

# Dianthus

Tough targets. Confident decisions.

The Dianthus™ system provides high-confidence, in-solution binding data with the speed needed to advance hit-to-lead workflows and expand access to hard-to-drug targets. The Dianthus  $\alpha$  applications package extends the platform with capabilities such as *Optical Unfolding* and *Slow Kinetics*, giving researchers deeper insight into complex binding behaviours. With a workflow-focused design and 384-well format, the platform adapts easily to diverse targets and modalities in drug discovery.



Figure 1. Dianthus

## Key benefits

**De-risk early development.** Accelerate time to insight by generating a 12-point binding curve in 1 minute or screen 2,000 fragments in 3 hours. Identify aggregation and folding issues early with reliable in-solution data, even from fragile targets. Use as little as 10–20  $\mu\text{L}$  per data point and detect binding at target concentrations down to 250 pM.

**Validate hits with confidence.** With three orthogonal fluorescence methods, achieve reliable hit validation even in assays that challenge biochemical or FRET approaches. Confirm true binders, rule out false negatives, and ensure targets remain properly folded under near-native conditions.

**Address the undruggable.** Work with targets that were once out of reach using a binding site-agnostic approach. Study degrader-induced ternary complexes, characterise covalent modifications, and resolve slow binding events with measurable kinetics of  $k_{\text{obs}} \leq 1 \times 10^{-2} \text{ s}^{-1}$ .

## System components

The Dianthus system consists of a benchtop instrument and software for control and analysis, with a Dianthus  $\alpha$  applications package that extends its functionality. Samples are introduced through a plate gate at the front and controlled via a dedicated computer. The system has no fluidics, pumps, or valves and includes active temperature control between 20–25 °C.

Dianthus can operate as a standalone instrument or be integrated into automation systems using the gRPC framework. The Dianthus  $\alpha$  applications package adds additional measurement modes, with data analysis carried out through the NanoTemper App Hub.

## Characterization technologies

Dianthus is powered by two biophysical characterisation technologies: Spectral Shift<sup>1</sup> and Temperature-related intensity change (TRIC). Both offer in-solution, equilibrium interaction measurements enabling the study of interactions in near to native conditions, independent of their size and minimising sample buffer restrictions.

### Spectral Shift

Spectral Shift detection quantifies molecular interactions between one binding partner (target) that is labelled with a specific fluorophore optimised to report subtle environmental changes, and another unlabelled binding partner (ligand). Spectral shift detects ligand induced changes in the hydrophobicity of the target biomolecule's surface by measuring picometer shifts in the fluorescence emission at two wavelengths, 650nm and 670nm. A ratiometric signal of the labelled target as a function of ligand concentration is measured to obtain a dose-response curve.

As a rapid, isothermal measurement, it is non-destructive, permitting sample re-analysis, temperature-dependent studies and investigations of fragile molecules that destabilise quickly. The highly sensitive and robust detection allows work with small amounts of labelled target and suboptimal samples containing aggregation or precipitation.

### Additional Spectral Shift capabilities in the Dianthus $\alpha$ package

The Dianthus hardware enables the *Optical Unfolding*<sup>a</sup> application, extending Spectral Shift beyond binding measurements to protein stability. *Optical Unfolding*<sup>a</sup> detects unfolding and early aggregation by heating samples up to 80 °C while monitoring spectral shifts. Unfolding alters the fluorophore's environment, producing a clear sigmoidal curve. Ligand binding shifts this curve, showing stabilisation through non-covalent interactions. Comparing thermal melting profiles with and without ligand enables orthogonal hit confirmation and deeper insight into binding.

### Temperature-related intensity change (TRIC)

TRIC also measures the interaction between the fluorescently labelled target and unlabelled ligand, using the same samples as Spectral Shift. TRIC detects ligand induced changes in the target's shape and structural flexibility by measuring the fluorophore response to a small temperature perturbation. Beyond  $K_d$  determination, TRIC is sensitive to aggregation or compound induced precipitation, providing additional insights into sample quality.

