When protein stability matters.

Dr. Pierre Soule

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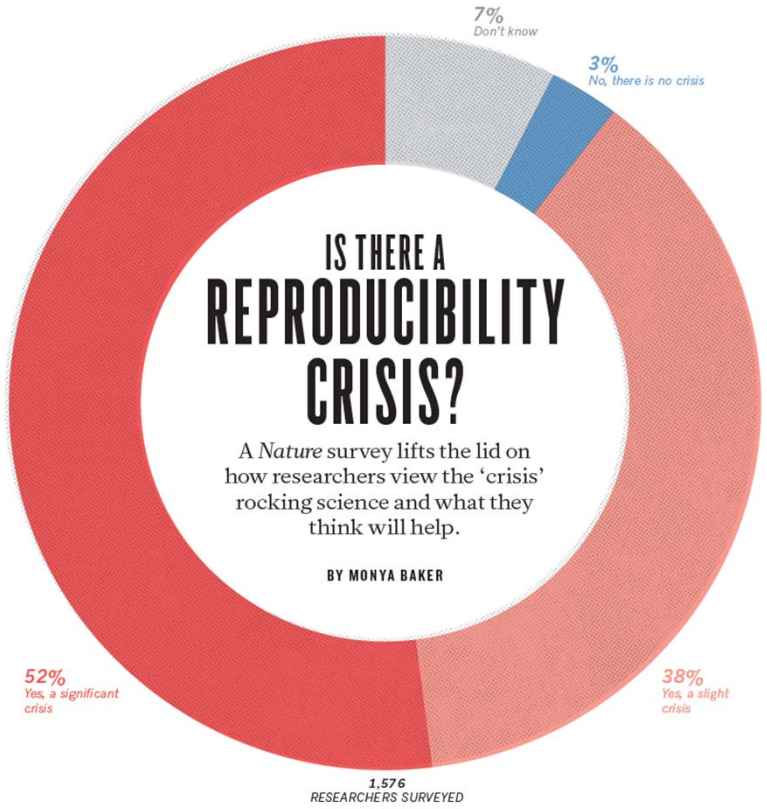
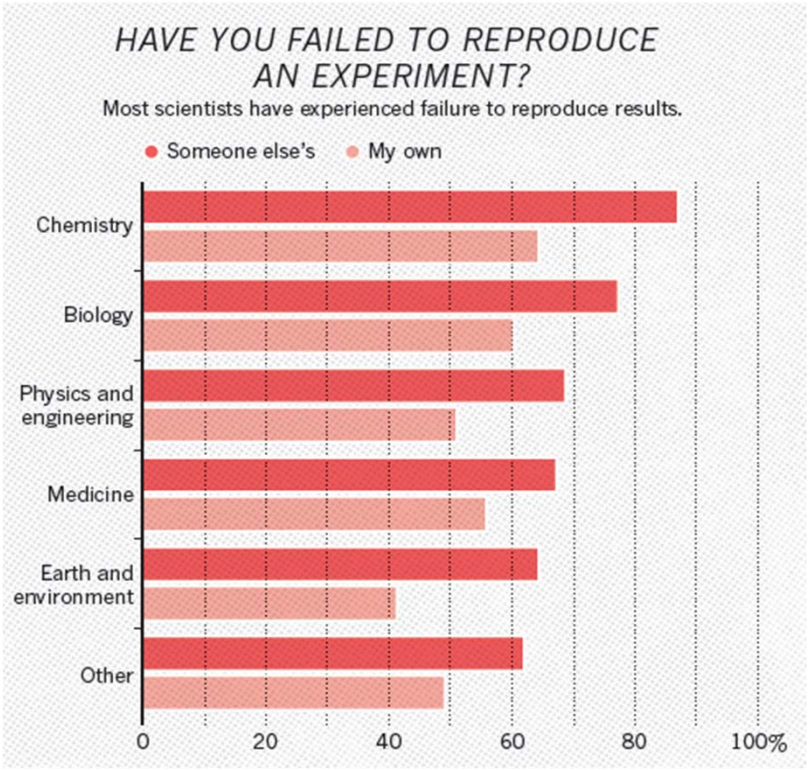
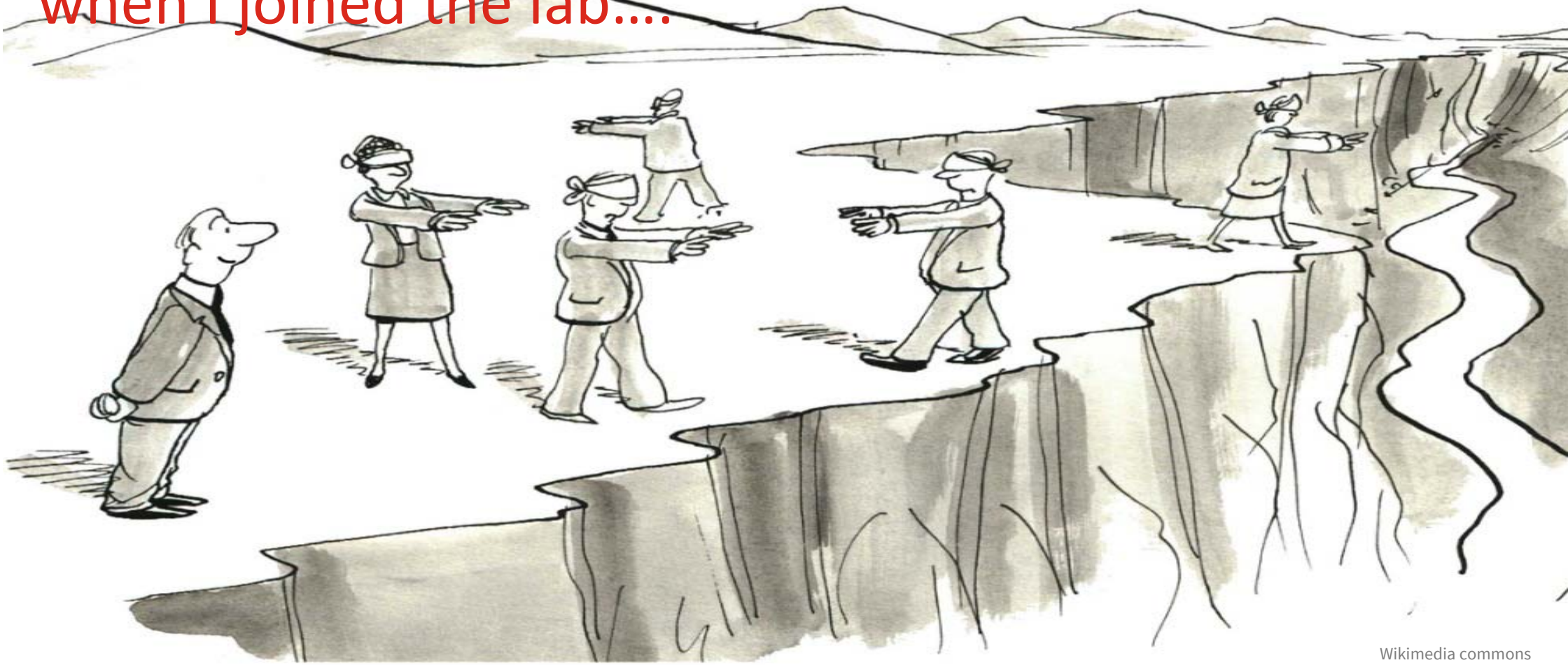
There is a reproducibility crisis in research





Nature 533, 452–454 (26 May 2016)

“I’m just following the protocol the way I was shown when I joined the lab….”



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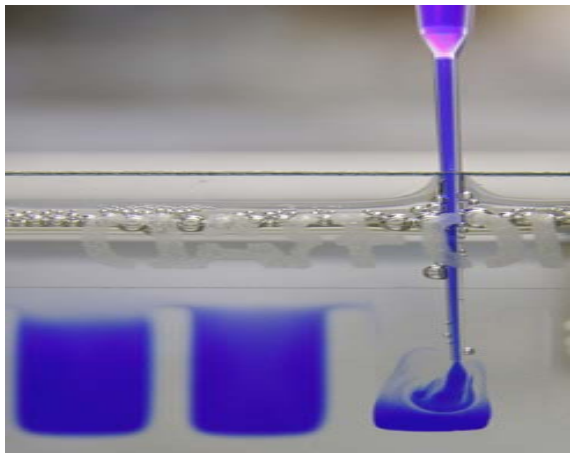
Traditional tools for protein research have been around for a LONG time…

The spectrophotometer is over 77 years old



Gel electrophoresis is almost 67 years old

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Column chromatography has been around for the last 60 years

Are you learning everything you could about the quality and functionality of your protein with these methods?

