Precision Meets Throughput in Targeted Protein Degradation

Accelerate Degrader Screening with High-Throughput, Reproducible Assays on the Leo System

Introduction

Targeted protein degradation (TPD) is rapidly evolving, opening new therapeutic opportunities against traditionally undruggable targets.[1] Efficient and precise protein quantification remains essential yet challenging.[2] **Simple Western™ Technology** offers quantitative, reproducible data with minimal hands-on time. With the **Leo™ System**, the newest innovative platform in Simple Western Technology, TPD research is now more efficient and reliable, accelerating therapeutic discovery and development.

The Need for Precision in TPD

TPD strategies, including PROTAC® Degraders and molecular glues, rely heavily on accurate protein quantification to determine degradation efficiency.[2] Plate based assays offer high-throughput library screening, but every TPD project requires follow-up quantification of the endogenous target. Traditional western blots are labor-intensive and lack precision, complicating measurement of dynamic protein level changes. Therefore, an automated, high-throughput approach delivering consistent results is essential.

Introducing Leo: Redefining Throughput and Efficiency

Leo elevates automation and throughput in Simple Western workflows. It supports up to 96 unique samples per run, providing unmatched flexibility and efficiency. The system's capabilities are especially advantageous for high-throughput scenarios, like dose-response and time course experiments for degrader characterization.

To illustrate Leo's effectiveness, we characterized androgen receptor (AR) degradation by ARCC 4 degrader, clinically relevant for prostate cancer therapy.[1,3] Our 2-pronged analytical approach encompassed dose-response and time-course analyses (**Figure 1**), showcasing Leo’s precision, throughput, and reproducibility. Comparisons with other Simple Western systems (Jess™ System, Peggy Sue™ System, and a second Leo System) affirmed assay transferability and highlighted Leo's substantial savings in time, reagents, and antibodies.

Leo Run 1: Dose-Response
2 compounds × **8** concentrations × **2** biological replicates per compound × **3** technical replicates = **96** samples

Leo Run 2: Time Course
2 compounds × **6** time points × **3** biological replicates per compound × **2 or 3** technical replicates = **96** samples

Figure 1. Experimental setup to characterize AR degradation by ARCC 4 on Leo. Degradation was characterized with 2 Leo runs at full 96-sample capacity: (1) dose-response analysis to determine DC_{50} and D_{max} and (2) time course analysis to determine degradation half-life ($t_{1/2}$) and degradation constant (k_{deg}). Results from Leo were also compared to Simple Western systems—Jess, Peggy Sue, and a second Leo.

Materials and Methods

All materials used in this study are provided in **Table 1**. Dose-response and time course analyses focused on clinically significant AR target degradation by ARCC 4 in MDA-MB-453 cells.

Dose-Response Cell Treatment Conditions

MDA-MB-453 cells were treated in biological duplicate with a 7-point, 4-fold titration (40–0.0098 µM) of ARCC 4 and ARCC 4 Negative Control and 0.4% DMSO vehicle only control for 24 hours. Cell pellets were lysed with 75 µL of RIPA Lysis Buffer with DMSO and Aqueous inhibitors. The total protein concentration was determined with BCA and lysates were aliquoted and snap-frozen for Simple Western analysis.

Time Course Cell Treatment Conditions

MDA-MB-453 cells were treated in biological triplicate with 10 µM ARCC 4 and 10 µM ARCC 4 Negative Control for 1 h, 3 h, 6 h, 15 h, and 24 h, including a 0.4% DMSO vehicle only control for 1 h. Cell pellets were lysed with 75 µL of RIPA Lysis Buffer with DMSO and Aqueous inhibitors. The total protein concentration was determined with BCA and lysates were aliquoted and snap-frozen prior to Simple Western analysis.

Name	Cat. #
Jess 12-230 kDa Separation Module	SM-W001
Leo 12-230 kDa Separation Module	SWSM-W014
Anti-Rabbit Detection Module	DM-001
RePlex™ Module	RP-001
Total Protein Detection Module	DM-TP01
Lysis Kit - RIPA Buffer	CBS401
Androgen R/NR3C4 Antibody	MAB58762
ARCC 4	7254
ARCC 4 Negative Control	7255

Table 1. Materials used in this study. All materials are available from Bio-Techne.

