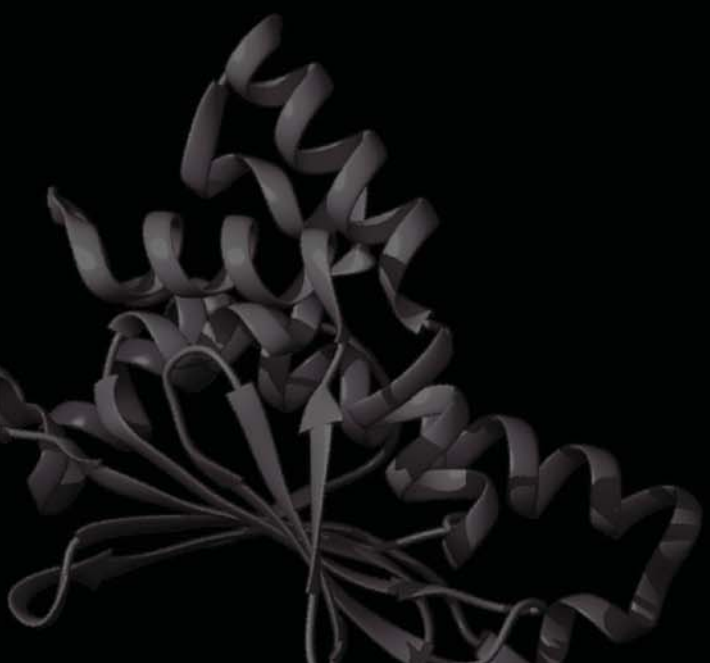
NanoTemper technologies

Product Catalog
Instruments

Affinity. Stability.
BIOPHYSICS in a new dimension



DongYoung 동영과학(주)
DONGYOUNG

MicroScale Thermophoresis

Monolith® Instruments for MicroScale Thermophoresis

Monolith Series

A technology by NanoTemper®

다양한 application을 지원하는 Dissociation constant (Kd) 분석 장비 입니다.

Monolith Series는 MST (Microscale Thermophoresis) 기술을 이용하여 protein–protein, protein–nucleic acid 뿐 아니라 protein–HMW complex, protein–ion, 더 나아가 cell lysate의 Kd 값을 측정할 수 있는 분석 장비입니다.

Enjoy the benefits of MST :

K_d plus

Optimize assays quickly :

▶ Judge and improve sample quality immediately

Measure previously unmeasurable targets :

▶ Work with very small amounts and sensitive samples

Benefit from close–to–native conditions :

▶ Analyze in all buffers and bioliquids (cell lysate, serum) – immobilization–free

Do your research efficiently :

▶ Enjoy perfect ease–of–use, purification–free measurements and get rid of maintenance downtime

Work flexibly :

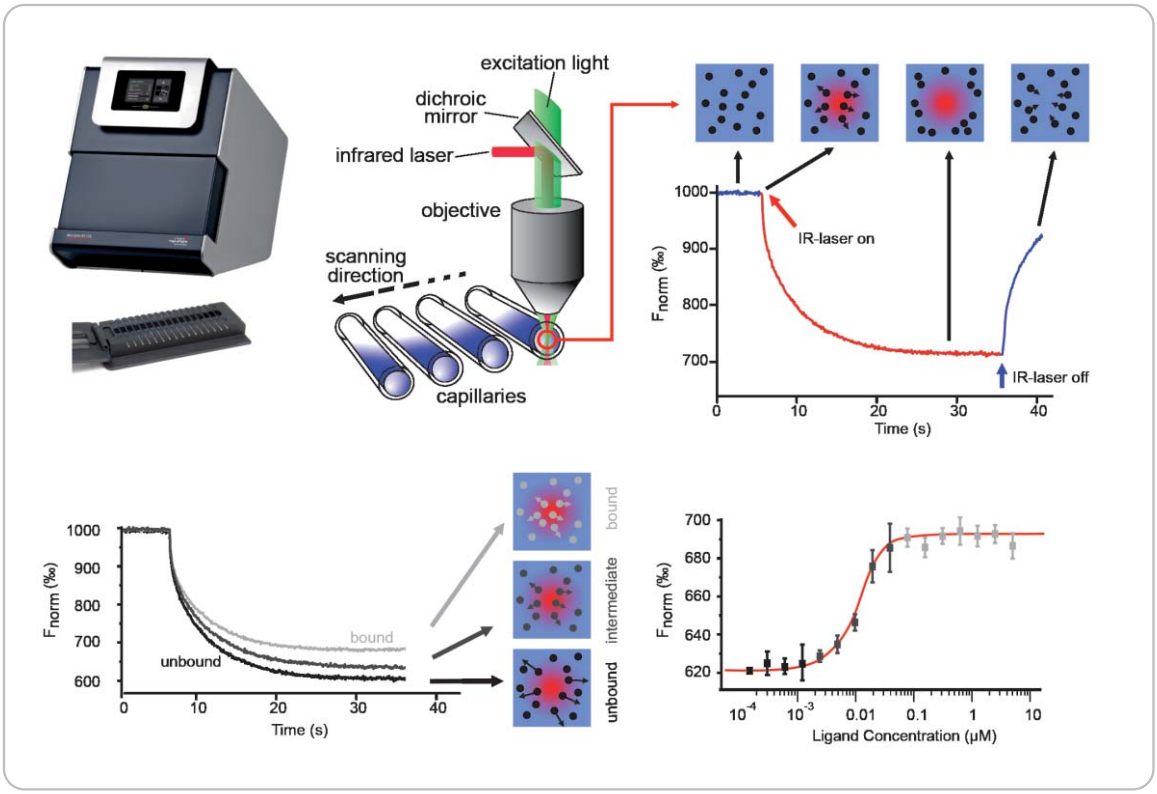
▶ K_d s for all molecular weights from ions to ribosomes and for pM to mM binding affinities

MicroScale Thermophoresis

A powerful technique

MicroScale Thermophoresis(MST)는 biomolecular interactions을 측정할 수 있는 빠르고 정확한 방법입니다. MST는 온도변화를 주었을 때 온도 차이에 따라 분자들이 이동하며, 분자들의 bound와 unbound 구간의 분자움직임 차이를 읽어 냅니다.

MST 수행을 위해 infrared laser를 이용하여 온도를 변화 (Microscopic temperature gradient) 시키며, 분자들의 직접적인 움직임은 공유결합된 dye, fluorescent fusion proteins, 또는 intrinsic fluorescence (LabelFree)의 형광을 이용하여 검출, 정량합니다.

