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,

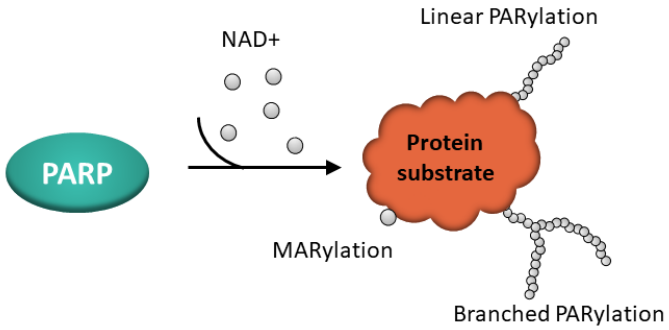

Figure 1: Mono and poly-ribosylation

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

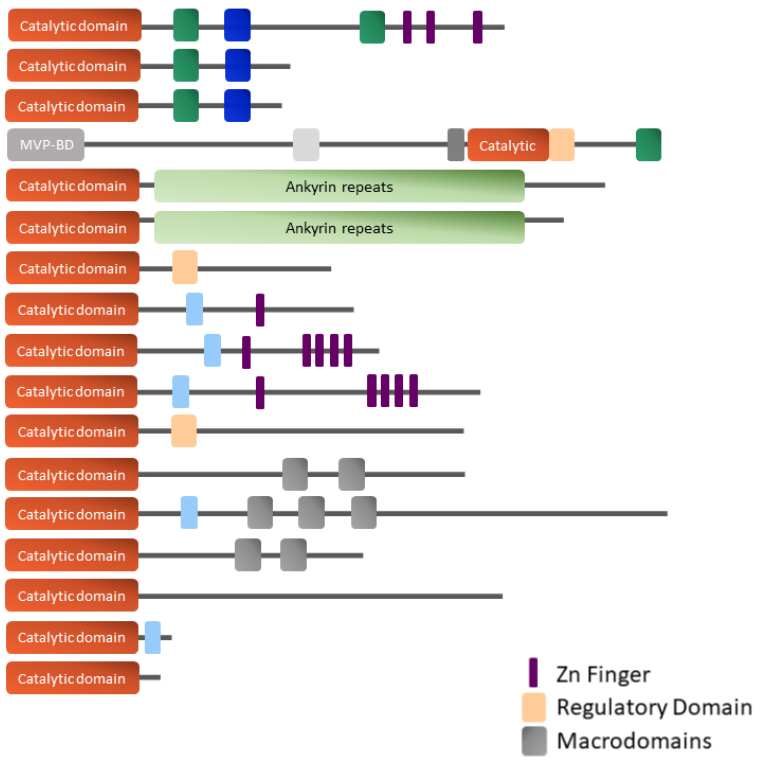

Figure 2: Structure and Characteristics of PARP family members, inspired from [2].

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).

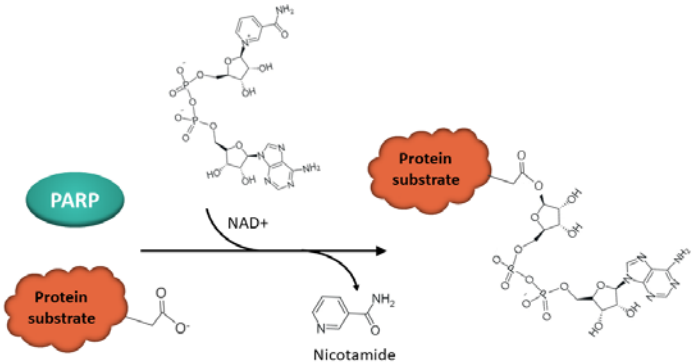

Figure 3: PARP-mediated ADP-ribosylation of a protein substrate.

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.