Simple Western Analysis

The assay for AR detection was optimized on Jess and transferred to Leo and Peggy Sue without modification. All samples were denatured under reducing conditions in 1X Master Mix for 5 minutes at 95 °C. The anti-AR primary antibody was diluted to 50 µg/mL (1:10) in Antibody Diluent 2 and the Anti-Rabbit Secondary Antibody and Total Protein SA were used at ready-to-use concentrations. Lysates were analyzed at 0.2 mg/mL final concentrations. For Leo and Jess analysis, AR expression was normalized to total protein measured using RePlex with chemiluminescence detection. Peggy Sue is not compatible with Total Protein Normalization using RePlex and therefore only raw (unnormalized) values are reported for Peggy Sue.

For the dose-response experiment, lysates from treated cells were analyzed on Simple Western systems, Leo, Jess, and Peggy Sue. All four sample sets were analyzed on Leo, one per cartridge, with cartridge normalization. On Jess, one sample set was performed per run for a total of four runs with normalization. A nonlinear regression 4-Parameter Logistic fit (unweighted) was used to calculate DC_{50} values. D_{max} was calculated from the percent signal change between the Vehicle Control and 24-hour time point where the vehicle only control is 100%.

For the time course experiment, all samples were analyzed on Leo and one time course per run (ARCC 4 or negative control) was run on Jess. A nonlinear regression One Phase Decay (unweighted) was used to establish half-life degradation ($t_{1/2}$). D_{max} was calculated with the percent signal change between the vehicle only control and the 24-hour time point, where the vehicle only control represents 100% AR expression.

Results

Dose-Response Analysis of AR Degradation

At a Glance

- AR degradation was evaluated across Leo, Jess, and Peggy Sue using a 7-point ARCC 4 titration. While raw AR peak areas varied by instrument (**Figure 2A**), normalized dose-response curves were nearly identical (**Figure 2B**), highlighting consistent assay performance.
- DC_{50}, D_{max}, and hillslope values were reproducible across instruments, with CVs ≤ 5.1% (**Table 2**).
- Total protein normalization using RePlex yielded nearly identical DC_{50} values (**Figure 3**).
- Biological replicates confirmed inter-run consistency (**Tables 3–4**, **Figures 4–5**).
- Leo outperformed Jess in runtime and antibody use, with 3–4 times greater efficiency (**Table 5**).

We characterized AR degradation activity by dose-response analysis and compared results across Simple Western systems, Leo, Jess, and Peggy Sue. To ensure accurate comparison of assay performance, the same assay for AR detection was run on each instrument without modification using default instrument settings. The results from each instrument showed that the AR peak area decreased with increasing ARCC 4 concentrations, with little to no response to dosing by the negative control, as expected (**Figure 2A**).

While each instrument showed similar response to degrader dosing, the raw peak AR area differed between instruments, namely Jess produced the highest peak area and Peggy Sue produced the lowest AR peak signal under each dosing condition tested (**Figure 2A**). It is important to note that differences in raw peak areas obtained between instruments is not a meaningful measure of assay performance, and it is not unexpected that each instrument produces a unique raw peak area value under identical experimental conditions.

