Monolith X

Made for affinity characterization.
Built for confidence.

Monolith X is a biophysical platform designed to perform precise measurements of a wide range of biomolecular interactions free in-solution, in any buffer, using orders of magnitude less sample than other technologies. Featuring dual technologies, Spectral Shift and Temperature Related Intensity Change (TRIC), researchers can accurately characterize interactions between proteins, nucleic acids, small molecules, PROTACs, receptors, and other biomolecules. The system's intuitive software and user-friendly interface enable easy setup, data collection, and binding analysis, allowing anyone in academia or industry to innovate and accelerate biotherapeutic discoveries.

Figure 1. Monolith X

Key benefits

Flexibility: Study diverse interactions including : proteins, nucleic acids, small molecules, peptides, and even complex mixtures. Perform in-solution, equilibrium interaction measurements which enable purification-free binding affinity determination.

Efficiency: Measure the dissociation constant (Kd) in less than 10 minutes, using only 10 µl of target - orders of magnitude less sample than other techniques. Use just picomoles of target sample without immobilization on costly biosensors

User-friendliness: Generate meaningful results after only one day of start-up and training. With a user-friendly interface and an intuitive software, adopt a technology accessible to users of varying expertise levels. This reduces the learning curve and increases productivity in the lab.

System components

The Monolith X system comprises a benchtop instrument, a laptop, and a single intuitive software suite for data acquisition and analysis. Capillaries containing 10 µL of sample volume are loaded via a capillary tray gate in the front; this easy-to-load capillary-based system minimizes system maintenance and user error by eliminating the need for fluidics.

Characterization technology

Monolith X is powered by two biophysical characterization technologies to quantify molecular interactions between a fluorescently labeled biomolecule (target) and an unlabeled binding partner (ligand): Spectral Shift[2] and Temperature Related Intensity Change (TRIC). Employing dual technologies in the same instrument provides for the broadest range of measurements and offers additional structural insights based on the acquired data.

Spectral Shift

Monolith X Spectral Shift Technology harnesses the ability to detect very subtle fluorescence wavelength shifts upon binding events. The label has its own unique emission spectrum driven largely by the polarity of the environment; upon ligand binding, the polarity of the fluorescent label changes resulting in a shift of the emission spectrum that can be detected down picometer resolution. The carefully designed optics measure fluorescence at 650 and 670nm, then the ratio of 670/650nm is plotted versus the ligand concentration. This ratiometric measurement is used to derive the affinity constant (K_d) which is automatically determined at the end of each run without any additional data analysis.

Spectral Shift is a rapid, isothermal measurement that is non-destructive; this permits sample re-analysis, temperature-sensitive studies, and studies of time-sensitive molecules that destabilize quickly or require long incubation periods. The highly sensitive and precise detection allows researchers to work with small amounts of labeled target and even suboptimal samples containing aggregation or precipitation

Temperature Related Intensity Change (TRIC)

TRIC is an advancement on NanoTemper's long utilized Microscale Thermophoresis (MST) technology and is prepared and measured using the same samples as Spectral Shift during a single experiment. The TRIC technology applies a brief, precise laser-induced temperature change to the sample, creating two fluorescence intensity readings: one at the set temperature of the device, and another at a slightly elevated temperature, allowing for a ratiometric analysis of the fluorescence intensity change. By using the information from these two readings, fluorescence variations from pipetting errors are eliminated, enabling a more accurate analysis of the effects on the fluorescence intensity caused by ligand binding, such as those resulting from changes in the target's shape or flexibility. The Kd is then calculated by plotting the change in fluorescence against ligand concentration. Beyond Kd determination, TRIC is also sensitive to the presence of aggregation, providing additional insights regarding sample quality.

[2] Langer, A., Bartoschik, T., Cehlar, O., Duhr, S., Baaske, P., & Streicher, W. (2022). A new spectral shiftbased method to characterize molecular interactions. Assay and Drug Development Technologies, 20(2), 83-94. https://doi.org/10.1089/adt.2021.133s

Software

The Monolith X's software consists of an integrated control and analysis module, designed with an intuitive user interface that minimizes the time required for experimental set up and analysis. Such a straightforward, single-platform system allows for installation and training to be completed in just one day, with extensive support information easily accessible at support.nanotempertech.com.

Monolith Control (MO.Control)

The control software enables design and execution of binding affinity experiments. This includes assay setup, optimization, single-dose, or dose-response experiments with just a few clicks. The software allows to test up to 6 different buffer conditions in less than 10 minutes.

Figure 2. Dose Response Curve: The Dose Response analysis displays concentration dependent changes of Ratio 670/650 nm, which are used to calculate K_d or EC_{50} values. The signal of each capillary is plotted against the ligand concentration in the capillary

MO.Control is built to support the user — not only does it provide step-by-step experimental planning and assay setup guidelines, but it also provides immediate feedback on assay optimization based on the results. In addition, data can be easily exported, data sets can be merged and grouped for comparison purposes. MO.Control automatically generates publication quality data including binding curves with error bars.

