Technical Overview

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Analysis of Protein Crude Lysates on the Agilent ProteoAnalyzer System

Introduction

Protein extraction from cells or tissues generates a crude protein lysate that can be further purified for downstream use. Traditionally, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) has been used for the analysis of crude protein lysates. However, this method faces obstacles such as extensive processing and analysis time, coupled with an absence of full automation. To overcome these limitations, analytical instruments, such as automated sodium dodecyl sulfate capillary electrophoresis (CE-SDS), are being used.

The Agilent ProteoAnalyzer system is an automated parallel CE-SDS instrument equipped with a 12-channel capillary array that requires only 1 µL of sample and has a run time of 30 minutes.[1] The ProteoAnalyzer is designed to perform runs consecutively by completing a cleaning and conditioning step between each run. These steps eliminate any material buildup or sample carryover and rejuvenate the capillaries for subsequent injections, providing reliable analysis. Additionally, samples are electrokinetically injected, which minimizes the chances of an unhomogenized sample from entering the capillary. Combined, these features allow the ProteoAnalyzer to analyze challenging samples such as protein crude lysates.

In this technical overview, the sizing and quantification of protein crude lysates from various tissue types were assessed across multiple runs. The consistent results generated in these experiments with the ProteoAnalyzer demonstrate the ability of the system to provide reliable protein measurements.

Methods

Chicken heart, porcine liver, and porcine kidney tissues were obtained locally and homogenized in RIPA buffer (sodium chloride 150 mM, Tris-HCl 50 mM, sodium deoxycholate 0.5%, SDS 0.1%, PMSF 0.005%). The samples were clarified by centrifugation (20,000 x g for 20 minutes at 4 °C). Each sample was analyzed on the Agilent ProteoAnalyzer system using the Protein Broad Range P240 kit (part number 5191-6640) under reduced conditions, then evaluated using the Agilent ProSize data analysis software.

Samples were also analyzed with SDS-PAGE using precast gels (Bio-Rad, part number 4569036) under reduced conditions. Each sample was diluted 3:1 with 4x Laemmli buffer (Bio-Rad, part number 161-0747), with a final concentration of 50 mM DTT. The samples were heat denatured at 90 °C for 5 minutes, then 10 µL of each concentration was loaded onto the SDS-PAGE gels, and Bio-Rad Precision Plus Protein Dual Color Standard (part number 161-0374) was loaded in the wells flanking the sample lanes.

Separation was conducted at 200 V for approximately 40 minutes. The gels were fixed (10% acetic acid, 40% ethanol, 50% water) for 15 minutes with rocking, then rinsed with water. The gels were stained overnight in Bio-Rad QC Colloidal Coomassie stain (part number 1610803) and destained with de-ionized (DI) water for 3 hours. Gel image analysis from SDS-PAGE was performed using GelAnalyzer software[2] for sizing.

Results and discussion

Equivalency of the ProteoAnalyzer and SDS-PAGE

Following electrophoretic separation on the ProteoAnalyzer system, the data was automatically processed using the ProSize data analysis software. Samples are visualized as a digital gel image or an individual electropherogram of each sample, along with a results table that provides valuable information such as size and concentration. The digital gel image is analogous to an SDS-PAGE gel, while the electropherogram provides more detailed insight into the sample's integrity, with different peaks corresponding to different protein species (Figure 1).