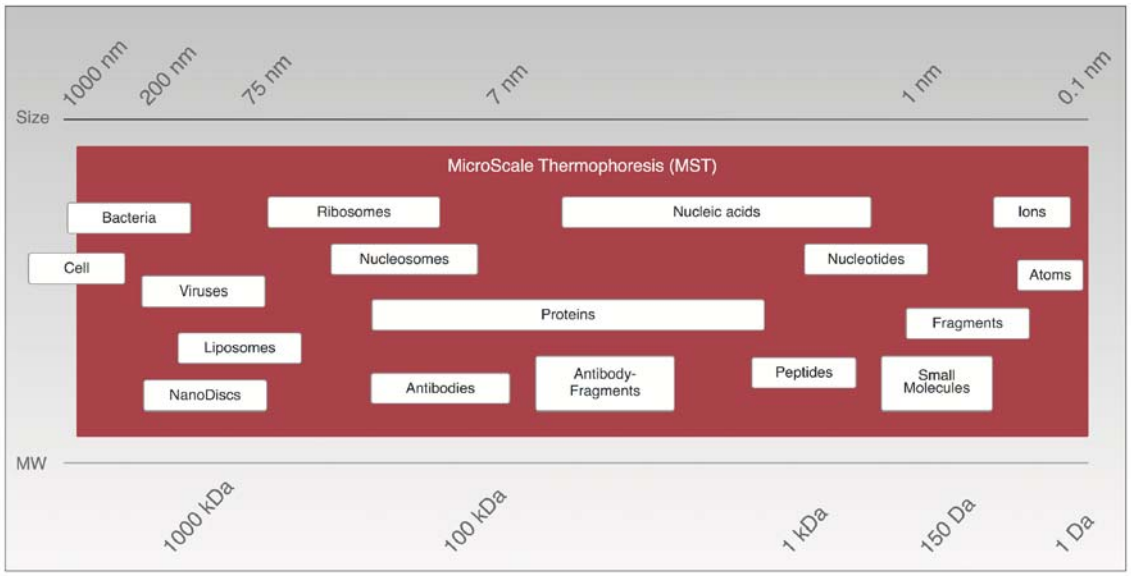


Wide Application Range

MicroScale Thermophoresis는 ion이나 small molecules부터 high molecular weight, multi-protein complex까지 다양한 종류의 biomolecules interactions의 측정이 가능합니다.

온도 변화에 따라 분자가 이동하는 원리인 Thermophoresis는 size나 charge의 변화 뿐 아니라 hydration shell의 변화에도 영향을 받습니다. 그러므로 binding event는 complex 형태에서 size나 mass의 변화가 없는 구조변화도 측정이 가능합니다.

MST는 surface immobilization 이 필요없으므로 liposomes, nanodiscs 또는 membrane proteins과 같은 크기가 크거나 민감한 분자를 이용하여서도 측정이 가능합니다.



Monolith® NT.115 Series
Product Information

Monolith® Instruments for MicroScale Thermophoresis

Monolith® NT.115 and NT.115^{Pico}

The NT.115 Series는 fluorescent dyes나 fluorescent fusion proteins (GFP, RFP 등)의 검출을 이용하여 biomolecular interaction을 측정할 수 있습니다.

- Immobilization–free affinity determination from 1 pM to mM
- Broad application range
- Buffer independency : including serum or cell lysate
- Purification free : fluorescent fusion proteins

Cat #	Instrument	Channel 1	Channel 2	Affinity Range (K_d)	Sample Consumption (per K_d)
G006	NT.115^{Pico}	Pico – RED	–	1 pM to mM	120 pg[1]
G007	NT.115^{Pico} Blue/Green	Nano – BLUE	Nano – GREEN	1 nM to mM	120 ng[2]
G008	NT.115 Blue/Red	Nano – BLUE	Nano – RED	1 nM to mM	120 ng[2]
G009	NT.115 Green/Red	Nano – GREEN	Nano – RED	1 nM to mM	120 ng[2]

[1] calculated for a standard protein of 50 kDa, 12 data points per K_d and 10 pM fluorescently labeled protein.

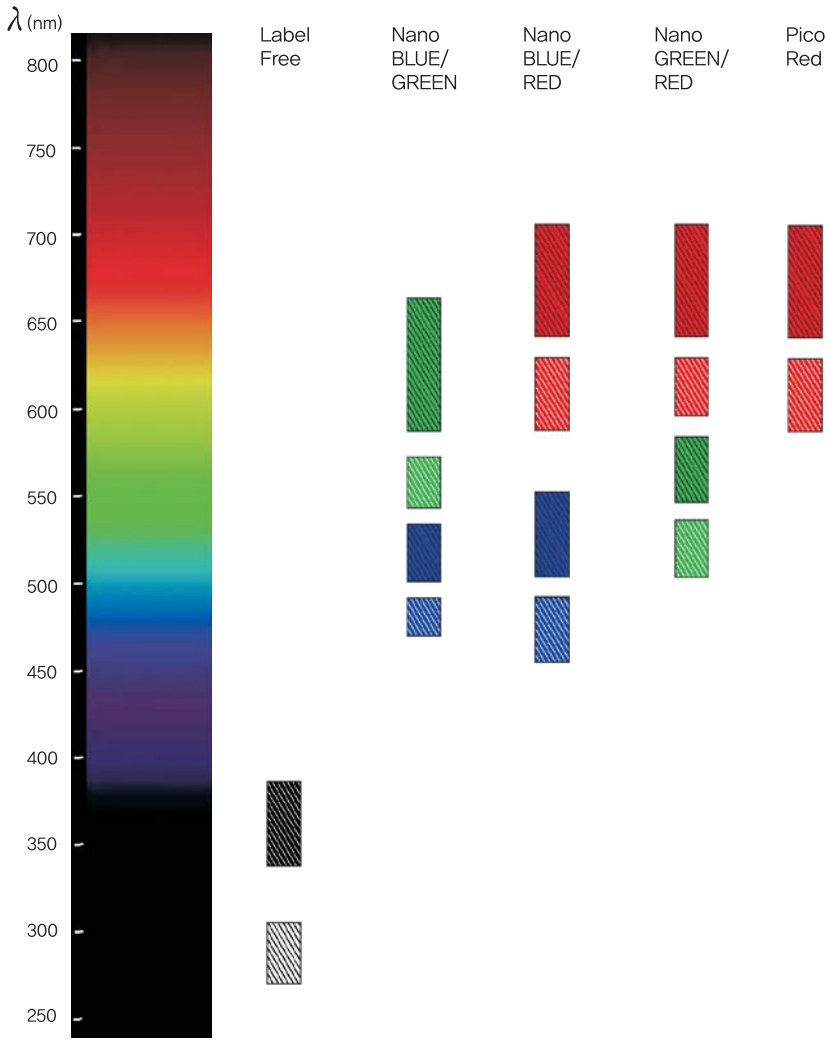
[2] calculated for a standard protein of 50 kDa, 12 data points per K_d and 10 nM fluorescently labeled protein.

