A Bespoke Screening Platform to Study Mono-ADP-Ribosylation

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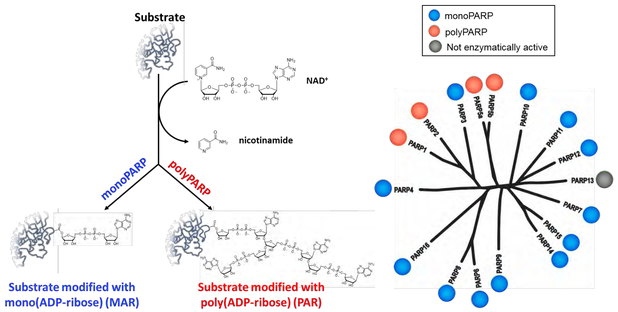
1. Mono-ADP-Ribosylation Primer



- The PARP enzyme family is sub-divided based on the type of ADP-ribosylation performed; at least 6 different nucleophilic amino acids can be modified with MAR derived from nicotinamide adenine dinucleotide (NAD^+)
- PARPs are activated under conditions of cellular stress such as viral infections and cancer

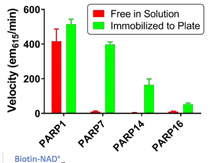
2. MonoPARP Assay Development Challenges

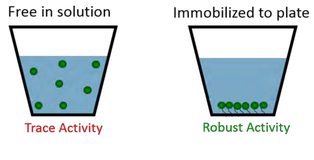
Lack of validated substrates for in vitro enzyme assays

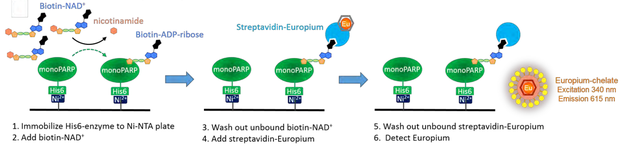
No selective anti-MAR antibodies for assay development

Unclear how monoPARPs are activated

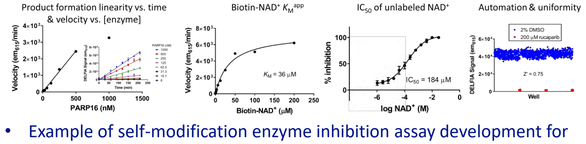
3. Forced Self-Modification of Immobilized PARPs





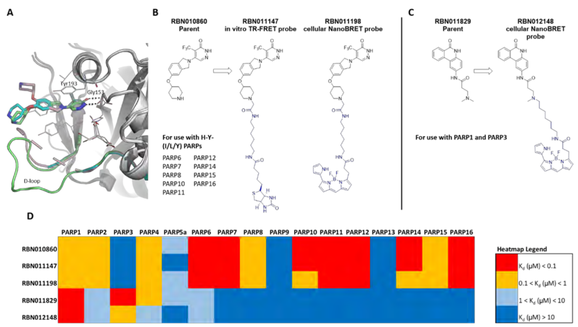


- Immobilization overcomes weak K_M for self-modification
- Dissociation Enhanced Lanthanide Fluorescence Immunoassay (DELFIA) assays developed for all PARPs



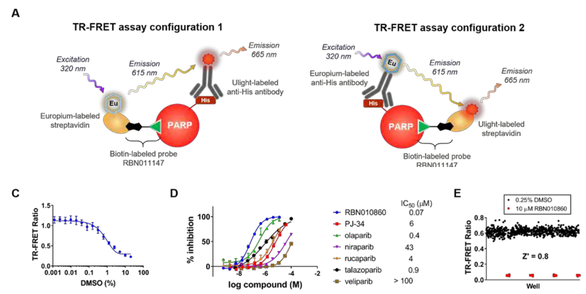
- Example of self-modification enzyme inhibition assay development for PARP16
- DELFIA assays developed for all PARPs

4. NAD+-Competitive Probes for Assay Development

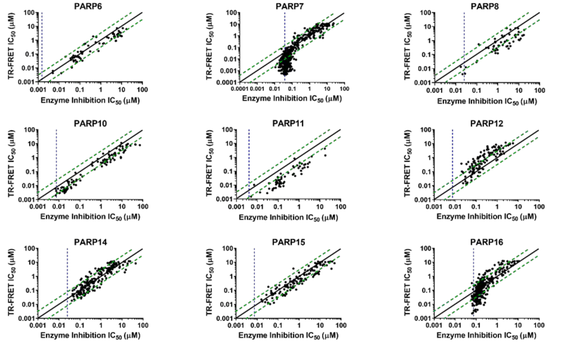


- Pan-PARP inhibitors were modified with a linker + fluorophore or biotin to generate probes for assay development
- Characterization of probe binding by SPR indicates they retain binding affinity

