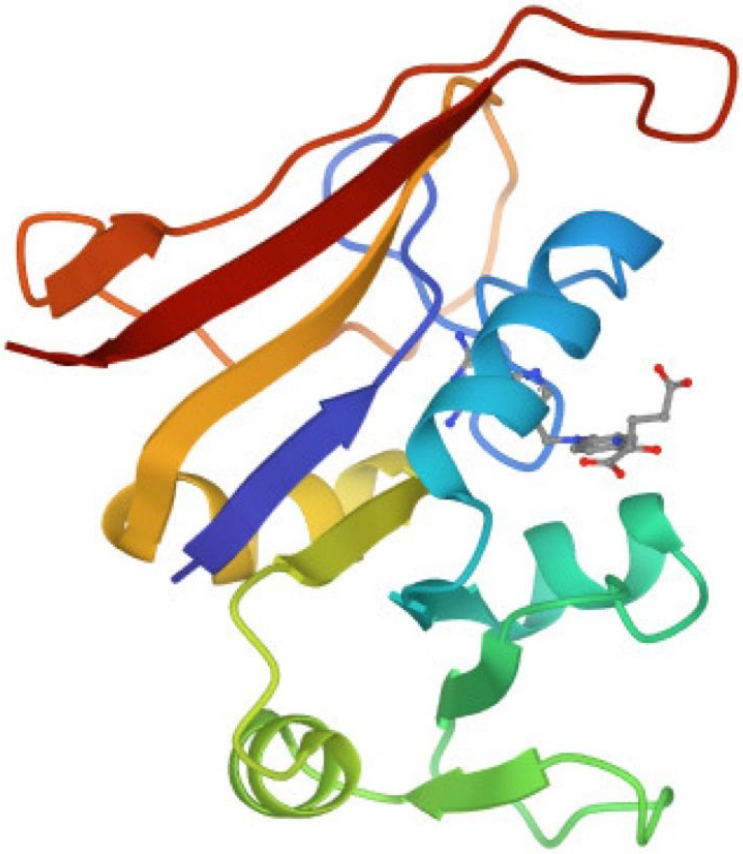
DHFR

Dihydrofolate reductase (Homo sapiens)



Case study

V2.0

About DHFR

Folate metabolism has long been recognized as an important and attractive target for the development of therapeutic agents against bacteria, parasitic infections, and cancer therapy. Dihydrofolate reductase (DHFR) is an essential enzyme, which catalyzes the reduction of dihydrofolate acid (7,8-dihydrofolate, DHF) to tetrahydrofolic acid (5,6,7,8-THF) using reduced nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor.

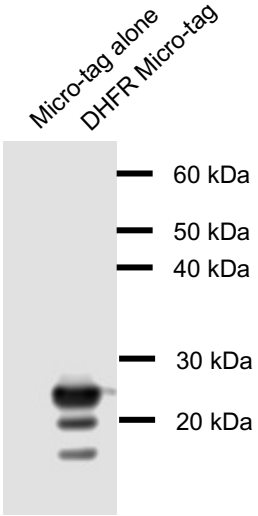
Discovered in 1958, DHFR is an attractive pharmaceutical target for inhibition due to its pivotal role in DNA precursor synthesis. The crucial role of DHFR is related to biosynthesis of thymidylate and purines. Its loss, or inhibition, results in starvation of DNA synthesis precursors. Folate is necessary for cell growth and proliferation. This makes the folate metabolism pathway, and specifically DHFR, an attractive and viable target in developing improved treatments for cancer, improved anti-bacterial agents, and compounds that will prevent parasitic infections.

Therapeutic challenges of DHFR

The crystal structure of DHFR has been reported and several compounds have been identified that bind to DHFR and inhibit its activity. Methotrexate (MTX), a competitive inhibitor of DHFR, is one such anticancer drug that inhibits DHFR. Trimethoprim and pyrimethamine are two other DHFR inhibitor compounds. These three are widely used as antitumor and antimicrobial agents. The drug methotrexate is designed to mimic a folate molecule, so that it will bind in the active site of the enzyme and block its action. Trimethoprim (TMP) has demonstrated activity against a variety of Gram-positive bacterial pathogens.