Technical Details

Monolith Instruments NT.115Series	NT.115	NT.115Pico
Samples per run	16 samples	16 samples
Fluorescence channels per instrument	2 (BLUE, RED or GREEN)	1 (RED)
Affinity range	1 nM to mM	1 pM to mM
Labeling required	Yes	Yes
Concentration of fluorescent molecule	10^{-9} – 10^{-3} M	10^{-11} – 10^{-3} M
Range of accessible interactions	■ ■ ■ ■ ■	■ ■ ■ ■ ■
Biophysical parameters	Affinity, Stoichiometry, Enthalpy	Affinity, Stoichiometry, Enthalpy
Tryptophan fluorescence required	No	No
Measurements in complex bioliquids (serum, cell lysate)	Yes	Yes
Volume per measurement	〈 4㎕	〈 4㎕
Molecular weight range (Da)	10^{1} – 10^{7}	10^{1} – 10^{7}
Time for experiment & analysis	Minutes	Minutes
Immobilization required	No	No
Temperature controlled	22 – 45℃	22 – 45℃
Maintenance required	No	No

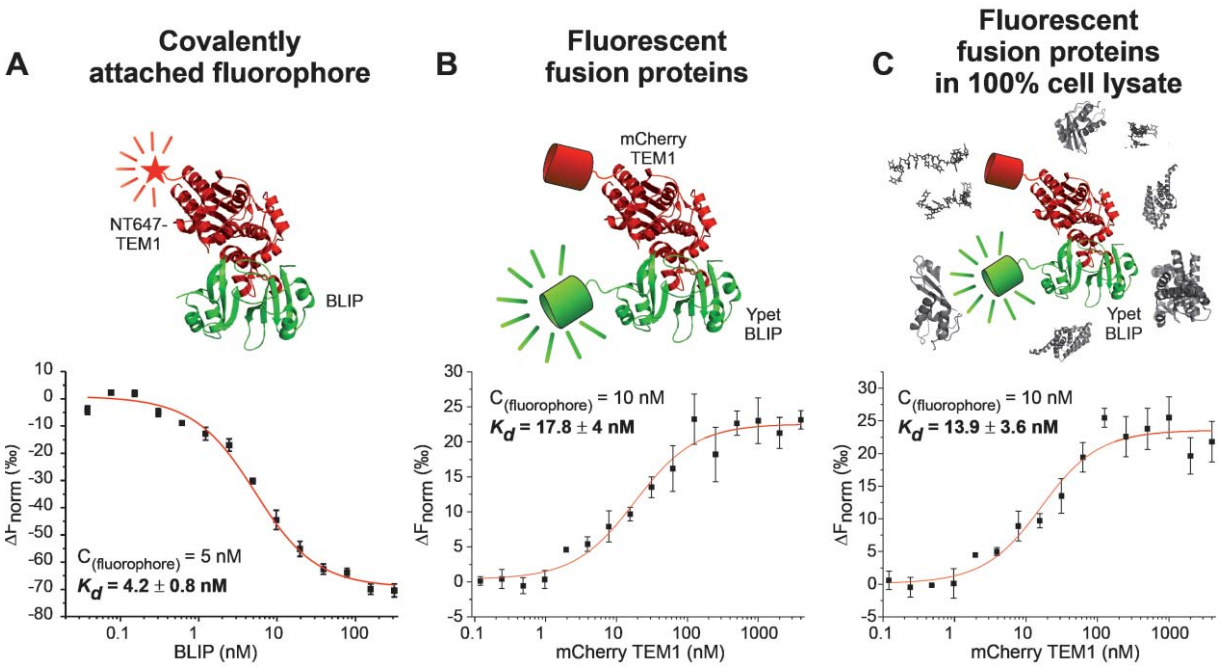
Detectors and Spectra

The excitation/emission wavelengths for the corresponding Monolith NT.115 Series detectors are illustrated below.



The light and dark colored boxes correspond to the excitation and emission wavelength spectra, respectively, of the different fluorescence detectors.

Protein–Protein Interaction



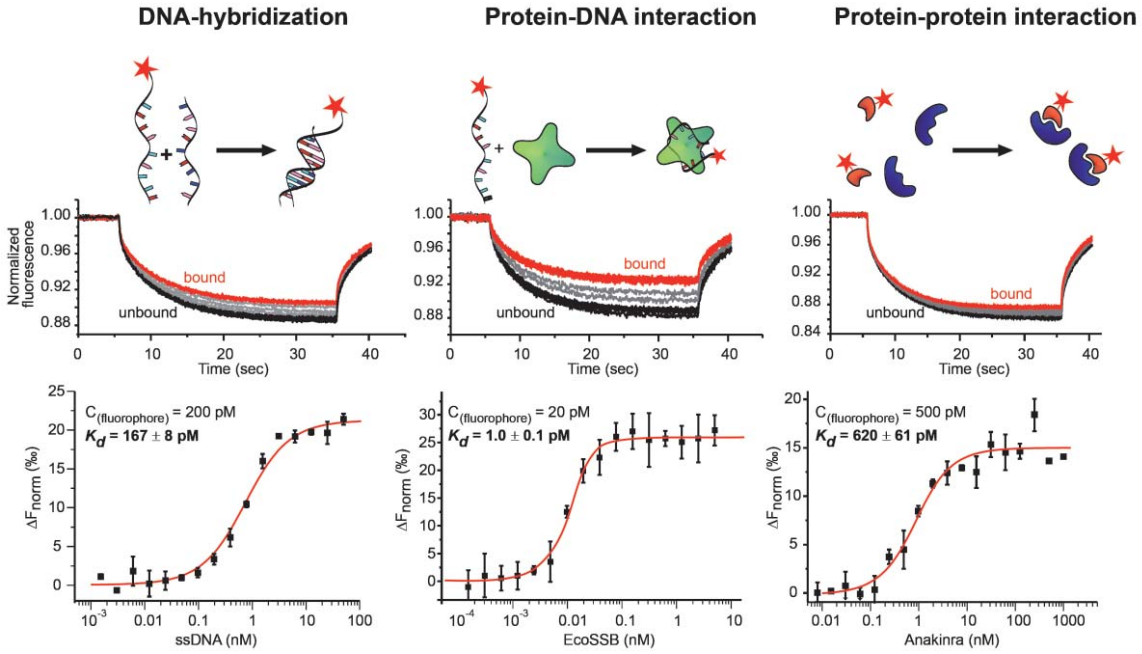
Thermophoretic analysis of a protein–protein interaction

This interaction was investigated in a purified system using a covalently attached fluorophore (A) and using fluorescent fusion proteins in buffer (B) as well as in pure eukaryotic cell lysate (C). Comparable binding affinities were determined for this interaction in all three setups.