<sup>1</sup> 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>

<sup>a</sup> Features marked with  $\alpha$  are part of the Dianthus  $\alpha$  package.

## Software

Dianthus software combines intuitive control and analysis modules that streamline assay setup, execution, and data interpretation. Single-dose or dose-response experiments can be designed in just a few clicks, while built-in algorithms flag outliers such as precipitation or aggregation. Data exports in standard formats ensure seamless integration into enterprise workflows.

### On device software

#### Dianthus Control

The control software enables easy assay design and execution. It allows for set up of single-dose, or dose-response experiments with just a few clicks. The system can create assay templates or import sample IDs from .xls when working with large datasets. Raw data can also be exported as .json files for data integration into enterprise software solutions for data analysis and automated workflows.

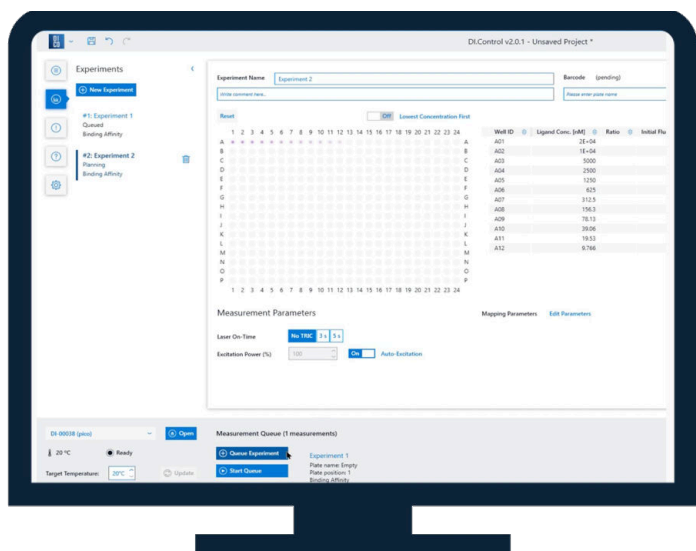


Figure 2. Intuitive experimental set-up with Dianthus Control.

#### Dianthus Screening Analysis

Dianthus Screening Analysis Software provides easy analysis, interpretation and visualisation of data from single-dose screening or dose-response curves using algorithms that also flag outliers, such as precipitation or aggregation. Data can be exported as either .csv or .xlsx for external analyses or formatted for report filing.

#### NanoTemper App Hub<sup>®</sup>

The NanoTemper App Hub is a server-based platform that provides a collection of assay applications and analysis tools. It serves as a central workspace for transforming raw Dianthus data into clear, actionable results with dedicated, easy-to-use apps. Continuously expanding with new applications, it evolves to streamline workflows and enhance data analysis.

The *Optical Unfolding* app analyses thermal unfolding profiles, calculates inflection points, and delivers precise stability and aggregation insights for binder validation.

The *Slow Kinetics* app evaluates fluorescence shifts over time, calculates spectral shift ratios, and extracts kinetic parameters ( $k_{obs}$ ,  $v_i$ ), while supporting analyses such as covalent modification monitoring.

## Consumables

### 384-well microplates

Dianthus uses dedicated 384-well, low profile, black walled, sealable, optically clear, flat-bottomed microplates. This plate design enables direct bottom reading of fluorescence signal avoiding well-to-well crosstalk. The plates are polymer coated to prevent analyte adsorption and are uniquely barcoded for identification. The microplates have a standard SBS format for compatibility with manual or automated liquid handlers, as well as automated plate handling devices.

### Labeling kits



Figure 3. Manual loading of 384-well microplate.

NanoTemper provides a range of kits specifically optimised for the highest sensitivity and optimal signal to noise ratios. The kits are designed for simple and quick labelling of the target binding partner, permitting rapid assay development, optimisation and time to data. The kits contain labelling reagents that attach fluorophores to a specific functional group or fusion tag via covalent or affinity binding. Labelling kits come in multiple size options to accommodate throughput requirements.