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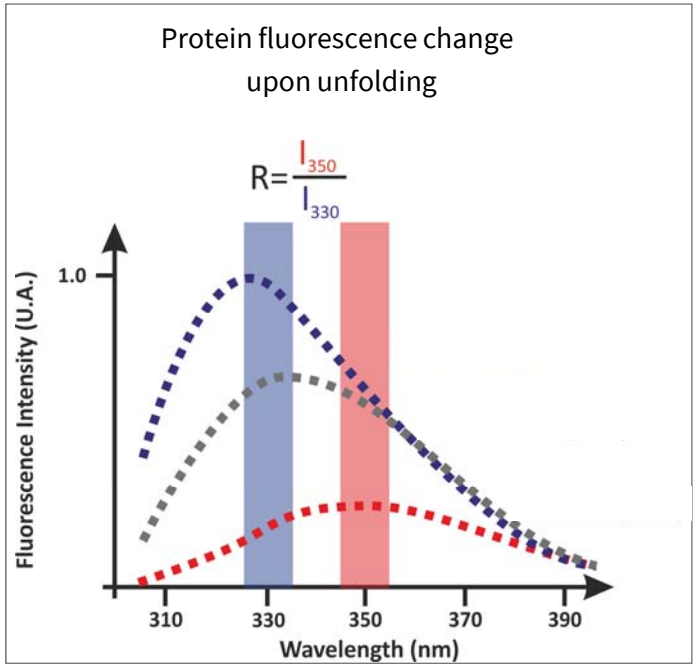
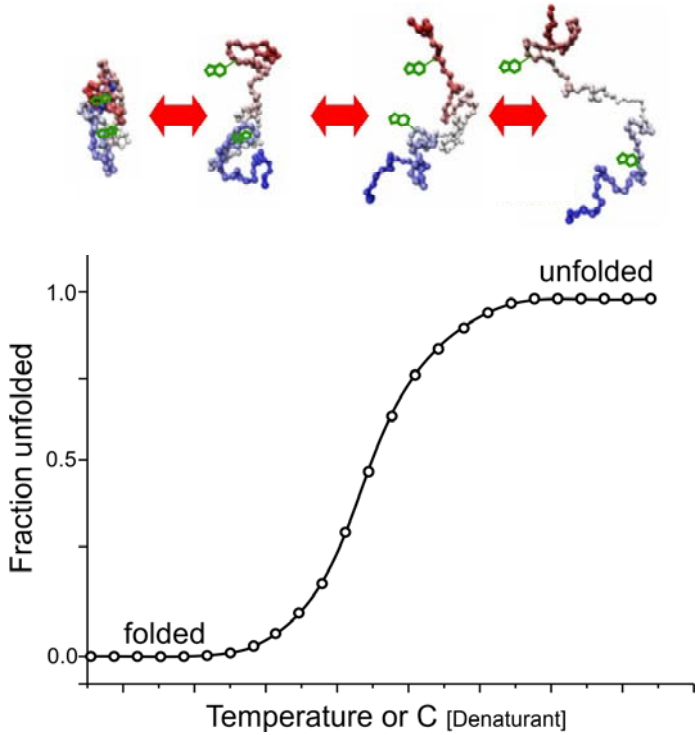
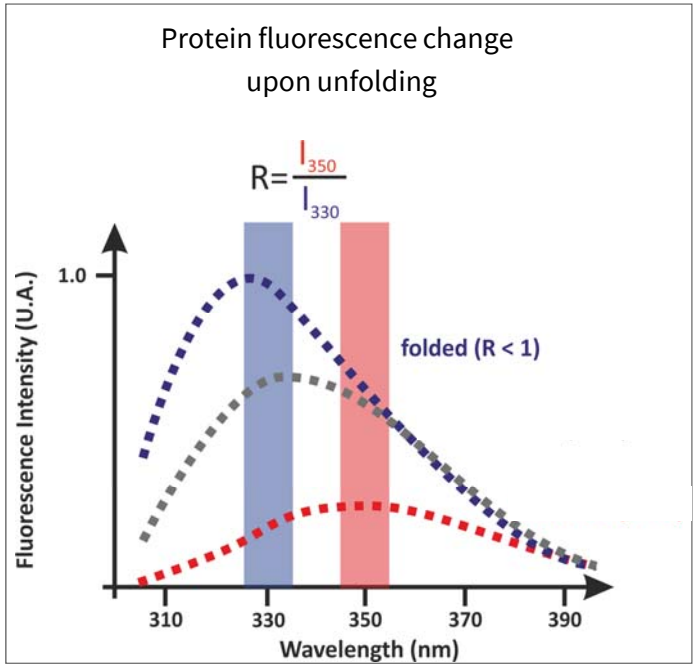
nanoDSF technology introduction
Prometheus NT.48