Material was kindly provided by Prof. Gideon Schreiber, Weizmann Institute, Israel

Jerabek–Willemsen, M., Andre, T., Wanner, R., Roth, H. M., Duhr, S., Baaske, P., and Breitsprecher, D. (2014) MicroScale Thermophoresis: Interaction analysis and beyond. Journal of Molecular Structure

High–Affinity Interactions



Detection of picomolar K_ds of different experimental systems using the Monolith NT.115^{Pico}.

DNA–DNA interaction (left). The hybridization of a 16mer with a complementary Cy5–labeled DNA strand was monitored with MST. The change in MST signals was fitted (redline) to yield a K_d of 167 ± 8 pM.

Protein–DNA interaction (center). Association of single strand binding protein from E. coli (EcoSSB) with a Cy5–labeled oligo–nucleotide (dT)70 was monitored by titrating EcoSSB against 20 pM Cy5–labeled DNA. Changes in thermophoresis were plotted, yielding a K_d of 1 ± 0.1 pM.

Protein–protein interaction (right). Native Anakinra was titrated against NT647–labeled, soluble fragment of the Interleukin–1 receptor. Plotting of the change in thermophoresis and concomitant fitting of the data yielded a K_d of 620 ± 61 pM.

Material was kindly provided by Dr. Ute Curth, Medical Unitersity Hanover, and Dr. Ahmed Besheer, Novartis, Basel.

Jerabek–Willemsen, M., Andre, T., Wanner, R., Roth, H. M., Duhr, S., Baaske, P., and Breitsprecher, D. (2014) MicroScale Thermophoresis: Interaction analysis and beyond. Journal of Molecular Structure

Monolith NT.LabelFree
Product Information

Monolith® Instruments
for MicroScale Thermophoresis

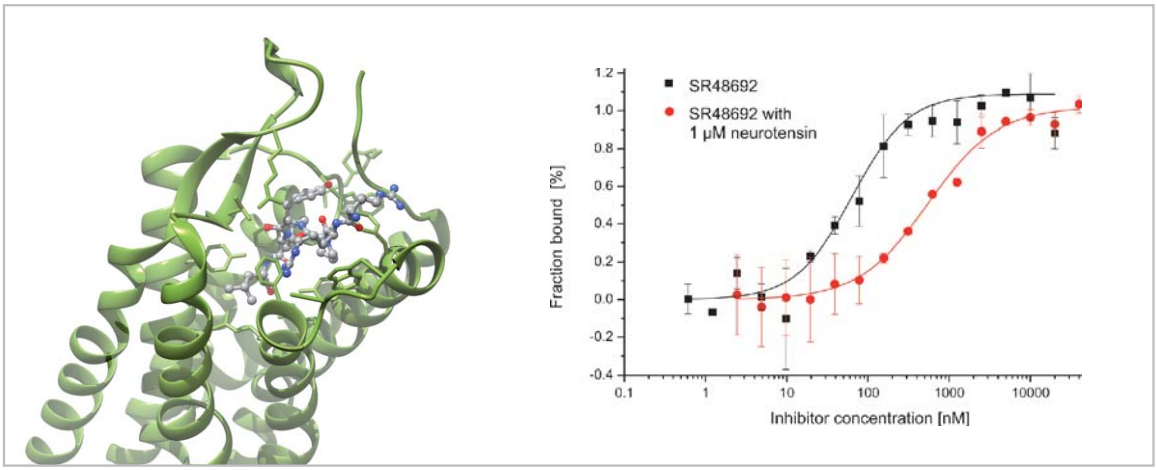
Product Details

The Monolith NT.LabelFree 는 intrinsic tryptophan fluorescence [280 nm (ex) and 360 nm (em)]를 이용하여MST를 측정하므로 label이 필요없으며 membrane protein과 같은 어려운 샘플에 매우 유용한 방법입니다.
NT.LabelFree는 단백질과 small molecules (fragments, inhibitors 또는 ions)의 binding을 측정하기 위한 매우 sensitive한 장비입니다. (* protein–protein 측정은 불가능)

Monolith Instruments NT.Series	NT.115
Samples per run	16 samples
Fluorescence channels per instrument	1 (UV)
Fluorescence multiplexing	No
Affinity range	10 nM to mM
Labeling required	No
Concentration of fluorescent molecule	10^{-8} – 10^{-3} M
Sample consumption	1.2μg[1]
Biophysical parameters	Affinity, Stoichiometry, Enthalpy
Tryptophan fluorescence required	Yes
Measurements in complex bioliquids (serum, cell lysate)	〈 4μℓ
Volume per measurement	No
Molecular weight range (Da)	10^{1} – 10^{7} Da
Time for experiment & analysis	Minutes
Immobilization required	No
Temperature controlled	22 – 45℃
Maintenance required	No

[1]calculated for a standard protein of 50 kDa, 12 data points per Kd and 100 nM tryptophan–containing protein.

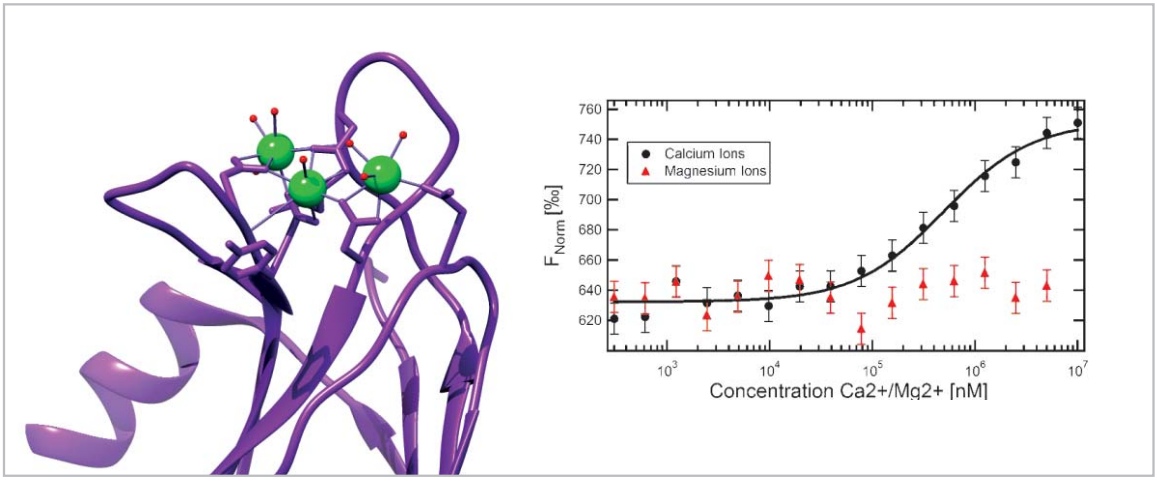
Protein – Small Molecule Interaction



MST binding experiment of a GPCR Receptor binding to an inhibitor in the presence and absence of the natural ligand Neurotensin.

