

Rapid small-scale protein purification and characterization using the JANUS G3 BioTx, LabChip GXII Touch platform and the AlphaLISA assay.

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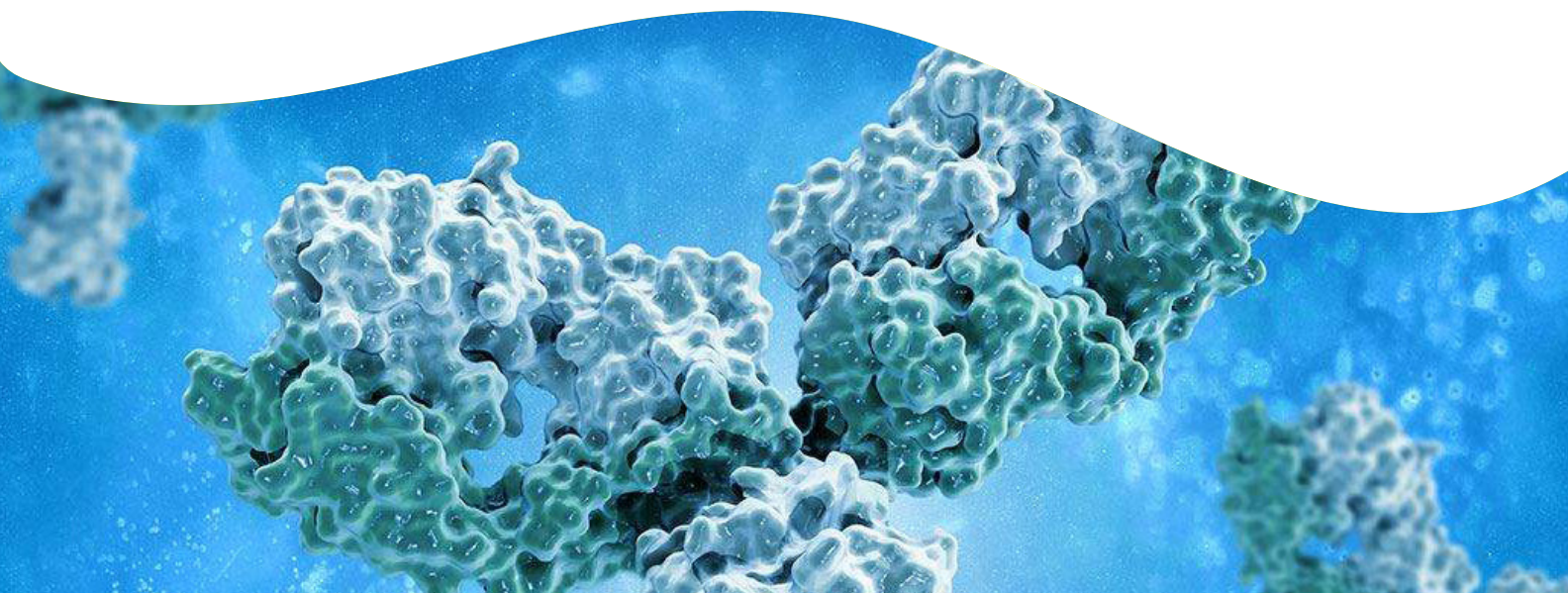
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Introduction

Antibody-based therapies are one of the largest areas of therapeutics. The developmental stages require researchers to prepare, purify and analyze thousands of samples. Small-scale purification reduces the time needed to purify samples and accelerates the development of biotherapeutics. However, since small-scale purification increases throughput, protein characterization and analysis become the bottlenecks in the workflow. Methods such as ELISA, SDS-PAGE, capillary electrophoresis and HPLC require manual sample handling and/or analysis times of up to 30 minutes per sample. The need to analyze more samples creates the demand for a high-throughput analytical platform with superior precision, automation and ease of use.

Purolite partnered with Revvity to show that, even when using different types of protein A, there would not be any significant difference in clearance performance under the process conditions typically used to purify full-length human antibodies.