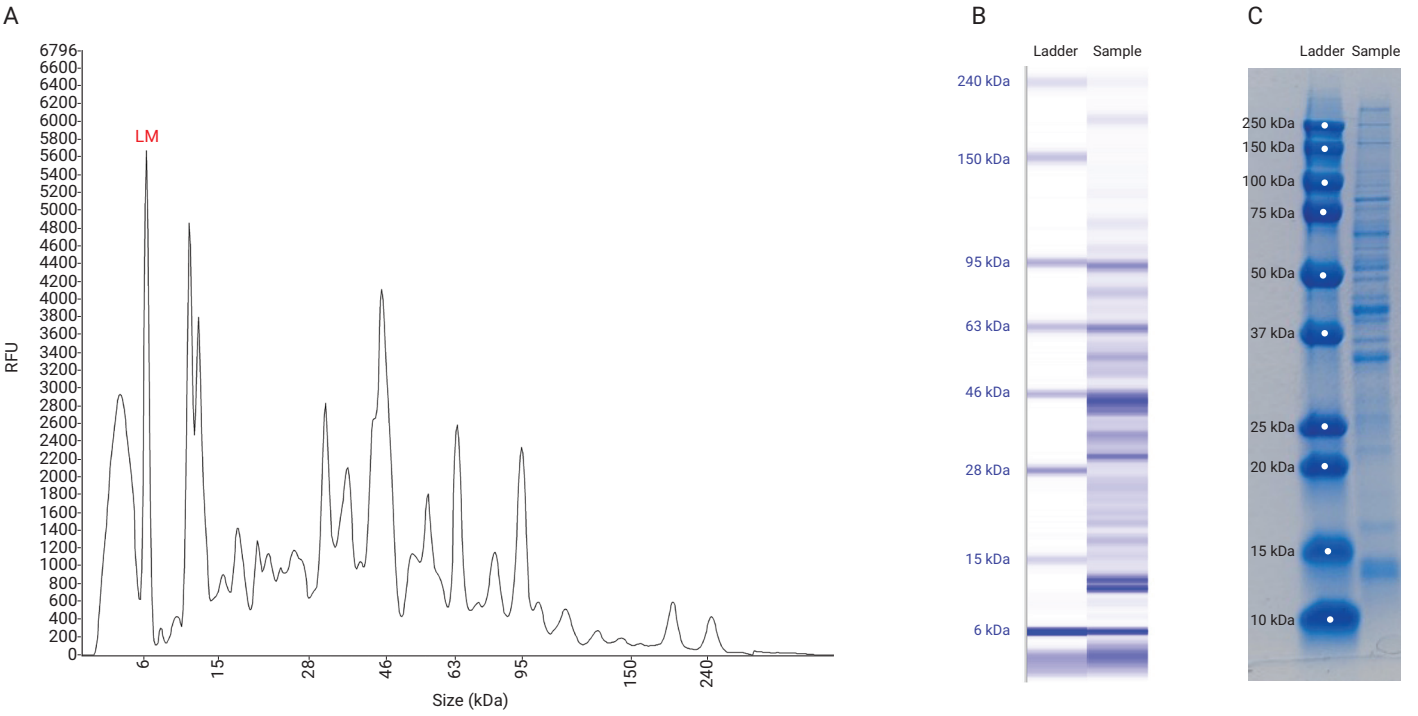
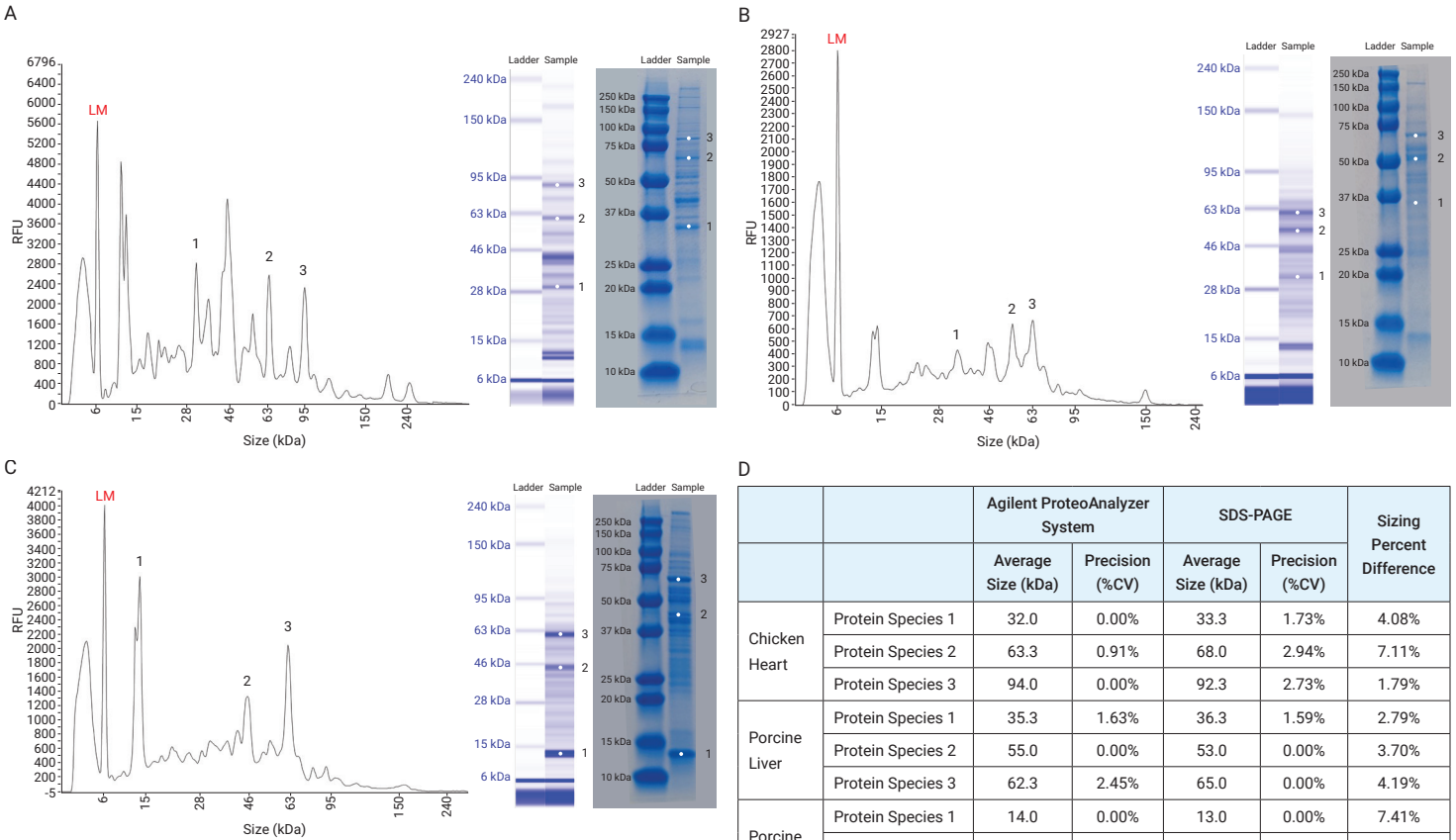