Prof. Anthony Watts, University of Oxford, Biochemistry, UK

Seidel et al, Angewandte Chemie, 2012



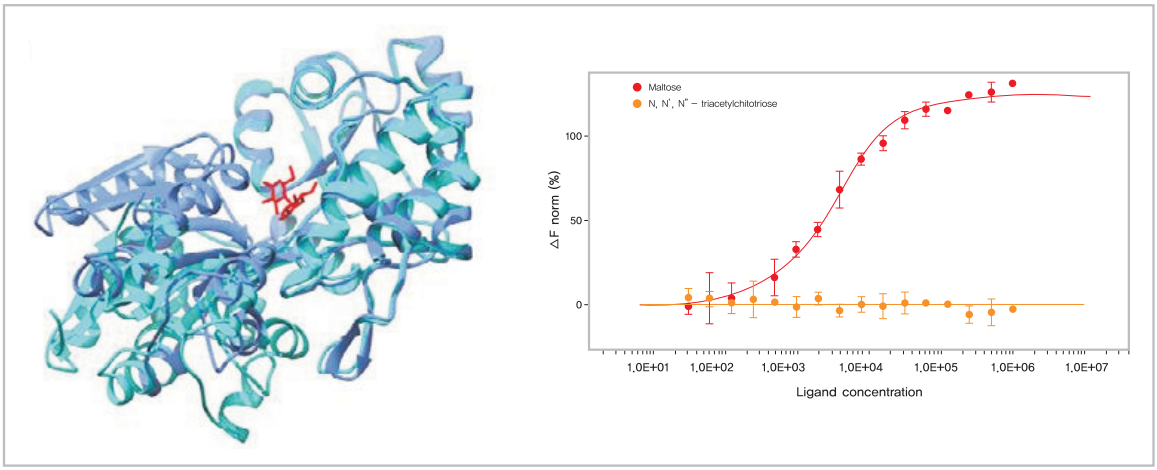
MST binding experiment of Synaptotagmin, a calcium sensor, to divalent cations.

Karsten Meyenberg1 & Geert van den Bogaart 2,

1 Institut fur Organische und Biomolekulare Chemie, University Gottingen, Germany

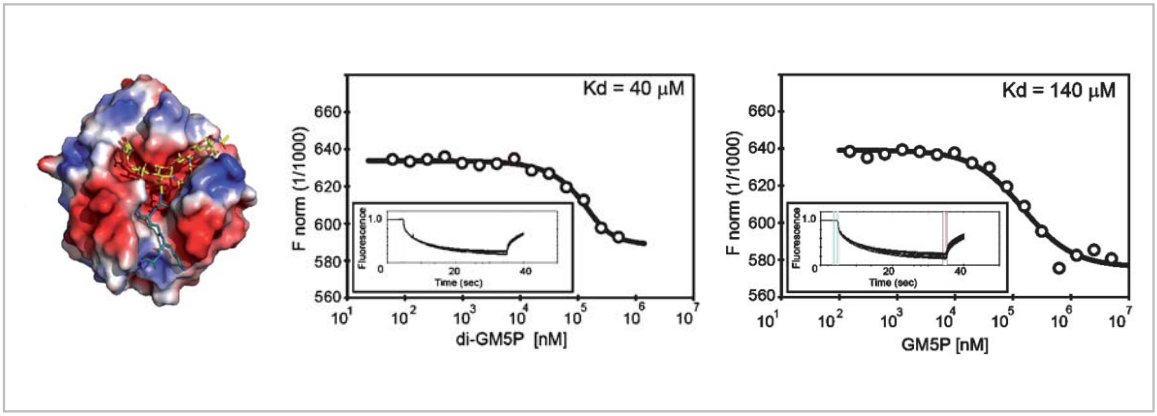
2 Max Planck Institute for Biophysical Chemistry, Gottingen, Germany

Protein – Sugar Interaction



MST experiment of maltose binding protein (MBP) binding to the sugars maltose and N, N', N" – triacetylchitotriose.

MBP was kindly provided by Susanna v. Gronau and Dr. Sabine Suppmann from the biochemistry core facility of the Max Planck Institute of Biochemistry, Martinsried.



Interaction of Lytic Amidase LytA with two different muropeptides

Mellroth, P., Sandalova, T., Kikhney, A., Vilaplana, F., Hesek, D., Lee, M., Mobashery, S., Normark, S., Svergun, D., Henriques–Normark, B., and Achour, A. (2014) Structural and Functional Insights into Peptidoglycan Access for the Lytic Amidase LytA of Streptococcus pneumoniae. MBio 5(1); doi:10.1128/mBio.01120–13.

amended with permission from the American Society for Microbiology

Prometheus® NT.48

Product Information

Prometheus Instruments®
for nanoDSF®

Prometheus® NT.48

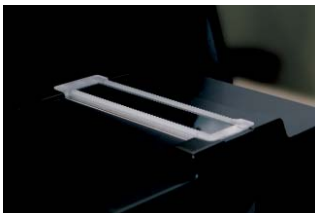
Prometheus 는 nanoDSF technology를 이용하여 쉽고, 빠르게 protein stability와 aggregation을 측정할 수 있으며, 한번에 48개의 sample을 *90분안에 분석할 수 있는 High-throughput 장비입니다. Protein engineering, membrane protein research, formulation development 그리고 quality control와 같은 application에서 사용 가능합니다.

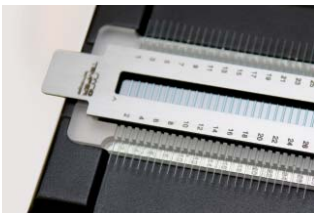
*온도 설정에 따라 시간은 단축 또는 연장 될 수 있습니다.

Enjoy the benefits of nanoDSF :

- Measure thermal and chemical stability – even for membrane proteins
- See more transitions – ultra-high resolution for antibody engineering
- Detect aggregates – long-term stability and storage of biologics
- Exploit the concentration range – for formulation of biopharmaceuticals
- Integrate into robotic platforms – for fully automated operation

