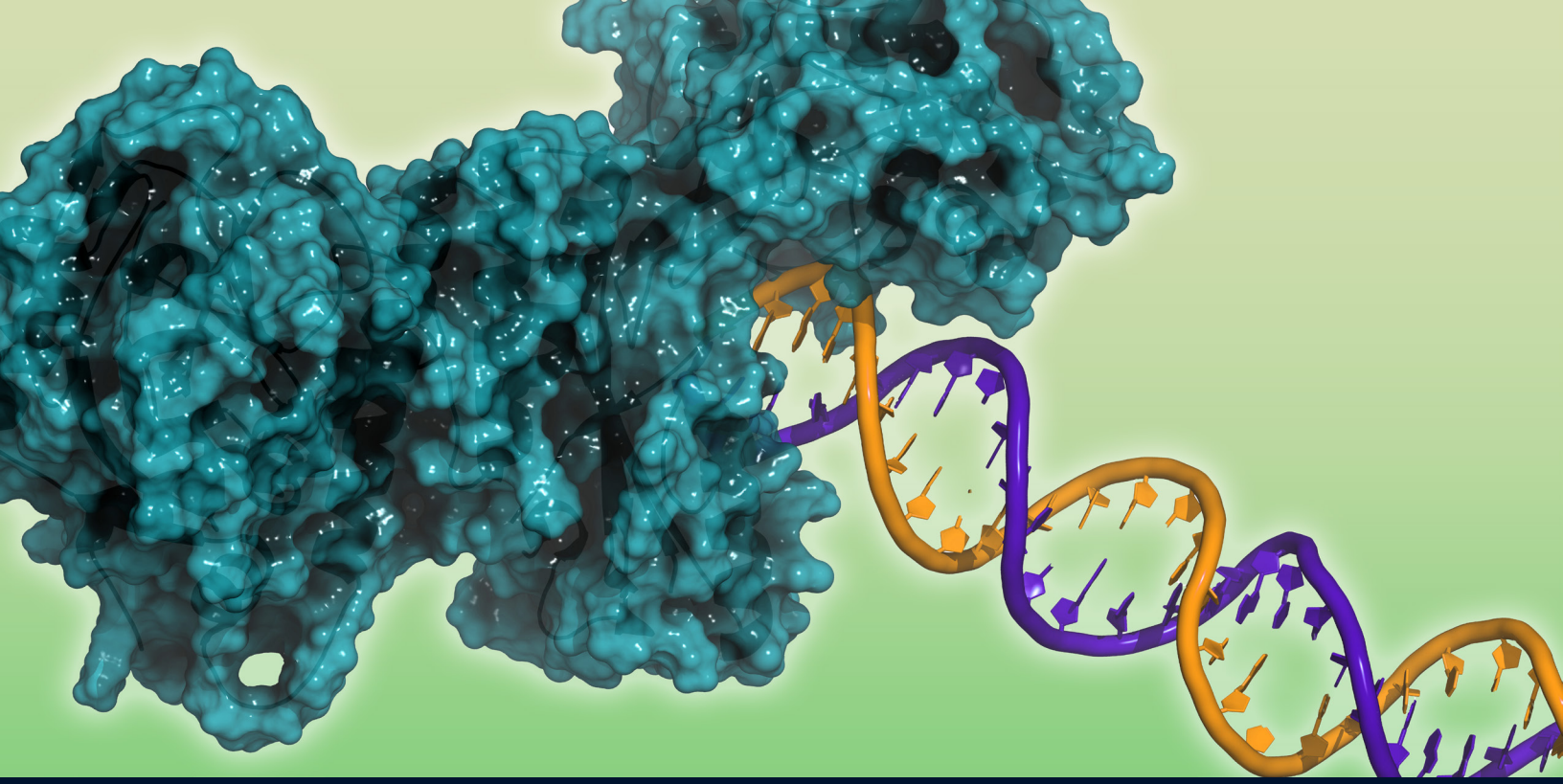


Choosing the Right Assay for Poly-ADP Ribose Polymerase (PARP)

BPS Bioscience

Introduction

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 (MARylation), while other PARPs catalyze poly-ribosylation (PARylation), which occurs in linear or branched patterns (Figure 1).