Figure 1. Crude protein lysate generated from chicken heart is shown as a representative example of analysis using the Agilent ProteoAnalyzer system, resulting in an A) electropherogram and B) digital gel image. Additionally, the C) gel image result from the same sample using SDS-PAGE, with the precision plus Protein Dual Color Standard bands and sizes noted is shown.

Sizing accuracy of the ProteoAnalyzer

Accuracy of the ProteoAnalyzer system was assessed by comparing the size of multiple peaks from each sample to the equivalent bands on SDS-PAGE. Figure 2 displays the average sizing results of three representative protein species for each sample type. The sizes of each species observed with the ProteoAnalyzer closely matched the results from SDS-PAGE. For example, chicken heart species 3 showed an average size of 94.0 kDa on the ProteoAnalyzer and 92.3 kDa with SDS-PAGE, a 1.79% difference between systems (Figure 2A).

Porcine kidney species 3 also displayed a 2.11% sizing difference between systems (Figure 2C). Among the remaining samples, the majority of the species had an average sizing difference compared to SDS-PAGE of 1 to 3 kDa, with percent differences of 4.20% or less. The largest sizing differences between the ProteoAnalyzer and SDS-PAGE were 7.11% and 7.41% (chicken heart species 2, and porcine kidney species 1). The data presented from this comparison demonstrates the accuracy of the ProteoAnalyzer in sizing.



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		Agilent ProteoAnalyzer System		SDS-PAGE		Sizing Percent Difference
		Average Size (kDa)	Precision (%CV)	Average Size (kDa)	Precision (%CV)	
Chicken Heart	Protein Species 1	32.0	0.00%	33.3	1.73%	4.08%
	Protein Species 2	63.3	0.91%	68.0	2.94%	7.11%
	Protein Species 3	94.0	0.00%	92.3	2.73%	1.79%
Porcine Liver	Protein Species 1	35.3	1.63%	36.3	1.59%	2.79%
	Protein Species 2	55.0	0.00%	53.0	0.00%	3.70%
	Protein Species 3	62.3	2.45%	65.0	0.00%	4.19%
Porcine Kidney	Protein Species 1	14.0	0.00%	13.0	0.00%	7.41%
	Protein Species 2	46.7	3.27%	48.7	1.19%	4.20%
	Protein Species 3	64.0	2.71%	62.7	2.44%	2.11%

Figure 2. Three individual protein species were chosen for sizing comparison on the Agilent ProteoAnalyzer system and SDS-PAGE for different tissue types: A) chicken heart, B) porcine liver, and C) porcine kidney. D) Average sizes, precision (%CV), and percent difference between the ProteoAnalyzer and SDS-PAGE is shown. n=3.