Prometheus NT.48



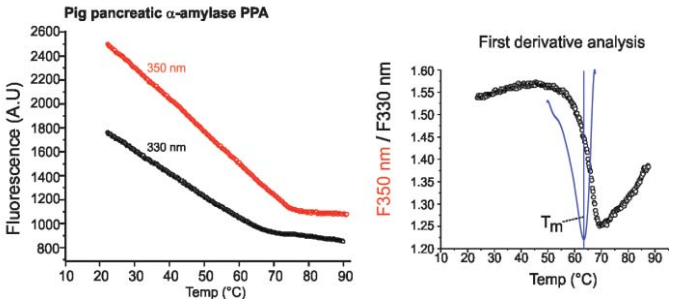


NanoDSF®

A technology by NanoTemper®

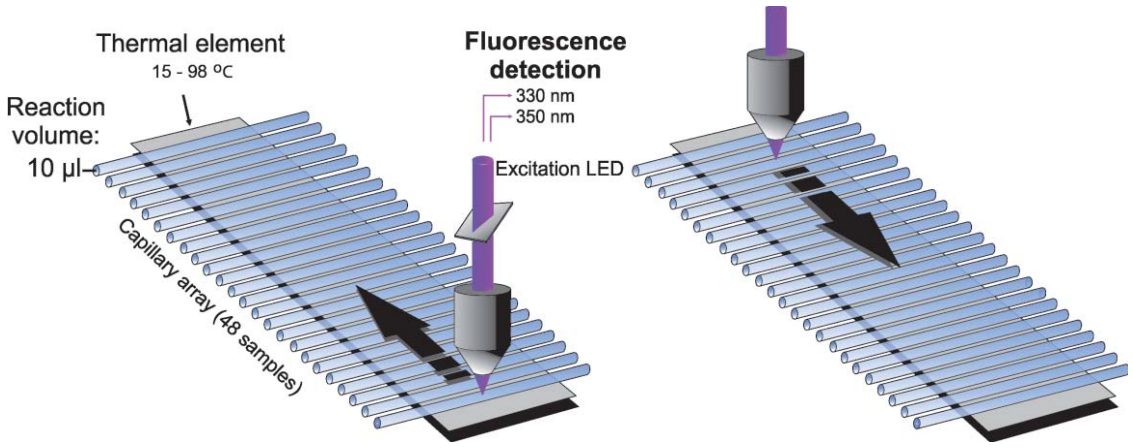
nanoDSF 는 advanced Differential Scanning Fluorimetry technology 입니다.
이 기술은 대부분의 단백질에 있는 tryptophan과 tyrosine 형광을 이용하며, 매우 작은 변화도 검출할 수 있습니다.

단백질에 있는 tryptophan과 tyrosine의 형광은 구조 또는 환경에 의해 영향을 많이 받습니다. 이러한 형광의 변화를 측정함으로써 labeling 과정 없이 protein 자체를 이용하여 chemical 그리고 thermal stability를 측정할 수 있습니다.



Thermal unfolding with nanoDSF
Two wavelenghts, 330 and 350 nm are recorded. The ratio of the two wavelengths is plotted against the temperature. The 350/330 nm ratio typically yields well-defined transitions, even if the single wavelengths do not exhibit a clear unfolding transition.

Dual-UV technology를 이용하여 빠른 형광 측정이 가능하며, 빠른 scanning speed와 data density를 제공하여 한번에 48개의 sample를 측정할 수 있습니다.



Conventional DSF와 다르게 secondary reporter fluorophores가 필요 없기 때문에 protein solutions은 buffer 조성과 상관없이 분석가능하며, 농도는 5 µ g/ml ~250 mg/ml까지 측정 가능합니다. 따라서 고농도의 antibody formulation 뿐만 아니라 detergent-solubilized membrane protein의 분석도 가능합니다.

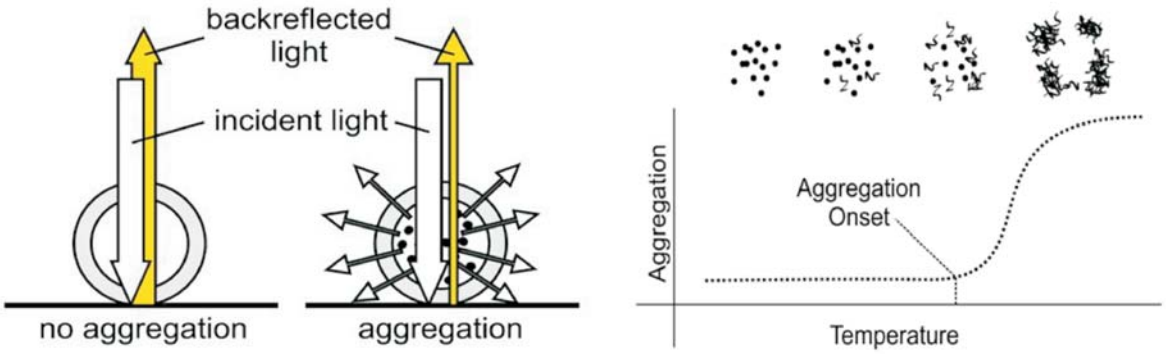
Optional Feature : Backreflection Optics

A technology by NanoTemper®

NanoTemper Technologies는 항체와 같은 단백질의 aggregation을 검출하기 위해 Backreflection optics기술을 발전시켰습니다.

검출 방법은 aggregation이 particle에 의해 빛의 산란을 야기시킨다는 것에 기초를 두었습니다. Prometheus series의 high precision capillary format과 automatic internal referencing을 이용하여 aggregation의 재현성과 감도는 일반적인 분석법보다 매우 우수합니다. Simultaneous dual-UV fluorescence detection의 high data point density를 유지하여 data quality의 변함 없이 protein unfolding을 함께 확인할 수 있습니다.

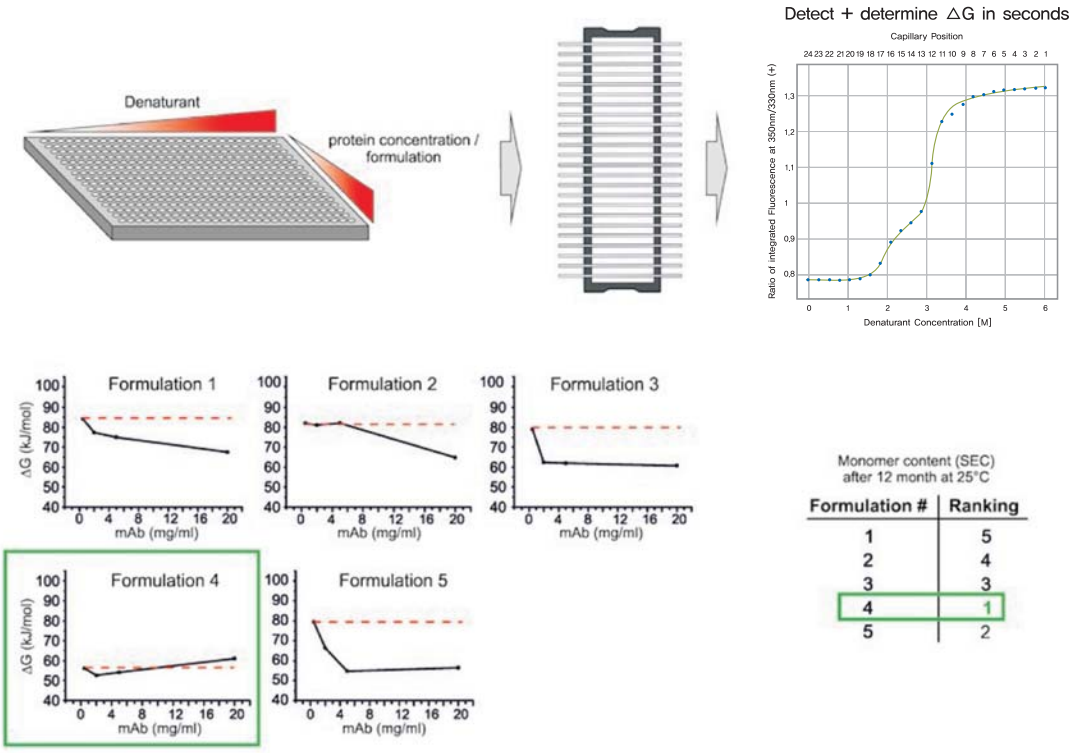
Thermal stability detection과 aggregation onset temperatures의 결합은 formulation screening이나 protein engineering project와 같은 연구에서 기존의 방법에 대비하여 더 많은 정보를 가진 분석을 가능하게 합니다.



