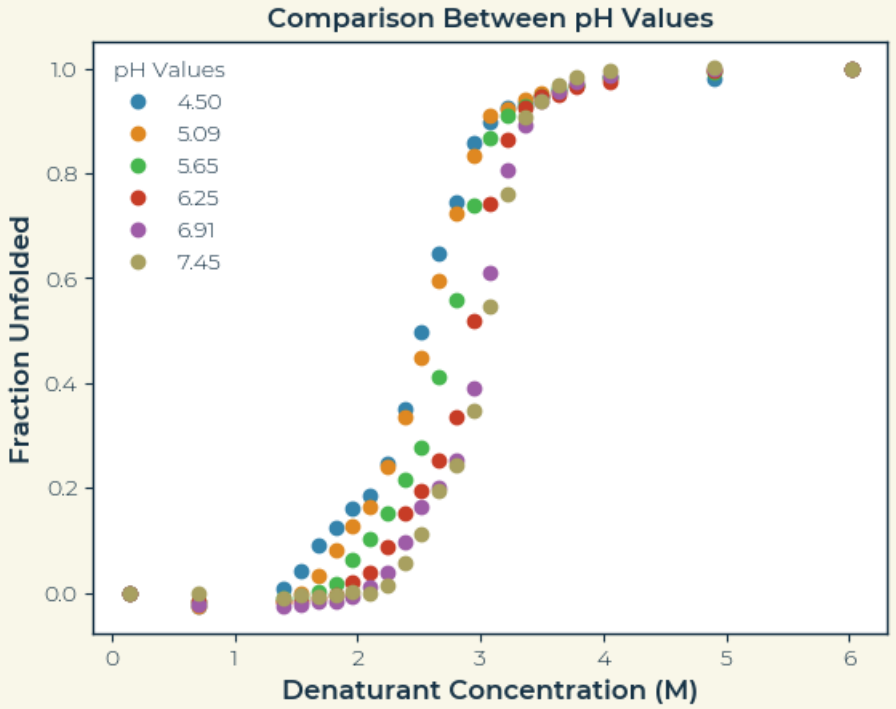
Formulation Example

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



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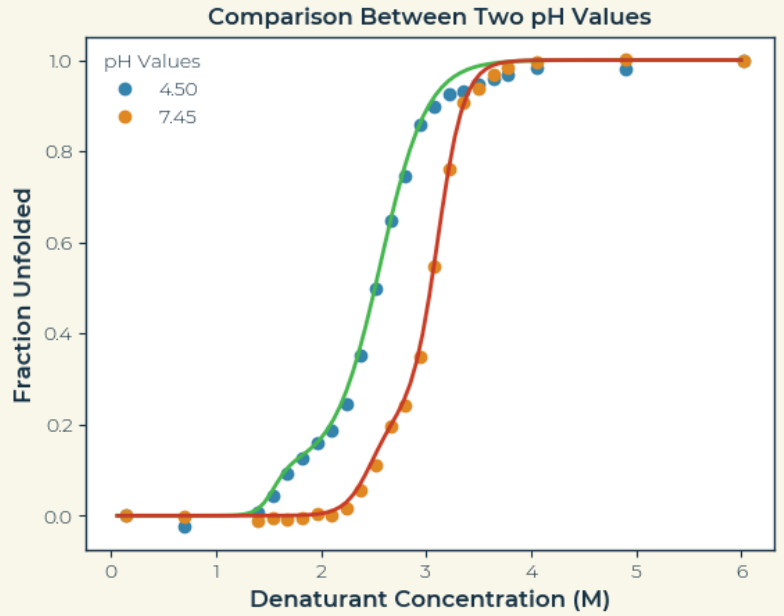
Formulation Example