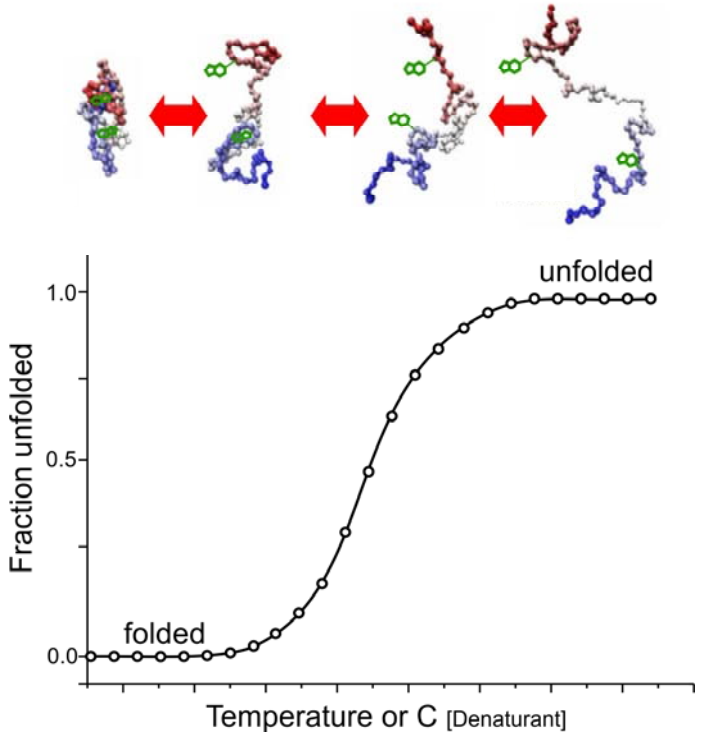
nanoDSF technology



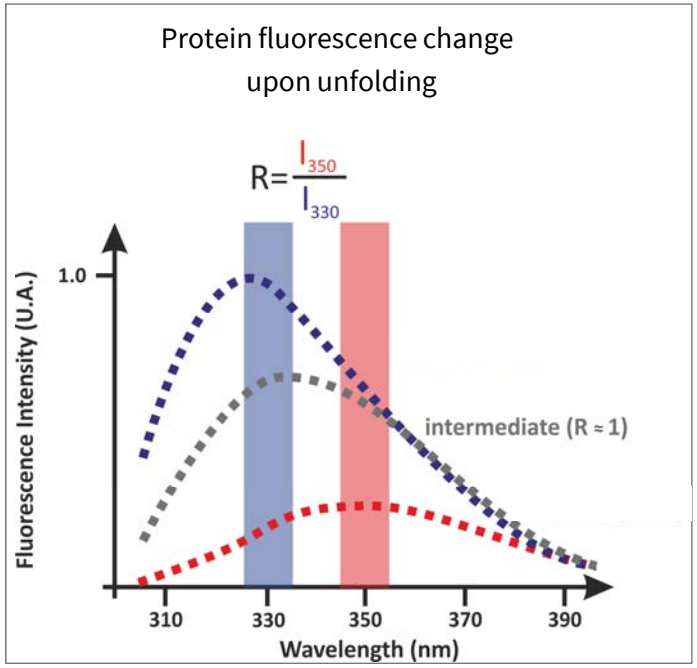


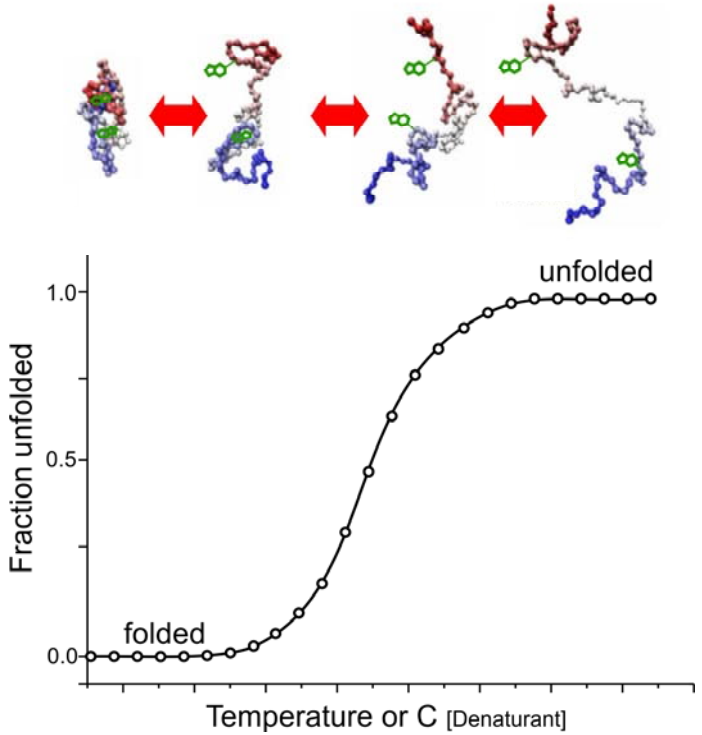
nanoDSF technology



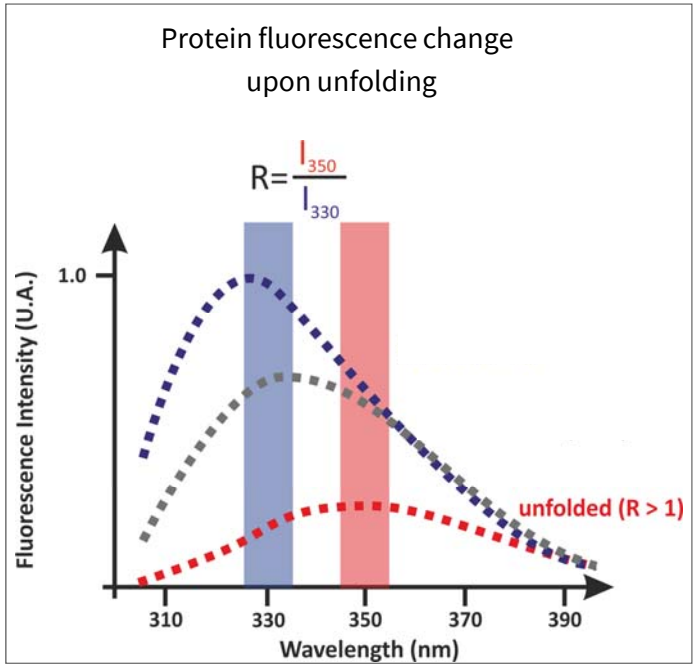


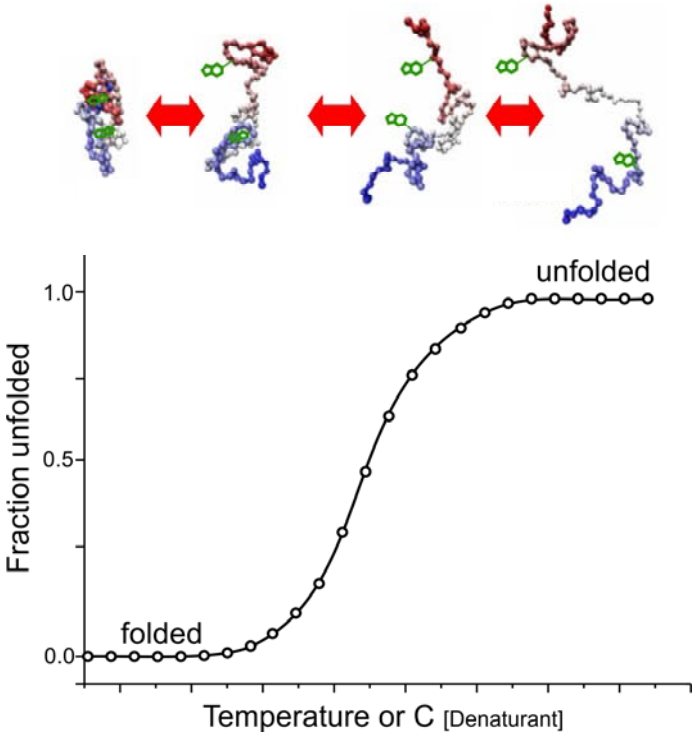
nanoDSF technology



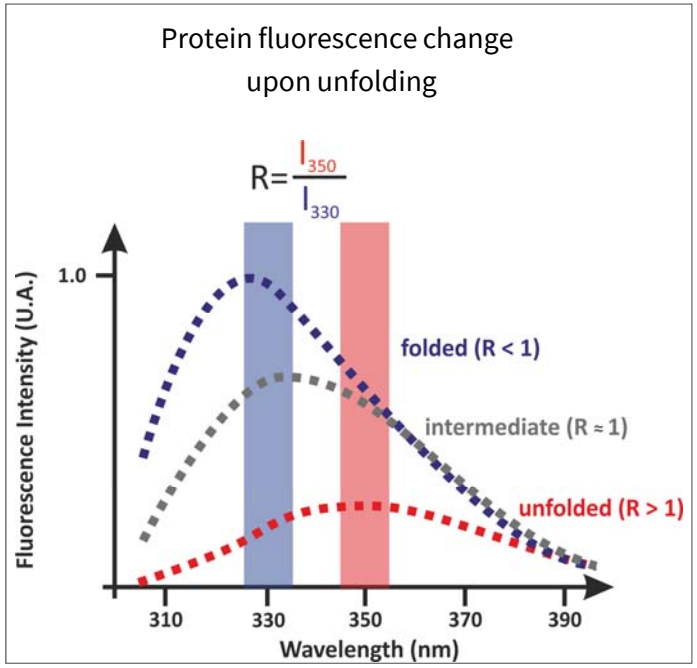


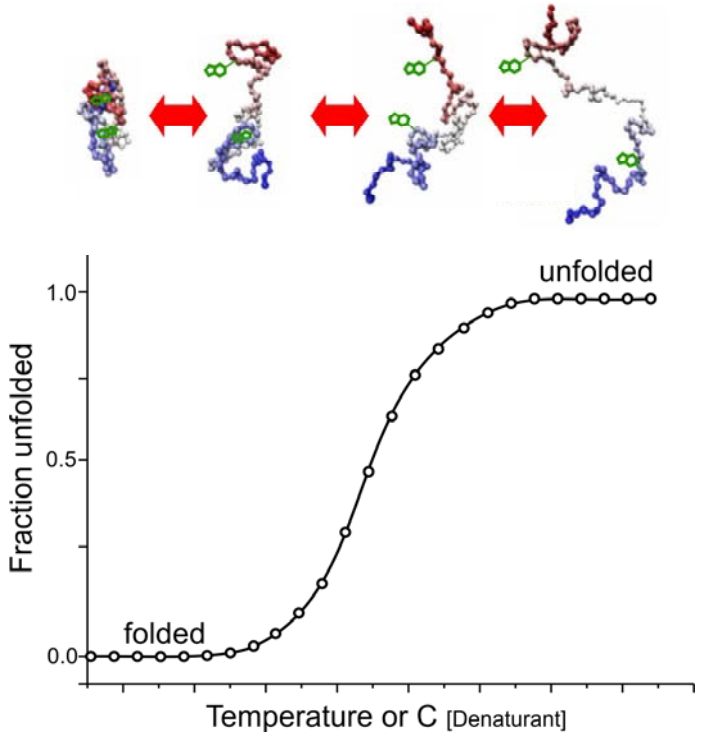
nanoDSF technology



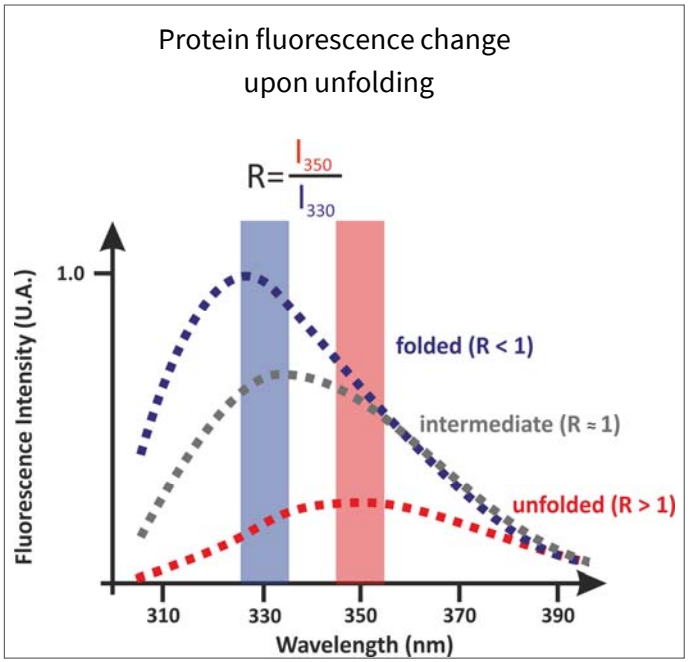


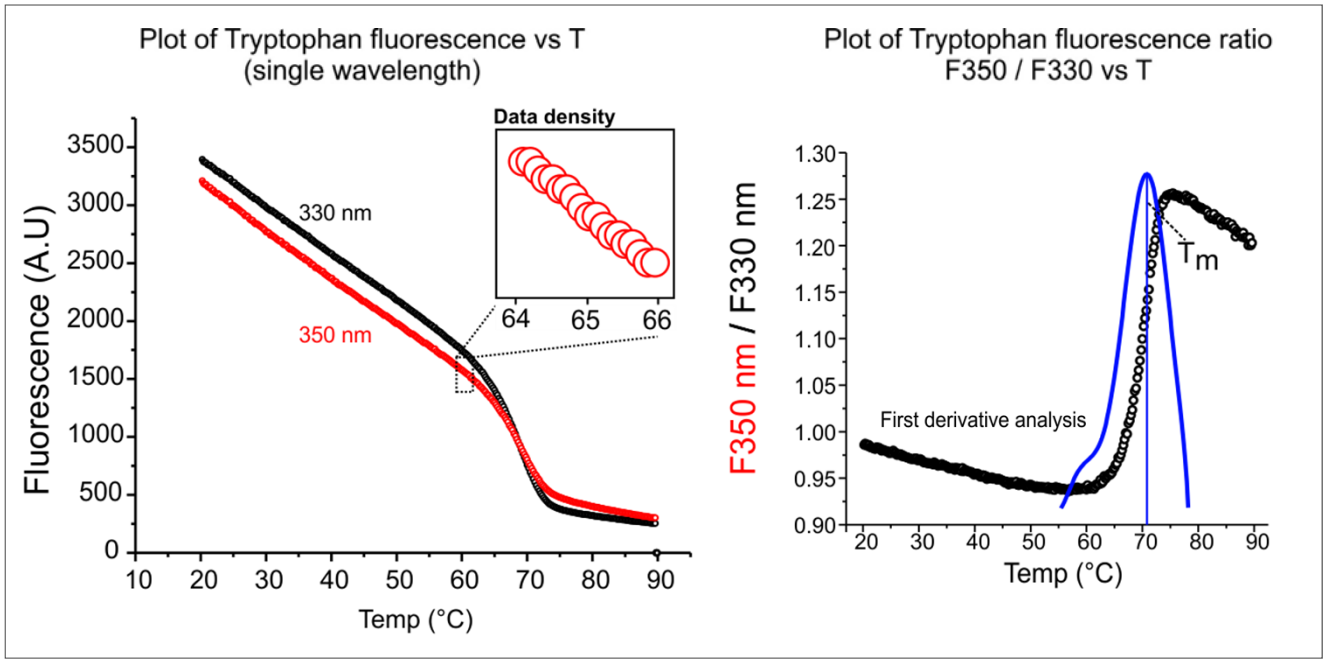