Chemical Unfolding in Antibody Development

The Prometheus software package detects chemical unfolding and determines ⊿G within seconds. Since concentration-dependence of ⊿G is a measure for aggregation propensity, nanoDSF is able to predict long-term stability of biopharmaceuticals in different formulations.

The fully automated nanoDSF solution with the Prometheus NT.Plex allows for unattended measurement of hundreds of chemical unfolding reactions per day.



Monomer content (SEC) after 12 month at 25°C

Formulation #	Ranking
1	5
2	4
3	3
4	1
5	2

Determine ⊿G of folding to predict aggregation propensities

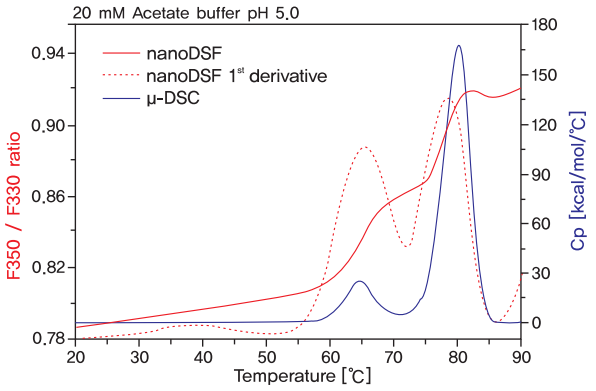
Upper panel: Schematic representation of chemical unfolding experiments. Denaturant dilutions in formulation buffer are prepared in 384-well MTPs, and loaded into Prometheus capillary chips. Chemical denaturation curves are recorded and analyzed automatically in seconds.

Lower panel: Concentration-dependence of ⊿G of a mAb in different formulations. A decrease in ⊿G indicates high aggregation propensity of the unfolded state. Formulation 4 shows a constant ⊿G, and also shows the highest monomer content after 12 months at 25℃ by HPSEC.

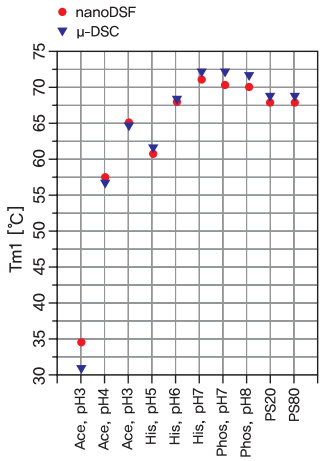
Comparison: nanoDSF and µDSC

A collaborative study with a leading CRO demonstrates that nanoDSF overcomes critical drawbacks associated with µ DSC since it provides perfect ease of use, is 100 times faster and requires 40 times less sample.

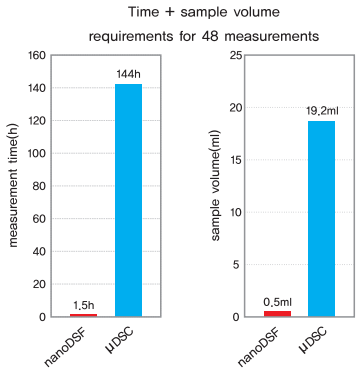
A



B



C



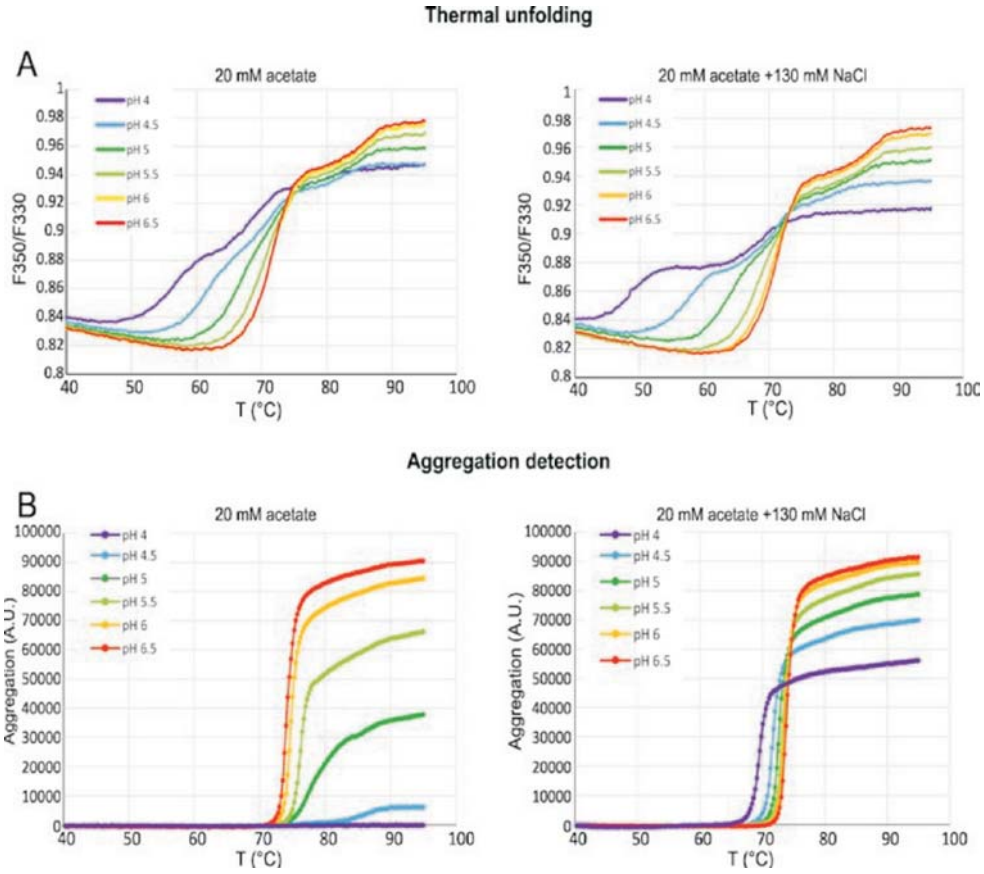
D

	µDSC	nanoDSF
Drawbacks / limitations	▪ sample and time consumption. ▪ high protein concentrations. ▪ incompatile detergents. ▪ aggregation. ▪ equilibration + maintenance. ▪ sample preparation required	proteins without aromatic amino acids.
Major benefits	▪ high resolution of all transitions. ▪ thermodynamics. ▪ (optional) full automation.	▪ parallel measurement of 48 samples. ▪ low sample consumption. ▪ high reproducibility. ▪ high resolution of transitions. ▪ calibration + maintenance free. ▪ no buffer restrictions. ▪ (optional) simultaneous detection of aggregation.