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



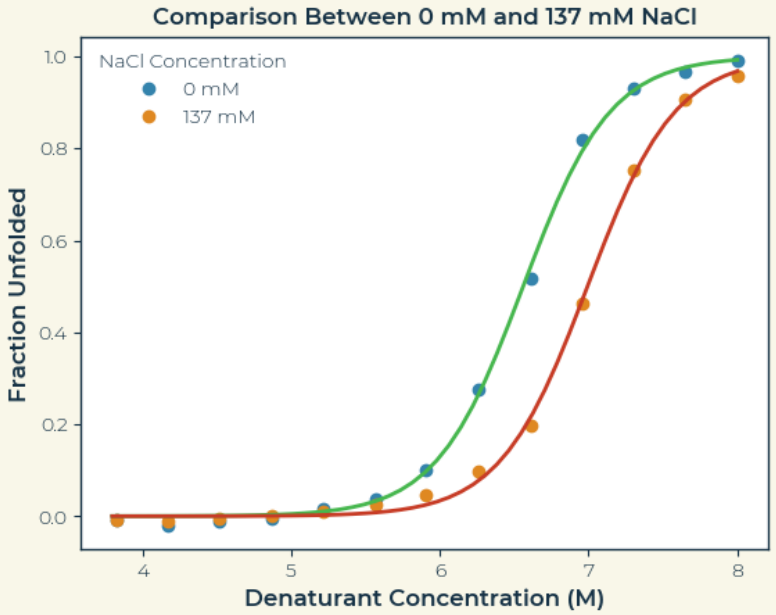
- The fitted parameters can be compared and demonstrate which formulation improved stability the most

Sample pH	$\Delta G°_1$ (kJ mol^{-1})	C_{m1} (M)	$\Delta G°_2$ (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**

Protein Stable

Formulation Example

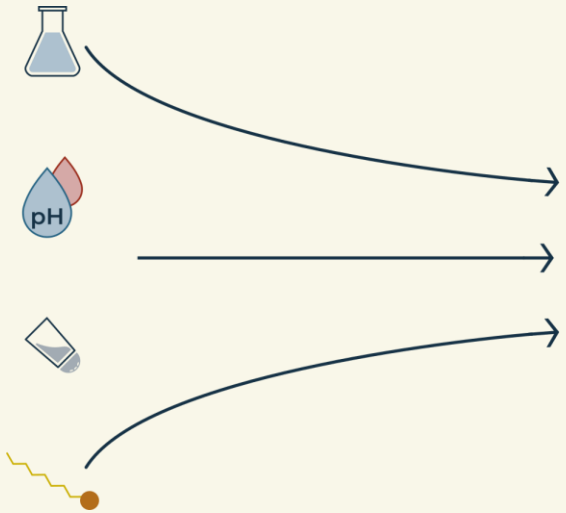
- Study extended to test NaCl effect on antibody stability



- 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

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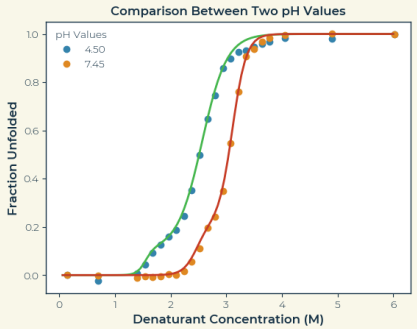

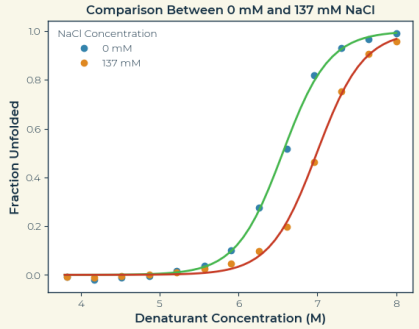

- Highest throughputs - wide range of solution conditions tested with ease
- Best data quality – confidence in decision making and regulatory submissions
- Microplates throughout simplify sample handling and minimise risk of errors

