Biocompare

The Buyer's Guide for Life Scientists

Biochemical and Cell-Based Assays for Targeted Cancer Drug Discovery and Development

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designed for drug discovery screening

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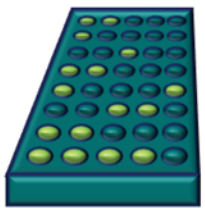

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Targeted Therapeutics for Cancer Treatment

A better understanding of underlying molecular mechanisms of disease is resulting in more specific drugs.

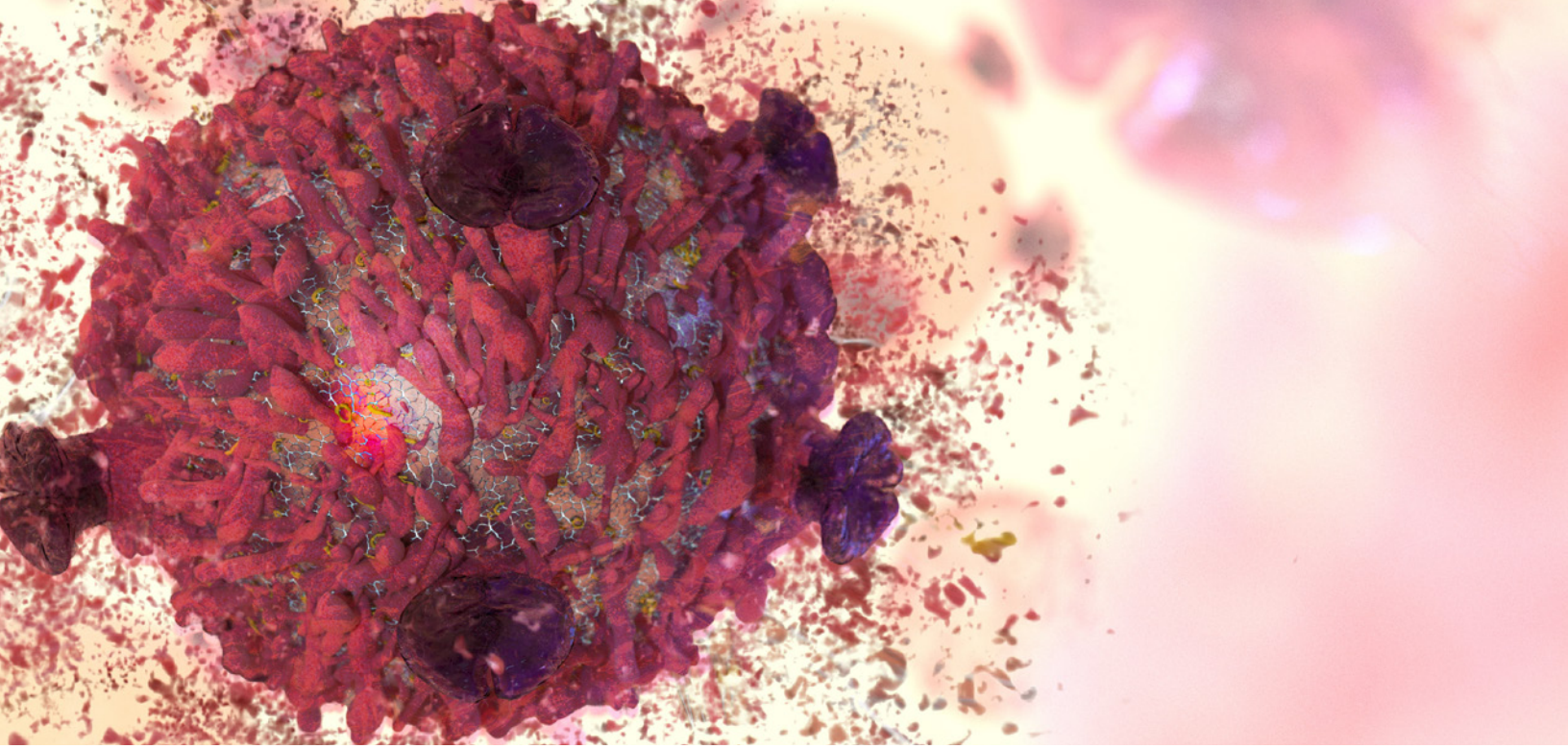
While many existing medicines can be described as one-size-fits-all, the past few decades have seen a shift toward targeted therapeutics. Nowhere is this more apparent than within the realm of oncology, where it is increasingly common for drugs to be developed against specific oncogenes. Biochemical and cell-based assays are essential tools for targeted drug discovery and development, being widely used for identifying proteins of interest, investigating the molecular mechanisms of disease, and refining the efficacy of potential clinical candidates. This ebook discusses some of the different types of assays available to researchers and includes a deep dive into the methods used for studying the Poly-ADP Ribose Polymerases (PARPs), a protein family that has been targeted successfully with multiple oncology therapeutics.

The Drug Discovery and Development Landscape Is Changing

Until relatively recently, most patients receiving a cancer diagnosis would be treated with one or more broad-spectrum chemotherapeutics. Such drugs kill dividing cells via mechanisms that include causing DNA damage and preventing replication. However, because broad-spectrum chemotherapeutics are unable to discriminate between cancer cells and healthy cells, they often cause debilitating side effects, meaning there has long been a need for alternative treatment options.

The FDA's approval for the humanized monoclonal antibody Herceptin (trastuzumab) in 1998 marked a major turning point for drug discovery and development. This was because Herceptin was specifically indicated for patients with metastatic breast cancer bearing tumors overexpressing human epidermal growth factor receptor 2 (HER2). In these individuals, Herceptin binds HER2 to downregulate PI3K pathway signaling and limit cell cycle progression, thereby slowing or stopping tumor cell growth. Critically, Herceptin's success fostered the development of other targeted treatments.

The next targeted therapy to be approved was Gleevec (imatinib), which became available in 2001 for the treatment of chronic myeloid leukemia



(CML). Gleevec is a small molecule drug that blocks the activity of the BCR-ABL fusion protein, a constitutively active tyrosine kinase known to promote uncontrolled cell growth. BCR-ABL was considered a promising oncology target for being a product of the Philadelphia chromosome, a classic hallmark of CML—a strategy that paid off. Countless other targeted therapeutics have since reached the market and many others are being developed.

Biologics and Small Molecule Drugs

A major difference between Herceptin and Gleevec is that Herceptin is a biologic while Gleevec is a small molecule drug. Both are classed as targeted therapeutics, but there are some key distinctions, not least in terms of size. While Herceptin has a molecular weight of approximately 145 kDa, Gleevec is around 300 times smaller at just under 500 Daltons. In practice, small molecule drugs tend to travel quickly through the blood vessels and penetrate all tissues (both normal and tumor) before being rapidly excreted. Biologics remain in the circulation for longer.

Another important difference between small molecule drugs and biologics concerns how they are produced. Small molecule drugs are chemical compounds with a clearly defined structure, so they are often identified by screening large compound libraries and can be manufactured through chemical synthesis. In contrast, biologics are isolated from or produced in living systems—typically mammalian cell lines such as human embryonic kidney (HEK) or Chinese hamster ovary (CHO) cells—using recombinant DNA technology. Given that even small structural changes can impact the activity of a biologic, the development of these types of drugs is subject to rigorous quality control.

A further distinction between the two classes of targeted therapeutics lies in how they are delivered. Small molecule drugs are typically stable and orally bioavailable, so are usually supplied in tablet form. Biologics are instead administered intravenously, which is less convenient and can mean poorer patient compliance. Additionally, biologics are less likely to produce off-target effects or interact with other drugs due to their highly specific nature, but have an associated risk of

causing adverse immunogenicity. Ultimately, the market for targeted therapeutics remains strong, with an estimated 20 million cancer patients in 2022 expected to rise to more than 27 million new cancer cases by 2040.

Druggable Oncology Targets

Besides HER2 and BCR-ABL, countless proteins are now recognized as viable oncology targets. Well-known examples include the PARP proteins, which are involved in processes including DNA repair, chromatin remodeling, and mitotic spindle assembly; the Kirsten rat sarcoma viral oncogene homologue (KRAS), a GTPase with the highest mutation rate of all oncogenes among human cancers; and mammalian target of rapamycin (mTOR), a critical effector in many of the cell signaling pathways that are dysregulated during cancer onset and progression. Other proteins with proven links to cancer are the Janus kinases (JAKs), a family of non-receptor tyrosine kinases that transduce cytokine-mediated signals via the JAK-STAT pathway; vascular endothelial growth factor (VEGF), a key mediator of angiogenesis within the tumor microenvironment; and the Aurora kinases, which function as central regulators of mitosis.

In addition to these well-studied proteins, many emerging oncology targets are of interest to researchers and clinicians alike. These include protein regulator of cytokinesis 1 (PRC1), a microtubule binding protein that creates resistance as chromatids are pulled to either end of the cell during division; the apolipoprotein B mRNA editing catalytic polypeptide-like (APOBEC) proteins, which have been shown to induce tumor mutations that can accelerate the development of drug resistance; and the claudins, integral membrane proteins of tight junctions that exhibit altered patterns of expression in various cancers.

Future Perspectives

As researchers learn more about the underlying molecular mechanisms of disease, targeted therapeutics are becoming more specific. For example, it is increasingly the case for oncology drugs to be developed against known point mutations rather than to simply block ligand- or ATP-binding sites; two small molecule drugs targeting the KRAS mutant G12C are currently in clinical trials, and a drug to the G12D mutant is in development.[1,2] To ensure these efforts are supported, a growing range of tools and technologies is available from companies such as BPS Bioscience, including many products that are designed to improve the speed, reproducibility, or accessibility of established techniques. Read on for a more detailed look at some of these methods and the different ways they are being used for targeted drug discovery and development.

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Choosing the Right Assay for Poly-ADP Ribose Polymerase (PARP)

Studying PARP family members is a high priority in cancer drug development.

Maintenance of genome integrity is critical to proper cellular functioning. In humans, over 150 proteins form an intricate DNA damage response (DDR) network that constantly scans and repairs DNA.[1] The PARP (Poly ADP-Ribose Polymerase) protein family consists of 17 members, which catalyze the ADP-ribosylation of proteins. PARPs are involved in a wide range of biological functions: repair of DNA damage, genome stability, chromatin remodeling, mitotic spindle assembly, regulation of RNA turnover and of gene expression, and DNA methylation.

Although all members of the same family, these proteins reveal distinct features. A few PARPs are only capable of mono-ribosylation activity, while others catalyze poly-ribosylation, also termed PARylation, which occurs in linear or branched patterns (Figure 1).

PARPs may localize predominantly in the nucleus, in the cytoplasm, or both. They differ considerably in size and structure and may contain a diverse array of functional domains (Figure 2). For example,

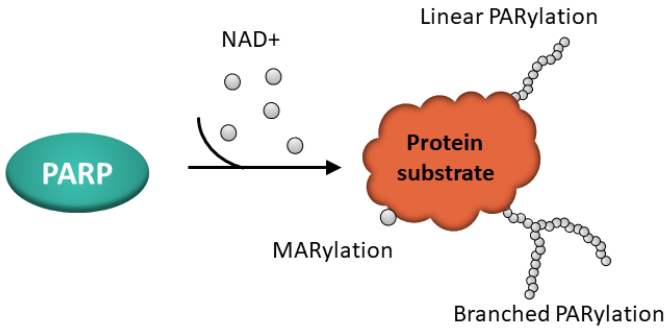

Figure 1: Mono and poly-ribosylation

PARP5A and PARP5B contain only one large ankyrin domain in addition to the catalytic domain (hence the name of tankyrase TNKS1 and TNKS2 corresponding to PARP5A and B, respectively). Other distinctive features include the strict DNA-dependency of PARP1-3, and the substrate specificity of each enzyme.