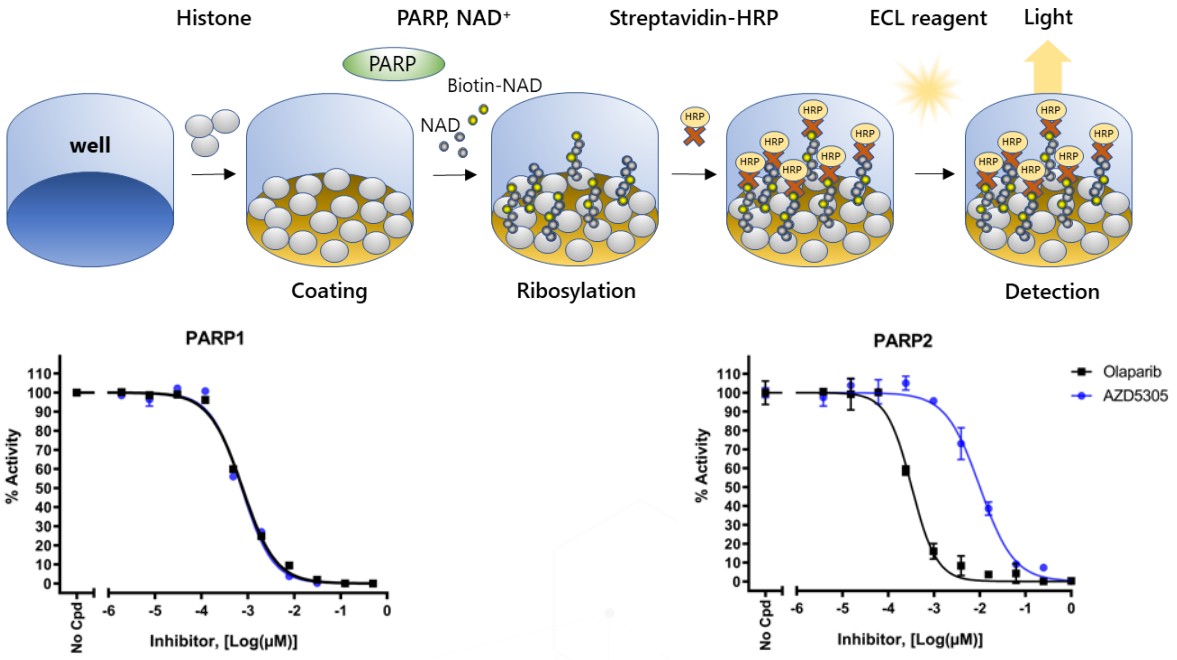

Figure 4: Principle of a chemiluminescent PARylation assay for PARP1-3 and TNKS1-2, which catalyze poly-ribosylation (upper panel); representative results using PARP1 and PARP2 chemiluminescent assay kits BPS Bioscience #80551 and #80552 (lower panels).

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.