5. In Vitro Probe Displacement Assays

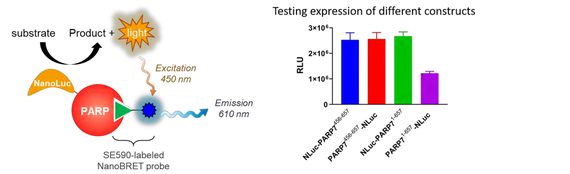


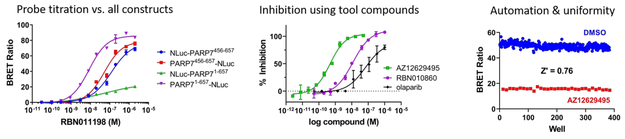
- Example of PARP7 TR-FRET probe displacement assay development
- TR-FRET assays developed for nearly all monoPARPs
- In many cases TR-FRET assays require far less enzyme than DELFIA self-modification assays to observe robust signal



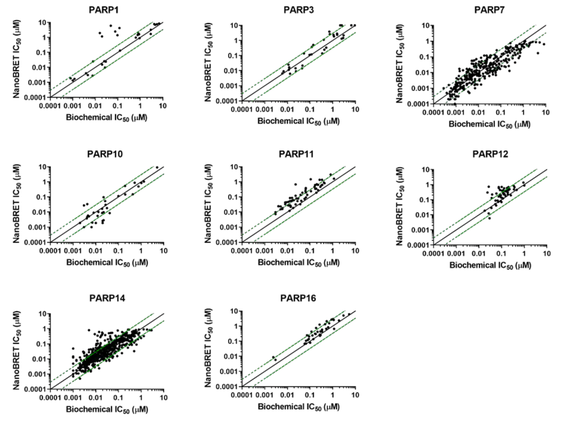
- TR-FRET probe displacement and DELFIA self-modification assays correlate within 3-fold
- TR-FRET extends the potency limit (blue lines) for PARP7 and PARP16, which need high amounts of enzyme to stimulate self-modification

6. Cellular Probe Displacement Assays



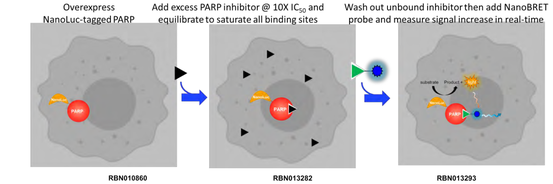


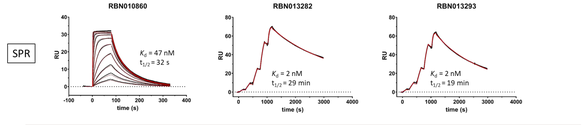
- Multi-step assay development of cellular probe displacement assays successfully applied to multiple PARP enzymes

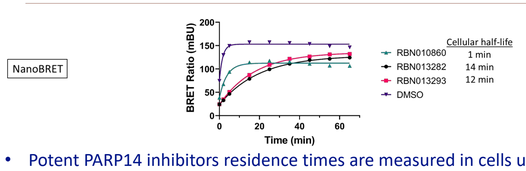


- Cellular probe displacement correlates with enzyme inhibition assays

7. Cellular Inhibitor-Target Residence Time

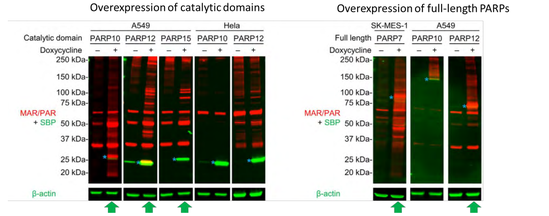




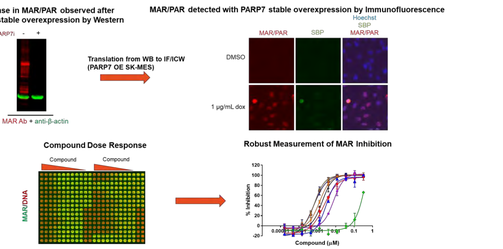


- Potent PARP14 inhibitors residence times are measured in cells using a NanoBRET assay

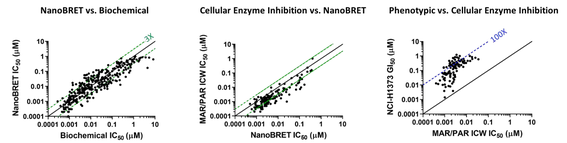
8. Cellular MARylation Assays



- Novel MAR/PAR binding antibody used to detect global MARylation changes after overexpressing PARPs in cell lines
- Effects on MARylation display cell-line dependence



- Cellular MARylation is observed in PARP7 stable overexpression cell line and detection can be measured in high-throughput plate-based format using in-cell Western



- Example of correlation of PARP7 inhibition assays; biochemical inhibition correlates to cellular probe displacement, cellular MARylation and proliferation inhibition in NCI-H1373 cells

9. Conclusions

- Suite of biochemical & cellular assays developed enable family-wide profiling of PARP inhibitors and generation of potent and selective tool compounds for multiple PARP enzymes
- Assays do not rely on knowledge of PARP substrates
- Tool compounds being used at Ribon to investigate role of PARP enzymes in cancer cellular stress response and innate immunity
- RBN-2397, a potent and selective PARP7 inhibitor, was discovered using the assay platform described here. RBN-2397 is in a phase 1 trial in cancer patients.

REFERENCES:
Lu et al, *Biochem Pharmacol*. (2019)
Wigle et al. *SLAS Discovery* (2019)