As is the case for many protein families with essential roles, the PARP proteins functionally overlap. PARP1 and PARP2 are the main proteins involved in DNA repair. Both proteins regulate the DDR

Name	Features	Size (aa)	Ribosylation	Localization
PARP1	DNA-dependent	1014	Poly-	Nuclear (N)
PARP2	DNA-dependent	570	Poly-	N, Cytoplasmic (C)
PARP3	DNA-dependent	540	Poly-	N, C
PARP4		1724	Poly-	N, C
PARP5A	Tankyrase	1327	Poly-	C
PARP5B	Tankyrase	1166	Poly-	C
PARP6		630	Mono-	C
PARP7	CCCH-containing	657	Mono-	N, C
PARP12	CCCH-containing	701	Mono-	C
PARP13	CCCH-containing	902	n/a	C
PARP8		854	Mono-	C
PARP9	Macro domain-containing	854	n/a	N, C
PARP14	Macro domain-containing	1801	Mono-	N, C
PARP15	Macro domain-containing	678	Mono-	n/d
PARP10		1025	Mono-	C
PARP11		331	Mono-	N, C
PARP16		322	Mono-	C

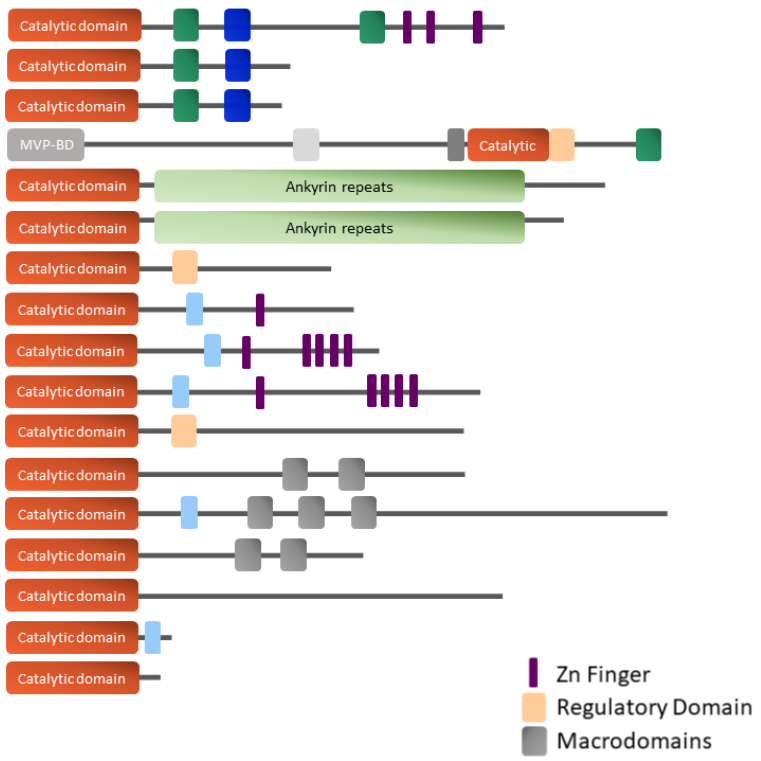

Figure 2: Structure and Characteristics of PARP family members, inspired from [2].

network, but PARP1 also regulates transcription and induces apoptosis when DNA is damaged beyond repair. In contrast, PARP2 has regulatory functions in epigenetic, proliferative, and inflammatory processes and is important for spermatogonia, thymus, and adipose tissue development.[3,4]

Normally, cells accumulating DNA damage that can't be repaired are killed. Defects in DDR pathways result in genomic instability and accumulation of mutations that support the emergence and evolution of tumor cells. For example, mutations in DNA damage repair and tumor suppressors BRCA1 or BRCA2 (Breast cancer type 1/2 susceptibility protein) impair the ability of a cell to repair double-stranded DNA breaks through homologous recombination (HR), and this increases an individual susceptibility to breast, ovarian, or prostate cancer.[5] However, the loss of a HR-dependent DNA repair system means that these tumor cells rely on other repair pathways for survival, exposing their therapeutic Achilles heel to scientists.

Interest for PARPs as therapeutic targets initially grew from the finding that PARP1/2 inhibition killed cancer cells with mutations in BRCA1 or BRCA2. This observation demonstrated for the first time the concept of synthetic lethality, which is the cell death resulting from the simultaneous disruption of two proteins that do not cause loss of viability when lost individually.

PARP1 is a first responder to damaged DNA, and its importance is reflected in its abundance as it is one of the most common nuclear proteins. PARP1 is also the best studied family member, having been successfully translated into the clinic with targeted drugs for cancer therapy. Four PARP inhibitors (PARPi) are currently approved for use in the clinic, with many others making their way through (pre) clinical phases.[3] Applications are expanding as well, now that it is well established that blocking HR in tumor cells or naturally occurring HR defects (not limited to BRCA genes) confers "BRCAness".[6] Improving on existing inhibitors, targeting other PARP family members, and adding new inhibitors that will circumvent therapeutic resistance remain a high priority in cancer drug development.[7]

Assaying PARP Enzymatic Activity

ADP-ribosylation (termed PARylation for PARP-mediated ribosylation) is the reversible addition of ADP ribose units to carboxyl groups in Glu, Asp, or Lys residues present in protein substrates, using NAD+ as ribose donor (Figure 3).

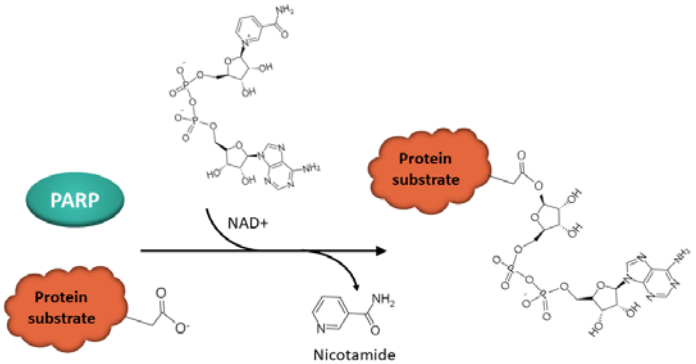

Figure 3: PARP-mediated ADP-ribosylation of a protein substrate.

Measuring PARP activity *in vitro* involves a PARP substrate, NAD+, a DNA probe for DNA-dependent PARP 1-3, and purified PARP enzymes. All these components must be carefully optimized to ensure the sensitivity, robustness, and reproducibility of the assay:

- The protein must be enzymatically active and purified, without contaminants that would alter its activity. Constructing the recombinant protein with a tag facilitates affinity purification.
- Lot-to-lot testing: Protein enzymatic activity should be tested for each new lot of protein to ensure assay consistency over time.
- Titration of the protein in the assay development phase determines the optimal protein concentration to be used in the assay, for each PARP.
- Identifying the best DNA probe for PARP1, PARP2, and PARP3 increases assay specificity.
- In assays based on labeled NAD+, identifying the appropriate NAD+ mix is critical to the sensitivity of the assay. This must be determined for each enzyme to account for mono- or poly-ribosylation and enzyme kinetics.

Throughput, number of steps, and low volumes are critical criteria of an assay designed to screen large compound libraries. Alternatively, overall assay cost or instrument availability may be the most important aspect of the assay for small research laboratories. What to consider when choosing an assay format:

- Instrument availability
- Ease of use

Table 1.							
Type of assay	**Homogeneous**	**High Throughput**	**Dynamic Range**	**Signal Stability**	**Smal Volumes**	**Cost**	**Time to Completion**
Chemiluminescent	No	No	Broad	No	No	Low	Slow
Colorimetric	No	No	Narrow	Yes	No	Low	Slow
TR-FRET	Yes	Yes	Broad	Yes	Yes	High	Fast
FP	Yes	Yes	Narrow	Yes	Yes	High	Fast
AlphaLISA®	Yes	Yes	Broad	No	Yes	High	Very Fast

- Cost
- Sensitivity
- Throughput
- Time to completion

ELISA-based Enzymatic Assays

ELISA-based chemiluminescent and colorimetric assay kits are designed to measure PARP activity for drug profiling applications. In these assays, substrate proteins are coated on a 384-well plate (Figure 4). Next, a biotinylated NAD+ mix is added with the PARP enzyme in an optimized assay buffer. The plate is treated with streptavidin-HRP (horseradish peroxidase) followed by addition of the HRP-ECL substrate or HRP-colorimetric substrate to produce chemiluminescence or color. The plate is washed after each step. The intensity of the signal is proportional to the amount of biotin-NAD+ attached to histones.

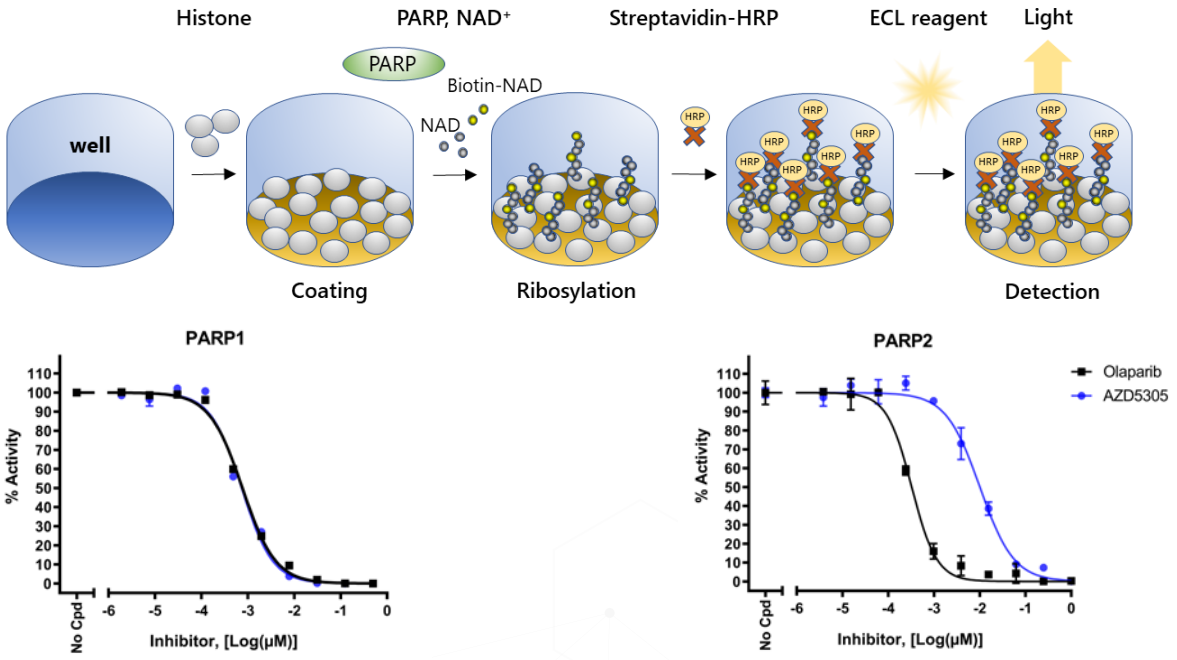

Figure 4: Principle of a chemiluminescent PARylation assay for PARP1-3 and TNKS1-2, which catalyze poly-ribosylation (upper panel); representative results using PARP1 and PARP2 chemiluminescent assay kits BPS Bioscience #80551 and #80552 (lower panels).