By using Revvity technologies, Purolite was able to save time and money while producing highly purified eluates that are straightforward to analyze.



Protein A affinity chromatography

Protein A affinity chromatography is widely used in biopharmaceutical manufacturing, with 95% of all commercially available monoclonal antibodies (mAbs) made using protein A purification. In this publication, we use the JANUS™ G3 BioTx™ Pro Plus, LabChip™ GXII Touch™ platform and AlphaLISA™ assay kits to evaluate eight different protein A resins with respect to removal of contaminating residual DNA and host cell proteins (HCPs), and protein A leakage.

Such parameters are important. Contaminating HCPs can elicit an undesired immunological response in a patient. In addition, lot-to-lot variability of these contaminants could cause commercial production of a biotherapeutic to be completely scrapped. Residual DNA contamination can come from either the production host cell line or microbial contamination. Determination of DNA contamination at each step of a purification process is necessary to ensure the removal of host cell DNA, and to evaluate the sterility of that purification process.

Study aim – comparability

To maintain the cost-effectiveness of large-scale manufacturing, it is important to reuse a resin for 100–200 cycles. Chromatography resin used for multiple cycles degrades over time – usually because of protein A leakage and resin fouling. The most common sign of degradation is loss of dynamic binding capacity, often a result of loss or inactivation of the ligand due to protease activity in the feedstock, irreversible fouling or hydrolysis of the protein A during cleaning under alkaline conditions. Thus, it is reasonable to assume that the difference in performance between the first cycle and the last cycle during the lifetime of a resin is larger than the difference between two new protein A resins with different amounts of ligand immobilized onto them.

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Purolite launched two resins that use the same protein A ligand but vary in density. *Praesto® AP* is for large-scale manufacturing. It offers a high ligand density, which allows its use for several hundred cycles. The less expensive *Praesto® APc* resin is for applications in which the anticipated use is less than 50 cycles (for example, clinical manufacturing). This study also included an inexpensive *Praesto® AC* resin for which the ligand is based on the native sequence of protein A.

Purolite Life Sciences brings Purolite's innovative thinking and distinguished history of resin technology expertise to the global Life Sciences marketplace. Purolite Life Sciences provides resins for purification and separation to support research and development, and production-scale applications in pharmaceuticals, protein purification, food production, bioprocessing, fine chemical and additional markets. With a team of world-class researchers and scientists, we develop novel, high-demand and customized products to meet customer needs.

(Global Sales and Marketing Director, Purolite).

The equipment – JANUS G3 BioTx and LabChip GXII Touch systems

The JANUS G3 BioTx liquid handler is a compact, affordable automated workstation that enables consistent small-scale protein purification and sample preparation for analytical protein characterization. It accommodates column, tip, and batch chromatography modes on the same platform.

The JANUS G3 BioTx platform is available in two sizes:

- The JANUS G3 BioTx Pro, which has 12 deck positions and a small footprint.
- JANUS G3 BioTx Pro Plus, which has 24 deck locations and a molecular dispense technology 96-tip head for parallel purification methods. It also uses an integrated gripper for labware positioning and includes an integrated vacuum manifold.

Both JANUS G3 BioTx models use a wizard-driven WinPREP™ Application Assistant, which has a simple, intuitive graph-based interface. This allows the user to easily modify the parameters according to the study's aims. This simple interface does not need to be programmed, but the collected fraction parameters and chromatography steps can be customized if desired. Use of the Application Assistant requires only basic training. The software produces a report file that couples the entered parameters with fraction collection locations. This allows seamless integration with the downstream analytical data collected from each fraction and so gives faster analysis.