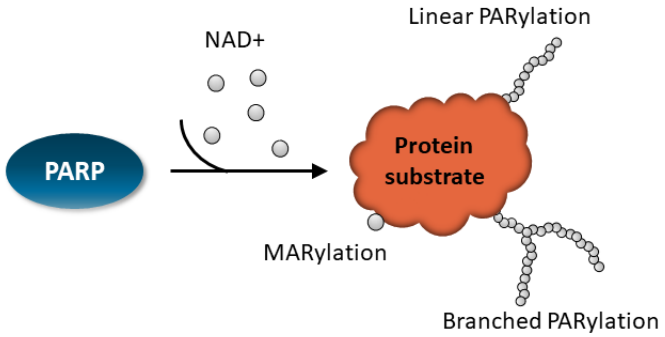
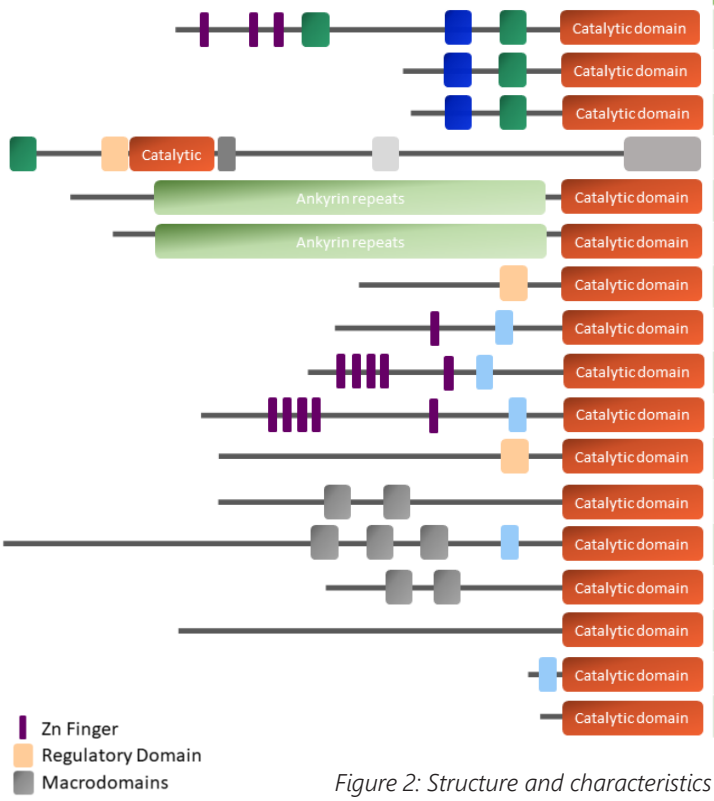


Figure 1: Mono and poly-ADP ribosylation

PARPs may localize predominantly in the nucleus, in the cytoplasm, or both. They differ considerably in size and structure and contain a diverse array of functional domains (Figure 2). Notably, PARP5A and PARP5B have only one large ankyrin domain in addition to the catalytic domain (hence the name of tankyrase TNKS1 and TNKS2 corresponding to



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)