Figure 3. MO.Control software can manage multiple files for review of large data sets. Replicates can be merged.

Consumables

Capillaries

Employing single-use capillaries provides several advantages and offers maximum flexibility for the Spectral Shift and TRIC measurements. Cross-contamination from re-use of sampling vessels or costly chips is avoided and no cleaning is required; even highly viscous samples can be easily loaded into the capillaries for more challenging measurements in a variety of solutions or serums. Two different capillary types are available for use with Monolith X: Standard grade, made of borosilicate glass, and Premium grade, made of glass with a polymer coating to prevent protein adsorption.

Figure 4. Easy sample introduction with capillaries.

Premium capillaries are used for targets that have a propensity to adhere to standard glass. They provide results with less background noise than standard capillaries.

Capillary chips

For convenient and fast sample loading directly from 384-well microtiter plates, Capillary chips, with 24 individual capillaries mounted on a frame spaced to fit into wells, can be utilized. For additional convenience, a Capillary chip filling station can be used.

Labeling Kits

One of the binding partners, the "target", must be fluorescently labeled to measure the Spectral Shift and TRIC signals in the Monolith X. NanoTemper Technologies offers labeling kits with different coupling chemistries, such as covalent, affinity-based, site-specific, or amino acid-specific. The fluorophores used in the kits have been specifically engineered to detect subtle changes in the chemical environment, enabling sensitive and reproducible measurements.

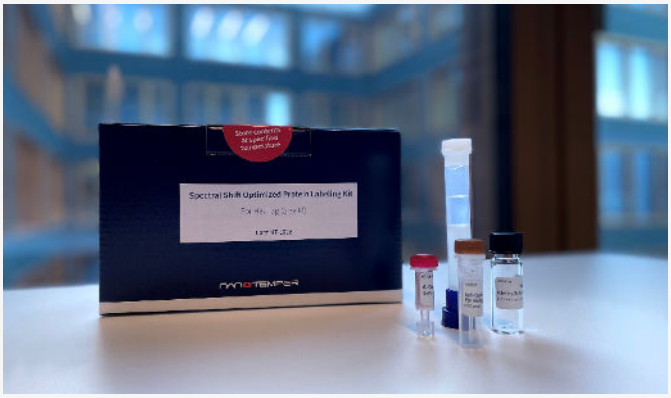

Figure 5. Easy-to-use labelling kits from NanoTemper support your success.

Applications

Monolith X can measure a broad spectrum of biomolecular interactions, detecting binding events in-solution, at equilibrium, and in a manner that is massindependent, eliminating complications associated with size differences between interacting partners. Integrating two complementary technologies—Spectral Shift and TRIC within a single device, Monolith X enables the evaluation of (i) sample quality, identifying issues such as aggregation, adsorption, and fluorescence inhomogeneity, (ii) qualitative characterization of molecular interactions in a single-dose measurement, and (iii) quantitative assessment in a dose-response assay, covering a detectable affinity range from weak (mM) to tight (nM) binders. Examples of use cases are seen below, with in-depth applications found online in our resource center, under the Monolith tab: resources.nanotempertech.com/application-notes

1. Small molecule binding interactions

Characterize interactions between small molecules, proteins and nucleic acids.

- Study small molecule interactions with proteins, antibodies, or peptides across a broad dynamic range, from nM to mM.
- Characterize interactions of RNAs to ligands such as small molecules, proteins and peptides.
- Investigate weak binders and assess low Dalton-sized compounds, e.g., fragments or ions, problematic for other biophysical methods.
- Measure covalent inhibitor binding at equilibrium via direct biophysical readout, eliminating surface regeneration issues.

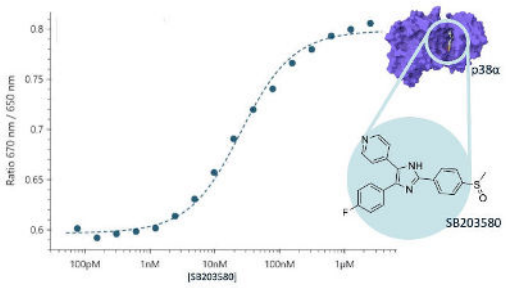

Figure 6. Small molecule SB203580 titrated against labelled p38α (MAPK14), K_d = 21.6nM.

2. Characterization of membrane proteins

Characterize interactions between membrane proteins (including GPCRs) and a binding partner.

- Study membrane protein interactions when solubilized in detergents or within synthetic membrane models such as nanodiscs.
- Work with minimal sample amount: 10ul of target at low nM concentrations.
- Measure unpurified proteins in complex matrices such as lysates.