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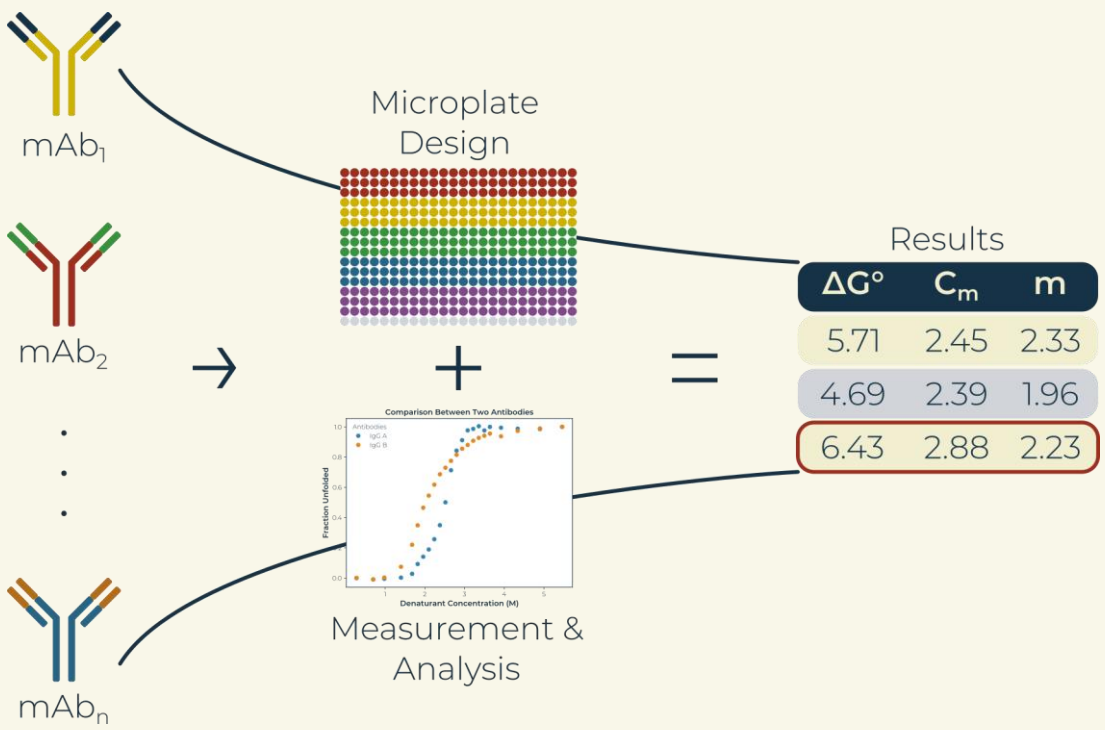
Antibody Engineering Example



- **Reagents:**
  - 2 x IgG antibody (IgG A & IgG B)
  - Guanidine Hydrochloride (GuHCl)
  - Phosphate Buffer Solution (pH 7.2)
- **384 Microwell plate Set-Up:**
  - 50 $\mu g/ml$ 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

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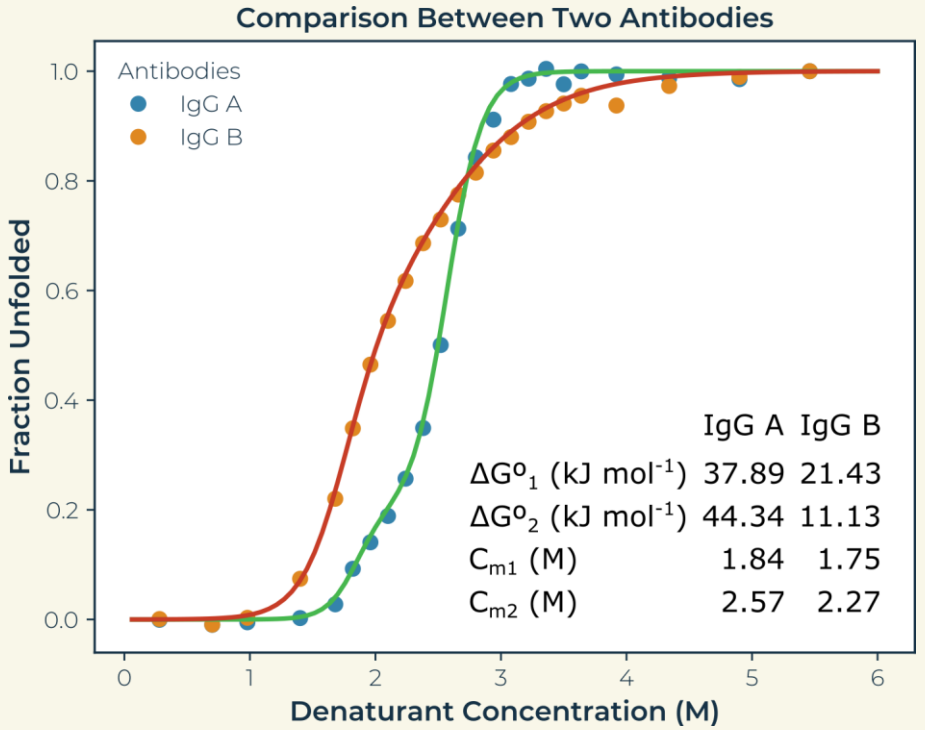
Antibody Engineering Example