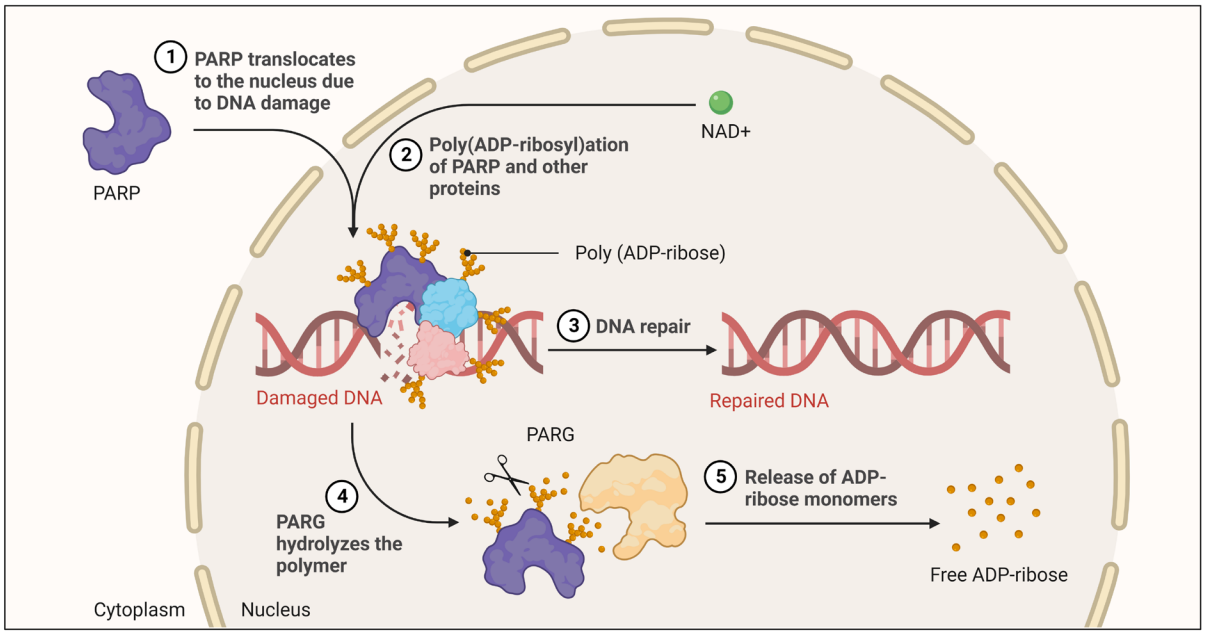
PARP5A and B, respectively). Other distinctive features include the strict DNA-dependency of PARP1, PARP2 and PARP3, and the substrate specificity of each enzyme.

As is the case for many protein families with essential roles, the PARP proteins functionally overlap. PARP1 and PARP2 are mainly involved in DNA repair, and both proteins regulate the DDR network. PARP2 also regulates epigenetic, proliferative, and inflammatory processes and is important for spermatogonia, thymus, and adipose tissue development (3, 4). In contrast, PARP1 alters transcription and induces apoptosis when DNA is damaged beyond repair. It 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.

Defects in DDR pathways result in genomic instability and accumulation of mutations that support the emergence and evolution of tumor cells. Thus, 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's 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 Achille's heel.

Indeed, 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 impaired individually.

Several PARP inhibitors 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 established that blocking any HR pathway in tumor cells (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 remains a high priority in cancer drug development (7).



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Assaying PARP Enzymatic Activity

ADP-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). 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. Here are a few things to keep in mind:

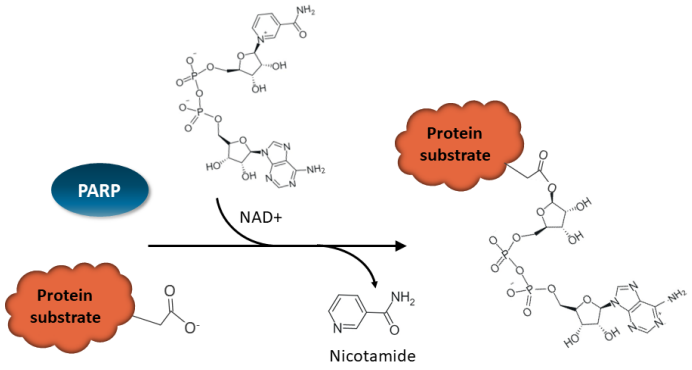


Figure 3: PARP-mediated ADP-ribosylation of a protein substrate

- 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 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.

Type of Assay	Homogeneous	High Throughput	Dynamic Range	Signal Stability	Small 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
Fluorescence Polarization	yes	yes	narrow	yes	yes	high	fast
AlphaLISA®	yes	yes	broad	no	yes	high	very fast

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
- Cost
- Sensitivity
- Throughput
- Time to completion

ELISA-based Enzymatic Assays

Enzyme-linked immunosorbent assay (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 plate (Figure 4). Next, a biotinylated NAD+ mix is added with the purified PARP enzyme in an optimized assay buffer. The plate is treated with streptavidin-HRP (horseradish peroxidase) followed by addition of the appropriate HRP 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.

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), whereas the IC_{50} was 0.3 nM for Olaparib and 100 nM for AZD5305 when assaying PARP2, demonstrating the exquisite specificity and sensitivity of the assays.