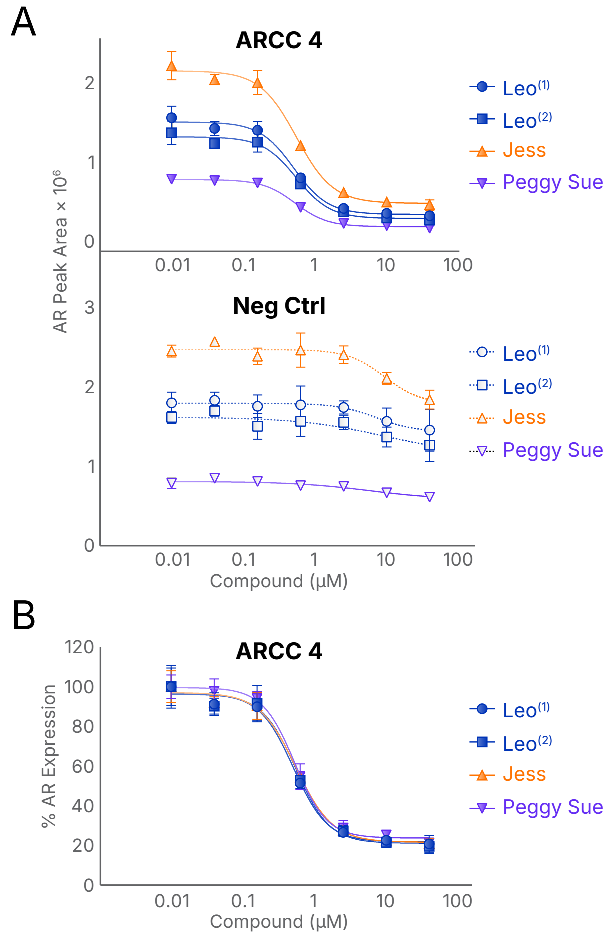

Figure 2. Dose-response analysis of AR degradation by (A) peak area and (B) peak area normalized to the lowest dose. Values represent averages of 3 biological replicates and 3 technical replicates (n = 6). Error bars represent standard deviations from the means. Leo(1) and Leo(2) are 2 separate Leo instruments.

Nearly identical dose-response to degrader treatment was observed when normalizing raw peak area from cells treated with the lowest compound dose (**Figure 2B**). The DC_{50} values obtained from each instrument were within 0.05 µM of each other or closer (**Figure 3**, top panel). For analysis on Jess and Leo, we measured total protein in the same run using the RePlex feature, which is not compatible with Peggy Sue. When normalized to total protein, the DC_{50} values between Leo and Jess were within 0.04 µM of each other or closer. (**Figure 3**, bottom panel). Although raw peak areas varied by instrument with a CV of 38.6%, the key parameters for characterizing degrader activity (DC_{50}, D_{max}, and hillslope) were reproducible between the instruments, with a CV of 5.1% or less across instruments (**Table 2**).

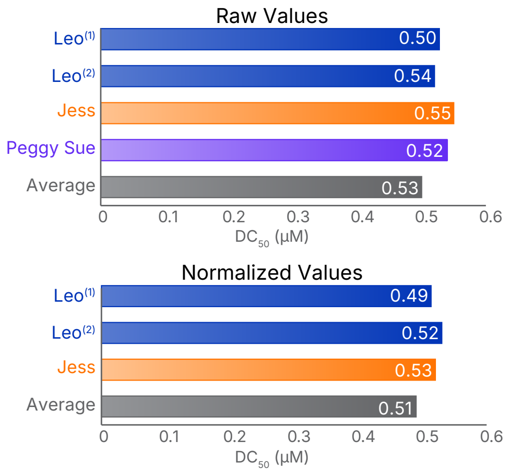

Figure 3. DC_{50} values (raw and normalized by total protein) across instruments (n = 6). Only raw values are reported for Peggy Sue due to incompatibility with total protein detection. Leo[(1)] and Leo[(2)] are 2 separate Leo instruments.

Instrument	Peak Area	D_{max} (%)	Hillslope
Leo[(1)]	9.3×10^5	78.1	-1.82
Leo[(2)]	8.1×10^5	79.5	-1.84
Jess	1.3×10^6	76.7	-1.69
Peggy Sue	4.9×10^5	78.7	-1.92
CV	**38.6%**	**1.5%**	**5.1%**

Table 2. Inter-instrument variability. Values represent averages of 3 biological replicates and 3 technical replicates (n = 6). Leo[(1)] and Leo[(2)] are 2 separate Leo instruments.

These results indicate that dose-response assays confirmed AR degradation efficacy across instruments (Leo, Jess, Peggy Sue), highlighting robust assay transferability between instruments with consistent DC_{50}, D_{max}, and hillslope values.