Considering the high degree of homology between PARP1 and PARP2, it may be difficult to find drugs that have better affinity for PARP1 than for PARP2, which is desirable due to the harsher side effects caused by PARP2 inhibition.[8] To reduce off-target activity, researchers are screening for molecules that target PARP1 with better affinity than PARP2. In a set of experiments comparing the efficacy of AZD5305 and Olaparib, scientists at BPS Bioscience were able to show a distinctive inhibition profile for PARP1 and PARP2. Indeed, the two inhibitors displayed a similar IC_{50} for PARP1 (7 and 8 nM, bottom left panel), whereas the IC_{50} was 0.3 nM for Olaparib and 100 nM for AZD5305 when assaying PARP2 (bottom right panel), demonstrating the exquisite specificity and sensitivity of the assays.

AlphaLISA® Homogeneous Assays

AlphaLISA® is a technology developed by PerkinElmer that enables the quantitation of protein-protein binding, in a bead-based, no wash assay system. AlphaLISA® PARP Homogeneous Assay Kits take advantage of a highly specific antibody that recognizes PARylated substrates. The assay protocol is quite simple: first, the enzyme is incubated with a biotinylated substrate. Next, acceptor beads and primary antibody are added, then donor beads. These no-wash steps are followed by a direct reading of the Alpha-counts (Figure 5). This assay design is very advantageous for its short time to completion and is highly amenable to high-throughput applications such as drug library screening.

PARPtrap™ Assays

When PARP1 or PARP2 bind damaged DNA, they add PAR chains to their own protein backbone (auto-PARylation), then to other DDR proteins to recruit and activate them.[8] PARylated PARP1 or PARP2 next detaches from the DNA so that the other PARylated partners can initiate the repair process.

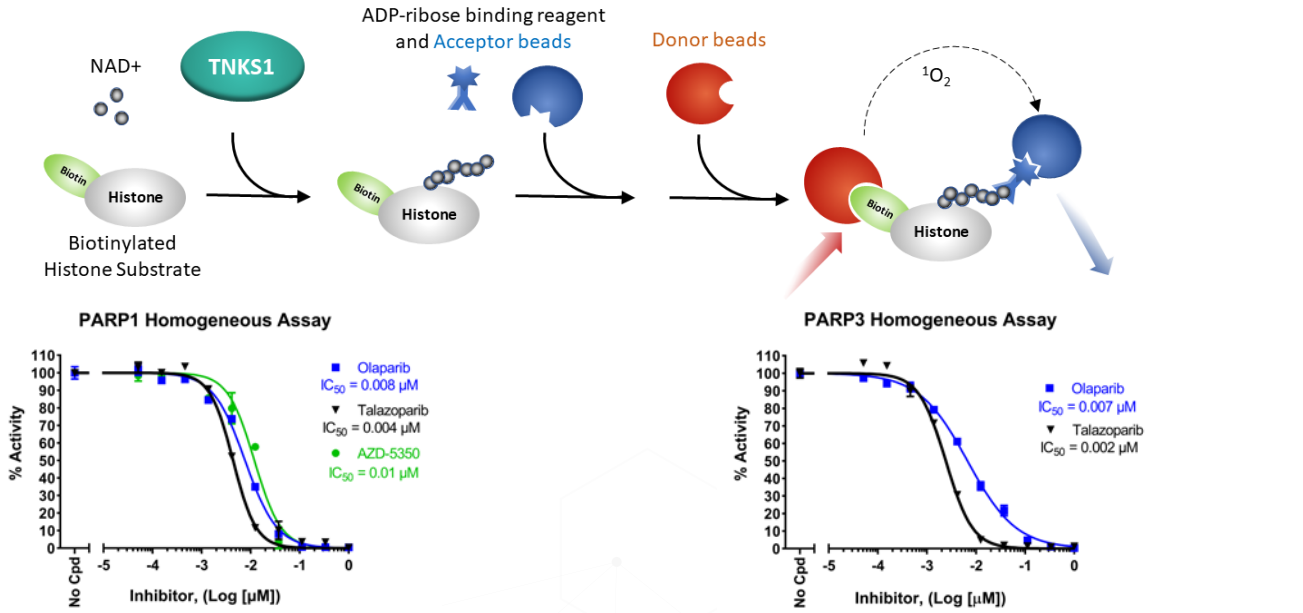

Figure 5: Principle of the AlphaLISA® PARP homogeneous assay (upper panel) and inhibition of PARP1 or PARP3 activity by specific inhibitors measured using Homogenous Assay Kits # 78438 and #78491 (lower panels).

Some currently approved drugs reduce the activity of PARP1 and PARP2 by competing with NAD+ for binding to the catalytic site. Without NAD+, PARP fails to PARylate and remains bound to the damaged DNA, shielding it from other DDR proteins. This prevents DNA repair and increases cellular toxicity, potentiating the effect of these drugs. Thus, the cytotoxic effects of this class of drugs depend primarily on how efficiently they trap PARP1 on damaged DNA.[9]

Scientists recently found that trapping PARP1, but not PARP2, to DNA with PARPi resulted in increased cytotoxicity towards cancer cells. Therefore, PARPi drug screens should include assays that quantify PARP-trapping ability and distinguish an inhibitor's selectivity to PARP1 or PARP2.

Most commercially available PARP activity assays quantify PARylation of target proteins, such as histones, and test only one PARP enzyme at a time. In contrast, the PARPtrap™ Combo Assay Kit for PARP1 and PARP2 compares a molecule's ability to trap PARP1 versus PARP2 in the same assay. The assay uses fluorescently labeled DNA probes that emit polarized light depending on PARP1 or PARP2 binding. These probes have high fluorescence polarization (FP) when PARP1 or PARP2 is bound. When scientists add NAD+ to the assay, PARylated enzymes detach from the probe, reducing FP levels. If, instead, they add NAD+ and a PARPi, the inhibitor's trapping ability increases FP in a dose-dependent manner. This homogeneous, simple assay can be incorporated into high-throughput drug discovery screens for molecules that enhance PARP1 or PARP2 trapping on DNA. This PARPtrap™ assay allows researchers to efficiently screen their libraries for the most specific and effective PARPis.

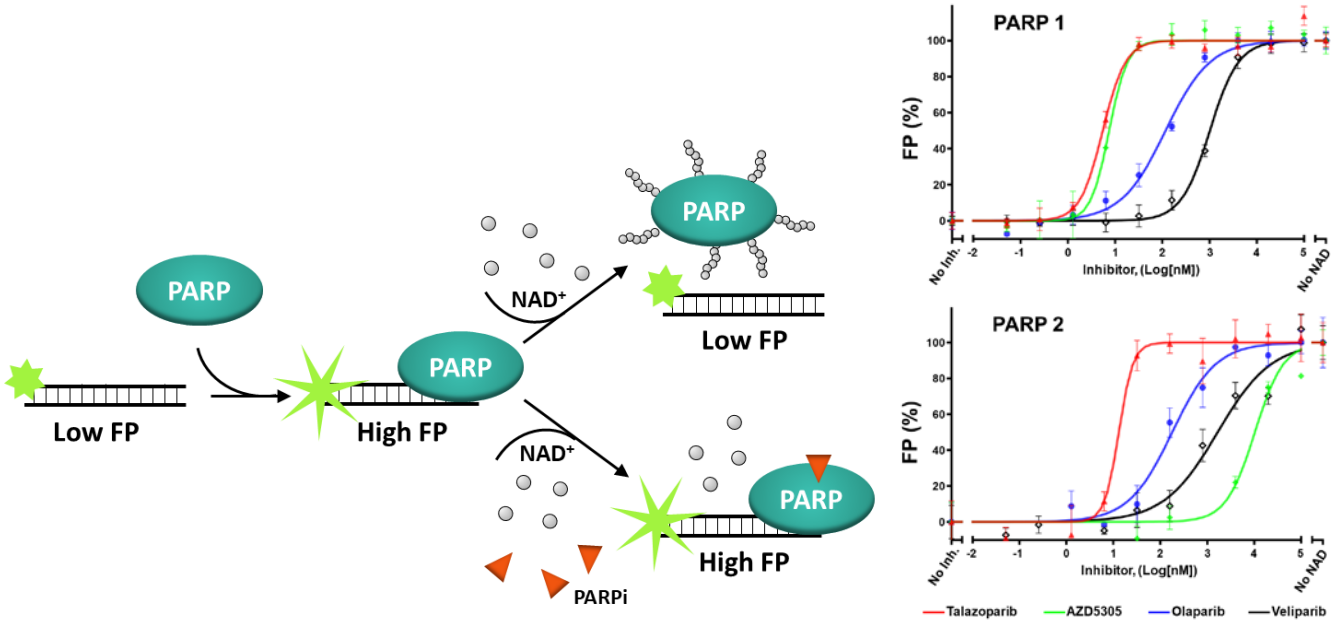

Figure 6: Assay principle (left panel) and representative results (right panels) showing drug-induced trapping of PARP1 and PARP2 (BPS Bioscience #80584 and #78296). This innovative assay was designed to screen for small molecule PARP inhibitors that can trap the enzyme on DNA, a feature unique to PARP1 and PARP2.

Conclusion

Sensitive, robust assays provide high-quality data within a short amount of time. Whether you are comparing a drug IC_{50} on all PARP family members or screening for PARP-trapping drugs, our team will support your research needs. We can also develop custom assays for your desired targets using your preferred platforms.

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Fluorescence Polarization Assays

Principles and applications

Fluorescence Polarization (FP) technology measures changes in light polarization emitted by a fluorescent tracer in a sample and is quite different from fluorescence intensity, which measures the intensity of emitted light at a specific wavelength. FP is widely used to monitor molecular interactions in solution as it is easily amenable to high-throughput formats and screening applications. FP is a complex technique that requires careful design and uses a specific instrument (a plate reader with fluorescence filters capable of polarized light excitation and capture of emitted light on two planes). This note explores the principles underlying the technology and FP-based experiments.

Principles of FP

In FP technology, the fluorescent dye is excited by polarized light. Although the initial light source emits light in all directions, a polarizer filters the light and limits it to a single plane along the direction of propagation. When a fluorescent dye is excited by the polarized single plane light, it re-emits light in all directions (i.e., it depolarizes the excitation light), because it moves around and rotates between the time of excitation and the time of emission. The molecular rotation is due to Brownian movement, which happens within nanoseconds, and the extent of light depolarization is proportional to this movement.

Indeed, the polarization of the light re-emitted by a fluorophore decreases as increasing temperature accelerates Brownian movement, whereas it increases as high solvent viscosity slows the movement of the fluorophore. Similarly, Brownian movement decreases as the size of the fluorophore increases, and this increases the retention of polarization (history of FP and theoretical foundations are reviewed in [1]).