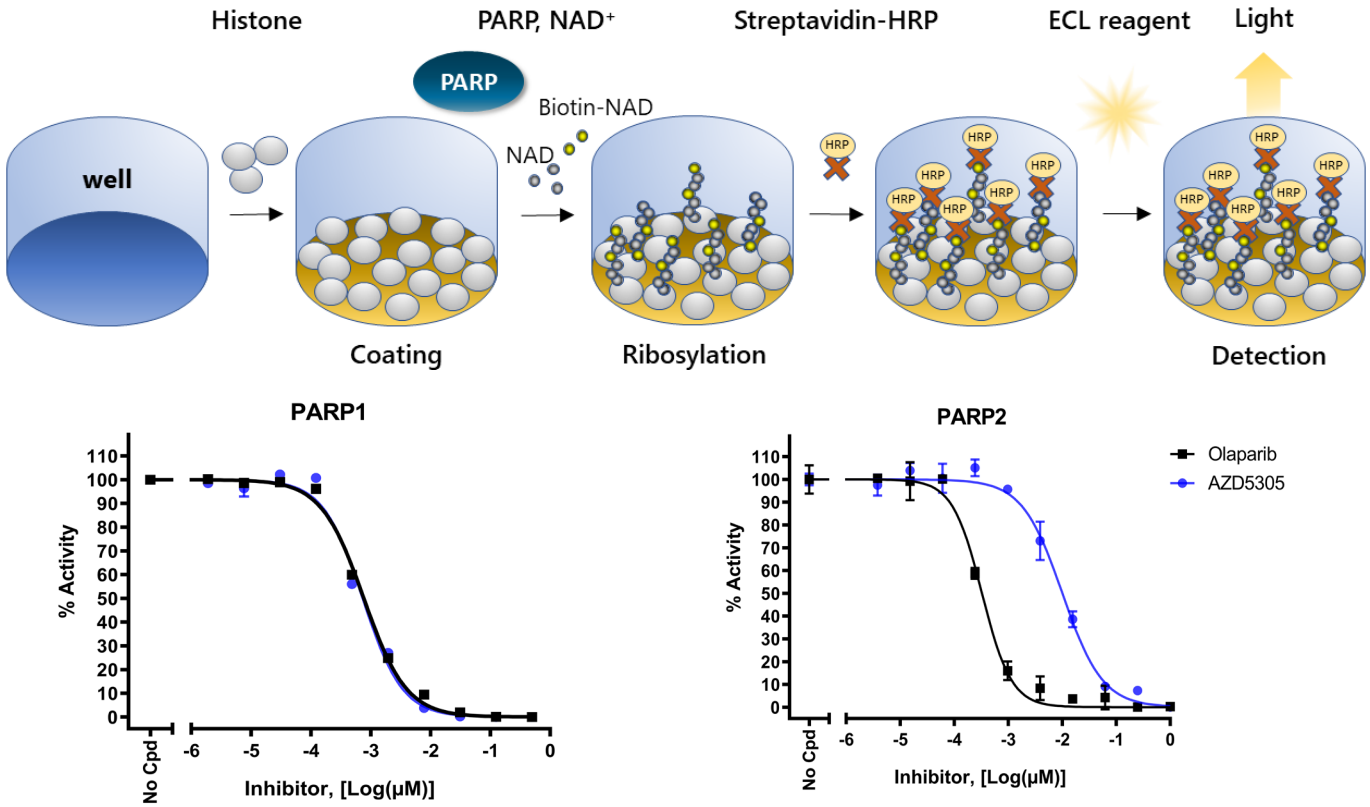


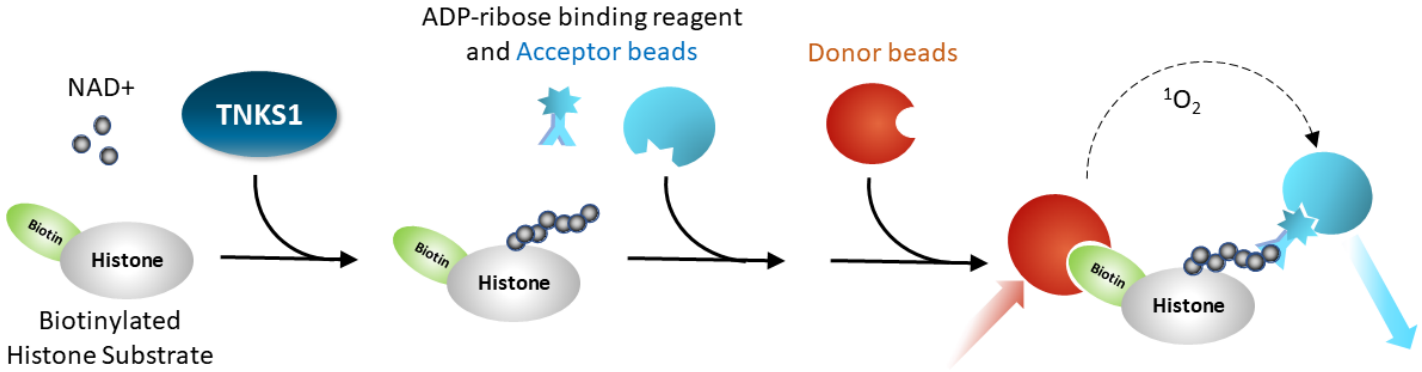
Figure 4: Principle of a chemiluminescent ELISA for PARP1-3 and TNKS1-2, which catalyze poly-ribosylation (upper panel). Representative results using PARP1 and PARP2 chemiluminescent assay kits BPS Bioscience #80551 and #80552 (lower panels).

AlphaLISA® Homogeneous Assays

AlphaLISA® is a bead-based, no wash technology developed by PerkinElmer that enables the quantitation of protein-protein binding or the quantification of a new enzymatic product. AlphaLISA® PARP Homogeneous Assay Kits take advantage of a highly specific antibody that recognizes PARylated substrates. Therefore, they