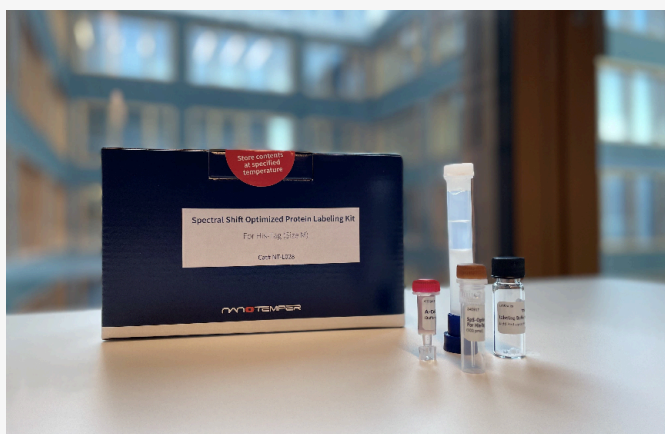


Figure 4. Protein labeling kit.

## Services and training

Service contracts and user trainings are available to support consistent operation and effective use of the platform. Options include extended warranty and service coverage to minimise downtime, as well as training programs delivered online or onsite to ensure users gain proficiency in system operation, assay design and development. Extensive supporting information is provided online at [support.nanotempertech.com](http://support.nanotempertech.com).

# Applications

Get binding data at multiple stages of discovery, from single-point screens and dose-response curves to quantitative  $K_d$  or  $EC_{50}$  values for SAR and hit-to-lead optimisation. In-solution measurements enable studies across a broad range of targets and therapeutic modalities, including those difficult to study with immobilisation, while also providing insight into thermal stability, aggregation, and slow kinetics.

Detailed application data is available at [resources.nanotempertech.com/application-notes](https://resources.nanotempertech.com/application-notes), under the Dianthus tab.

## 1. Hit identification

Adopt Dianthus biophysical technology for affinity-based screening of libraries ranging from hundreds to thousands of compounds, including targeted, focused, and fragment libraries. Achieve reproducible, reliable results, with assays consistently delivering  $Z'$  scores above 0.8, and easily above 0.5 once assay development is completed.

- Measure binding directly for targets lacking functional or biochemical read-outs, e.g., transcription factors or scaffold proteins.
- Monitor target engagement directly, irrespective of ligand binding site where FRET based approaches are unsuitable.
- Investigate very low affinity (mM) interactions and assess low Dalton sized compounds, e.g., fragments or ions, problematic for other biophysical methods.

- Maintain the conformational plasticity of intrinsically disordered proteins (IDPs) by direct in-solution measurement to provide binding studies with data integrity.
- Study membrane protein interactions when solubilised in detergents or within synthetic membrane models such as nanodiscs.

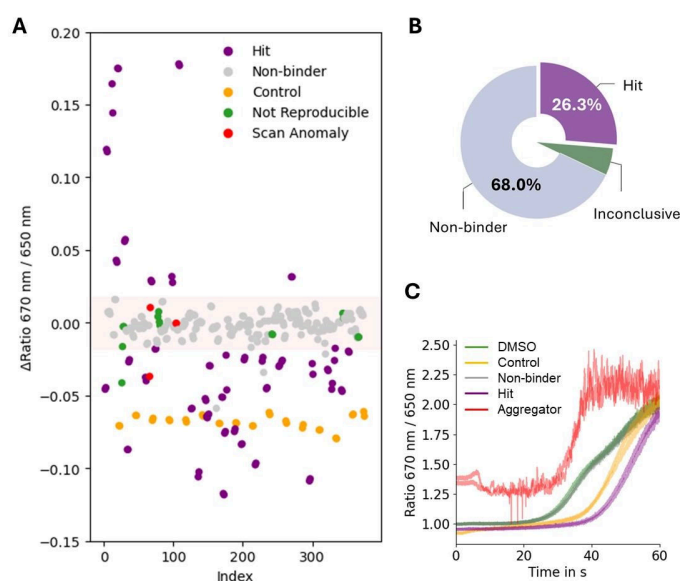


Figure 5. A. Screen results are automatically evaluated, and data plotted to quickly gain insight. B. Summarized data allows for rapid decision making. C. Additional Optical unfolding profiles allow for deeper insights into target-ligand complex stability.