nanoDSF technology





nanoDSF technology

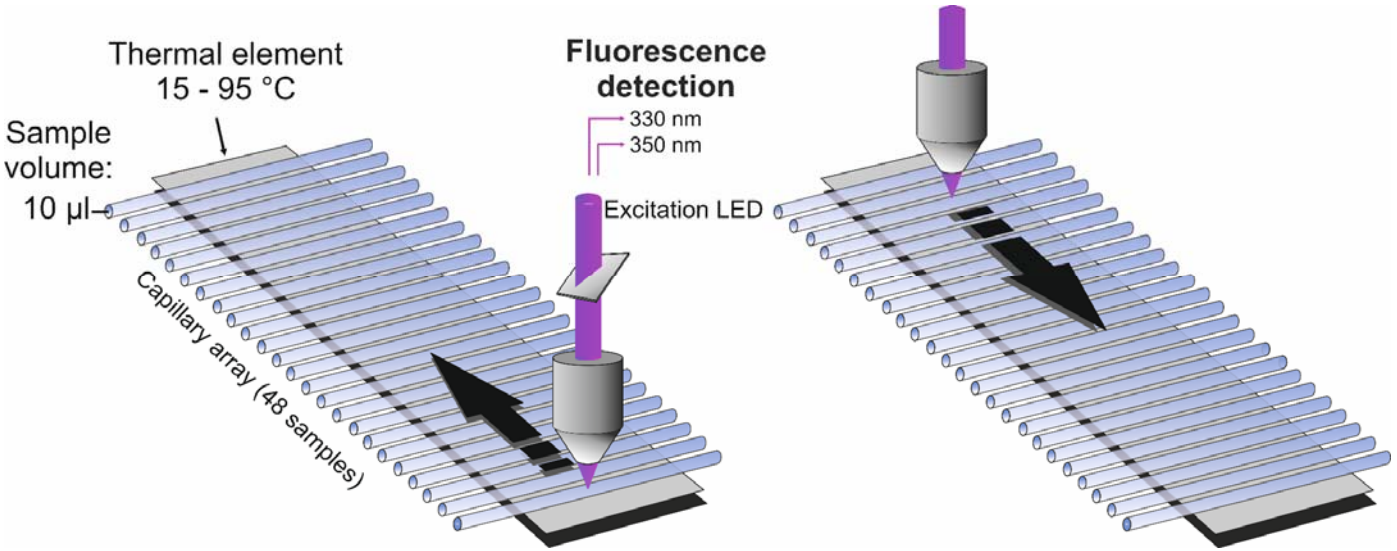




Dual UV-detection

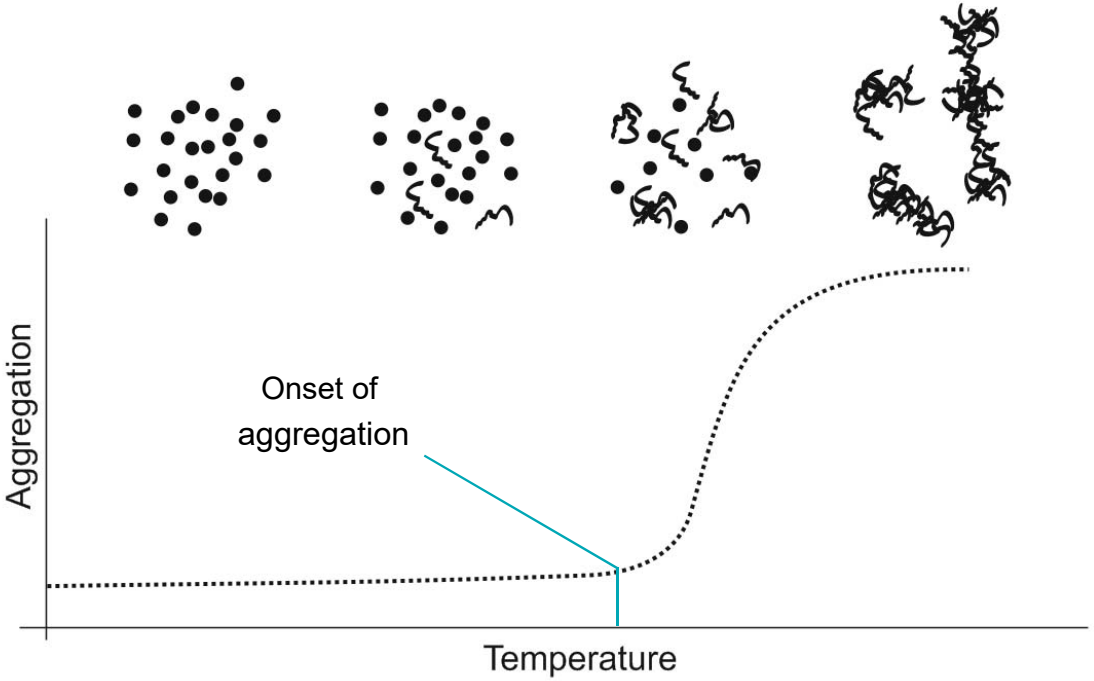
- Rapid detection of trytophan fluorescence without losing unfolding information

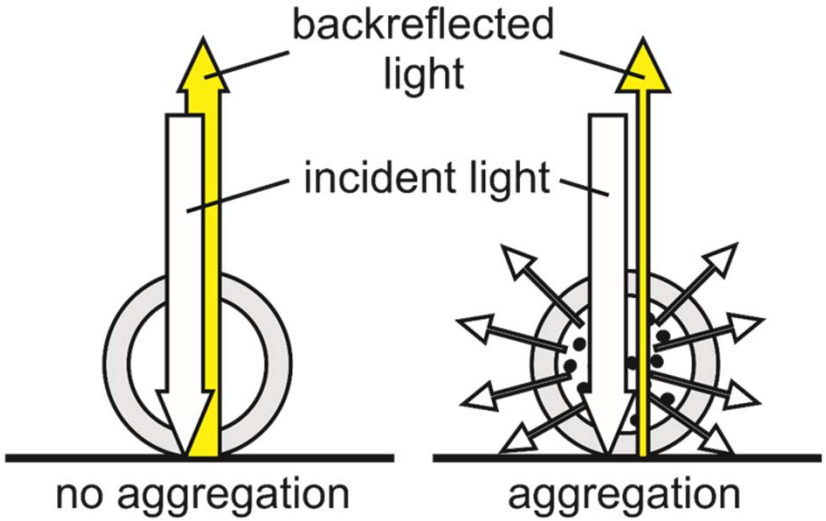
How does the Prometheus NT.48 work?



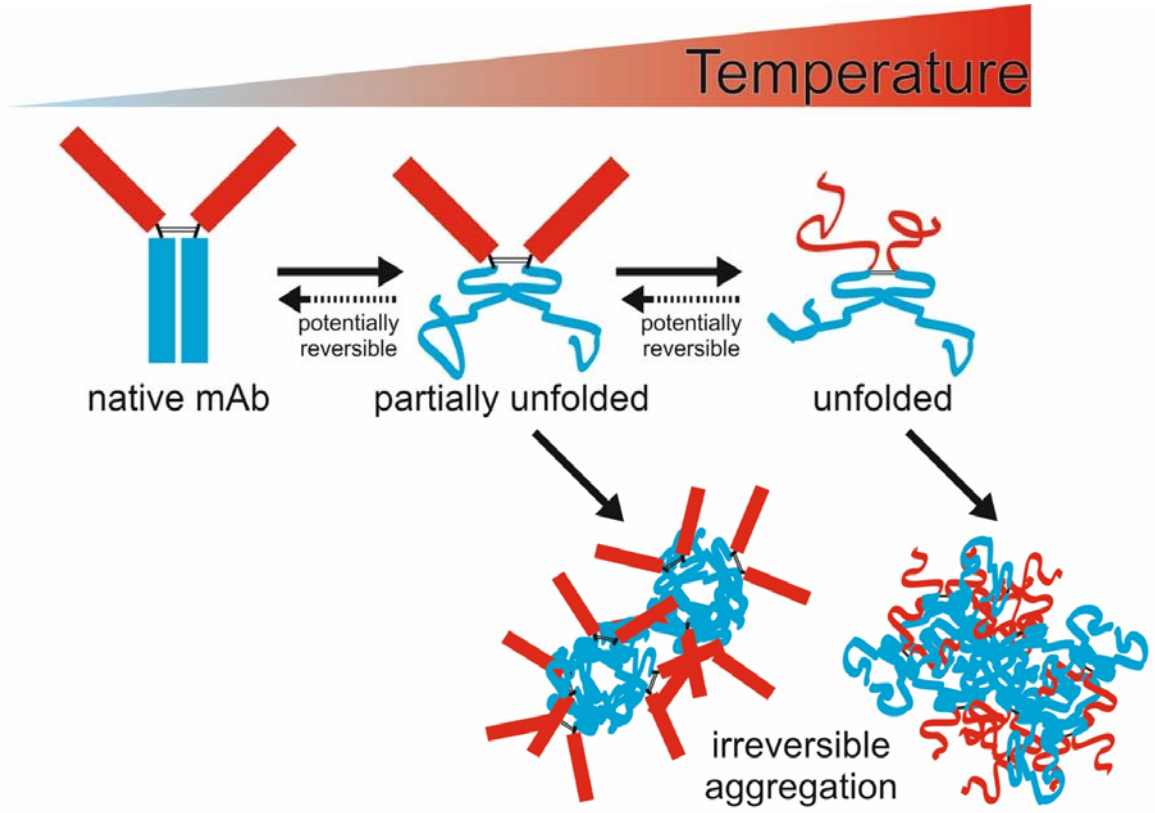
- **Heat and read**
  From 15 °C to 95 °C
  Heating rate: 0.1-7 °C/min
- **Measure 48 capillaries „on-the-fly“**
  Within 3 seconds