To take a closer look at degrader activity on Leo and Jess, we plotted individual biological replicates (**Figure 4**), as well as the averages across all replicates of each compound treatment (**Figure 5**).

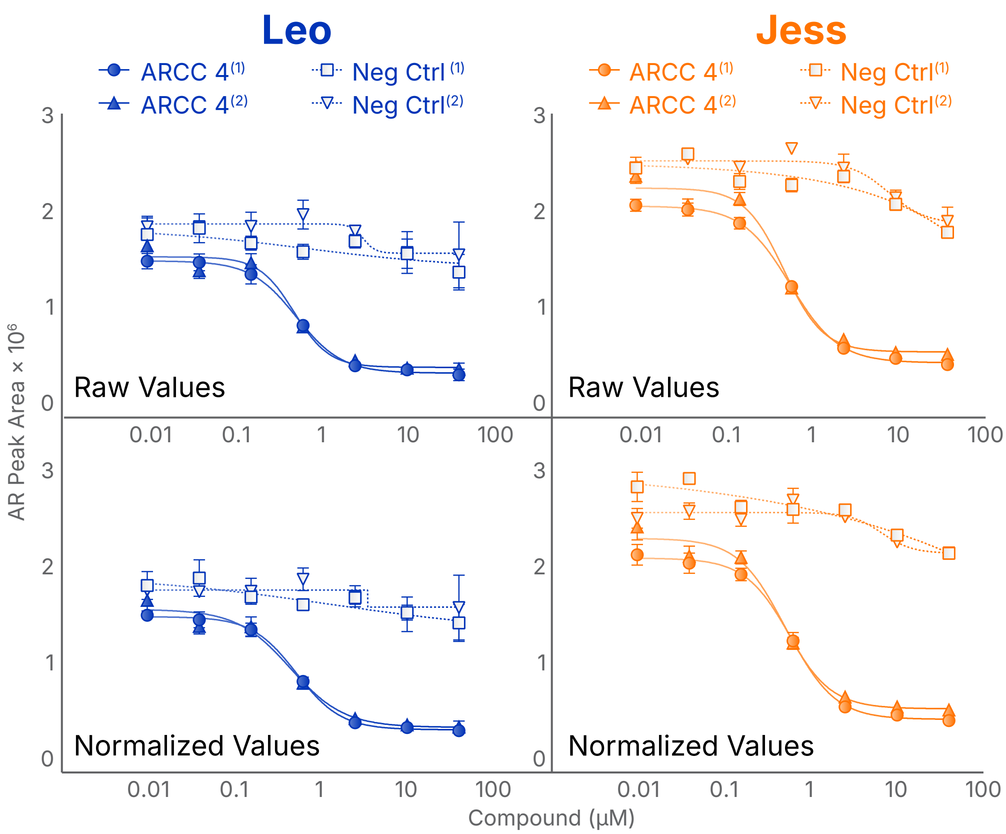

Figure 4. Dose response by treatment with ARCC 4 and Negative Control as represented by raw AR peak areas (top panels) and AR peak areas normalized by total protein (bottom panels). Biological replicate samples are indicated by [(1)] and [(2)]. Values represent averages from 3 technical replicates (n = 3) for both Leo (left panel) and Jess (right panel). Error bars represent standard deviations from the means.