Run to run reproducibility

The reproducibility of the ProteoAnalyzer was examined by comparing the results of each sample across multiple injections. Overlays of the resulting electropherograms from three independent runs are shown in Figure 3. The separation profiles overlap almost perfectly across the three runs for each sample. The high similarity between runs indicates the consistent and reproducible results achieved by the ProteoAnalyzer.

To further demonstrate the consistency of the ProteoAnalyzer, sizing precision (%CV) was assessed for three prominent protein species from each tissue type (Figure 2). For example, the three peaks assessed for sizing of chicken heart in Figure 2 showed excellent precision, with 0.00, 0.91, and 0.00 %CV, respectively (Figure 2). Low %CVs were also observed for the chosen porcine liver and kidney protein species, demonstrating that the ProteoAnalyzer delivers consistent sizing results when analyzing crude protein lysates.

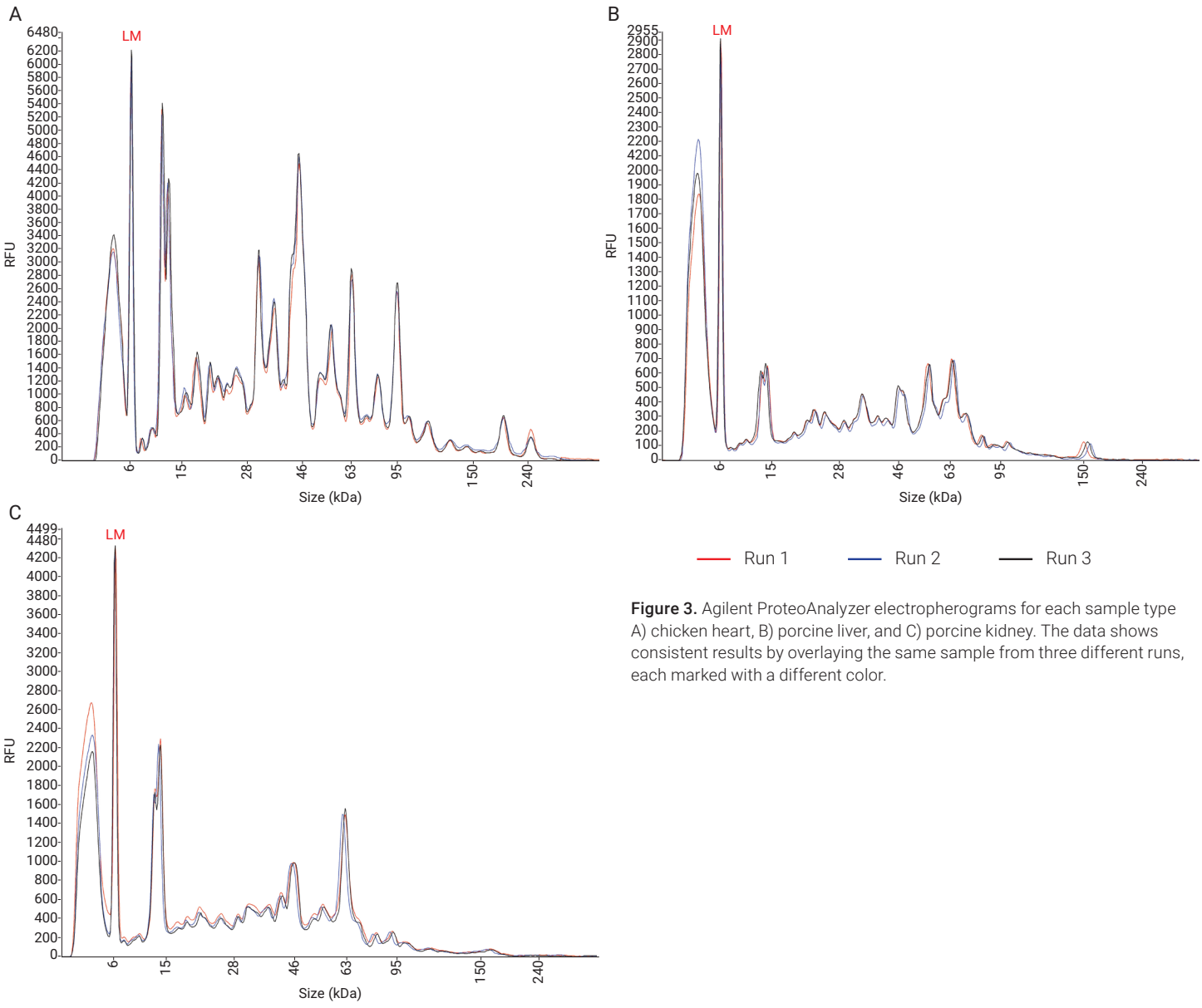

Figure 3. Agilent ProteoAnalyzer electropherograms for each sample type A) chicken heart, B) porcine liver, and C) porcine kidney. The data shows consistent results by overlaying the same sample from three different runs, each marked with a different color.

The concentration of the samples was also assessed to further demonstrate the reproducibility of the system. Using the ProteoAnalyzer for quantitation relies upon the lower marker (LM) as an internal quantitative standard for relative concentration calculations[1]. The concentrations of the individual protein species identified in Figure 2 were analyzed across multiple runs. All protein species concentrations measured had %CV values of 5% or less, with the lowest being chicken heart species 1 at 1.37% CV (Table 1). These low %CV values were indicative of the excellent quantitative precision achieved by the system.

The ProSize software can also be used to calculate the purity, or relative abundance, of an individual protein species. This calculation is done using the smear analysis function to report the "percent of total" of the sample present within a defined region. Each of the protein species previously chosen for analysis were analyzed using the smear analysis function to determine their relative abundance. For example, species 1 of the porcine kidney displayed an average of 22.2% of the total proteins, with a 0.26% CV indicating a high level of precision for the percent of total calculations. The rest of the proteins evaluated had good precisions of 7.90% CV or less (Table 2). Overall, the ProteoAnalyzer demonstrated excellent reproducibility for analysis of the relative abundance of protein crude lysates.