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



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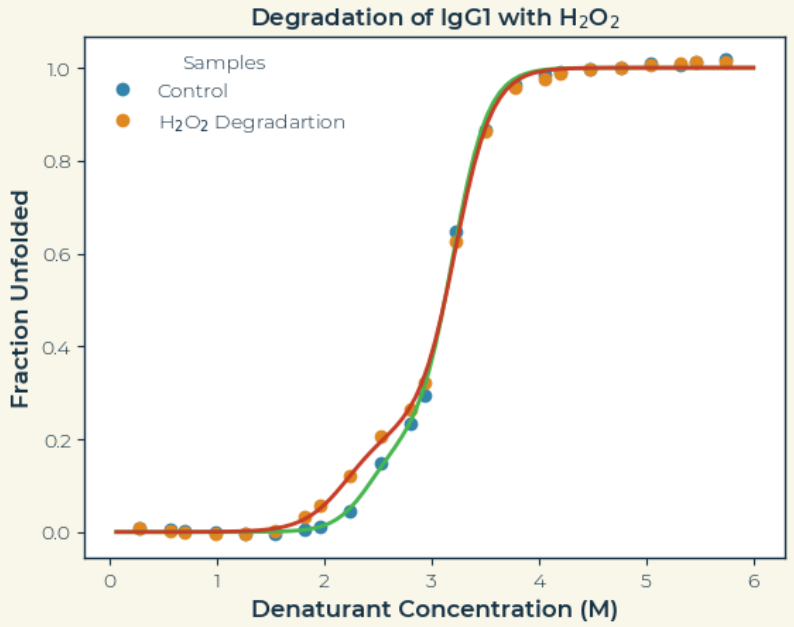
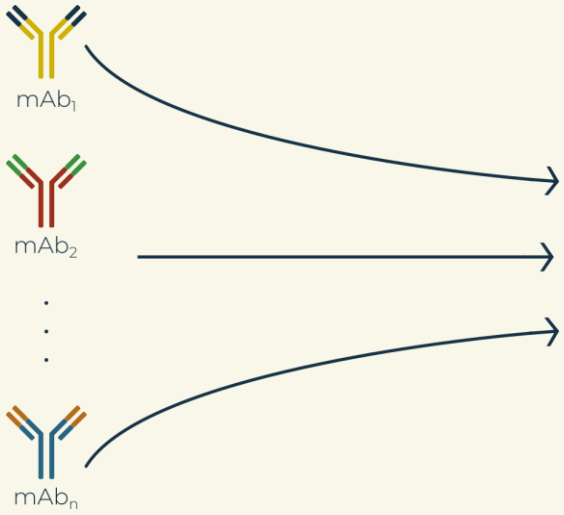
Antibody Engineering Example

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



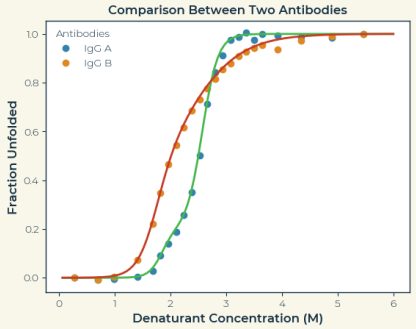
Samples	$\Delta G°_1$ (kJ mol^{-1})	C_{m1} (M)	$\Delta G°_2$ (kJ mol^{-1})	C_{m2} (M)
Control	**36.30**	**2.40**	47.45	3.20
H_2O_2	**26.82**	**2.20**	45.21	3.22

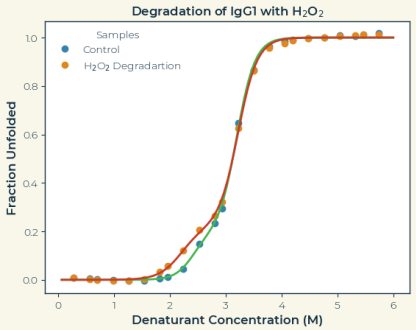
SUPR-CM for Antibody Engineering



Most Stable Candidate

?