However, resistance to TMP and other drugs aimed at DHFR can arise due to a variety of mechanisms, limiting the success of their therapeutic use. One mechanism leading to bacterial resistance to drugs such as TMP and methotrexate is through mutations in DHFR. Infections caused by multidrug-resistant bacteria represent a significant global healthcare problem.

Figure 1: Step 1A - Expression test of DHFR in HEK293 cells.



Immunoblot Antibody:
Micro-tag antibody

Targeting DHFR with MICRO-TAG target engagement[2]

Recent advances in drug discovery technologies open up new possibilities to discover more potent and selective inhibitors to DHFR. There is a growing need for such novel therapeutics that will target multiple-drug resistant infections, various cancers and auto-immune diseases such as rheumatoid arthritis.

TMP and other nonclassical antifolates are excellent templates to design new active, both antibacterial and anticancer drugs. Novel artificial intelligence platforms will enable not only optimization of the existing scaffolds, nut also discovery of completely new ones with novel pharmacological properties.

CellarisBio's MICRO-TAG cellular target engagement technology offers a unique way to approach to identify novel therapeutic candidates that directly engage with DHFR within the context of the cell.

The MICRO-TAG system designed to identify direct binders to DHFR has 4 steps:

Step 1: Design a MICRO-TAG reporter for the target.
Step 2: Establish thermal melting profile of the target.

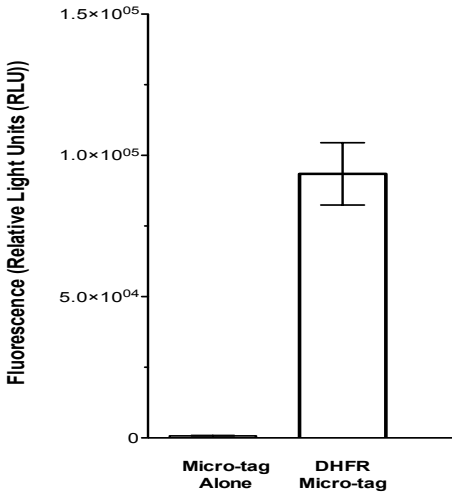
Step 3: Run a pilot test with a reference compound.
Step 4: Scale the system for compound discovery.

Step 1: Design MICRO-TAG reporter system specific for DHFR

DNA construct encoding for DHFR transfected into HEK293 cells and expression of the target was detected, as shown in **Figure 1**.

MICRO-TAG system works on the basis of enzyme complementation that generates fluorescence signal. Hence, following satisfactory expression of the target, enzyme complementation of the MICRO-TAG construct is tested, as shown in **Figure 2**.

Figure 2: Step 1B - Activity of MICRO-TAG target construct for DHFR.



Step 2: Establish the thermal melting profile

Once target expression and enzyme complementation steps are completed, the target in the MICRO-TAG system is tested for thermal profile. Intact HEK293 cells carrying the expression construct are subjected to short thermal gradient ranging typically from 37C to 75C.

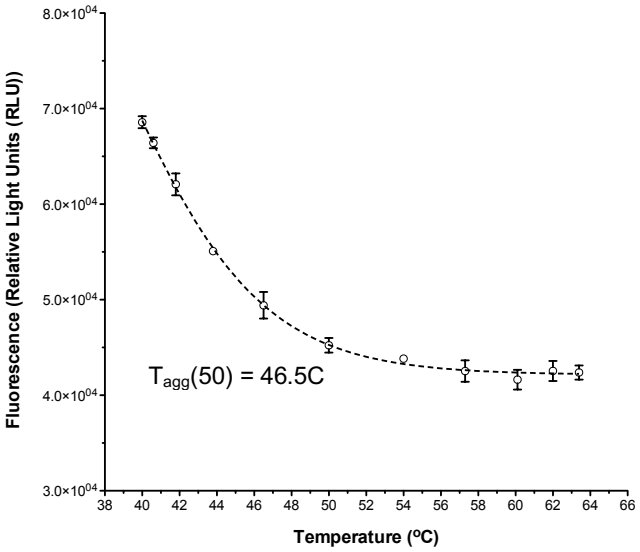
Fluorescence signal originating from DHFR is detected at each temperature point is detected and thermal melting profile of the target is established. This step has to be performed empirically in order to establish the unique fingerprint of the target. The temperature of aggregation (T_{agg}) for DHFR is calculated as 46.5C, as shown in **Figure 3**. At this temperature, the target in the cell is expected to be in half structured and half unwound state.