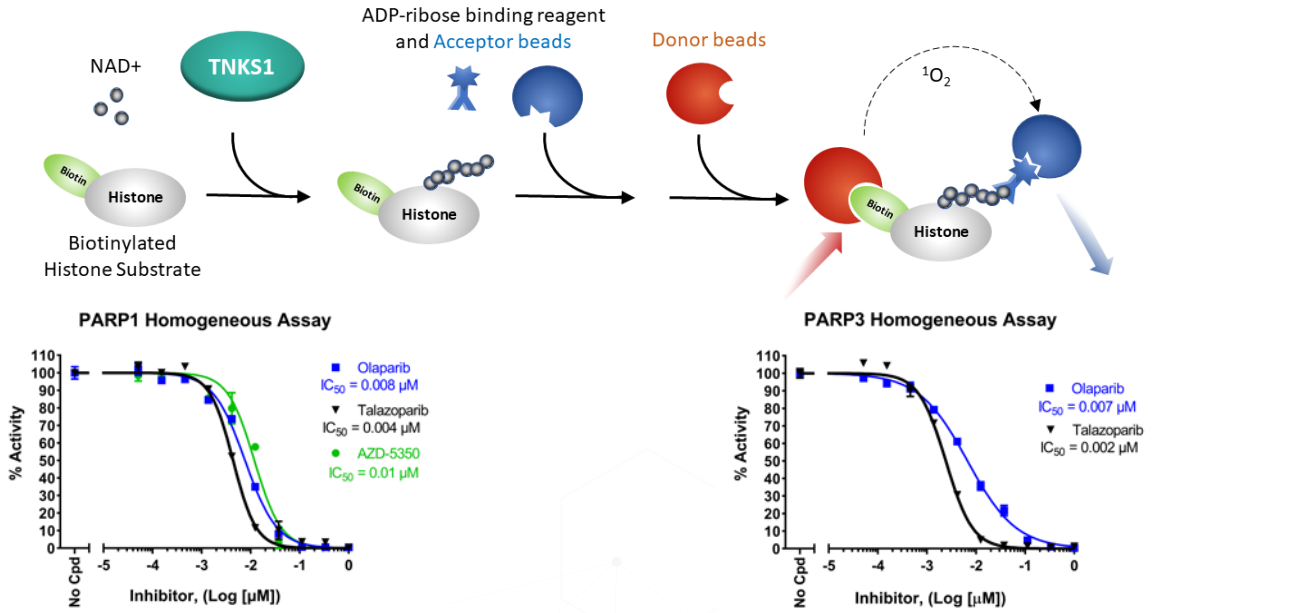

Figure 5: Principle of the AlphaLISA® PARP homogeneous assay (upper panel) and inhibition of PARP1 or PARP3 activity by specific inhibitors measured using Homogenous Assay Kits # 78438 and #78491 (lower panels).

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.

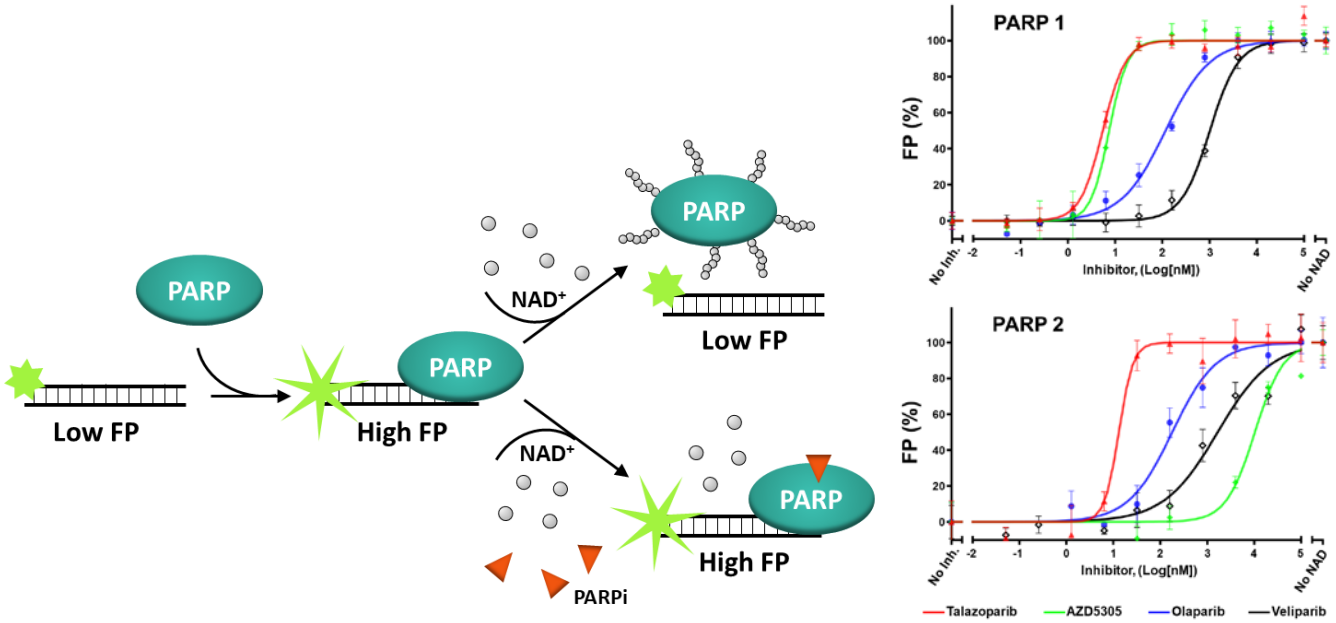

Figure 6: Assay principle (left panel) and representative results (right panels) showing drug-induced trapping of PARP1 and PARP2 (BPS Bioscience #80584 and #78296). This innovative assay was designed to screen for small molecule PARP inhibitors that can trap the enzyme on DNA, a feature unique to PARP1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

References

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