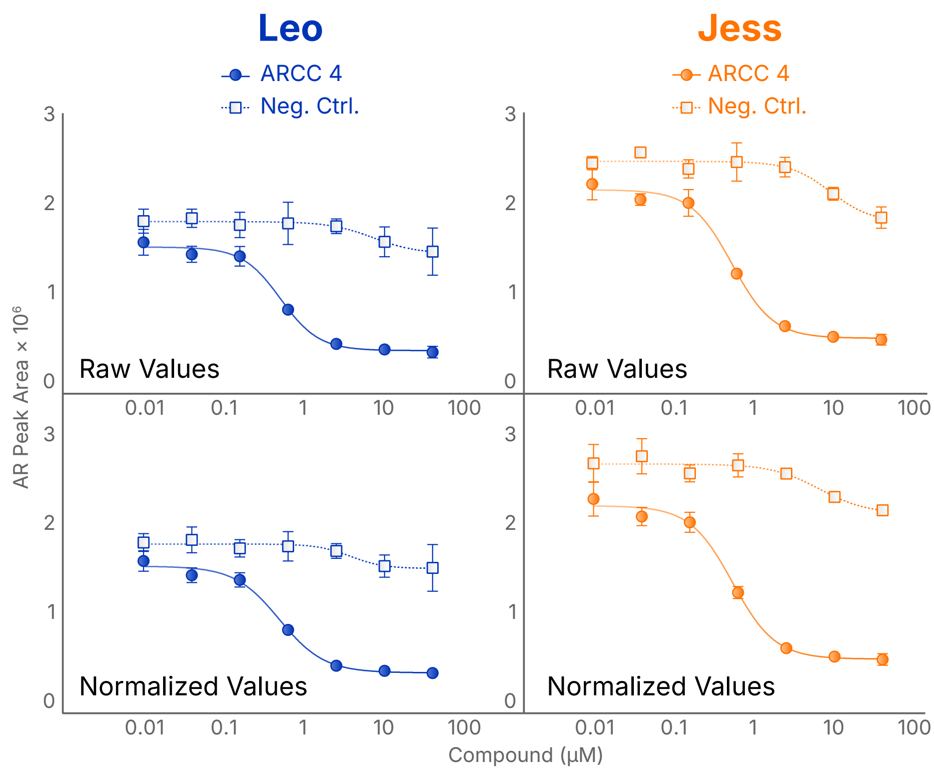

Figure 5. Dose response by treatment with ARCC 4 and Negative Control as represented by raw AR peak area (top panels) and AR peak area normalized by total protein (bottom panels). Values represent averages of all biological and technical replicates (n = 6) for both Leo (left) and Jess (right). Error bars represent standard deviations from the means.

As described above, the peak area values differed between Leo and Jess, but similar DC_{50} and D_{max} values were obtained between Leo and Jess (**Tables 3–4**). Again, these results show how differences in raw peak areas between instruments do not necessarily correlate with differences in assay performance.

While Jess and Leo provide reproducible results, Leo significantly improves throughput, reducing sample preparation and runtime by 3–4 times compared to Jess. This operational efficiency translates directly to accelerated degrader screening and optimization (**Table 5**).

	Raw Values (µM)			Normalized Values (µM)		
Replicates	Leo(1)	Leo(2)	Jess	Leo(1)	Leo(2)	Jess
Rep 1	0.52	0.58	0.60	0.52	0.55	0.60
Rep 2	0.49	0.52	0.51	0.45	0.49	0.48
All Reps	0.50	0.54	0.55	0.49	0.52	0.53

Table 3. DC_{50} values (raw and normalized by total protein) calculated from dose-response degradation curves. Biological replicate samples are indicated by Rep 1 and Rep 2 and represent averages from 3 technical replicates (n = 3). Values of all replicates represent averages of all biological and technical replicates (n = 6). Leo(1) and Leo(2) represent 2 separate Leo instruments.

	Raw Values (%)			Normalized Values (%)		
Replicates	Leo(1)	Leo(2)	Jess	Leo(1)	Leo(2)	Jess
Rep 1	79.7	79.4	78.6	80.5	80.8	85.1
Rep 2	76.6	79.6	76.1	79.2	80.7	82.9
All Reps	78.1	79.5	76.7	79.8	80.8	83.4

Table 4. D_{max} values (raw and normalized by total protein) calculated from dose-response degradation curves. Biological replicate samples are indicated by Rep 1 and Rep 2 and represent averages from 3 technical replicates (n = 3). Values of all replicates represent averages of all biological and technical replicates (n = 6). Leo(1) and Leo(2) represent 2 separate Leo instruments.