Aggregation detection with the backreflection technology





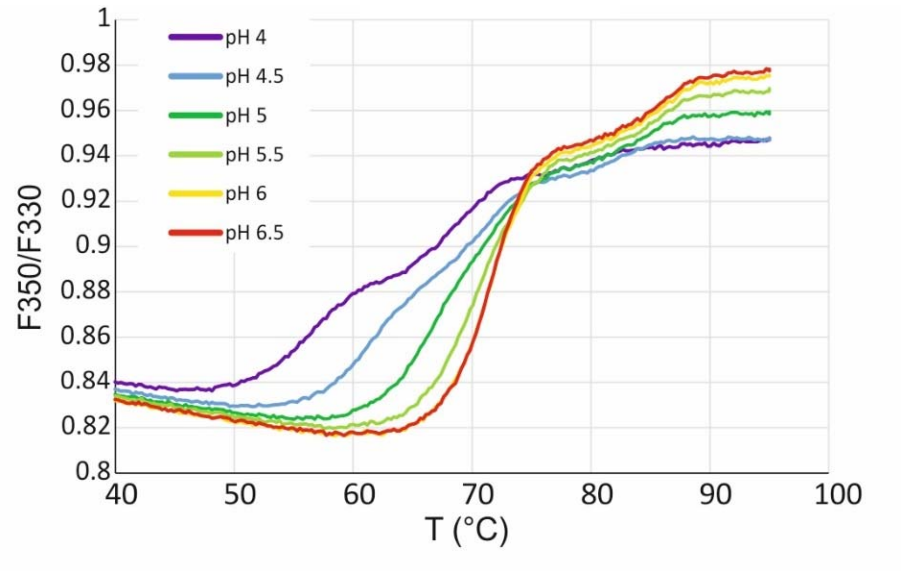
Combinatory evaluation of thermal unfolding and aggregation



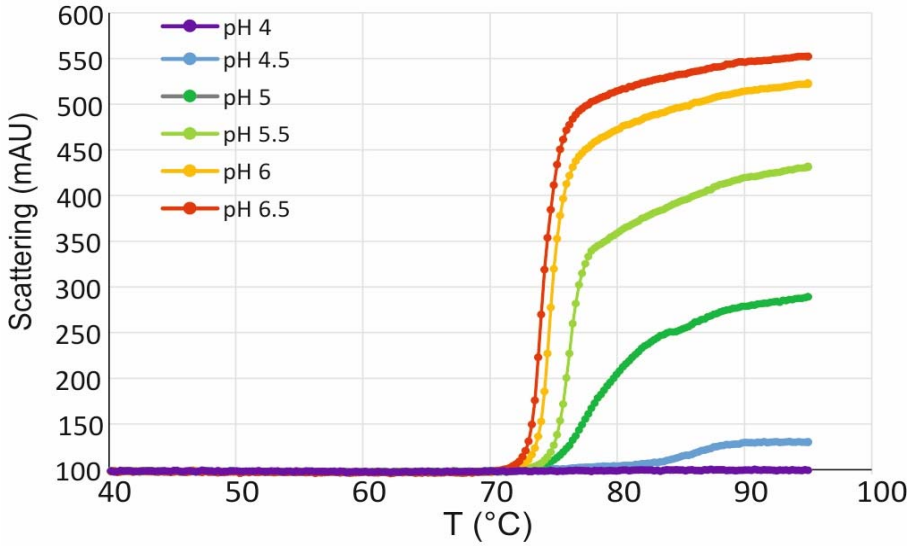
- **Proteins are in an equilibrium of folded and (partially) unfolded states**
- **Temperature-induced unfolding allows for rapid testing of aggregation of unfolded protein**
- **Long term stability depends on:**
  - Thermal stability
  - Degree of aggregation of the unfolded state

Combinatory evaluation of thermal unfolding and aggregation

Fluorescence

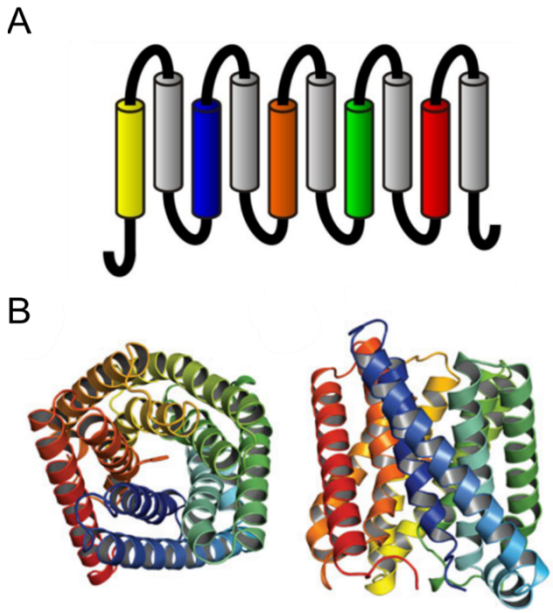


Aggregation



- Simultaneous detection of conformational and colloidal stability
- Decrease in pH destablizes antibody but prevents aggregation of the unfolded state

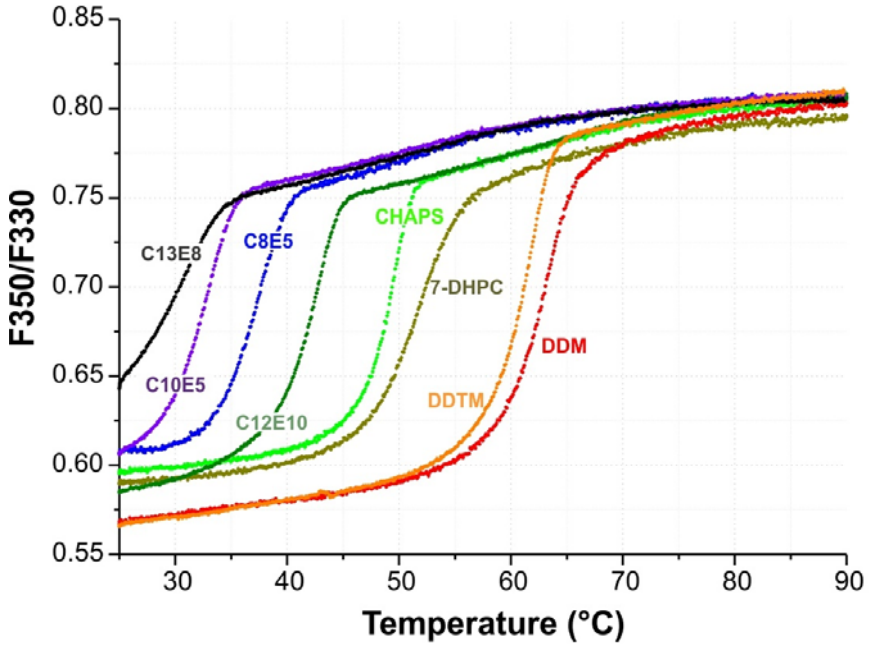
Detergent screens for integral membrane proteins

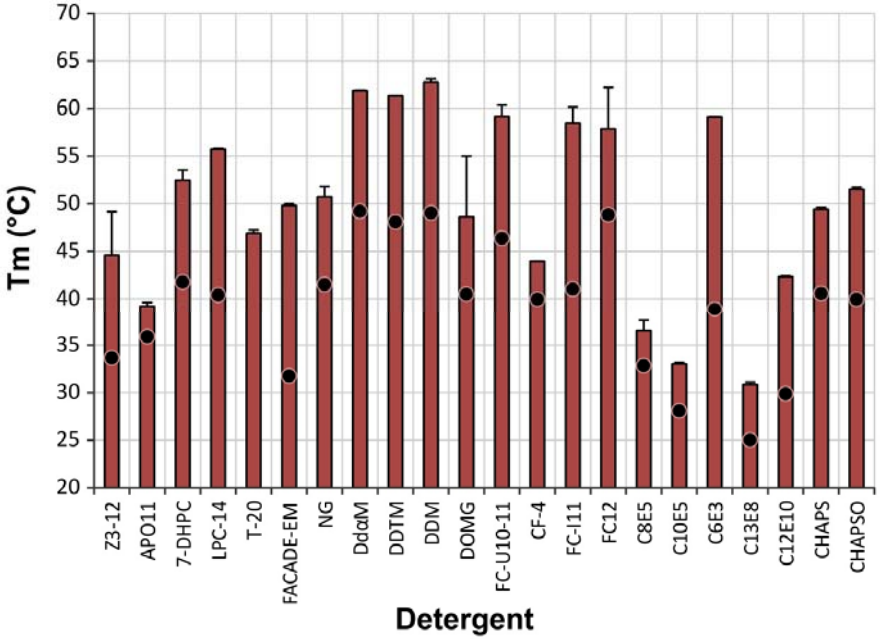


LPC-14	14:0 LysoPC (1-tetradecanoyl-2-hydroxy-sn-glycero-3-phosphocholine)	0.06	0.3
T-20	Tween 20 (Anapoe-20)	0.059	0.59
FACADE-EM	Façade-EM	0.1	1
NG	n-Nonyl-ß-D Glucopyranoside	6.5	1.625
DdαM	n-Dodecyl-α-D-maltopyranoside	0.15	0.75
DDTM	n-Dodecyl-ß-D-thiomaltopyranoside	0.05	0.5
DDM	n-Dodecyl-ß-D-maltopyranoside	0.17	0.85
DOMG	n-Dodecyl-N,N-dimethylglycine	1.5	0.45
CF-4	CYCLOFOS-4	14	2.8
FC-I11	FOS-CHOLINE-ISO-11	26.6	5.32
FC12	FOS-CHOLINE-12	1.5	0.45
C8E5	Pentaethylene glycol monooctyl ether	7.1	1.775
C10E5	Pentaethylene glycol monodecyl ether	0.81	0.81
C6E3	Triethylene glycol monohexyl ether	23	4.6
C13E8	Polyoxyethylene(8)tridecyl ether (Anapoe C13E8)	0.1	1
C12E10	Polyoxyethylene(10)dodecyl ether (Anapoe C12E10)	0.2	1
CHAPS	CHAPS	8	2

Only 10 µl with ~5 µg/ml protein per condition are required!