## 2. Hit confirmation and validation

Measure binding affinity curves for a range of biomolecular interactions to help determine Structure-Activity-Relationships (SAR). Enable hit-to-lead workflows and perform affinity-based lead optimisation. Validate hits from primary screens by orthogonal methods.

- Measure dose-response curves for any kind of interaction, regardless of biomolecule classes or buffer condition.
- Determine ligand-induced thermal shifts to orthogonally validate results from single dose screens.
- Understand large multi-complex protein-protein, or protein-nucleic interactions.
- Directly measure interactions between nucleic acids & small molecules. Cy5 can be used for RNA and DNA labeling.

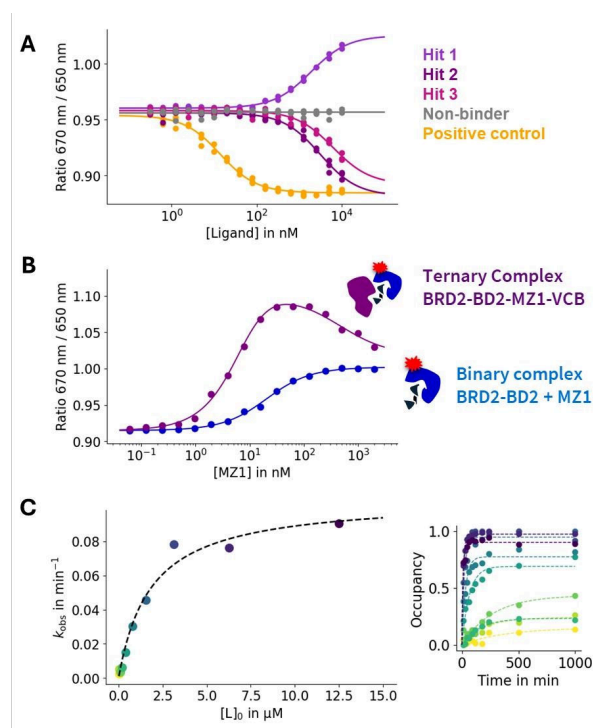


Figure 6. Examples show A. Hit validation B. The binary and ternary affinity measurements involving PROTAC MZ1 and C. The covalent binding of a compound to a target

## 3. Additional applications - research and development

Dianthus is a flexible tool for drug discovery and basic research. Its plate-based measurements and non-invasive Spectral Shift technology enable both simple binding studies and more advanced interaction analyses.

- Address the diverse range of target and ligand molecules, including interactions within complex matrices or samples containing aggregation.
- Provide simple assay development, and rapid buffer condition testing, to swiftly enable both novice or experienced users to obtain data with minimum fuss and avoid issues with sensor maintenance.
- Investigate covalent inhibitor binding directly with a biophysical readout: no surface = no regeneration.
- Analyse proximity inducers, such as heterobifunctional degraders (PROTACs) or molecular glues, for binary/ternary complex affinities, cooperativity, and hook effects. Use binary complex assay conditions to progress to ternary complex measurement
- Displacement assays with site-specific tracer molecules can add functional insights into ligand interactions.