	Leo *(1 run)*	Jess *(4 runs)*	Savings
Sample & Reagent Prep*	80 min	4 h *(60 min × 4 runs)*	3X faster
Plate Fill	40 min	2 h *(30 min × 4 runs)*	3X faster
1° & 2° Antibodies	480 µL	1920 µL *(480 µL × 4 runs)*	75% less antibody
Run Time	5 h	20 h *(5 h × 4 runs)*	4X faster (+1 day)
Data Points	100	100 *(25 × 4 runs)*	-

Table 5. Time and antibody savings on Leo versus Jess for the dose-response study performed here. The times listed are approximately based on typical sample and reagent preparation for the respective instruments. *This study used split-sample material for the best comparison instrument to instrument.

Time Course Analysis of Degradation Kinetics

At a Glance

- We tracked AR degradation over 1–24 hours using 10 µM ARCC 4 and negative control (**Figures 6–7**).
- Leo and Jess delivered comparable kinetic values ($t_{1/2}$, D_{max}, k_{deg}) across replicates (**Tables 6–8**).
- Leo reduced run time, antibody use, and prep effort, doubling throughput (**Table 9**).

Finally, we sought to characterize the kinetics of degradation by performing a time course study. From the dose-response analysis described previously, an ARCC 4 dosing concentration of 10 µM showed degradation 99% of D_{max} at 24 hours. Therefore, we tested various time points between 1–24 hours using 10 µM ARCC 4.

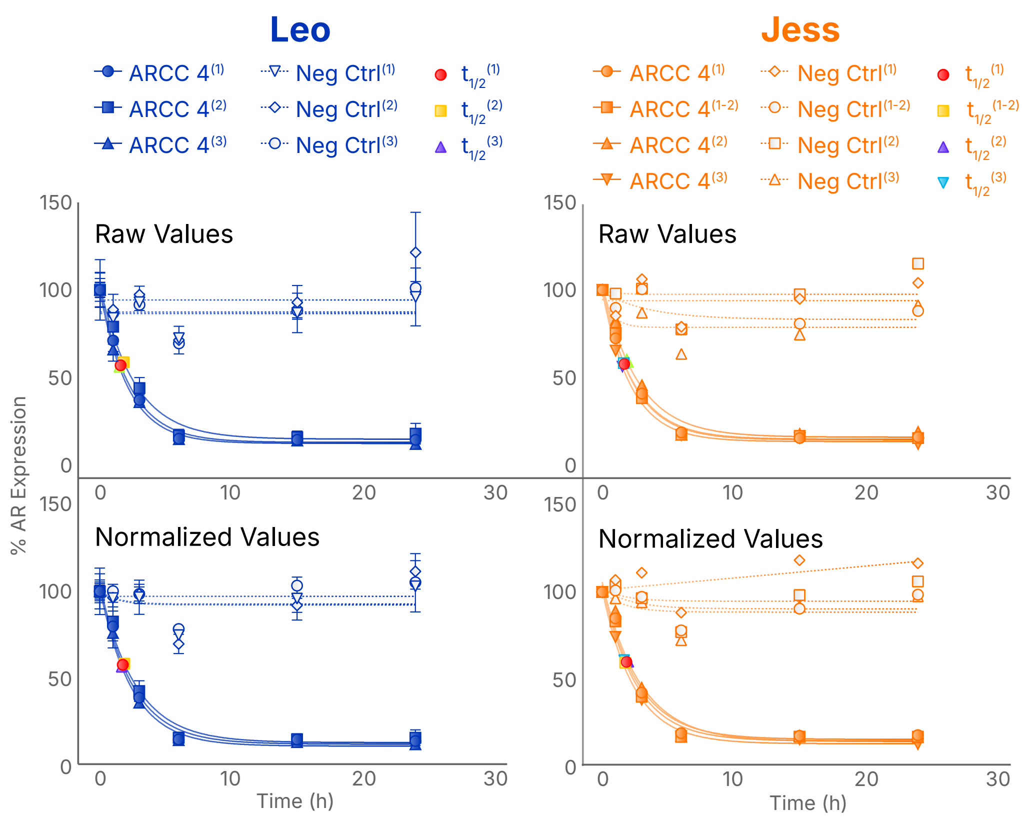

Figure 6. Time-course degradation by treatment with ARCC 4 and Negative Control. Biological replicate samples are indicated by (1), (2), and (3). Values for Leo (left panel) represent averages from 3 technical replicates (n = 3), except the third biological replicate is the average of 2 technical replicates (n = 2). Error bars represent standard deviations from the means. Values for Jess (right panel) represent single measurements. The first biological replicate was repeated and plotted separately, indicated by (1-2).