Detergent screens for integral membrane proteins

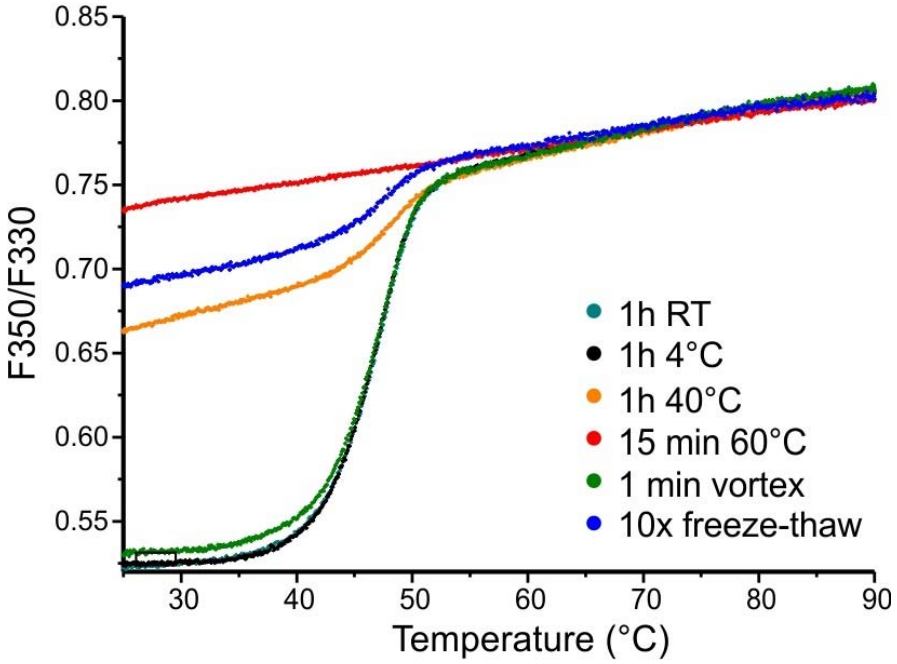


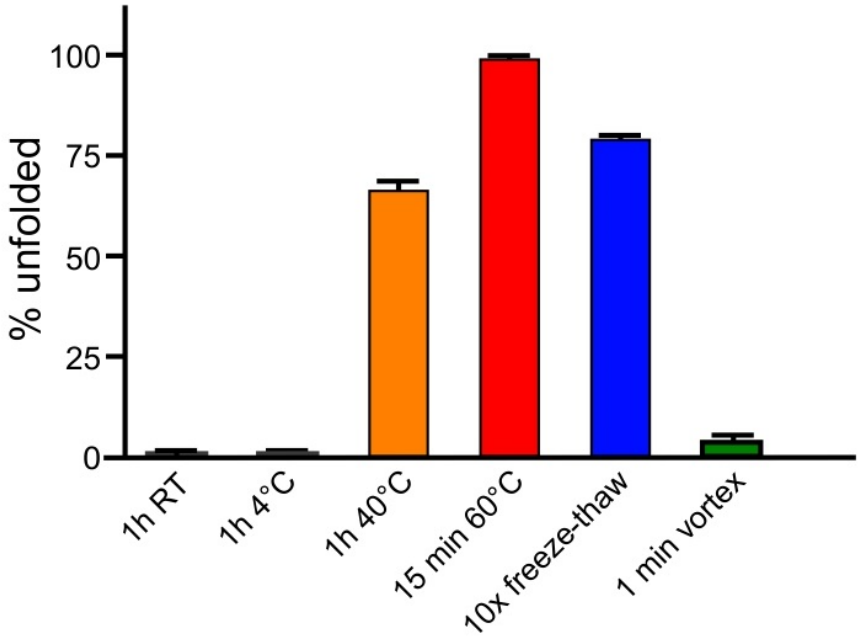


Detergent has strong effect on thermal stability with T_m varying between 31°C and 63°C

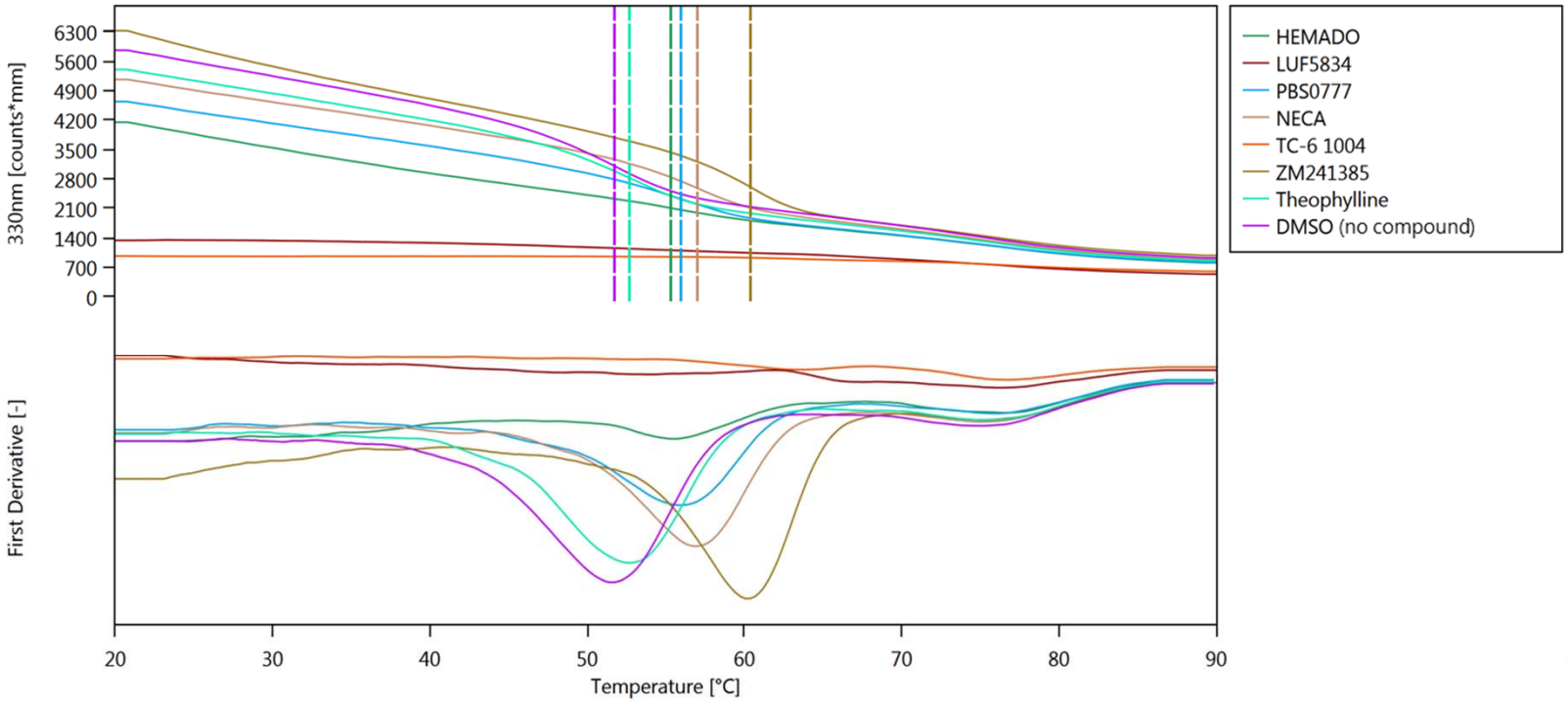
Forced degradation: testing protein sensitivity to different stresses

MEK1 is highly sensitive towards elevated temperatures and freeze-thaw cycles, but rather insensitive towards mechanical stress





Thermal shift compound screen with GPCRs



- Influence of various compounds on thermal stability
- Dye-free ligand screen with high-throughput potential

NOVARTIS INSTITUTES FOR BIOMEDICAL RESEARCH

Center for Proteomic Chemistry, Novartis Institutes for Biomedical Research, Basel, Switzerland

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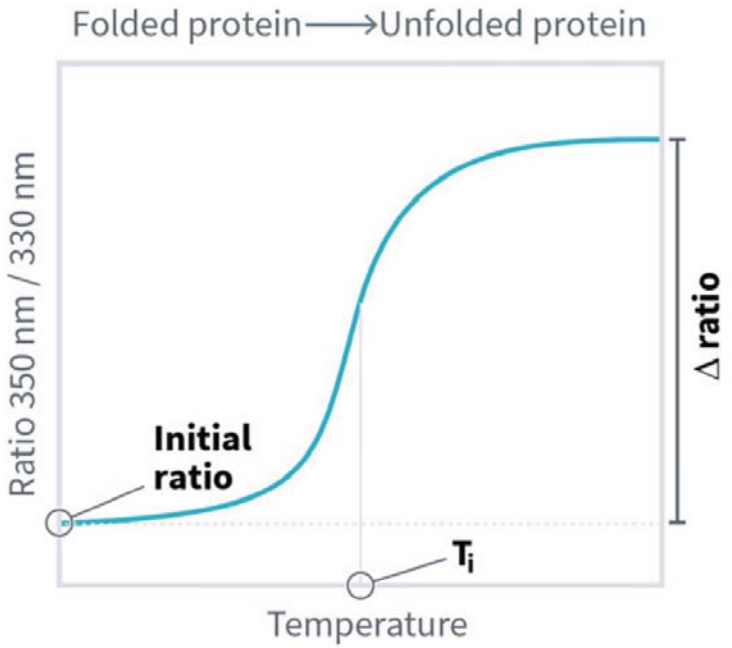
Tycho NT.6
3 minutes quality cont

Tycho NT.6 results



Initial ratio

Δ ratio

Inflection temperature (T_i)