Step 3: Run a pilot test with a reference compound

Once the thermal melting profile of the target is empirically established, response of the target can be tested in a pilot test using a reference or a tool compound. Reference compounds specific to the target are preferred.

To test response of DHFR, we used the reference compound Methotrexate (MTX). Intact cells carrying MICRO-TAG constructs for DHFR were treated with MTX in dose-dependent manner, then exposed to thermal challenge at 46.5C. The ligand stabilized the target in dose-dependent manner with EC50 of 75nM, as shown in **Figure 4**.

Figure 3: Step 2 – Thermal melting profile of DHFR.



The pilot phase also provides an opportunity to optimize the assay to capture more response from the cellular drug-target engagement.

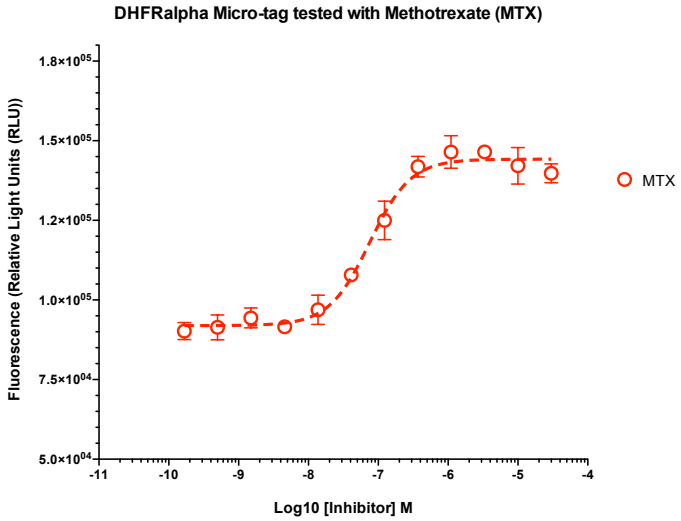
Step 4: Scale the system for compound discovery

Once the pilot step is accomplished, the reporter assay is ready for scale-up. Depending on requirements, there are several options to consider.

Transient expression of the MICRO-TAG target can be used for low-throughput screens aiming at validation of advanced drug candidates. Stable MICRO-TAG reporter assay can be generated using lentiviral knock-in or CRISPR approach. This latter approach is more tuned for high-throughput primary screens aimed at discovering of novel candidates.

In the case of DHFR, we proceeded with transient-expression system and set up a medium-throughput screen for validation of already identified small molecule candidates.

Figure 4: Step 3 – Cell target engagement of reference compound MTX with DHFR.



Accelerate and De-Risk Drug Discovery with CellarisBio

CellarisBio's is a drug discovery technology company, based in San Diego, California. Our MICRO-TAG cell target engagement platform is built to tackle challenging drug targets.

We work across various therapeutic targets classes such as:

- Enzymes
- Membrane proteins
- Transcription factors
- Other challenging proteins

We work with multiple therapeutic modalities:

- Small molecules
- Peptide
- PROTACS
- Antibodies

Accelerate your drug discovery with CellarisBio:

- Discover drug candidates using DNA-Encoded Libraries or plated libraries,
- Validate drug candidates for potency and selectivity,
- Analyze for in-cell kinetics and live-cell imaging.

Some of the drug targets we worked with:

What drug targets are you interested in?

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