Exploring the Landscape of PARP Inhibitor Selectivity in Live Cells Using NanoBRET™ Target Engagement Assays

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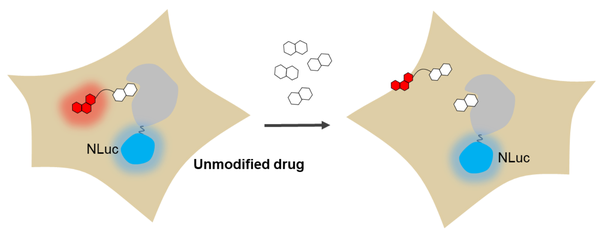
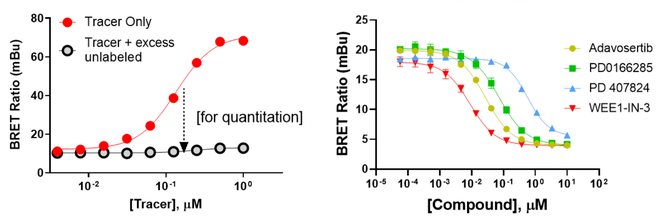
1. Abstract

The 17-member poly(ADP-ribose) polymerase (PARP) family of proteins mediate a number of cellular processes including gene transcription and DNA repair, and are widely implicated in human disease. However, a number of family members (especially those mediating mono ADP ribosylation) are considered understudied and generally lack described substrates and chemical inhibitor annotation. Consequently, majority of PARP proteins lack chemical probes as selective tools to modulate and study PARP function in cells and tissues.

Here we describe the development and application of a novel target engagement method for characterizing PARP inhibitor selectivity and affinity in live cells. This method is based on the NanoBRET™ assay platform, which is a proximity-based probe displacement assay that operates in live cells. This method is based on biophysical engagement and is therefore independent of target function. NanoBRET™ assays are therefore ideally suited for proteins that lack functional annotation. Using NanoBRET™ technology, we have explored the family-wide selectivity of a diverse panel of clinical and preclinical PARP inhibitors in live cells. We observe a surprising spectrum of inhibitory activity for some "PARP1/PARP2" inhibitors against lesser-studied mono-PARPs, offering chemical starting points for further optimization. This method can also be used to quantify drug-target residence time in live cells. Using the residence time configuration, we uncover opportunities for achieving kinetic selectivity for PARP family members that is not readily observed under thermodynamic equilibrium. This represents the first family-wide, cell-based method for profiling PARP selectivity under both equilibrium and non-equilibrium conditions.

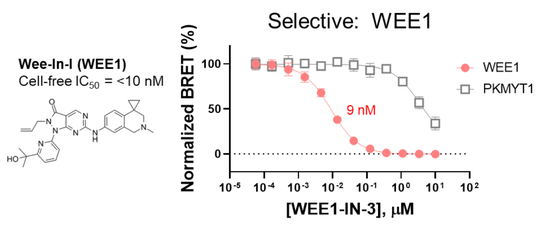
2. NanoBRET™ Target Engagement (TE): A quantitative probe displacement assay in live cells

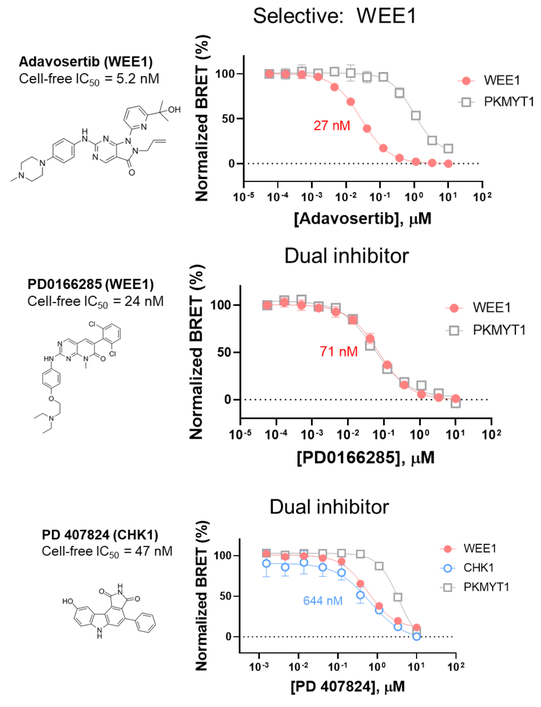




- The NanoBRET™ assay is specific for the target fused to NanoLuc® luciferase, since BRET assays are governed by tight distance constraints between energy donor (NanoLuc® luciferase) and energy acceptor (tracer).
- NanoBRET™ assays are conducted in live cells that allow equilibrium binding analysis and real time binding analysis.