Both nanoDSF and µDSC generate precise and highly comparable T_m values (Figure A and B) in a small formulation screen using a commercially available therapeutic mAb. A total of ten different formulations with varying buffers and pH–values were tested in addition to polysorbate 20 and 80, which are common surfactants in mAb formulations, but preclude the analysis by orthogonal fluorescence methods such as DSF assays.

The integrated nanoDSF protocol and innovative capillary sample format of the Prometheus NT.48 overcomes many key limitations of µDSC (Figure C and D). In addition to its speed, precision and throughput, nanoDSF is a robust method that does not require cumbersome instrument maintenance and time–consuming sample preparation such as dialysis or filtration. Therefore, the Prometheus NT.48 is the ideal instrument for rapid and precise thermal stability screening in biopharmaceutical development.

Thermal and Colloidal Stability of Antibodies

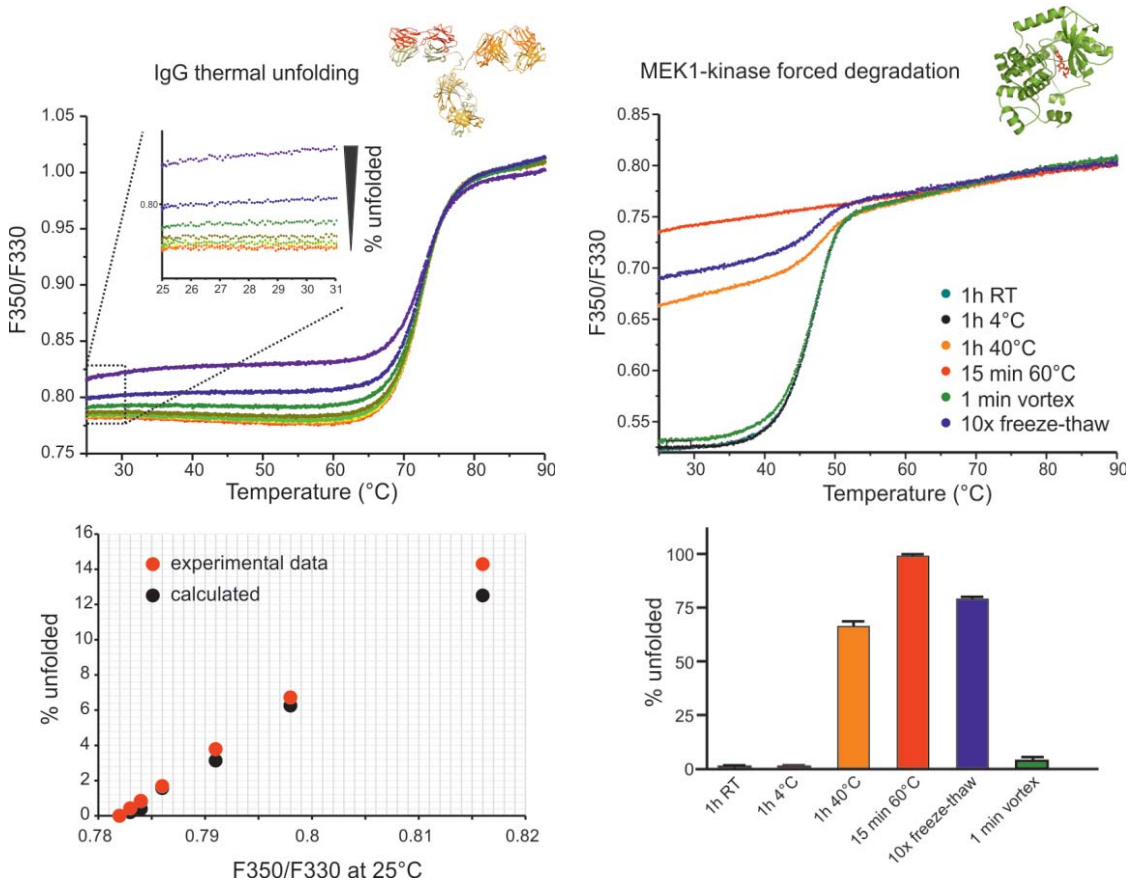


Conformational stability and aggregation of a monoclonal antibody (mAb) under different buffer conditions.
(A)Thermal unfolding monitored by detection of shifts in the fluorescence ratio (F350/F330) in dependence of different buffer pH values and NaCl concentrations.
(B)Aggregation detected by changes in backreflection.

The Prometheus NT.48 delivers high quality thermal unfolding data for antibody buffer screening campaigns and detects unfolding of single antibody domains (A). The large dynamic range of the Prometheus NT.48 allows for analyzing thermal unfolding in solutions containing antibody concentrations between 250 mg/ml down to few μ g/ml. In addition, the colloidal stability (B) and aggregation onset temperatures (C) of antibodies can be assessed simultaneously employing the backreflection optics.

The results from our parallel investigation of thermal unfolding and aggregation using the Prometheus NT.48 suggest that a slight thermal instability might be acceptable and even favourable for long-term stability of the antibody, due to reduced aggregation under these conditions (D). Future screening approaches could therefore be designed t o find excipients which thermally stabilize the mAb at lower pH values while maintaining low aggregation of the unfolded state.

Quality Control



Establishing a protein unfolding standard.
Unfolded IgG at different concentrations was mixed with folded IgG and subjected to thermal unfolding. The percentage of unfolded IgG in the solution was quantified based on the F350/F330 ratio measured at 25°C.

Forced-degradation stress-test on MEK1
MEK1 protein was subjected to the indicated stresses, and the fraction of unfolded protein was calculated based on the F350/F330 ratio at 25°C. Error bars are s.d. from three measurements.

nanoDSF can be employed to quickly detect and quantify unfolded proteins for quality control purposes with unmatched speed, at the same time offering unique ease of use.

The presented quality control experiments can be performed by filling capillaries directly from stock solutions without laborious sample preparation. F350/F330 values of up to 48 samples are then recorded in parallel using a one-button routine, providing stability data within seconds.

nano TEMPER technologies

동영과학(주)
DONGYOUNG

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