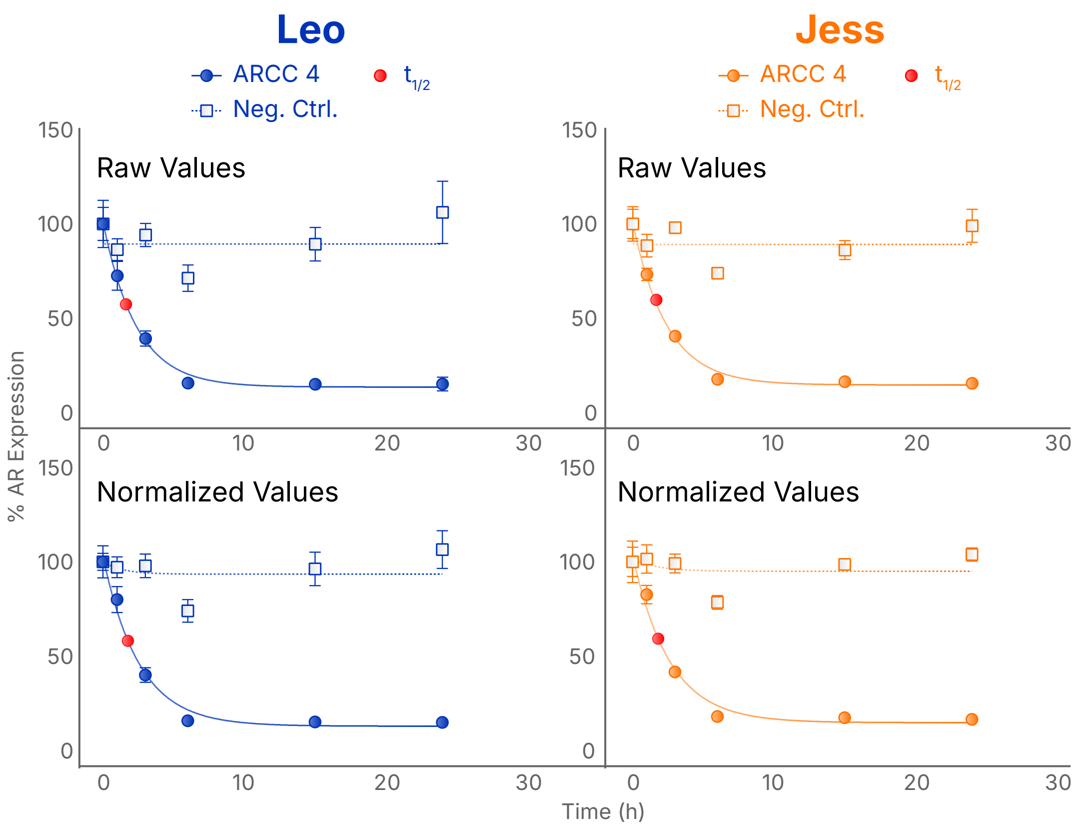

Figure 7. Time-course degradation by treatment with ARCC 4 and Negative Control. Values represent averages of all replicates, n = 8 and n = 4 on Leo and Jess, respectively. Error bars represent standard deviations from the means.

We examined the kinetics of degrader activity on Leo and Jess by plotting individual biological replicates at each time point (**Figure 6**), and by plotting the averages across all biological and technical replicates of degrader treatment (**Figure 7**). We calculated the $t_{1/2}$, D_{max}, and k_{deg} for Leo and Jess (**Table 6–8**), which demonstrate that the assay performs similarly on Leo and Jess. These results underscore Leo's accuracy in capturing essential kinetic parameters critical for degrader optimization.