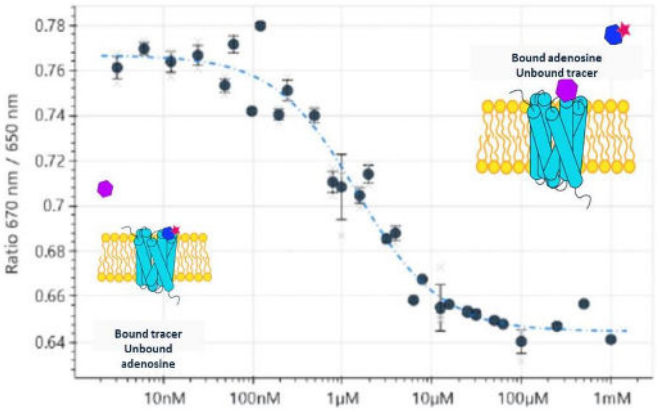

Figure 7. Interaction between A2A membrane extract and fluorescent tracer CELT-300 (EC_{50} = 1.46+/- 0.26 µM).

3. Additional Applications - Basic Research

- **Purification-free binding affinity determination:**

  Characterize molecular interactions in crude lysate to maintain nearphysiological conditions, including natural ligands, substrates and ions.

- **Bivalent analytes:**

  Analyze heterobifunctional degraders (molecular glues, PROTACs) for binary/ ternary complex affinities, cooperativity, and hook effects.

- **Intrinsically disordered proteins (IDPs):**

  Preserve IDP conformational flexibility with in-solution measurements for accurate binding data.

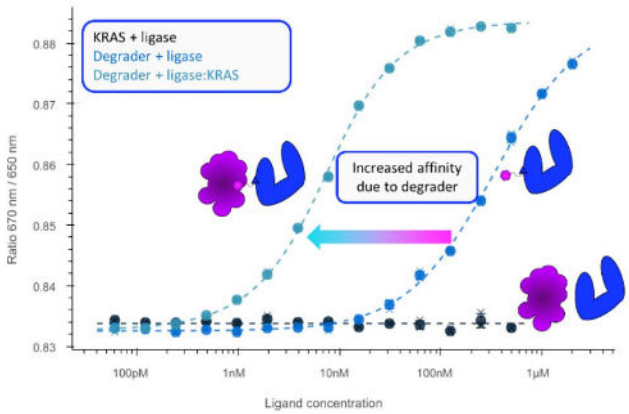

Figure 8. PROTAC targeting KRAS shows a positive cooperativity value as indicated by increased affinity of the ternary complex.

Specifications

General specifications

Time it takes to get a K_d	2 min with Spectral Shift ~5.5 min with Spectral Shift and TRIC
Dynamic Range	1 nM - mM
Molecular Weight Range	10^1 -10^7 Daltons
Sample Volume Required (per Capillary)	10 µL
Capillaries per Run	Up to 24
Temperature Control	20-40 °C ± 0.5 °C (active control)
Fluorescence wavelengths	Excitation: 592 nm Emission: 650 & 670 nm
Dimensions	Width: 36 cm Height: 40 cm Depth: 58 cm
Weight	27 kg

Computer requirements

Operating system	Windows 10 64 bit or higher, English language
CPU	12th Gen Intel Core i5 or better
RAM	≥8 GB
Hard drive	≥60 GB free disk space
Display resolution	1920 x 1080 or better
Software	Microsoft.NET 4.7.0 & Microsoft.NET Core 3.1
Network	1000 Mbps Ethernet connection

Compliance

Compliant with	CE, CB, NRTL/UL, CSA
Safety	IEC 61010-1:2010/AMD1 :2016 Part 1, IEC 61010-2-010:2019 Part 2-010, IEC 60825-1:2014, 21 CFR 1040.10 and 1040.11[3]
Electromagnetic compatibility (EMC)	IEC 61326-1:2012 EMC IEC 61000-3-2:2006 EMC IEC 61000-3-3:2008
Overvoltage category	CAT I
Laser classification	Laser Product Class I
Environmental	Pollution degree 2

Ordering information

Product	Code
Monolith X *incl. Dell Mobile Precision Workstation, SW MO.Control*	MO-G039
Standard capillaries	MO-K02
Premium capillaries	MO-K25
Premium capillary chips	MO-MK025
Capillary chip filling station	NT-AT100
Spectral Shift Optimized Protein Labeling Kit — For His-Tag (Size M)	NT-L028
Spectral Shift Optimized Protein Labeling Kit — Lysine-Reactive (Size L)	NT-L021
Spectral Shift Optimized Protein Labeling Kit — Cysteine-Reactive (Size L)	NT-L024
Biotinylated Target Labeling Kit	NT-L020
His-Tag Labeling Kit RED-tris-NTA 2nd Generation	MO-L018
Protein Labeling Kit RED-NHS 2nd Generation	MO-L011
Protein Labeling Kit RED MALEIMIDE 2nd Generation	MO-L014
SNAP-Tag® Labeling Kit RED 2nd Generation	MO-L019
Human Fc Labeling kit	NT-L030

Note: all NanoTemper labeling kits are compatible with the Monolith X, Dianthus, and Dianthus uHTS.

Scan the QR code to open the Monolith product page.
nanotempertech.com/monolith

For local office contact information, visit
nanotempertech.com/offices

[3] Exception: conformance with IEC 60825-1 Ed 3, as described in Laser Notice No 56, May 8, 2019.