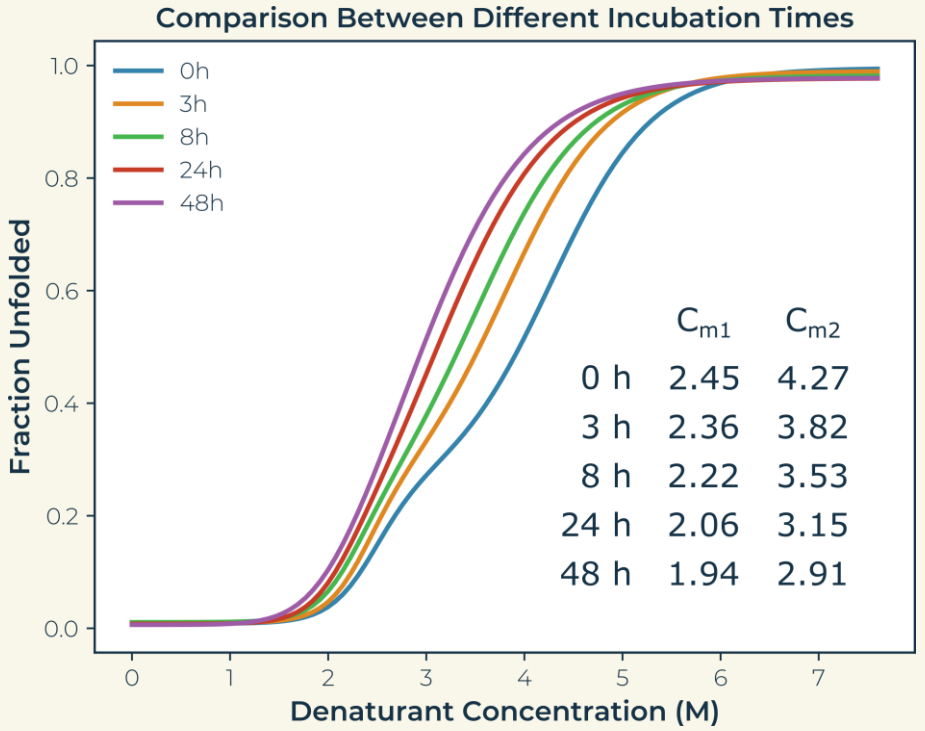


- Which candidate to progress?
- Measure the stability of many antibodies in minutes
- Chemical denaturing able to capture complex antibody unfolding.
- Able to quantify subtle changes to the stability of antibodies

Protein Stable

Advantages Of SUPR-CM

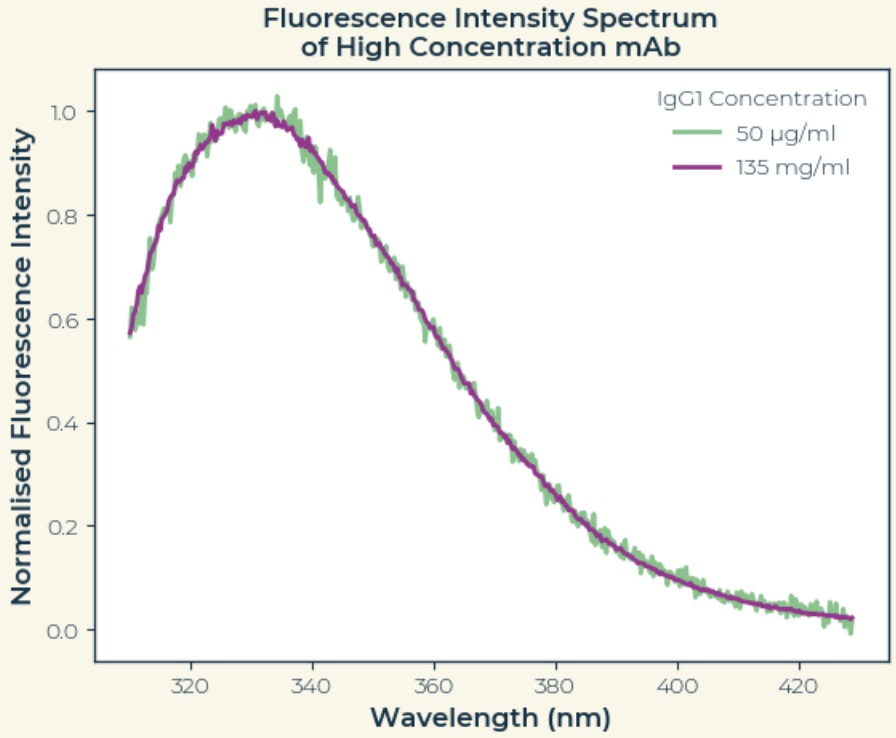
- Modular approach means no restrictions on incubation times
- Allow samples to reach equilibrium and improve quality of data