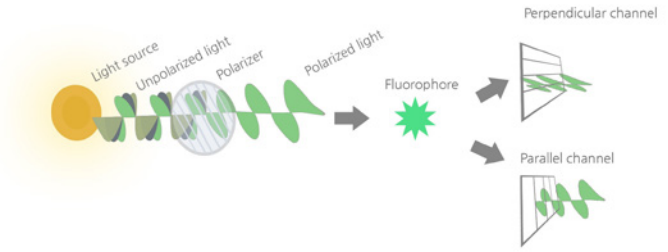

Figure 1: Illustration of fluorescence polarization principle

The degree of light polarization of a fluorescent probe is therefore inversely proportional to Brownian movements. Consequently, FP is affected by all parameters that alter the rotation and random movement of a molecule such as size, shape (a sphere rotates faster), solvent viscosity and temperature. When temperature and viscosity are kept constant, size becomes the main factor driving FP, which is then directly proportional to the size of the fluorescent probe.

To summarize, the degree of polarization of a fluorescent probe is a term that indicates to what extent the light of excitation remains polarized. When a small fluorophore is excited by polarized light, its movement is fast, and it re-emits light in all directions: it depolarizes the excitation light and the degree of polarization is low. On the contrary, reorientation of a large fluorophore excited by polarized light is limited: the light remains mostly polarized at re-emission and the degree of polarization is high.

Experimentally, FP technology measures fluorescence intensity emitted by the fluorophore in the two planes of light that are parallel and perpendicular relative to the plane of excitation. The degree of fluorescence polarization (P) is defined as the difference between the fluorescence intensity parallel and perpendicular relative to the plane of excitation, divided by the total fluorescence intensity:

$$P = \left(\frac{I_{II} - I_{\perp}}{I_{II} + I_{\perp}}\right)$$

$I_{|}$ = Fluorescence Intensity parallel to plane of excitation

$I_{\perp}$ = Fluorescence Intensity perpendicular to plane of excitation

Most instruments display fluorescence polarization in units of mP in which 1 mP = 1000 P

$$mP = \left(\frac{I_{II} - I_{\perp}}{I_{II} + I_{\perp}}\right) x\ 1000$$

Since (P) is a ratio of light intensities, it is a number without dimension. Theoretical (P) values range from –0.33 to 0.5 (–330 to 500 mP). Experimental data typically range from 10 mP to around 300 mP. Instruments achieve very precise measurements (P ± 0.002 or ± 2 mP).

The equation above assumes that light is transmitted equally well through both parallel and perpendicular channels. In practice, this is not true and a correction must be made. The correction factor is called the "G Factor".

$$mP = \left(\frac{I_{II} - G(I_{\perp})}{I_{II} + G(I_{\perp})}\right) x\ 1000$$

OR

$$mP = \left(\frac{G(I_{II}) - I_{\perp}}{G(I_{II}) + I_{\perp}}\right) x\ 1000$$

The G-factor is instrument-dependent and needs to be determined by the investigator. The instrument manual will contain information about how to establish the **G-factor**.

FP Assays

FP assays measure changes in light polarization that occur when a small fluorophore interacts with, or dissociates from, a much larger partner. Any small molecule which can be covalently labeled with a fluorophore and can form a complex with a larger partner is amenable to FP.

Several controls are necessary to conduct a successful experiment:

- "Blank" contains buffers and solvent but does not contain the tracer or the binding partner. It measures the small auto-fluorescence generated by the reagents and should be lower than the "Reference". It is subtracted from all measurements.

- "Reference" is an internal control that contains the tracer but does not contain the binding partner. It is the lowest FP allowed by the experimental conditions since all of the fluorescent tracer is present in free form.
- "High FP" is an internal control corresponding to the highest FP allowed by the experimental conditions, in which most or all available tracer is present in its bound state. In many (but not all) types of assays, this is the same as the "Positive Control".
- "Positive Control" is the experimental control. For example, if an inhibitor is being tested, the positive control is the condition without the inhibitor.

Depending on the experimental setting and type of assay, the "high FP" control can be omitted if it is the same as the positive control.

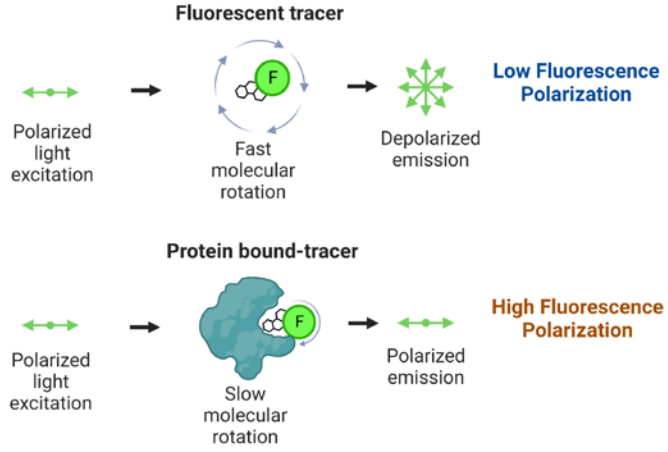

Figure 2: Upon binding to a much larger partner, the rotation of a small fluorophore becomes limited. When excited with polarized light it now emits mostly polarized light.

Applications

FP is applicable to any experiment that involves the molecular binding of two entities where one is much smaller than the other, as long as the smaller entity can be fluorescently labelled.[2] It is rather straightforward to establish a competition or an inhibition assay; proteolysis and other enzymatic assays have been developed as well. A general procedure for the development of FP assays can be found in [3].

- Binding of an antibody to a small antigen or an epitope
- Receptor–ligand and protein-protein interaction
- Protein–DNA and protein-RNA interaction
- Enzymatic reaction: substrate binding to the enzyme or formation of a new product
- Proteolysis
- Formation of a new product that is distinguished using specific fluorescent-beads. For example, measure of phosphorylation using a fluorescent antibody specific for the phosphorylated product
- Detection of specific PCR products[4]
- Competition studies in which the tracer is displaced, determination of EC_{50}
- High-throughput screening of small molecule inhibitors
- Screening for inhibitors of alpha-synuclein oligomerization[5]
- Study of muscle function[6]

Advantages and Limitations

Although FP assays are complex to develop, a well-designed assay is simple to use and highly amenable to high-throughput formats. Here are a few characteristics that make FP assays particularly attractive.

Advantages

- In-solution
- Tolerate very small volumes
- Homogenous, no wash steps
- Non-radioactive
- Real-time
- Less dye dependent and less susceptible to pH interference than fluorescence intensity assays

Limitations

- No kinetic constants
- The fluorescent tag, if not properly designed, may alter the binding properties of the tracer
- Sensitivity to changes in temperature

Considerations

Factors that do not interfere significantly

- Buffer pH
- Dye concentration
- Color additives produce relatively minor interference

Critical factors

- A change in size, ideally by a factor ≥5 times, is the most critical factor. The higher the difference, the more robust the measurements will be.
- Fluorescent molecules have an excited state, and the longer the molecule remains excited the more it rotates. This does not affect measurements as it is an intrinsic property of the fluorophore that remains constant throughout the experiment. However, it influences the choice of fluorophore since stable fluorophores allow for more robust measurements.
- The performance of an assay depends on the extent to which the biological activity of the small tracer is disrupted by the labeling. The choice of fluorophore is a critical factor. Validation is critical to ensure that labeling does not alter the tracer's interaction with the molecule of interest or does not affect the enzymatic reaction under study.
- The tracer should be >90 % labeled and the free dye should be eliminated. If a high percentage of tracer is unlabeled, it will compete with the labeled tracer for binding to the partner, which will alter the apparent IC_{50}. If the free dye is not eliminated, it will result in high background that will decrease the sensitivity of the assay.
- The purity of the components influences assay quality. Potential interference in light scattering can be caused by large molecules, such as cell or membrane debris, therefore the partner protein must be pure. For this reason, crude cell lysates or cell culture supernatants should be avoided. The presence of contaminants with high background fluorescence, or large molecules with non-specific trapping ability such as albumin is likely to interfere with the signal. Cleanliness of glass vessels and uncontaminated buffers are all important factors when performing FP assays.

FP Assays—Therapeutic Targets

Cat number	Product	Size
50293	HSP90α N-terminal Domain Assay Kit	96 reactions
78111	JAK2 JH2 Pseudokinase Domain inhibitor Screening Assay Kit	384 reactions
78107	TYK2 JH2 Pseudokinase Domain Inhibitor Screening Assay Kit	384 reactions
79859	KRAS(G12C) Nucleotide Exchange Assay Kit	384 reactions
78355	KRAS(G12D) Nucleotide Exchange Assay Kit	384 reactions
60400	PDE10A Assay Kit	96 reactions
72020	KEAP-Nrf2 Inhibitor Screening Assay Kit	96 reactions
79899	Cereblon Binding Assay Kit	96 reactions
78317	PARPtrap™ Combo Assay Kit for PARP1 and PARP2	384 reactions
60350	PDE5A1 Assay Kit	96 reactions

Conclusion

BPS Bioscience offers validated FP-based assay kits, saving scientists time and money by eliminating the need for many steps of optimization such as experimental design, labeling and validation of the fluorescent tracer, or buffer composition. Our kits contain high-quality purified proteins and are provided with a validated protocol.

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Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET)

TR-FRET is well suited for studying a wide range of events, including many that characterize cellular signaling pathways.

Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET) is a powerful technique commonly used to analyze the binding of two interacting molecules. Since most biological responses involve an interaction between at least two partners, TR-FRET is well suited to study a wide range of events, including many that characterize cellular signaling pathways. The possible applications are limitless: interaction between two proteins, between a receptor and its ligand or between an enzyme and its substrate; binding of a drug to its target or binding of a nucleic acid to a protein; measure of post-transcriptional modifications; and more.

The technology is a combination of Time-Resolved Fluorescence and Förster's Resonance Energy Transfer, a phenomenon in which a light-excited fluorophore can transfer its absorbed energy to a nearby acceptor fluorophore.

FRET

Fluorophores absorb high-energy light and emit light of lower energy than the absorbed light. Fluorescence Resonance Energy Transfer (FRET) is a phenomenon in which two fluorophores emitting at different wavelengths are coupled: the donor fluorophore excited by a high energy source transfers energy (not light) to an acceptor fluorophore. This results in excitation of the acceptor and fluorescence emission at the wavelength inherent to the properties of the acceptor fluorophore.

The measurement of molecular interactions takes advantage of the fact that the transfer of energy between the donor and the acceptor depends on physical proximity (<10 nm) and decreases rapidly with distance. Thus, partner molecules distributed in a solution are sufficiently far apart and FRET does not occur. Upon interaction, the partners complete the FRET pairing as they are now in proximity of each other.

Thus, FRET is applicable to any two interacting molecules, or to reactions in which a new molecular form appears providing that the new molecular form can be distinguished from the initial molecule. Examples include post-transcriptional modifications such as ubiquitination, methylation,

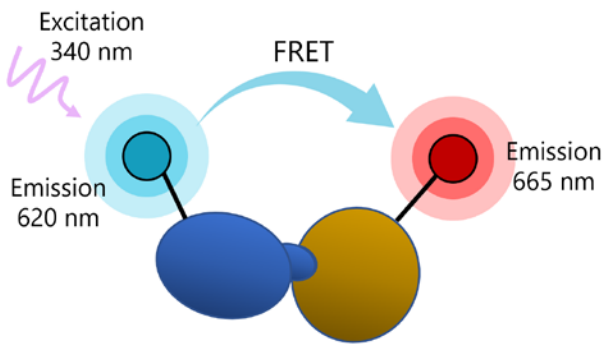

Figure 1: Illustration of FRET principle. Practically, one of the binding partners in the interaction of interest is labeled with a donor fluorophore such as a Europium chelate, whereas the other partner is labeled with an acceptor fluorophore. When direct labeling of the partners is not possible, the molecule of interest is tagged or biotinylated and the pairing is completed using streptavidin-coated donor or acceptor, or anti-tag antibodies.

or phosphorylation. The molecules under study can be directly labeled with a donor or acceptor fluorophore. Alternatively, a secondary reagent (for example, an antibody) that binds to the molecule of interest can be labeled with one of the fluorophores for indirect detection. This provides great flexibility on how to design an assay.