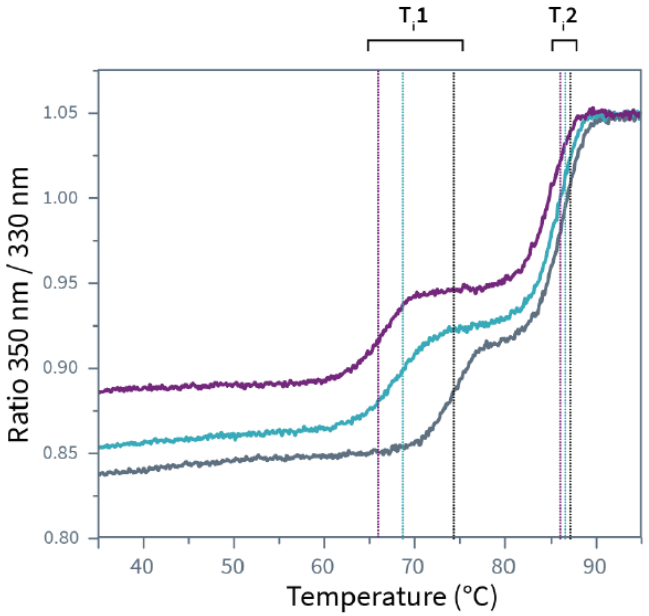
Sample brightness

Profile similarity (%)

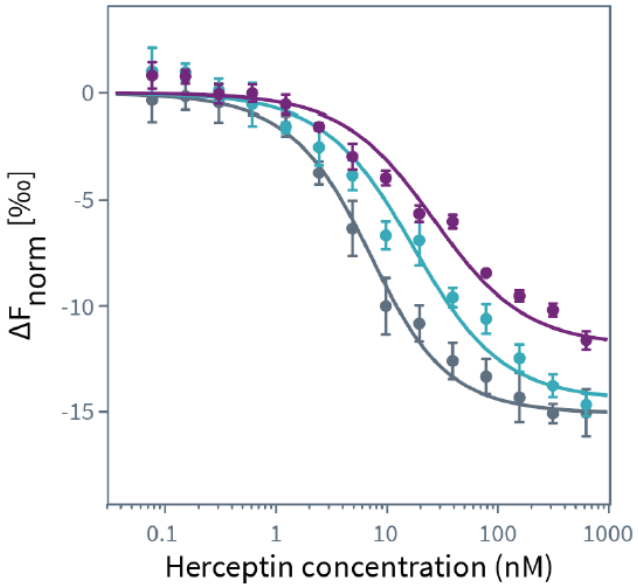
Specific application examples

Fast and accurate evaluation of oxidation-induced destabilization of mAbs

Oxidation of mAb results in reduced binding capabilities



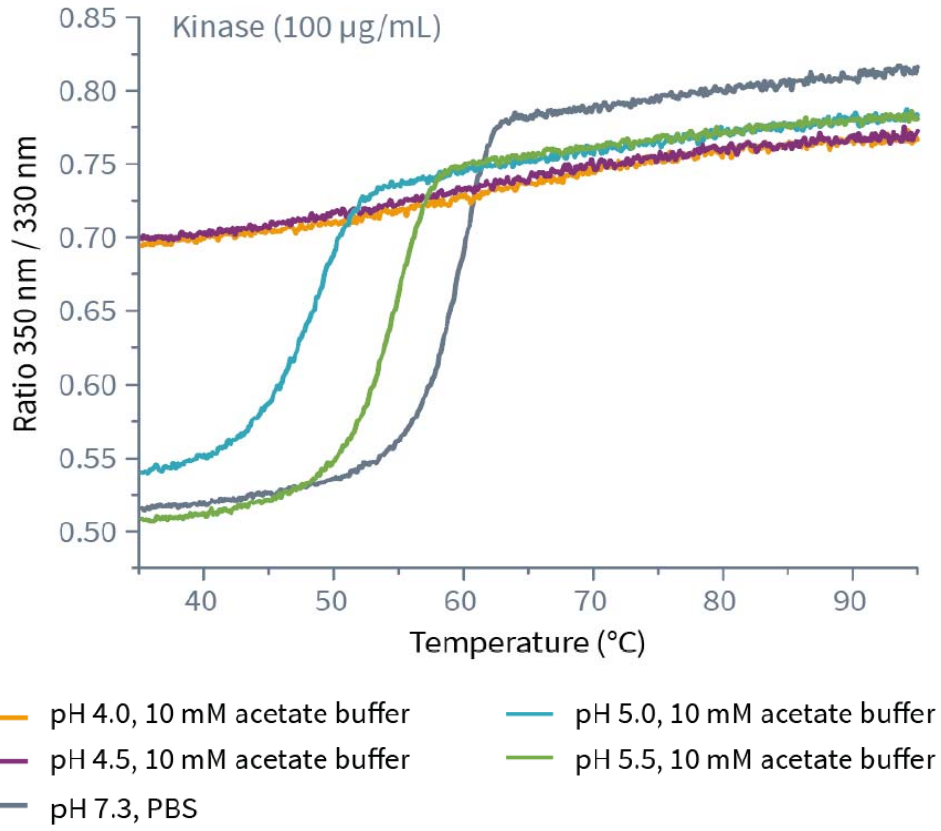
	Initial ratio	T_i1 (°C)	T_i2 (°C)
Native	0.837	74.2	87.4
3 h oxidation	0.853	68.0	86.9
18 h oxidation	0.885	66.5	86.3



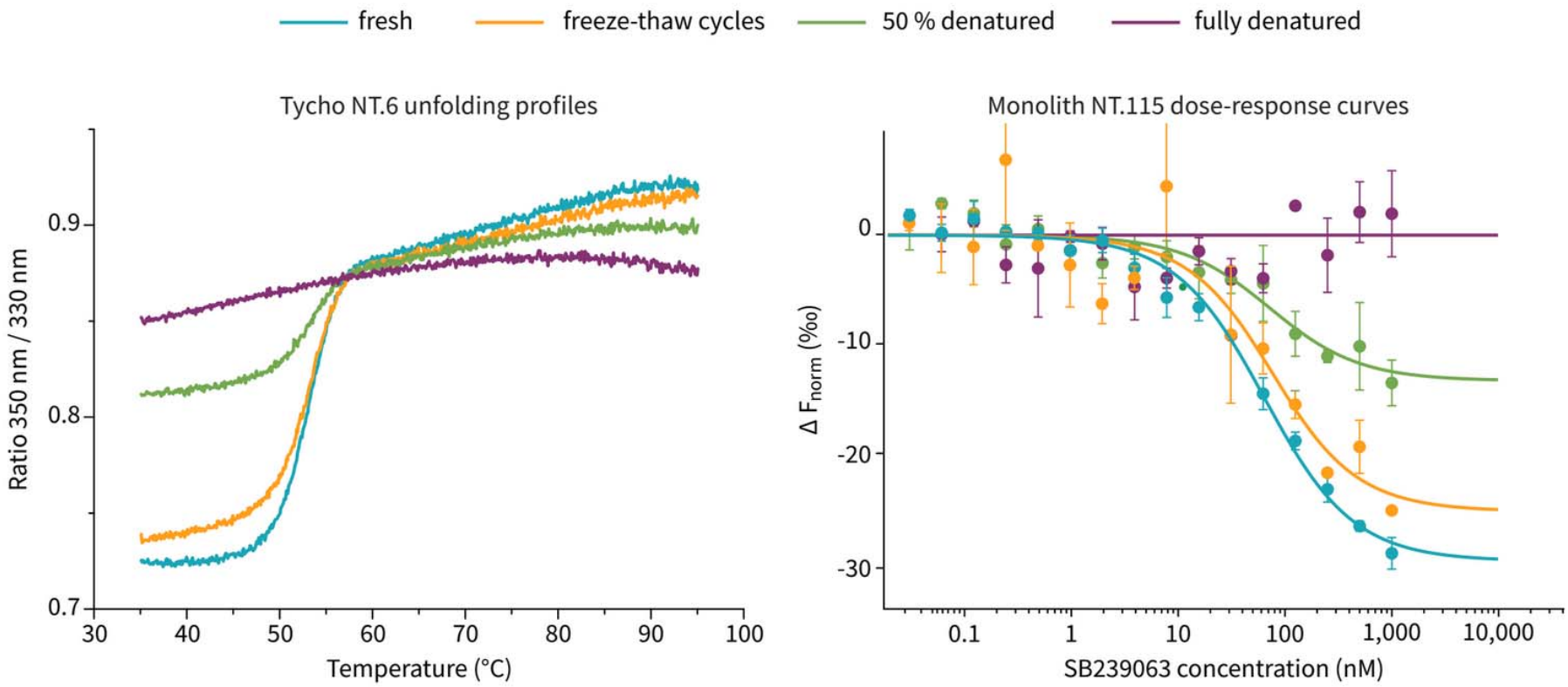
	K_d (nM)
Native	1.4
3 h oxidation	4.7
18 h oxidation	11.7