# Specifications

## General specifications

Detection technology	Spectral Shift, Temperature-related intensity change (TRIC)
Information obtained	Single dose ligand categories: binder, non-binder, outlier; Dose-response: $K_d$ , $EC_{50}$ ; Inflection time
Data presentation	Graphics and tables. Data export format: Excel or CSV (manual mode), JSON
Sample type	Small molecules, fragments, ions, heterobifunctional degraders, peptides, proteins, biologics, RNA/DNA
Sample volume	10-20 $\mu$ L / well
Sample format	Black, flat clear bottom, sealable, polystyrene 384-well barcoded plates
Instrument sample capacity	One 384-well plate
Typical run time per plate	33 min (Spectral Shift) 79 min (Spectral shift + TRIC) 3.5 h up to 7 h (Optical Unfolding, for 30 s and 60 s per well respectively)
Time to obtain a $K_d$ (12-point dilution series)	60 s (Spectral Shift); 132 s (Spectral Shift + TRIC)
Reproducibility of affinity measurement (32 biological replicates)	mean $EC_{50}$ = 29 $\mu$ M 95% CI [23.1 $\mu$ M to 36.6 $\mu$ M]
Automation	Integration with liquid handling systems and automation platforms via gRPC framework
Electric	Input Voltage: Single phase AC 100-240 V + 10% Input Current: AC 6-3.2 A Mains frequency: 50/60Hz

## Typical working ranges

Temperature control range	20 – 25 °C
Maximum difference to room temperature	$\pm$ 5 °C
Temperature control accuracy	$\pm$ 0.25 °C
Affinity range	250 pM to mM (Dianthus Pico) 5 nM to mM (Dianthus Nano)
Molecular weight range	101- 107 Da

## Compliance

Safety	IEC 61010-1:2010/AMD1:2016 Part 1 IEC 60825-1:2014 21 CFR 1040.10 and 1040.11 except for conformance with IEC 60825-1 Ed. 3., as described in Laser Notice No. 56, dated May 8, 2019 EMC IEC 61326-1:2021
Over-voltage category	CAT-II
Laser classification	Laser product class I

## Computer requirements

Operating system	Windows 10 64 bit or higher, English language
CPU	12 <sup>th</sup> Gen Intel Core i5 or better
RAM	$\geq$ 8 GB
Hard drive	$\geq$ 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

## Environmental and dimensions

Size	Width: 61cm (24.0") Height: 42cm (16.5") Depth (closed tray): 57cm (22.4") Depth (open tray): 69cm (27.2")
Weight	70kg (154.3 lbs) net
Operating temperature	20 – 30 °C (indoor only)
Pollution degree	2

## Ordering information

Product	Code
Dianthus Pico <i>incl. Control PC and Dianthus Software package (1 license)</i>	DI-011101
Dianthus Nano <i>incl. Control PC and Dianthus Software package (1 license)</i>	DI-011201
Optical Unfolding application <sup>a,2</sup>	DI-061110
Slow Kinetics application <sup>a,2</sup>	DI-061140
Dianthus Microwell plates	DI-P001
Large Vol. Spectral Shift Optimized Protein Labeling Kit – Lysine-Reactive	NT-L121
Large Vol. Spectral Shift Optimized Protein Labeling Kit – Cysteine-Reactive	NT-L124
Large Vol. Spectral Shift Optimized Protein Labeling Kit – For His-Tag	NT-L128
Large Vol. Labeling Kit RED-NHS 2nd Generation	NT-L111
Large Vol. Labeling Kit RED-MALEIMIDE 2nd Generation	NT-L114
Large Vol. His-Tag Labeling Kit RED-tris-NTA 2nd Generation	NT-L118
Large Volume Biotinylated Target Labeling Kit	NT-L120
Large Volume Human Fc Labeling Kit	NT-L130
SNAP-Tag <sup>®</sup> Labeling Kit RED 2nd Generation	MO-L019
Buffer Exploration Kit (96 plates)	NT-B001

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



Scan the QR code to open the Dianthus product page.  
[nanotempertech.com/dianthus](https://nanotempertech.com/dianthus)

<sup>2</sup> Existing Dianthus users can [contact us](#) to explore upgrade options to Dianthus  $\alpha$ .