Time-Resolved Fluorescence (TRF)

FRET has a lower background signal than classic fluorescence methods because the acceptor emission is further apart from and does not have a spectral overlap with the excitation pulse. The donor and the acceptor, however, must have good spectral overlap (the emission range of the first must overlap with the excitation range of the second), as well as good spectral resolution for a specific signal to be measured. However, it is the Time Resolved Fluorescence (TRF) technology element that allows for the ultra-low background advantage of TR-FRET.

Classic fluorescence intensity uses short-lived fluorophores such as fluorescein, with an emission speed in the order of the nanoseconds. Excitation and emission occur at specific wavelengths that can be differentiated by a fluorescence reader. However, excitation and emission happen at the same time. If there is any amount of spectral overlap between excitation and emission, as there usually is, the reader will capture some of the excitation fluorescence, resulting in background signal and low signal-to-noise ratios.

TRF solves this by using long-lived inorganic fluorophores as donors and adding a time delay between excitation and measurement, which means that the excitation signal is gone by the time of the measurement, which decreases background signals (Figure 2). TRF also uses excitation pulses (not continuous excitation), so that a series of measurements are repeated over time. TRF

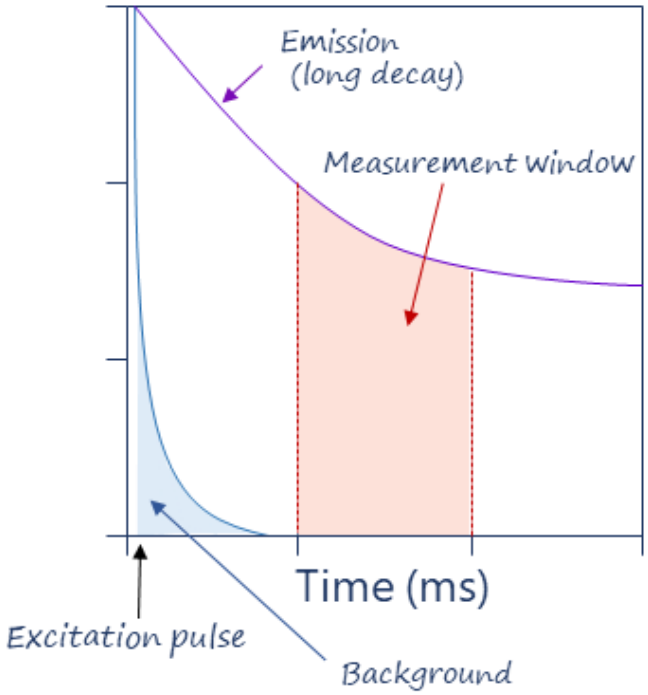

Figure 2: Illustration of TRF principle

also eliminates the very transient background fluorescence generated from sample components such as buffers, proteins, and chemicals, which hinders classic FRET methods.

Ideal fluorophores have high signal intensity, are highly stable, and offer excellent signal-to-noise ratios. Fluorophores commonly used are "Lanthanide probes" which are metal ions referring to elements Cerium to Lutetium in the periodic table. The most used are Europium and Terbium, which fluoresce over milliseconds instead of nanoseconds. In practice, a comparison measurement of the two emitted wavelengths over time is calculated for a TR-FRET response.

Monitoring BCL-2 Binding to Ligand

BCL-2 (B-cell lymphoma 2), a member of the BCL family, is an integral protein of the outer mitochondrial membrane. BCL-2 family members form hetero- or homodimers that regulate apoptosis. The main function of BCL-2 is to inhibit apoptosis and promote cell survival through control of mitochondrial membrane permeability, blocking the release of cytochrome c from mitochondria by pro-apoptotic BH3-containing proteins, and inhibition of caspase activity. However, BCL-2 may either promote or suppress apoptosis depending on context and partners. Constitutive expression of BCL-2 in B lymphocytes caused by translocation of the BCL-2 gene to Ig heavy chain locus promotes follicular lymphoma. The protein contributes to cancer resistance to treatment in leukemia, melanoma, and breast and prostate cancer, owing to its pro-survival effects. Inhibitors used in the clinic are BH3-mimetic molecules that prevent BCL-2 interaction with its BH3-type partners. Navitoclax (ABT-263) inhibits BCL-2, BCL-xL, and BCL-w, while venetoclax (ABT-199) is highly selective of BCL-2.

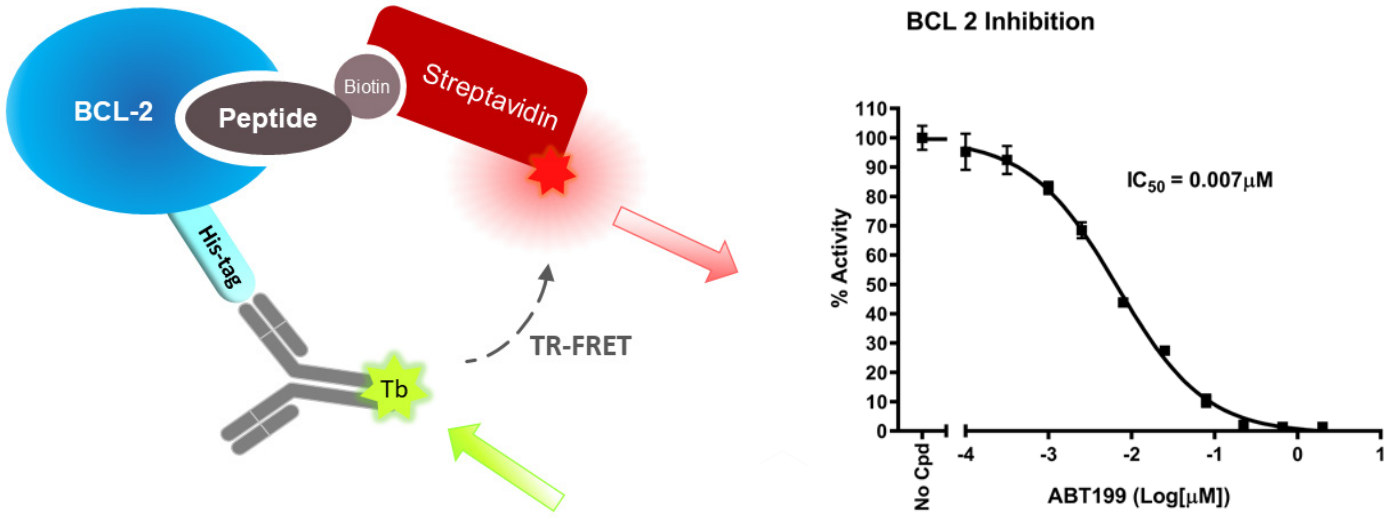

Figure 3, Left panel: Illustration of the assay principle (BPS Bioscience #50222). Right: Increasing concentrations of ABT199 were added to a terbium-labeled donor, a dye-labeled acceptor, purified His-tagged BCL-2 protein, peptide ligand, and incubated for 3 hours before TR-FRET reading. Two sequential measurements were performed: Tb-donor emission was measured at 620 nm followed by dye-acceptor emission at 665 nm.

A TR-FRET assay was designed to monitor BCL-2 binding to its peptide ligand in a homogeneous 384-reaction format. In this assay, a terbium-labeled anti-His-tag antibody is used as the donor fluorophore. It binds to His-tagged BCL-2, while the BCL-2 peptide ligand is labeled with biotin, which allows binding to the dye-labeled streptavidin acceptor. The TR-FRET signal is generated by proximity induced upon interaction of BCL-2 with the BCL-2 peptide ligand.

This assay can be used to screen for small molecules that inhibit the interaction of BCL-2 with its partner peptide or to determine the IC_{50} of candidate inhibitors.

Measuring Ubiquitination

Covalent conjugation of ubiquitin (Ub) to a protein is a common post-translational modification that regulates protein stability, function, and localization. Ubiquitination is the concerted action of three enzymes: a Ub-activating enzyme (E1), a Ub-conjugating enzyme (E2), and a Ub ligase (E3). The specificity and efficiency of ubiquitination are determined by the E3 enzyme, which directs the last step of the conjugating cascade by binding to both an E2~Ub conjugate and a substrate protein, leading to its mono- or poly-ubiquitination.

The Intrachain TR-FRET Assay kits were designed to measure the auto-ubiquitination activity of a

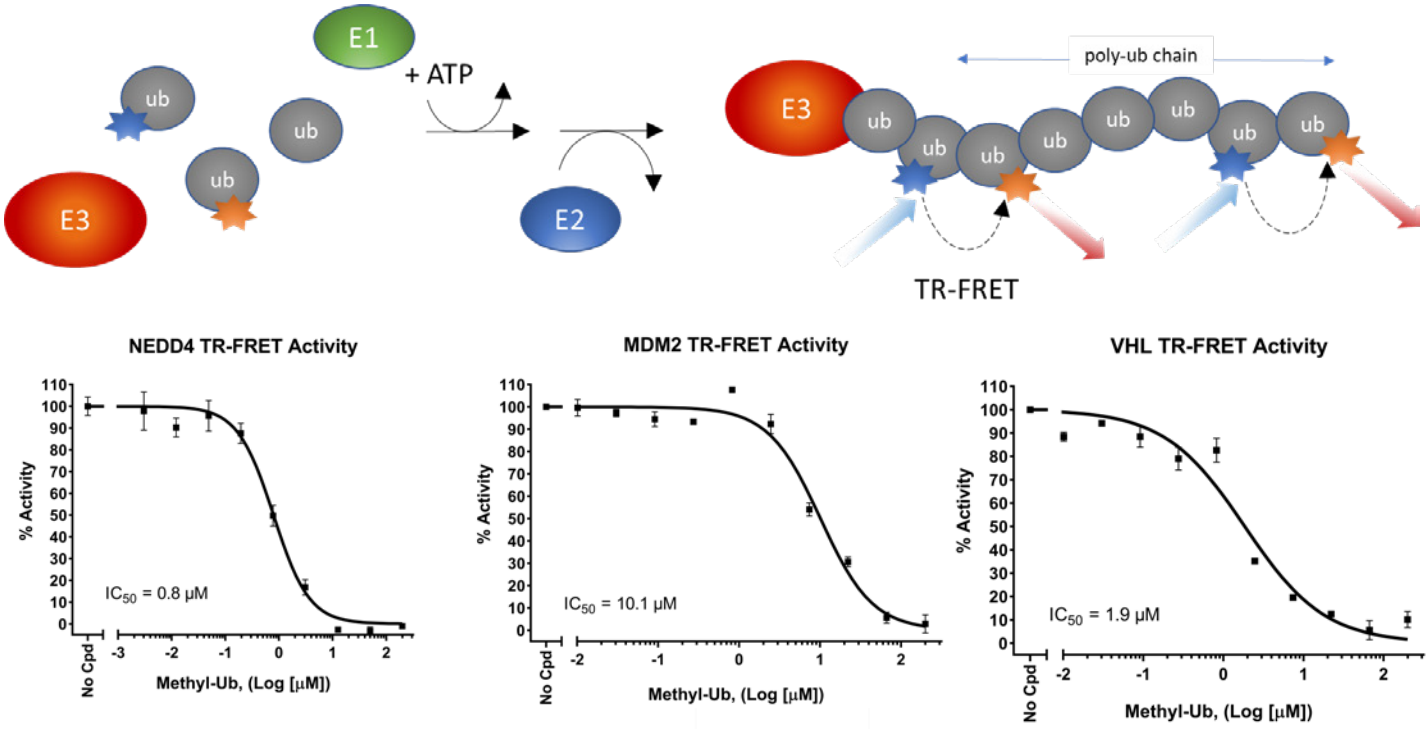

Figure 4, Upper panel: Illustration of the assay principle. Lower panels: Increasing concentrations of methyl-ubiquitin were added to a Europium-labeled donor, a Cy5-labeled acceptor, purified E1 and E2 proteins and a purified E3 ligase (NEDD4, MDM2 or VHL) in the presence of ATP, and incubated for 2 hours before TR-FRET reading. Two sequential measurements were performed: donor emission was measured at 620 nm followed by dye-acceptor emission at 665 nm.