Better optimization of biosensor assay conditions

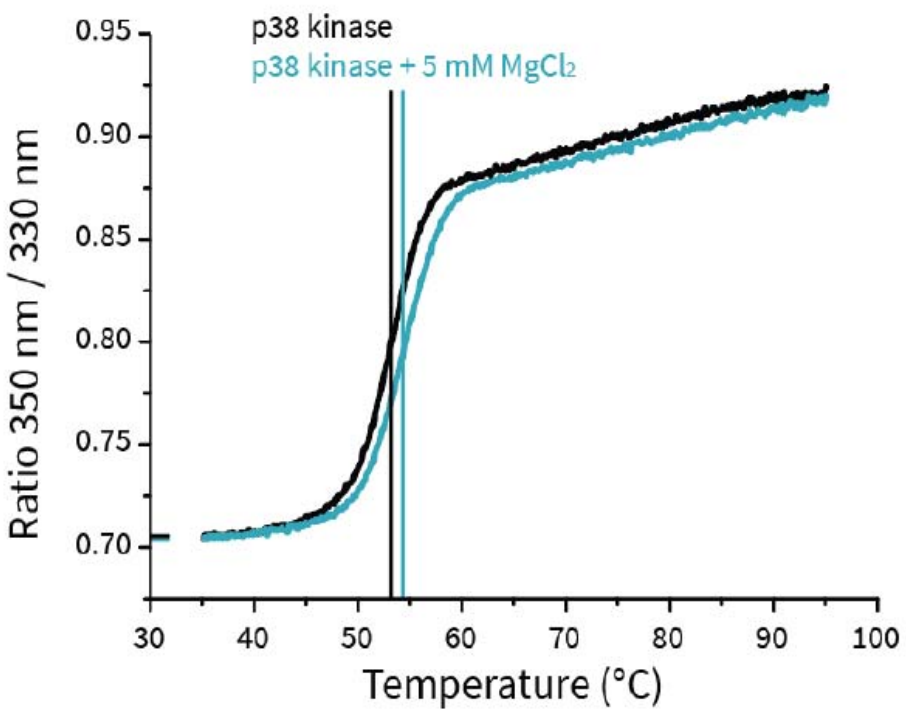
pH 5.5 recommended for immobilization

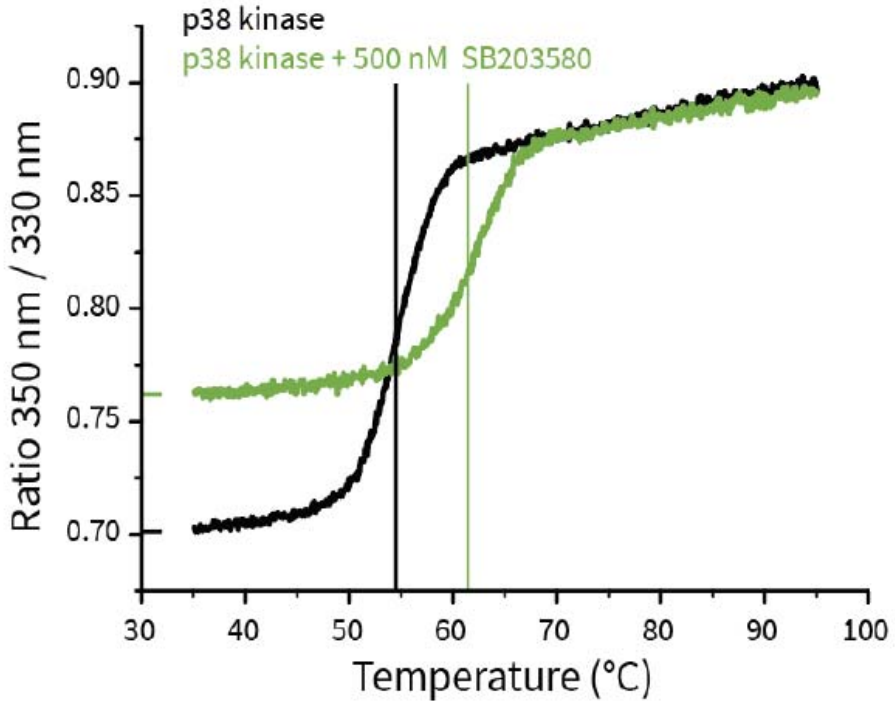


Storage-dependent denaturation has a direct effect on protein quality



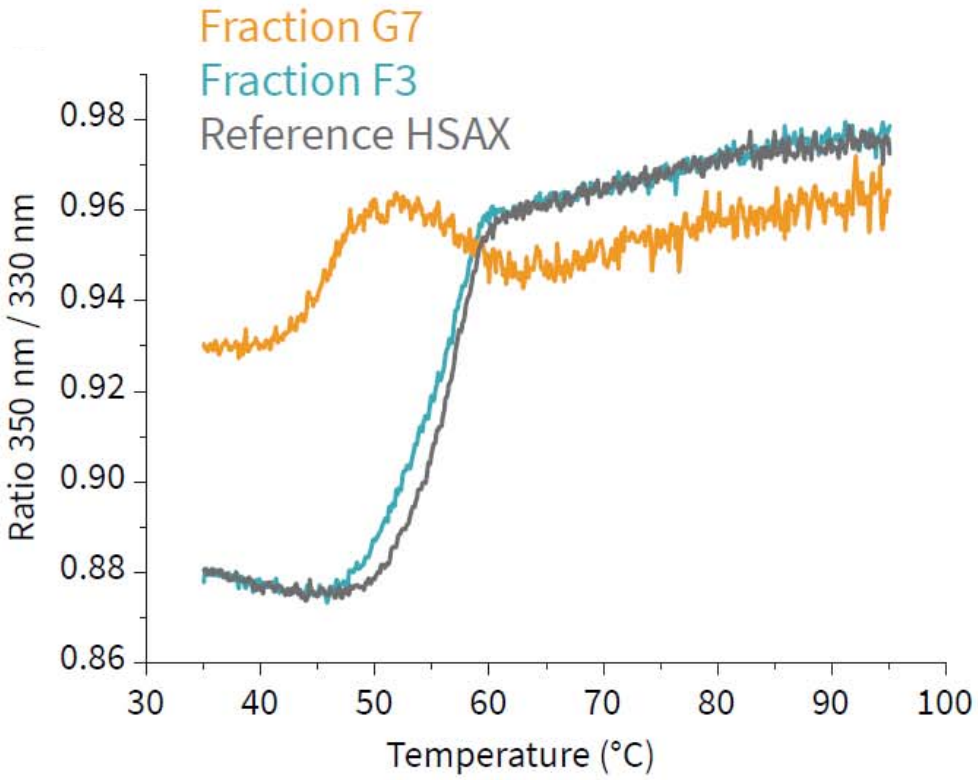
Quick protein binding tests by label-free thermal shift analysis





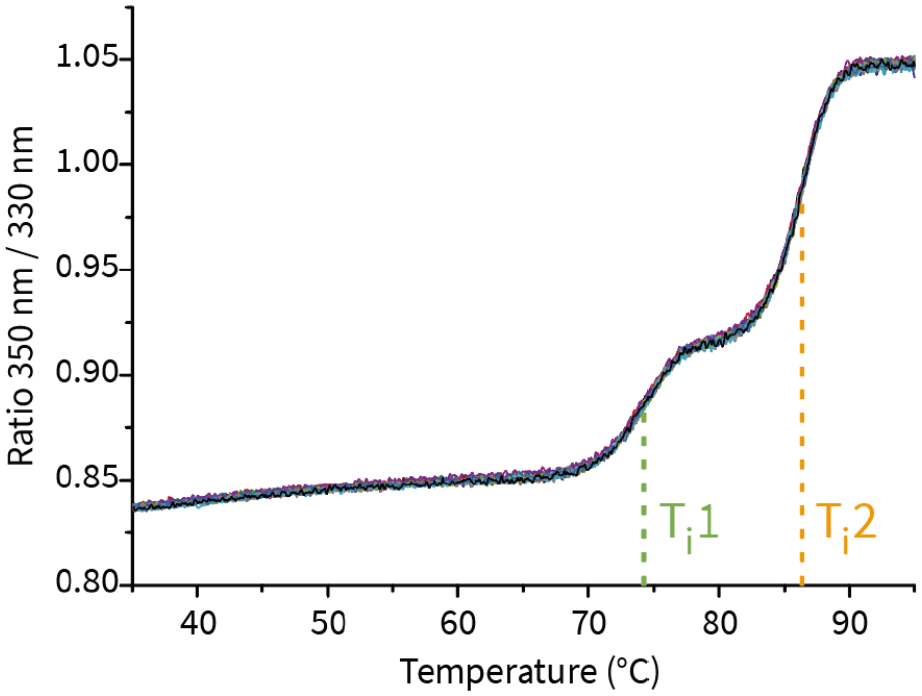
Efficient identification of chromatography fractions

Profile similarity easily determined after each run



Sample	Initial ratio	Δ Ratio	T_i (°C)	Profile similarity (%)
Reference	0.880	0.095	57.4	N/A
F3	0.879	0.097	57.0	89.5
G7	0.928	0.033	45.6 57.8	36.2

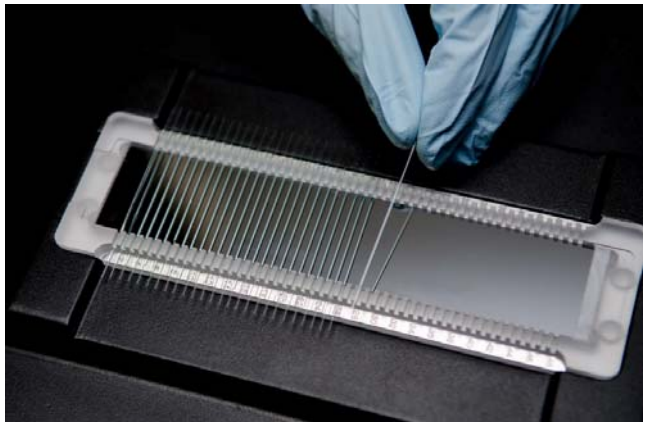
Tycho NT.6 repeatability assessment



	Average	SD	RSD (%)
T_i1	74.4 °C	+/- 0.07 °C	+/- 0.09
T_i2	86.2 °C	+/- 0.06 °C	+/- 0.07
Initial ratio	0.837	+/- 0.001	+/- 0.11

Benefits of nanoDSF

- **Native conditions**
  No dye, thus buffer & detergent independent
- **See more transitions**
  Ultra-high resolution
- **Broad concentration range**
  Detection of protein concentrations between 5 µg/ml and > 250 mg/ml
- **Simultaneous detection of protein aggregation**
  Get more information about your sample



Your advantage: Native conditions and quick results with lower sample consumption.

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Thank you for your attention!

Please feel free to contact us:

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