measure the enzymatic activity of a speciffic PARP family member. The specificity of the assay is linked to the identifty of the purified protein.

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. Of note, these assays require the availability of a specialized AlphaScreen microplate reader.

This assay design is very effective for its short time to completion, and is highly amenable to high throughput applications such as the screening of a small molecule library to identify new PARP inhibitors. Other applications include accurate measure of compound EC_{50}.



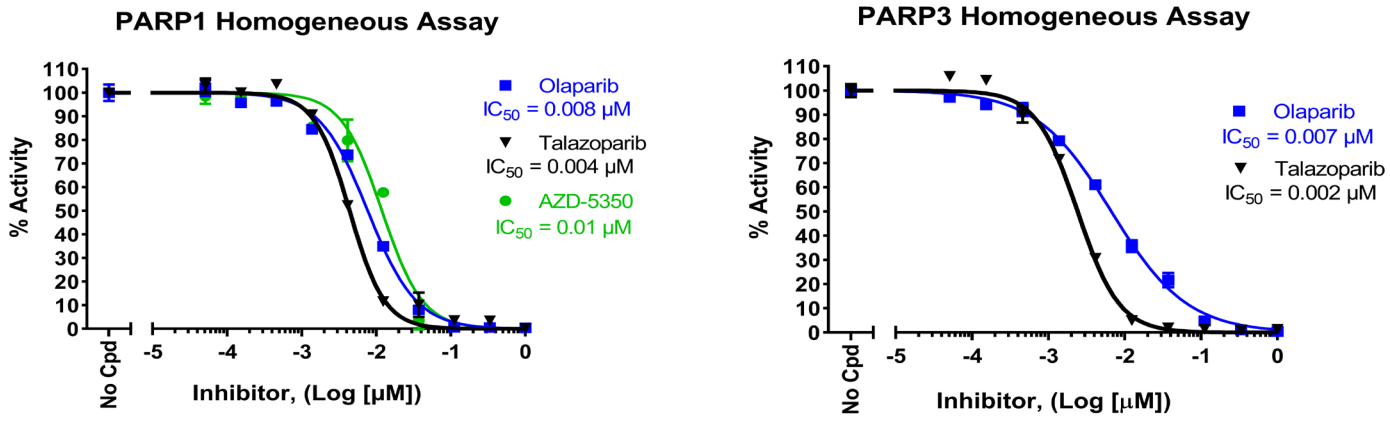
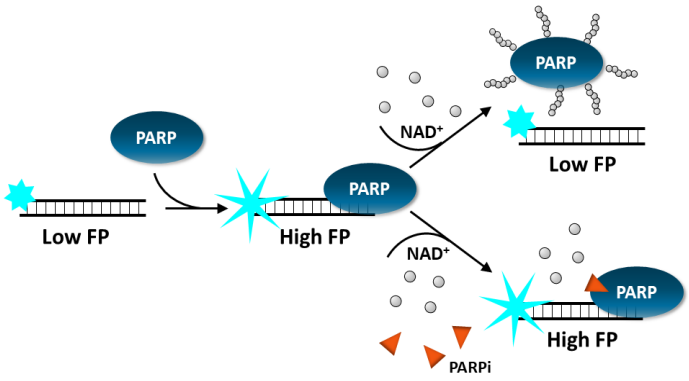