specific E3 enzyme in a homogeneous 384-well format. The E3 ligase, such as NEDD4, MDM2, VHL (Von Hippel Lindau), is affinity purified. The assays use a Europium-labeled Ub donor and a Cy5-labeled Ub acceptor to complete the TR-FRET pairing. Since both the donor and acceptor are incorporated into poly-ubiquitin chains forming on the E3 enzyme, the assays measure poly-ubiquitination and not mono-ubiquitination. They are used for high-throughput screening of E3 ligase inhibitors, to perform real-time kinetic analyses, or to accurately determine compound EC_{50}.

Measuring the Activity of SARS-CoV-2 RNA-Dependent RNA Polymerase (RdRp)

The pandemic coronavirus disease 2019 (COVID-19) is caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). One of the most promising druggable SARS-CoV-2 targets is the RNA-dependent RNA Polymerase (RdRp), a crucial enzyme in the life cycle of coronaviruses, which operates as a complex of NSP12, NSP7,

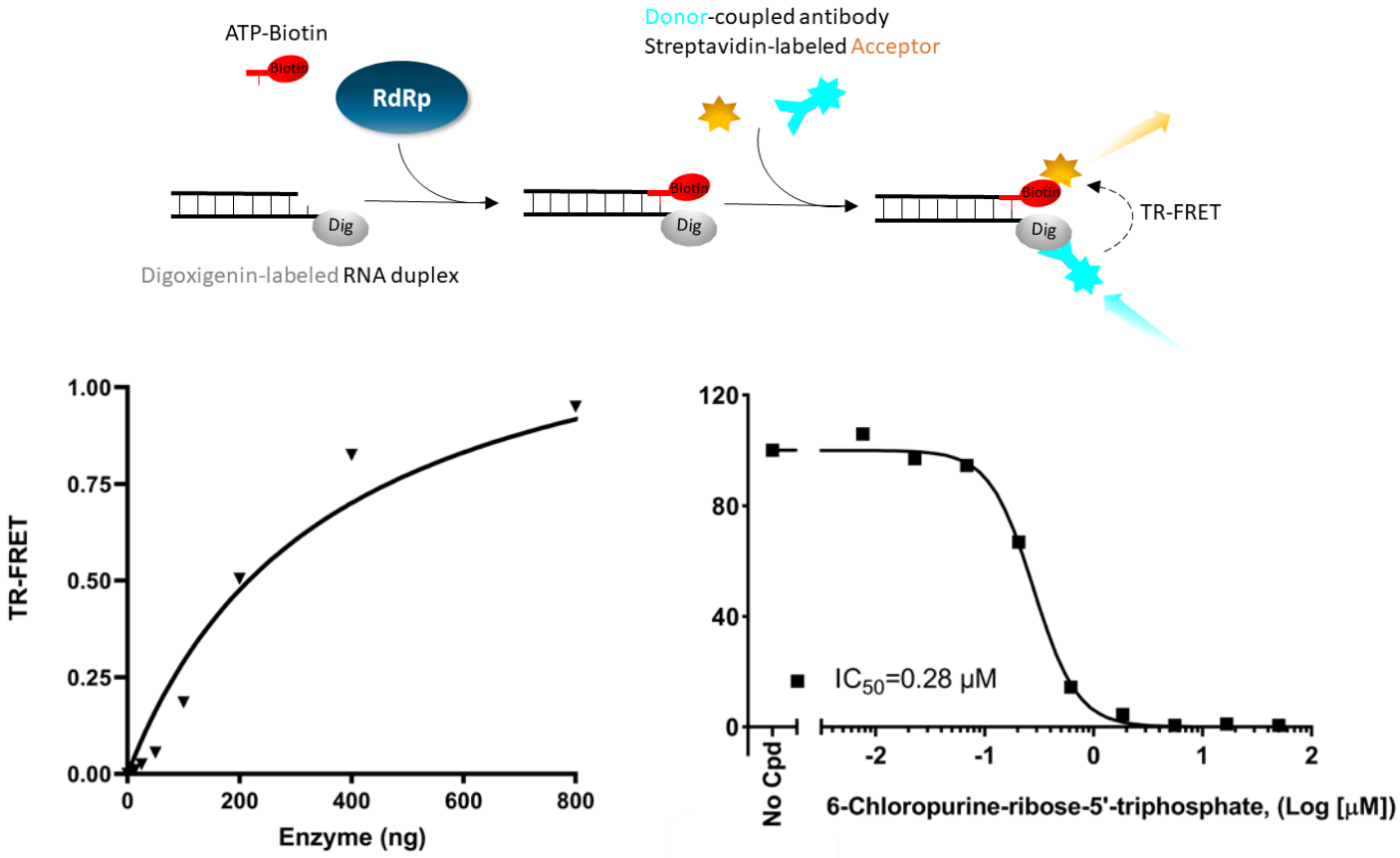

Figure 5, Upper panel: Illustration of the assay principle. Lower panels: the assay was performed in two steps. First, a test compound was incubated with the purified RdRp enzyme in a reaction mixture consisting of optimized buffers, substrate, and biotinylated ATP. Next, a dye-labeled acceptor and a Europium-labeled anti-Digoxigenin antibody were added for one hour before TR-FRET reading. Two sequential measurements were performed: donor emission was measured at 620 nm followed by dye-acceptor emission at 665 nm.

and NSP8 viral proteins. The RdRp TR-FRET Assay kit is designed to measure RdRp-mediated direct incorporation of biotinylated ATP into a double-stranded RNA substrate. The increase in TR-FRET signal is proportional to the amount of ATP incorporated in the RNA. This assay is ideal for high-throughput screening of enzyme inhibitors, to perform kinetic studies, or to accurately measure a drug EC_{50}.

Conclusion

TR-FRET is an ultra-low background technique allowing the measure of any reaction in which two labeled entities come in proximity. The main drawback of the technique is that it requires two optimized labeled entities, in addition to a low dynamic range. However, these drawbacks are offset by several advantages:

- Small volumes
- Homogeneous: no need for washing steps or for physical separation from the unbound entities
- Robust, sensitive signal
- Ultra-low background with high signal to noise ratio

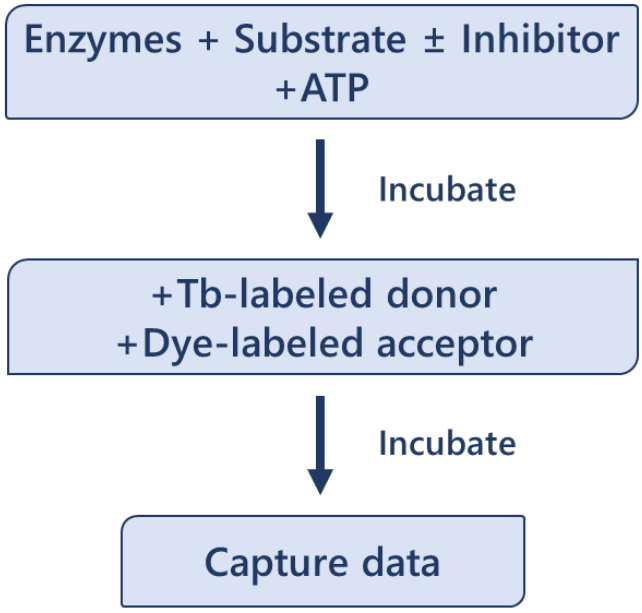

Figure 6: Simple Operation

- Stable signal: use of lanthanide donor fluorophores minimizes photobleaching

The growing commercial availability of ready-to-use TR-FRET immunoassay kits has opened the technique to mainstream use. BPS Bioscience offers over 80 assay kits for drug discovery in the TR-FRET format with new products developed regularly. Optimized, validated, high-quality assay kits ensure reliable results, quickly.

Quantitative Assays to Measure Therapeutic Antibody Binding to Fc Receptors

Interactions of antibody drugs with Fc receptors are important factors in therapeutics

Fc Receptors, expressed on the surface of immune cells, bind the Fc (fragment crystallizable) portion of antibodies, and play an essential role in modulating our immune defense system. They also impact the efficacy of therapeutic antibodies and therefore are of great interest in drug development.[1] Indeed, the effectiveness of a therapeutic antibody depends not only on how tightly it binds to the intended target, but also on how long it is present in the patient's blood and how well it engages an appropriate immune response through its interaction with Fc receptors.

Therapeutic antibodies represent a powerful class of drugs used for the treatment of cancer, immune disease, or viral infection. They include neutralizing antibodies targeting cytokines or cytokine receptors, as well as cytotoxic antibodies and antibody-drug conjugates that bind to cancer-specific targets and directly kill the tumor cells. Antibodies against immune targets such as anti-PD-1/PD-L1 or anti-CTLA4 antibodies are designed to enhance immune responses within the tumor microenvironment.

The half-life of an antibody is controlled by binding to the neonatal Fc receptor for IgG (FcRn), which regulates distinct functions in IgG transport and homeostasis. Other Fc receptors trigger immune responses through antibody-dependent cell mediated cytotoxicity (ADCC), a mechanism in which a natural killer (NK) cell is activated by antibodies bound to tumor cells, followed by the lysis of the tumor cell.

These Fc receptors are classified based on the type of immunoglobulin (Ig) that they recognize: Fcγ receptors bind to IgG, Fcα receptors bind to IgA, and Fcε receptors bind to IgE.

The development of new therapeutic antibodies (which are mostly IgGs) may require optimization of their interaction with FcRn or with the appropriate FcγR. Scientists must choose cell models and assays carefully depending on their intended goal.

Antibody Checkpoints

The Fcγ receptors function as antibody checkpoints. They contain multiple extracellular

immunoglobulin domains responsible for binding the Fc region of IgG. The FcγR family includes FcγRI (CD64), FcγRIIa (CD32a), FcγRIIb (CD32b), FcγRIIc (CD32c), FcγRIIIa (CD16a), and FcγRIIIb (CD16b), which differ in IgG affinity due to divergences in molecular structure.[2,3] Five of the receptors are activating and only FcγRIIb is inhibitory (Figure 1).