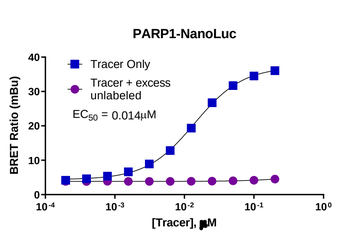
3. DDR Kinase Profiling WEE1, PKMYT1, and CHK1 Kinase Inhibitors

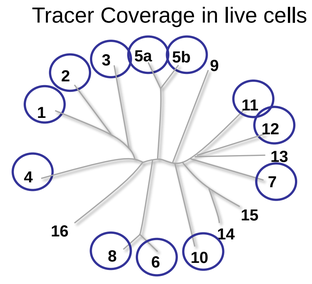




- Examples of highly selective WEE1 inhibitors (top panels) and of inhibitors with weaker selectivity (bottom panels)

4. Pan-PARP NanoBRET™ TE System





- HEK293 cells were transfected with PARP1-NanoLuc plasmid and were treated with serially diluted Pan-PARP NanoBRET™ Tracer in the presence or absence of a vast molar excess of unlabeled parental ligand.
- We've shown that the Pan-PARP Tracer can be used for NanoBRET™ TE assays for many PARP members.

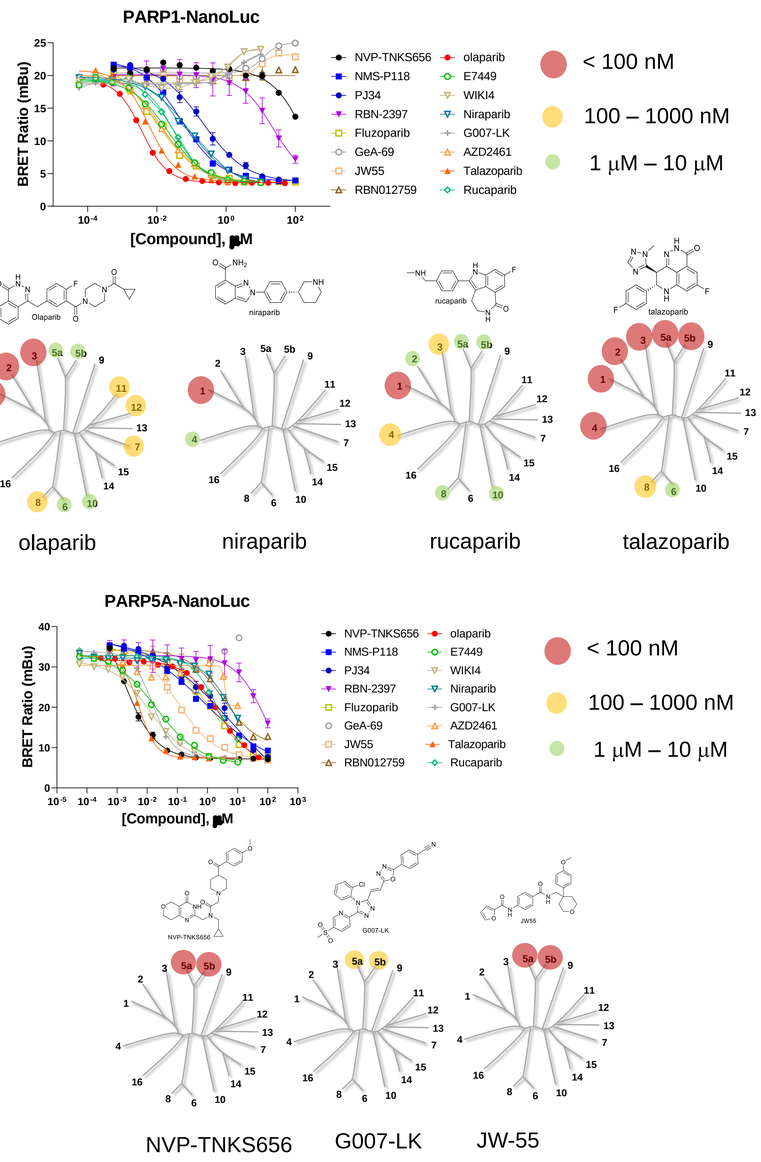
5. Mapping of Inhibitor-PARP Engagement in Cells