Figure 5: Principle of the AlphaLISA® PARP homogeneous assay (upper panel). Inhibition of PARP1 or PARP3 activity by increasing concentrations of the indicated inhibitors, measured using Homogenous Assay Kits BPS Bioscience #78438 and #78491 (lower panel).

PARPtrap™ Assays

When PARP1 and 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 and PARP2 next detach from the DNA so that the other PARylated partners can initiate the repair process. 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 the protein on damaged DNA (9), although scientists recently found that trapping PARP1, but not PARP2, to DNA with PARP inhibitors results in increased cytotoxicity. Therefore, screening for these drugs 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 to DNA. When scientists add NAD+ to the assay, PARylated enzymes detach from the probe, reducing FP levels. If, instead, they add NAD+ and a PARP inhibitor, 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. PARPtrap™ assays allows researchers to efficiently screen their libraries for the most specific and effective inhibitors.

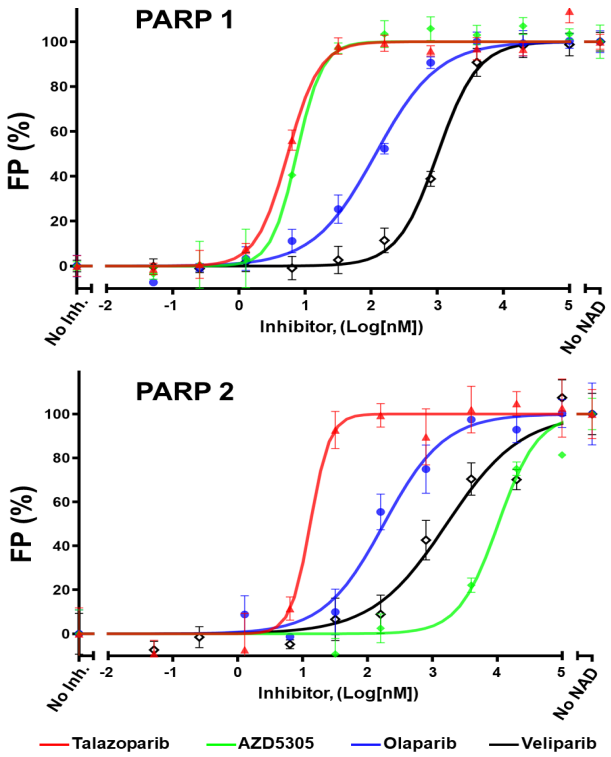
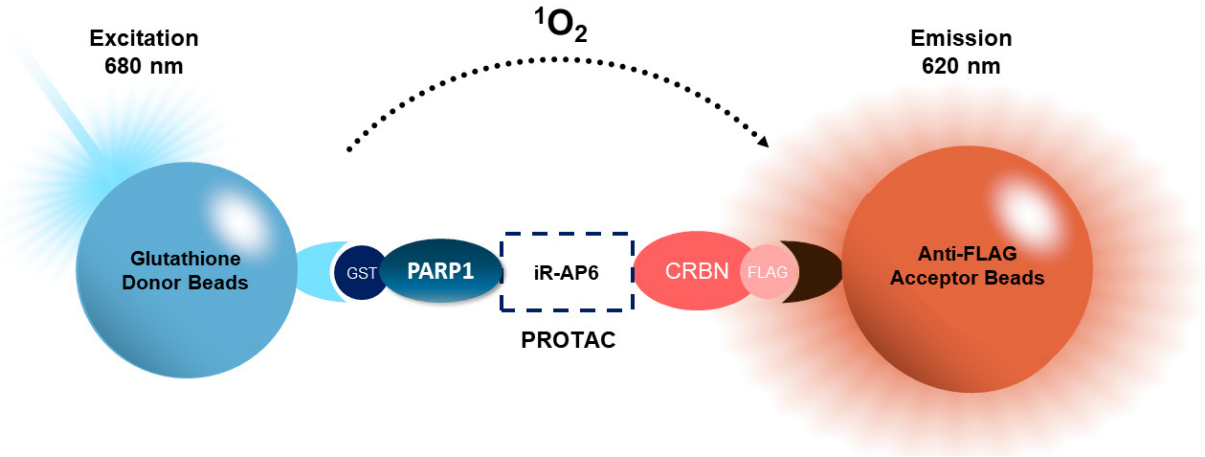