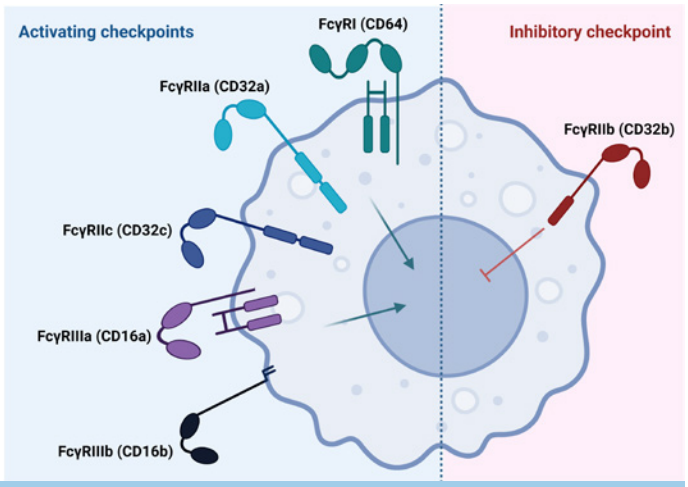

Figure 1: The antibody checkpoint family, expressed on the surface of innate immune cells and B cells, comprises activating and inhibitory Fcγ receptors. Inspired from [4]. Created with BioRender.com

Activating Fcγ receptors contain two ITAMs (immunoreceptor tyrosine-based activation motif) in their cytoplasmic domain, consisting of amino acid sequence YxxL/Ix(6-8)YxxL/I. FcγRI and FcγRIIIa do not contain an ITAM but signal through another ITAM-containing membrane-anchored subunit.

Upon activation of the receptor by IgG binding, the ITAM is phosphorylated on both tyrosine residues by an intracellular tyrosine kinase of the Src family. It becomes a docking site for SH2 domain-containing signaling proteins, initiating the signal transduction cascade necessary to generate a biological response (Figure 2).

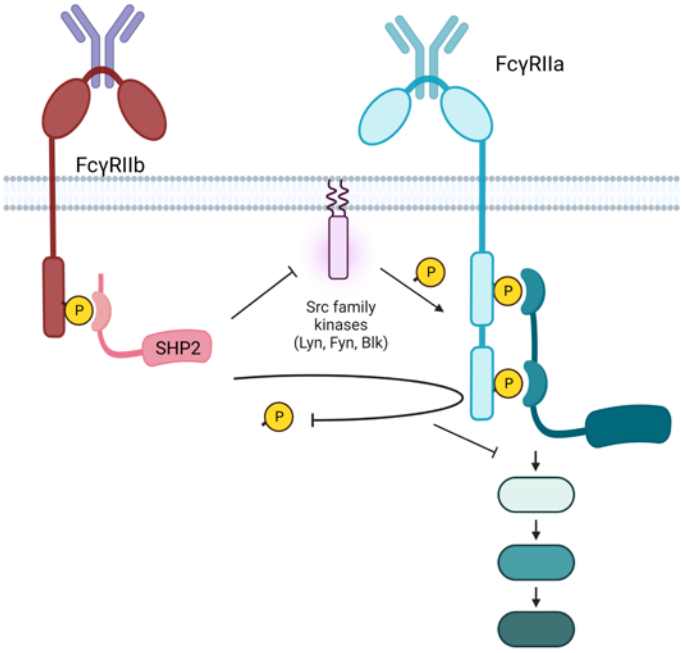

Figure 2: Activating and inhibitory signaling of FcγRIIIa (blue) and FcγRIIb (red), respectively.

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Inhibitory FcγRIIb, expressed mostly in B cells, provides a negative feedback loop that controls B cell stimulation. The receptor carries an intracellular ITIM sequence (immunoreceptor tyrosine-based inhibitory motif), which is a single I/VXXYXXL motif. Once phosphorylated, it engages SH2-containing tyrosine phosphatases such as SHP1, SHP2, or SHIP, which antagonize the phosphotyrosine signals.[5,6]

FcγRs participate in various biological functions depending on IgG specificity, cellular expression, and signaling. Receptors present on NK cells bind to antibodies that are attached to infected cells or invading pathogens to promote their lysis. FcγRs on phagocytes bind antibodies attached to invading bacteria to trigger phagocytosis of the bacterium, while FcγRs on eosinophils cause degranulation.

Therapeutic antibodies take advantage of antibody-dependent cell-mediated cytotoxicity (ADCC) activated by FcγRIIIa. Cell-based studies performed during the optimization of a therapeutic antibody will be facilitated by the engineering of target-expressing cells, the design of cell-based assays using reporter target or effector cells, or the design of co-culture assays. These research tools are especially useful in the field of immuno-therapeutics.

Activator of ADCC, FcRIIIa

The typical ADCC process involves the activation of NK cells, which express mostly FcγRIIIa (CD16a), and the release of cytotoxins that attack the target cells. Human FcγRIIIa exhibits a dimorphism at residue 158, in which variant Val-158 encodes a higher affinity receptor than variant Phe-158. FcγRIIIa potentiates the efficacy of therapeutic antibodies used to treat solid tumors and represents a direct therapeutic target in hematopoietic cancers.

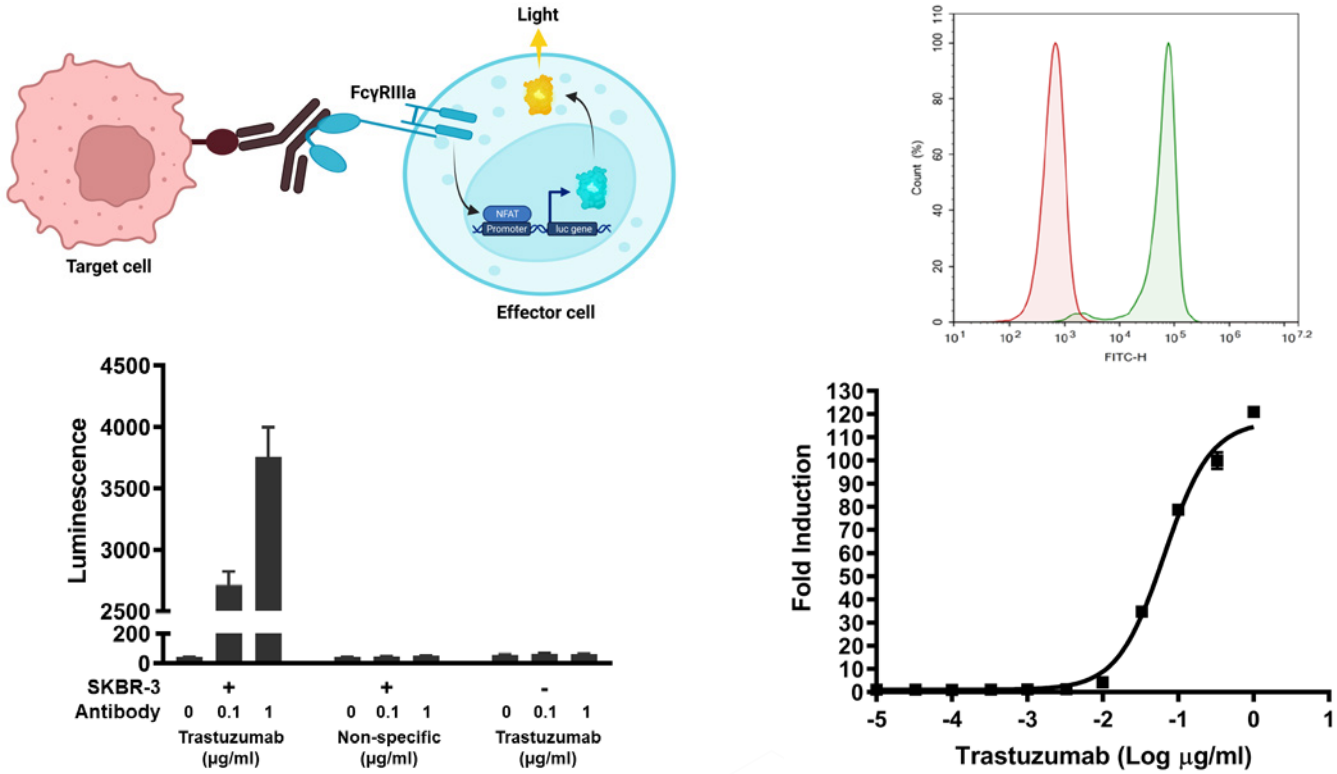

Figure 3, Top left: illustration of the ADCC bioassay principle. Top right: flow cytometry analysis of FcγRIIIa-F158 overexpression in the ADCC Bioassay Effector Jurkat Cell Line (F variant; BPS Bioscience #60540). Bottom left: ADCC response to anti-HER2 antibody drug Trastuzumab in the presence of HER2-expressing SKBR-3 breast cancer cells. Bottom right: ADCC response to increasing doses of Trastuzumab, in a co-culture of SKBR-3 and FcγRIIIa-F158/NFAT luciferase reporter Jurkat cells (EC_{50} = 28.1 ng/ml).

Illustration created with Biorender.com

Data shown in Figure 3 illustrate an effective ADCC bioassay using Jurkat cells that overexpress FcγRIIIa-F158. In the example shown here, a conditional luciferase reporter gene under the control of NFAT (Nuclear Factor of Activated T cells) response elements was introduced in the Jurkat cells to allow quantification of NFAT stimulation. The target cells were incubated with an antibody of interest. Upon addition of the Jurkat cells and binding of FcγRIIIa to the antibody, the NFAT signaling pathway was activated, resulting in a dose-dependent increase in luciferase activity. Thus, the ADCC efficacy of different antibodies can be compared directly.

Antibody Checkpoint Inhibitor FcRIIb

FcγRIIb (CD32b) operates as a negative regulator of B Cell Receptor (BCR)-induced activation of B cells.[7] The two isoforms FcγRIIb1 and FcγRIIb2, arising from mRNA splicing, differ in expression and function. The presence of exon C1 sequence in FcγRIIb1, which is highly expressed at the surface of B cells, tethers the receptor at the membrane and dramatically increases its half-life at the cell surface. The absence of exon C1 in FcγRIIb 2, expressed in myeloid cells, triggers rapid internalization of the receptor upon ligand binding. FcγRIIb induces the phagocytosis of aggregated immunoglobulins and may function as a "sink" for the removal of IgG immune complexes. Thus, the biological function of FcγRIIb is to tame antibody-dependent responses and to clear the circulation of spent immune complexes. Defects in FcγRIIb1 signaling lead to overt inflammation and are involved in autoimmune diseases.

FcγRIIb is an important therapeutic target for the treatment of B-cell malignancies. Therefore, FcγRIIb-expressing cells can be useful to identify and characterize anti-FcγRIIb antibodies, bi-specific T cell engagers, antibody-drug conjugates, or anti-FcγRIIb CAR (Chimeric Antigen Receptor) cells.

On the other hand, FcγRIIb contributes to the effectiveness of immunotherapy by cross-linking antibodies directed at T cell stimulatory checkpoints such as 4-1BB, OX40, and CD40. Co-culture assays have been designed to characterize the agonist activity of checkpoint antibodies using FcγRIIb-mediated crosslinking.