Protein Stable

Advantages Of SUPR-CM

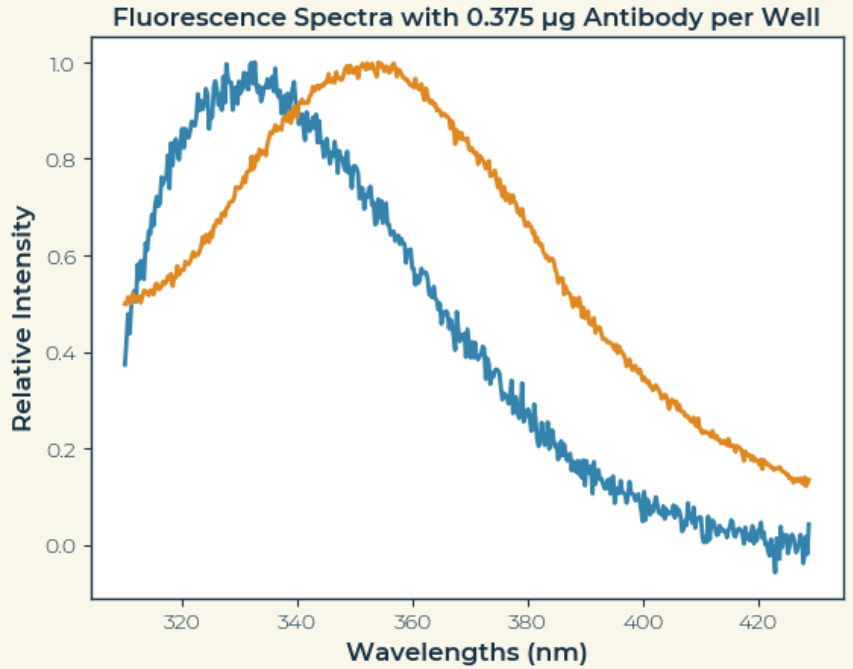
- Large range of antibody concentration can be used
- Can work at therapeutic concentration range (> 100 mg/ml)



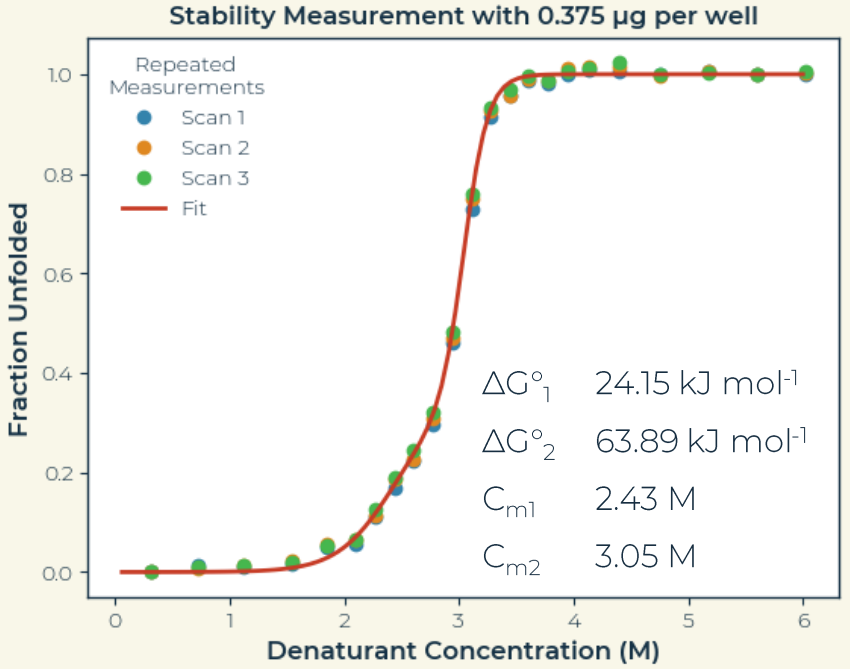
Protein Stable

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



Protein Stable

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

Protein Stable

www.proteinstable.com

sales@proteinstable.com