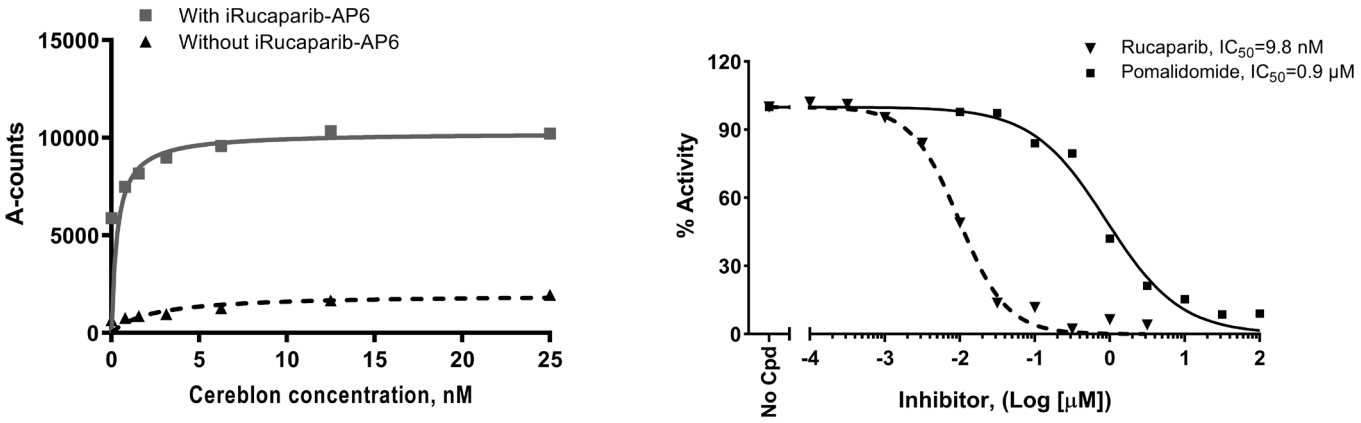
Figure 6: Representative results showing drug-induced trapping of PARP1 and PARP2 (BPS Bioscience #80584 and #78296). This innovative assay was designed to screen for small molecule PARP inhibitors that can trap the enzyme on DNA, a feature unique to PARP1/2.

PROTAC® Optimization Kit

Proteolysis Targeting Chimeras, or PROTACs, promote proteasome-mediated protein degradation by targeting the protein of interest to a ubiquitin E3 ligase. PROTAC-induced degradation of PARP may be an alternative strategy to eliminate the protein from target cells. A PROTAC molecule is composed of a ligand that binds to the E3 ligase, connected by a linker to a ligand that binds the protein of interest. This novel technology offers distinct advantages over traditional small molecule-mediated inhibition of a protein's activity. For example, a single PROTAC can promote the degradation of many proteins, as it is recycled upon degradation of its target. It has proven especially useful for "hard to drug" proteins because, at least in theory, any protein of interest may be targeted using this technique. However, PROTAC development requires several steps of optimization that are slowed by the technical difficulty of quantifying protein degradation. The PROTAC optimization assay bypasses these difficulties by directly measuring PROTAC-mediated complex formation.



Assay principle: A PROTAC of interest (here, positive control iRucaparib-AP6) interacts with both PARP1 and CRBN, bringing them in close proximity. PARP1 contains a GST tag, recognized by the GSH donor bead, while CRBN contains a FLAG tag that binds to an acceptor bead conjugated with an anti-FLAG antibody. Upon excitation of the donor bead, a singlet oxygen is generated by the donor bead. The singlet oxygen excites the acceptor bead and emits light proportionally to the level of interaction.