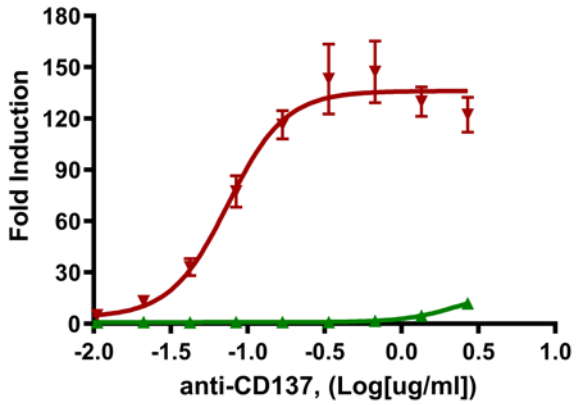

Figure 4: Dose response of anti-CD137 antibody in CD137/NF-κB-reporter HEK293 cells (BPS Bioscience #60650) co-cultured with FcγRIIb CHO cells (BPS Bioscience #79511). Cross-linking of the anti-CD137 antibody by FcγRIIb expressed at the surface of CHO cells potentiated the activation of NF-κB in CD137-expressing HEK293 cells (in red). Control CHO cells are shown in green.

As shown in Figure 4, FcγRIIb CHO (Chinese Hamster Ovary) cells placed in co-culture with CD137/NF-κB reporter cells validated the activating efficacy of an anti-CD137 antibody.

In another experiment, a TCR activator (TCRa) was expressed together with FcγRIIb in CHO cells. This cell line can be used in a co-culture assay to screen for regulators of antibody-mediated signaling

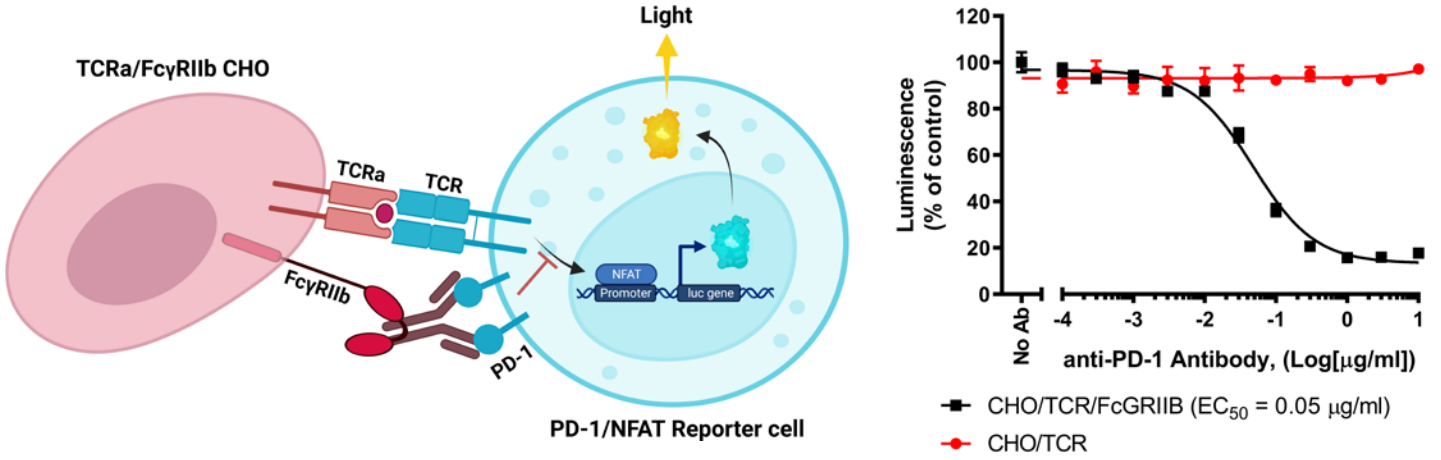

Figure 5, Left panel: illustration of the co-culture assay. Right: a co-culture assay was performed using the PD-1/NFAT Reporter Jurkat Cell Line (BPS Bioscience #60535) with either the TCRa/FcγRIIb CHO Cell Line (BPS Bioscience #78436) or the TCRa CHO Cell line (BPS Bioscience #60539), in the presence of increasing concentrations of anti-PD-1 antibody (BPS Bioscience #101178).

Illustration created with Biorender.com

and to identify or characterize agonists of FcγRIIb receptor-mediated crosslinking of checkpoint targets. As shown in Figure 5, FcγRIIb amplified the effect of an anti-PD-1 antibody, as indicated by PD-1-mediated inhibition of TCR activity observed in the presence of TCRa/FcγRIIb CHO cells, which was not observed in the presence of control TCRa CHO cells.

FcRn

Neonatal Fc receptor for IgG (FcRn) is a heterodimeric protein similar in structure to MHC class I.[8] It consists of the Fc Gamma Receptor and Transporter, encoded by the *FCGRT* gene, associated with beta-2-microglobulin. FcRn binds to the Fc region of monomeric IgG and transports the IgG from mother to fetus through the placenta. This receptor contributes to an effective humoral immunity by protecting the IgGs from degradation in the lysosome and recycling them, thereby extending their half-life in circulation. This can be exploited through the engineering of therapeutic antibodies to increase their binding to FcRn, thereby improving their half-life. Evusheld, a cocktail of mutated antibodies with extended half-lives, has been used to treat COVID-19, whereas first-in-class drug Enbrel contains an Fc domain fused to therapeutic protein TNFα to increase the drug's half-life.

Conversely, FcRn itself is a candidate target for autoimmune disease therapy since disrupting the FcRn/IgG interaction is expected to increase IgG clearance, including autoantibodies. The first FDA-approved drug targeting FcRn (efgartigimod), an Fc fragment decoy, provided proof-of-concept and is now used to treat the autoimmune disease myasthenia gravis.

The Fc:FcRn Inhibitor Screening Colorimetric Assay Kit was designed for the screening and profiling

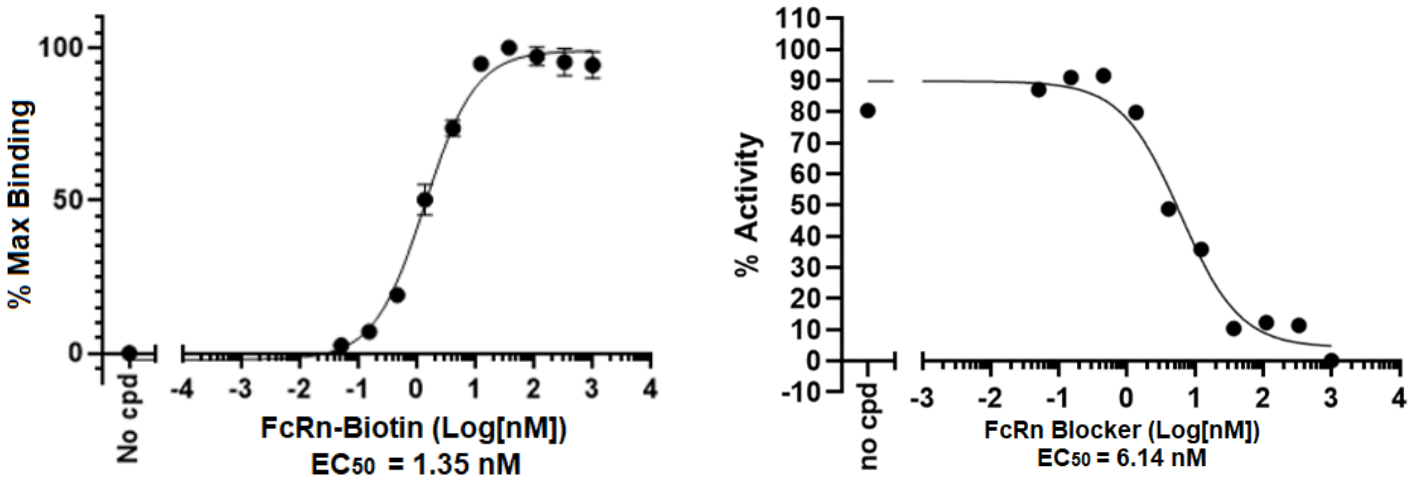

Figure 6, Left panel: direct binding of a decoy corresponding to an Fc region engineered for high affinity binding to FcRn (EC_{50}=1.35 nM). The decoy was tested using purified, biotinylated human FcRn ***(BPS Bioscience #71283).*** *Right: inhibition of FcRn binding to IgG1 by FcRn Blocker (BPS Bioscience #101468) using The Fc:FcRn Inhibitor Screening Colorimetric Assay Kit (BPS Bioscience #78501).*

Product	Product type	Cat number
ADCC Bioassay Effector Cell F variant Jurkat Cell Line	Cell Line	60540
ADCC Bioassay Effector Cell V variant Jurkat Cell Line	Cell Line	60541
ADCC Bioassay Effector Cell (Mouse) Jurkat Cell Line	Cell Line	79733
ADCP Bioassay Effector Cell FcγRIIa (H variant) NFAT Reporter Jurkat	Cell Line	71273
FcγRIIb CHO Recombinant Cell Line	Cell Line	79511
FcγRIIIa (CD16a) CHO Cell Line	Cell Line	78332
FcγRIIIb (CD16b) CHO Cell Line	Cell Line	78333
FcRL5 HEK293 Cell Line	Cell Line	78374
FcRL5 CHO Cell Line	Cell Line	78375
TCR Activator/ FcγRIIb CHO Cell Line	Cell Line	78436
Fc (IgG1):FcRn Inhibitor Screening Colorimetric Assay Kit	Assay Kit	78501
FcγRIIa (Human) CRISPR/Cas9 Lentivirus (Integrating)	Lentivirus	78207
FcγRIIa (Human) CRISPR/Cas9 Lentivirus (Non-Integrating)	Lentivirus	78199
FcγRIIIa (CD16a) Lentivirus	Lentivirus	79876
FcγRIIb (CD32b) Lentivirus	Lentivirus	79877

of neutralizing antibodies or inhibitors of the interaction between IgG-Fc and human FcRn (Figure 6).

Conclusion

Interactions of antibody drugs with Fc receptors are important factors in therapeutics. We have developed a suite of proteins, assay kits, lentiviruses, and engineered cell lines that enable quantitative Fc receptor activation, ADCC measurement, FcRn binding, and more. BPS Bioscience will continue to accelerate research by developing innovative tools to support optimization of therapeutic antibodies and Fc receptor research.

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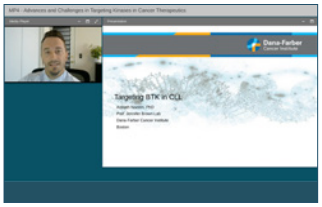
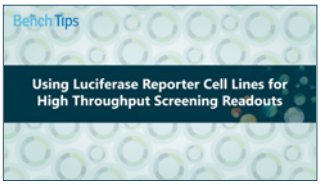
Resources

Assay Kit Frequently Asked Questions

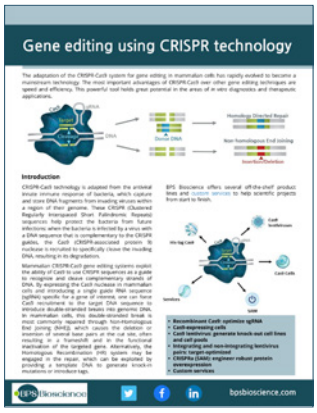


Advances and Challenges in Targeting Kinases in Cancer Therapeutics



Using Luciferase Reporter Cell Lines for High Throughput Screening Readouts

Poster: KRAS Nucleotide Exchange Assays for Inhibitor Screening and Profiling



Gene Editing Using CRISPR Technology

Advances in CAR T Cell Therapy Research