Leo significantly improves throughput for time course studies, reducing sample preparation and runtime compared to Jess (**Table 9**).

$t_{1/2}$	Raw Values		Normalized Values	
Replicate	Leo	Jess	Leo	Jess
Rep 1	1.57	1.67, 1.63	1.75	1.85, 1.76
Rep 2	1.81	1.86	1.87	1.98
Rep 3	1.47	1.52	1.67	1.68
All Reps	1.63	1.67	1.78	1.82

Table 6. $t_{1/2}$ values (raw and normalized by total protein) calculated from time course degradation curves. Biological replicate samples are indicated by Rep 1–3. Leo Rep 1–2 represent averages from 3 technical replicates (n = 3), and Rep 3 is the average of 2 technical replicates (n = 2). Jess Rep 1–3 represent single measurements (n = 1). Jess Rep 1 was repeated and both measurements are shown.

D_{max} (%)	Raw Values		Normalized Values	
Replicate	Leo	Jess	Leo	Jess
Rep 1	88.5	83.8, 84.5	85.3	81.6, 82.5
Rep 2	81.9	80.9	83.4	82.8
Rep 3	87.8	87.8	87.2	86.7
All Reps	84.8	84.4	85.1	83.5

Table 7. D_{max} values (raw and normalized by total protein) calculated from time course degradation curves. Biological replicate samples are indicated by Rep 1–3. Leo Rep 1–2 represent averages from 3 technical replicates (n = 3), and Rep 3 is the average of 2 technical replicates (n = 2). Jess Rep 1–3 represent single measurements (n = 1). Jess Rep 1 was repeated and both measurements are shown.

k_{deg}	Raw Values		Normalized Values	
Replicate	Leo	Jess	Leo	Jess
Rep 1	0.442	0.414, 0.426	0.396	0.375, 0.393
Rep 2	0.382	0.374	0.371	0.349
Rep 3	0.470	0.457	0.415	0.412
All Reps	0.425	0.416	0.391	0.381

Table 8. k_{deg} values (raw and normalized by total protein) calculated from time course degradation curves. Biological replicate samples are indicated by Rep 1–3. Leo Rep 1–2 represent averages from 3 technical replicates (n = 3), and Rep 3 is the average of 2 technical replicates (n = 2). Jess Rep 1–3 represent single measurements (n = 1). Jess Rep 1 was repeated and both measurements are shown.

	Leo *(1 run)*	**Jess** *(2 runs)*	**Savings**
Sample & Reagent Prep*	80 min	2 h *(60 min × 2 runs)*	1.5X faster
Plate Fill	40 min	1 h *(30 min × 2 runs)*	1.5X faster
1° & 2° Antibodies	480 µL	960 µL *(480 µL x 2 runs)*	50% less
Run Time	5 h	10 h *(5 h × 2 runs)*	2X faster
Data Points	100	50 *(25 × 2 runs)*	2X more

Table 9. Time and antibody savings on Leo versus Jess for the time course study performed here. The times listed are approximately based on typical sample & reagent preparation for the respective instruments. *This study used split-sample material for the best comparison instrument to instrument.

Conclusion

Leo: A New Standard for TPD Assays

Leo sets a new benchmark in automated protein analysis by combining exceptional throughput with analytical-grade precision. Capable of processing up to 96 samples in a single 3-hour run, Leo enables researchers to conduct comprehensive dose-response and time-course studies with unprecedented efficiency.

Designed for high-resolution quantification, Leo Simple Western Systems delivers consistent and reproducible results across experiments, with single-digit CV values. This level of precision ensures reliable data critical for PROTAC and molecular glue screening.

Moreover, Leo's compatibility with existing Simple Western assays facilitates seamless assay transferability between laboratories and instruments, supporting collaborative research and multi-site studies, while offering significant time and antibody savings.

By streamlining complex workflows and delivering rapid, high-quality data, Leo empowers scientists to accelerate the development of targeted protein degradation therapeutics.

References

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