	PARP1	PARP2	PARP3	PARP4	PARP5a	PARP5b	PARP6	PARP7	PARP8	PARP10	PARP11	PARP12
NVP-TNKS656	100	100	100	100	0.00552	0.00102	100	100	100	21.35	100	100
NMS-P118	0.05105	100	3.306	100	0.8191	0.2184	100	100	100	100	100	100
PJ34	0.2139	7.206	11.69	100	6.696	0.9635	100	100	100	33.23	100	100
RBN-2397	100	7.681	100	100	100	100	100	0.00132	100	100	0.9603	0.003407
Fluzoparib	0.008168	0.00796	0.08137	4.109	1.22	100	11.94	0.07686	1.686	1.493	0.09323	0.1054
GeA-69	100	100	35.26	100	100	100	100	100	100	100	100	100
JW55	100	100	100	100	0.1013	0.08395	100	100	100	100	100	100
RBN012759	100	100	100	100	3.243	100	100	1.846	100	0.6031	0.2196	100
Rucaparib	0.03101	1.229	0.2426	0.548	1.445	0.7792	100	100	100	2.182	10	100
Talazoparib	0.00598	0.00701	0.05023	0.003275	0.005374	0.002555	4.289	10	0.4154	10	10	10
AZD2461	0.01485	0.03359	0.4246	100	10	10	10	10	1.439	10	10	10
G007-LK	10	10	10	100	0.01531	0.00743	10	10	100	10	10	10
Niraparib	0.09055	10	10	1.847	10	10	10	10	100	10	10	10
WIKI4	10	10	10	100	0.01203	0.004128	10	10	100	10	10	10
E7449	0.02047	0.03279	0.7722	0.1891	0.02038	0.009454	10	10	1.696	10	10	10
Olaparib	0.003642	0.002626	0.01068	0.3122	2.424	1.209	3.987	0.259	0.9858	5.931	0.414	0.4723

IC50, uM	
0.00015-0.015	Red
0.015-0.15	dark orange
0.15-1.5	yellow
1.5-15.0	light green
15-150	green

- NanoBRET™ PARP TE assays were performed using HEK293 cells transiently expressing, PARPs C-terminally tagged NanoLuc all except for PARP4 (which used N-terminal Nanoluc).
- Pan-PARP NanoBRET tracer was added at the apparent tracer affinity for each PARP.
- BRET was quantified after 2h equilibration, and IC50 determined.

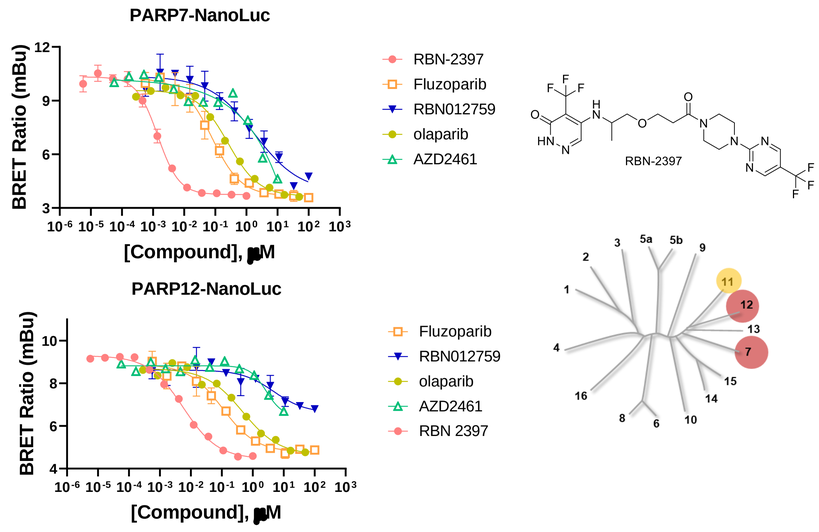
6. Profiling Examples for FDA-approved PARP Inhibitors