Firgure 7, left panel: Titration of cereblon at fixed concentration of PARP1 in the presence or in the absence of a fixed concentration of iRucaparib-AP6 (PROTAC) BPS Bioscience #78441. Right panel: Inhibition of iRucaparib-AP6-mediated interaction of Cereblon with PARP1 by increasing concentrations of Rucaparib (PARP1 inhibitor) or Pomalidomide (CRBN inhibitor).

Custom Services

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. High quality data are provided in a timely manner with protocols, raw and analyzed data. A broad portfolio of assays and PARP enzymes facilitate compound library screening, new assay development and optimization, and IC_{50} determination across the entire PARP family. BPS Bioscience's services include our unique PARPtrap™ assays, which allow to specifically assess the efficacy to a drug to trap PARP1 or PARP2 to the DNA.

Application example

Published in: Wang H. et al., Discovery of Pamiparib (BGB-290), a potent and selective Poly (ADP-ribose) polymerase (PARP) inhibitor in clinical development. *J. Med. Chem.* 2020; 63: 15541-15563.

Pamiparib is a selective oral PARP1 /2 inhibitor that has demonstrated PARP-DNA trapping ability as well as strong anti-tumor activity and CNS penetration in pre-clinical models. In this study, pamiparib was titrated against individual PARP proteins using corresponding assay buffers and solutions for each chemiluminescent assay kit: PARP1/2/3, PARP5A and PARP5B. In addition, PARP6, PARP7, PARP8, PARP10, PARP11 and PARP12 were tested at BPS Bioscience.

Pamiparib selectivity profile shows that it is equally potent at inhibiting PARP1 and PARP2, with an EC_{50} of 1.3 nM and 0.92 nM, respectively. It is about 50 times less potent against PARP3, and displays very low affinity toward other PARP family members. Thus, pamiparib is a potent and selective inhibitor of PARP1 and PARP2.

PARP Member	PARP1	PARP2	PARP3	PARP5A	PARP5B	PARP6	PARP7	PARP8	PARP10	PARP11	PARP12
EC_{50} (nM)	1.3	0.92	68	230	140	>100,000	11,000	8,400	11,000	2,700	2,400

Supporting products

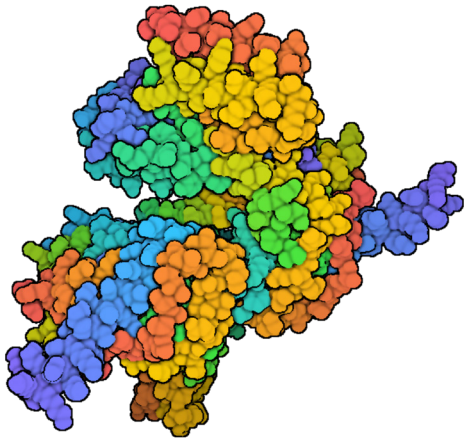
Assay kit components, such as substrates and buffers, can be purchased separately for convenience. About 30 PARP inhibitors are available to use as control or to optimize an assay.

In addition, we offer a sampling set of eight PARP inhibitors including those with a broad binding specificity such as Olaparib, Niraparib, Rucaparib, Talazoparib, Veliparib. AZD5305 is specific toward PARP1, XAV939 is specific toward PARP5A and PARP5B, and RBN-2397 is specific toward PARP7 (Set of PARP Inhibitors, BPS Bioscience #78318).

Conclusion

BPS Bioscience offers the largest available panel of recombinant PARP family members. Purified, tagged recombinant proteins are enzymatically active and suitable for assay development and inhibitor screening or profiling (for example, IC_{50} determination).

Our extensive line of PARP assay kits and services, including unique PARPtrap assays, facilitates the evaluation of drug specificity and effectiveness, and the study of its mechanism of action.



Structure of human PARP1 domains (Zn1, Zn3, WGR, HD) bound to a DNA double strand break. PDB ID: 7s81, Rouleau-Turcotte et al, Mol Cell (2022), PMID: 35793673. Created with BioRender.com

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