Table 1. The average concentration and precision of three different protein species across three separate runs for all the sample types (chicken heart, porcine liver, and porcine kidney). n = 3

Concentration						
	Protein Species 1		Protein Species 2		Protein Species 3	
	Average (ng/µL)	Precision (%CV)	Average (ng/µL)	Precision (%CV)	Average (ng/µL)	Precision (%CV)
Chicken Heart	118.4	1.37%	108.9	4.98%	242.0	2.57%
Porcine Liver	37.4	3.39%	55.1	2.60%	58.4	3.59%
Porcine Kidney	127.8	1.72%	80.8	4.36%	127.3	4.04%

Table 2. The average relative abundance (% of total), and precision for three different protein species across three separate runs for all the sample types (chicken heart, porcine liver, and porcine kidney). n = 3

Relative Abundance						
	Protein Species 1		Protein Species 2		Protein Species 3	
	Average (% of total)	Precision (%CV)	Average (% of total)	Precision (%CV)	Average (% of total)	Precision (%CV)
Chicken Heart	8.1%	1.43%	5.2%	7.72%	5.2%	2.92%
Porcine Liver	7.7%	7.90%	10.4%	5.28%	11.0%	5.90%
Porcine Kidney	22.2%	0.26%	8.8%	1.74%	12.9%	0.45%

Conclusion

In this technical overview, the robustness and reliability of the Agilent ProteoAnalyzer system for protein crude lysate analysis is demonstrated by the reproducibility of the sizing, quantification, and relative abundance results across multiple runs. Additionally, the ProteoAnalyzer was shown to deliver accurate sizing results compared to SDS-PAGE. The ProteoAnalyzer has been shown to be a valuable tool for analysis of protein crude lysates and offers the potential to streamline workflows and conserve resources.

References

1. Best Practices for Protein Analysis with the Agilent ProteoAnalyzer System, *Agilent Technologies technical overview*, product number 5994-7451EN, **2024**.
2. Istvan Lazar Jr., PhD and Istvan Lazar Sr., PhD, CSc. GelAnalyzer 23.1. Gelanalyzer.com. http://www.gelanalyzer.com/index.html (accessed 2023-10-06).

www.agilent.com/genomics/proteoanalyzer

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