NVP-TNKS656 G007-LK JW-55

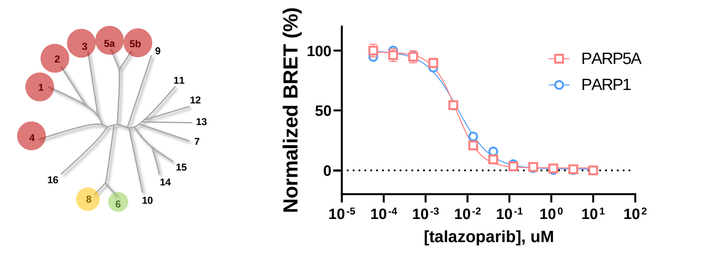
- PARP1 inhibitors are promiscuous against lesser-studied PARPs (upper).
- PARP5 inhibitors are highly selective in live cells (lower).

7. Patterns of Chemical Phylogeny for PARP7 and PARP12



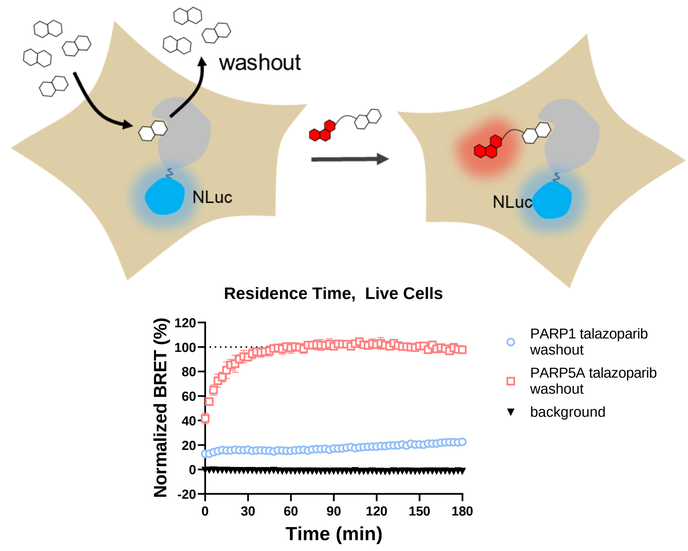
- A number of PARP inhibitors including RBN-2397 engage PARP7 and PARP12 with similar engagement patterns, supporting a phylogenetic relationship between these PARP family members
- Achieving selectivity between these PARPs may represent a technical challenge

8. Residence Time Analysis Reveals Kinetic Selectivity



Residence Time Protocol

1. Drug affinity determined at steady state (above)
2. IC90 concentration calculated
3. Drug added to cells IC90 and equilibrated
4. After binding, drug removed and washed
5. Tracer added immediately after drug removal
6. BRET recorded in real time
7. Residence time determined from observed association rate of tracer



- Similar affinities observed for PARP1 vs PARP5, supporting weak selectivity for PARP1 under equilibrium conditions
- Talazoparib has kinetic selectivity for PARP1 over PARP5, supporting the potential for selectivity under *in vivo* conditions

9. Conclusions

NanoBRET™ TE assays broadly enable the quantitative determination of compound affinity for specific targets in live cells

- A cell permeable NanoBRET™ Tracer has been developed that allow TE assays for PARP family members.
- PARP1/2 selectivity inhibitors react with a number of lesser-studied mono-PARPs.
- Patterns of chemical phylogeny can be established among some of the lesser studied family members.

Residence time for specific PARPs in live cells is measured with NanoBRET™ TE assay

- Using both equilibrium & residence time methods, selectivity may be revealed- offering unique inhibitor development opportunities.
- Residence time may be selectivity determinant for PARP inhibitors.

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