Monolith X

Made for affinity characterization.
Built for confidence.

Monolith[1] X is a biophysical tool designed to perform precise measurements of biomolecular interactions free in solution. Monolith X is suited for interaction characterization of a wide range of biomolecules. The system's intuitive software and user-friendly interface enable easy setup and data analysis, streamlined workflows and enhanced productivity. Whether in academic research labs or pharmaceutical companies, Monolith X offers reliable and reproducible results, helping scientists to better develop innovative therapies.

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 (K_d) 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 single software suite for control and analysis, and single-use capillaries for sample loading. No fluidics are needed, which minimizes system maintenance. The system is operated from an interactive touchscreen and a laptop. The samples are introduced via a capillary tray gate in the front.

Characterization technologies

Monolith X is powered by two biophysical characterization technologies: Spectral Shift[2] and Temperature Related Intensity Change (TRIC). With Spectral Shift and TRIC in the same instrument, you have two modalities that can complement each other, allowing for additional insight into the quality of your sample such as aggregation.

Spectral Shift

Monolith X uses Spectral Shift Technology to quantify molecular interactions between a fluorescently labeled target molecule (target) and an unlabeled binding partner (ligand). Monolith X optics detect binding events by monitoring subtle shifts in the emission wavelength of a fluorescent probe down to picometer resolution, which occurs largely due to changes in the polarity of the environment that surrounds a fluorophore. Monolith X records fluorescence at 650 and 670 nm, then the ratio of 670/650 nm is plotted versus 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 or lengthy data analysis.

Spectral Shift is a rapid, isothermal measurement, it is non-destructive permitting sample re-analysis, temperature-sensitive studies and studies of sensitive molecules that destabilize quickly. The highly sensitive and robust detection allows work with small amounts of labeled target and suboptimal samples containing aggregation or precipitation.

Temperature Related Intensity Change (TRIC)

Much like Spectral Shift Technology, TRIC is an advancement on Microscale Thermophoresis (MST) technology. TRIC measurements are prepared and taken using the same samples as Spectral Shift. During a TRIC experiment, a brief, precise laser-induced temperature change is applied to the sample. This creates two fluorescence intensity readings—one at the set temperature of the device and another one at a slightly elevated temperature—allowing for a ratiometric analysis of the data. 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 K_d is then calculated by plotting the change in fluorescence against ligand concentration. Beyond K_d 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 shift-based 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 system software consists of an integrated Control and Analysis module. The modules are designed with intuitive user interfaces that minimize time required for experimental set up and analysis. System installation and introductory training is performed in just one day. Extensive supporting information is found online 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 Kd or EC50 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

The use of single-use capillaries provides several advantages and offers maximum flexibility for the assay. Cross-contamination from re-used sample vessels is avoided and no cleaning is required. Even highly viscous samples can be measured with capillaries. NanoTemper's capillaries are manufactured with high precision which allows determination of pm resolution in Spectral Shift.

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, can be used. The capillaries in the chip are spaced to fit with the wells in the plate. For additional convenience, a Capillary chip filling station is available as an option.

Labeling Kits

One of the interacting partners must be fluorescently labeled. 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

The Monolith™ X system is a powerful tool for measuring a broad spectrum of biomolecular interactions, detecting binding events in solution and at equilibrium. Its mass-independent affinity determination eliminates complications associated with size differences between interacting partners. Monolith X enhances efficiency through the integration of two complementary technologies—Spectral Shift and TRIC—within a single device. The system enables the evaluation of sample quality, identifying issues such as aggregates, adsorption, and fluorescence inhomogeneity. Monolith X facilitates qualitative characterization of molecular interactions in a single-dose measurement and allows 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. In-depth application data can be 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.
- Asses covalent inhibitor binding via direct biophysical readout; eliminate surface regeneration issues.

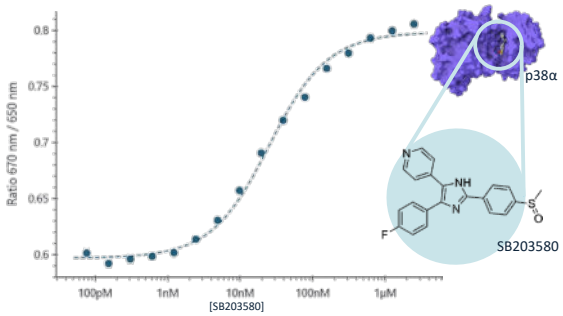

Figure 6. Small molecule SB203580 titrated against labelled p38α (MAPK14), K_d= 21.6nM.

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.

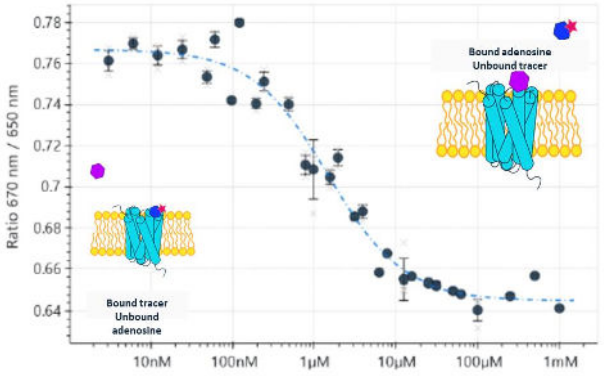

Figure 7. Interaction between A2A membrane extract and fluorescent tracer CELT-300 (EC_{50} = 1.46+/- 0.26 µM).

3. Additional Applications - Basic Research

- **Purification-free binding affinity determination:** Characterize molecular interactions in crude lysate to maintain near-physiological 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.

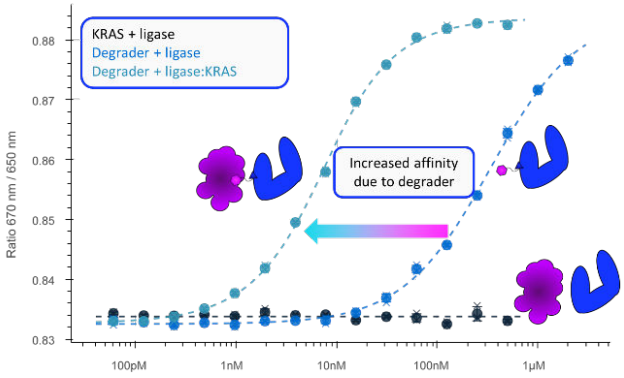

Figure 8. PROTAC targeting KRAS shows a positive cooperativity value as indicated by increased affinity of the ternary complex.

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 wavelenghts	Excitation: 592 nm Emission: 650 & 670 nm
Dimensions	Width: 36 cm x Height: 40 cm x 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

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

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, Dianthus, Dianthus uHTS.

Scan the QR code to open the Monolith product page.

nanotempertech.com/monolith

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