Optimization of HTS Assay Development with Integration of SpyTag/SpyCatcher Technology

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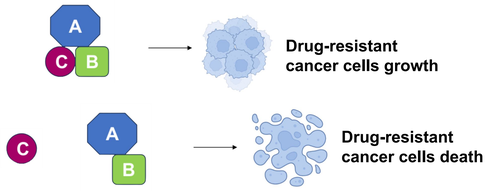
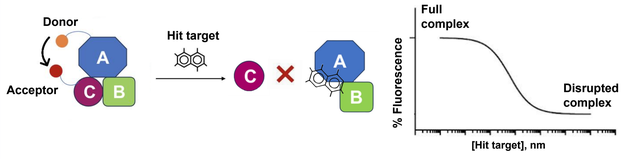
ABSTRACT

The Cancer Dependency Map[1] identified protein A as a synthetic lethal target in drug-resistant cancer cells. We aim to identify small molecules that disrupt the A-B-C complex.



A **Time-resolved Fluorescence Energy Transfer (TR-FRET)** assay was developed to do high-throughput screening (HTS) for hit identification. When protein C is displaced by a small molecule, the signal will decrease.



An ideal labeling strategy would need to bring the donor/acceptor pair close enough to signal, be small enough to minimize background fluorescence, and be cost-effective for HTS. Labeling strategies are used because the donor/acceptor fluorophores cannot bind directly to the proteins.

Past commercially available labeling strategies did not meet these parameters, but **SpyTag/SpyCatcher technology** was made in-house to favorably link protein C and the acceptor (ATTO647) fluorophore. SpyCatcher covalently binds to SpyTag which is linked to protein C, increasing the signal-to-background ratio in the assay.

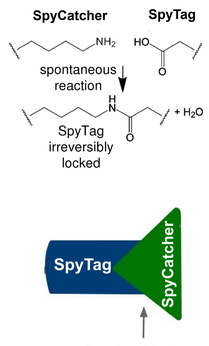
OBJECTIVES

1. **Express, purify and characterize the SpyTag/SpyCatcher technology.**
2. **Optimize the labeling reaction of protein C-SpyTag-SpyCatcher and the ATTO647 acceptor dye for HTS.**

EXPRESSION AND VALIDATION OF SPYTAG/SPYCATCHER

1. How do we characterize the covalent attachment of SpyTag/SpyCatcher to protein C?

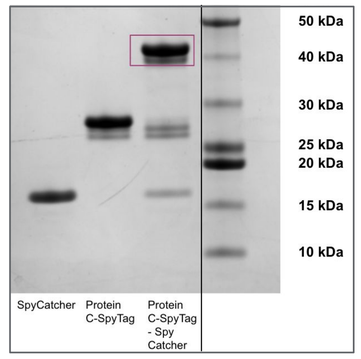
SpyTag/SpyCatcher System[2]



Methods[3]

Covalent binding allows for more consistent dye labeling and stronger TR-FRET signal

Covalent binding between Protein C-SpyTag-SpyCatcher

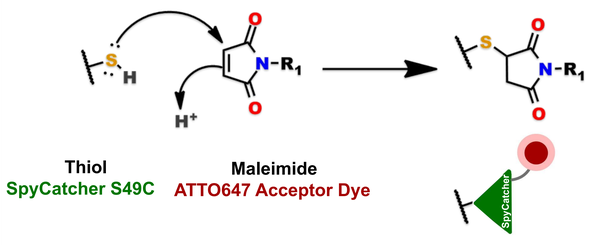


SDS-PAGE gel stained with Coomassie Blue

OPTIMIZATION OF ACCEPTOR DYE LABELING

2. How can we optimize the conditions of ATTO647 labeling to be more time and cost-effective in a high-throughput screening format?

Thiol-Maleimide Reaction[4]

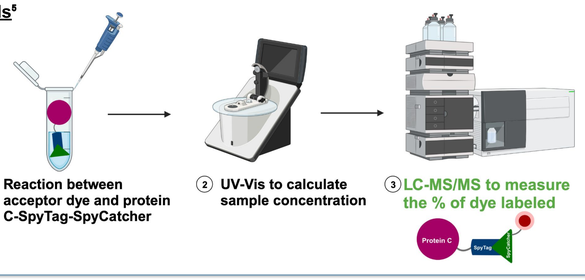


Thiol — SpyCatcher S49C

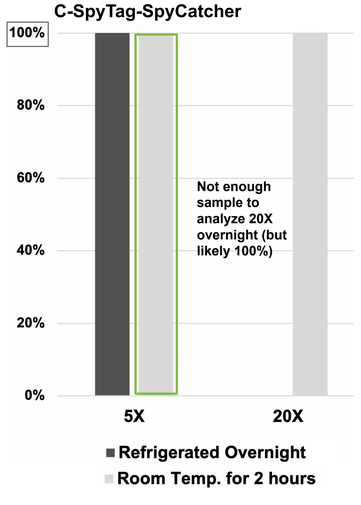
Maleimide — ATTO647 Acceptor Dye

Cysteine mutation (SpyCatcher S49C) allows for a thiol-maleimide reaction, labeling ATTO647 on protein C-SpyTag-SpyCatcher

Methods[5]



ATTO647 Labeling of Protein C-SpyTag-SpyCatcher



Acceptor dye use reduced four fold
Time of reaction reduced by a factor of 8

Data interpreted from LC-MS/MS

FUTURE DIRECTIONS

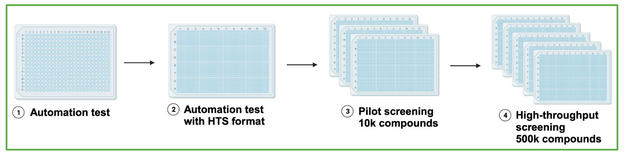
TR-FRET and SpyTag/SpyCatcher technology is effectively utilized in hit identification and optimization.

The next steps in the **cancer drug discovery process** will be applying these techniques to an HTS assay in order to screen 500k compounds.

The TR-FRET assay with its current parameters remains high quality in automation, technology used for high-throughput screening.

This assay optimization will limit the number of molecules screened and reduce false positive hits.

Drug Discovery Process[5]



REFERENCES

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