The LabChip GXII Touch protein characterization system rapidly characterizes eluted peaks at each respective purification step within the workflow. The single-sipper microfluidic chip technology automatically stains, destains, and electrophoretically separates and analyzes the protein samples. Denatured proteins are moved onto the chip from a microtiter plate through a capillary sipper. Samples are then electrokinetically loaded and injected into the separation channel. The protein-SDS complex and free SDS micelles within the separation channel are immediately stained and electrokinetically separated in the sieving matrix. At the end of the separation channel, the sample is diluted to reduce the SDS concentration to below its critical micelle concentration; this reduces the background fluorescence so that protein-SDS-dye complexes can be detected. This platform supports multiple assays for characterizing proteins in reduced and nonreduced forms. Figure 1 shows the workflow when using the JANUS G3 BioTx workstation and LabChip GXII Touch protein characterization system.

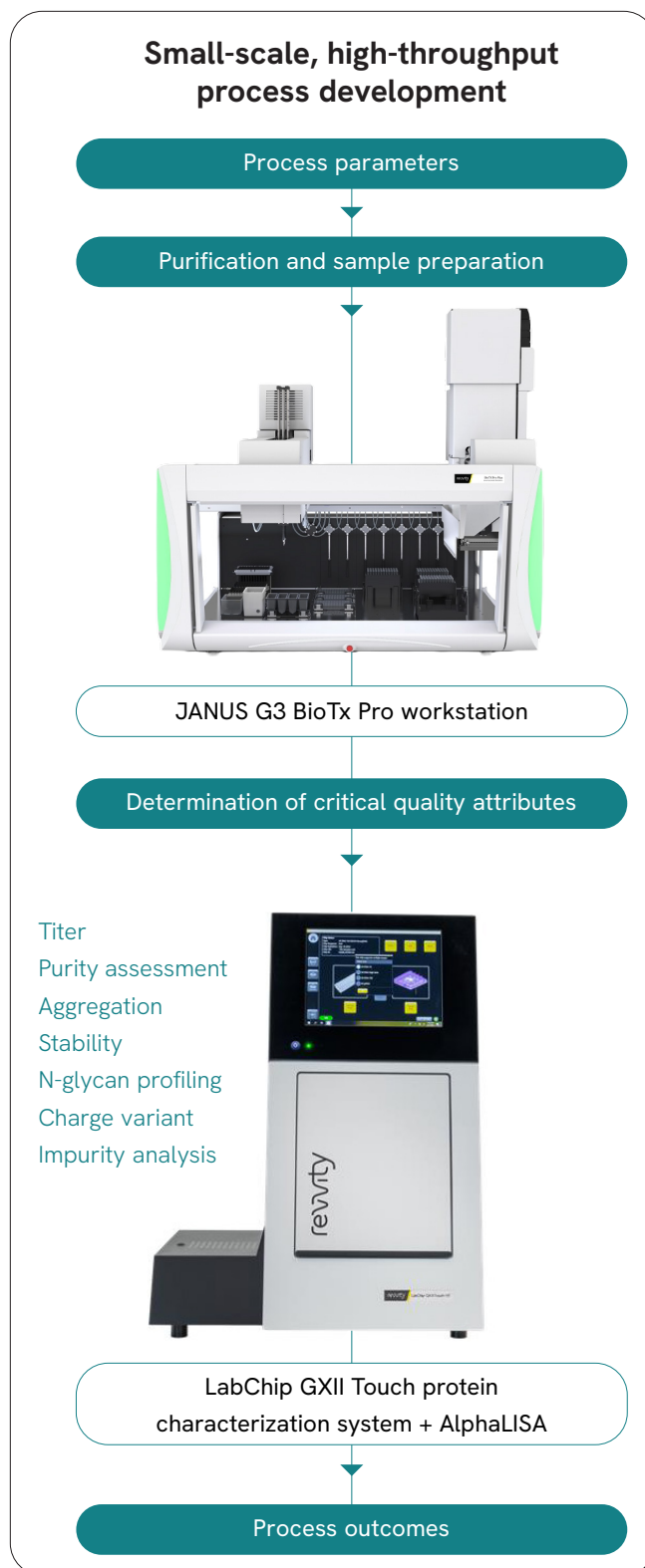


Figure 1: Workflow for the JANUS G3 BioTx workstation and LabChip GXII Touch protein characterization system.

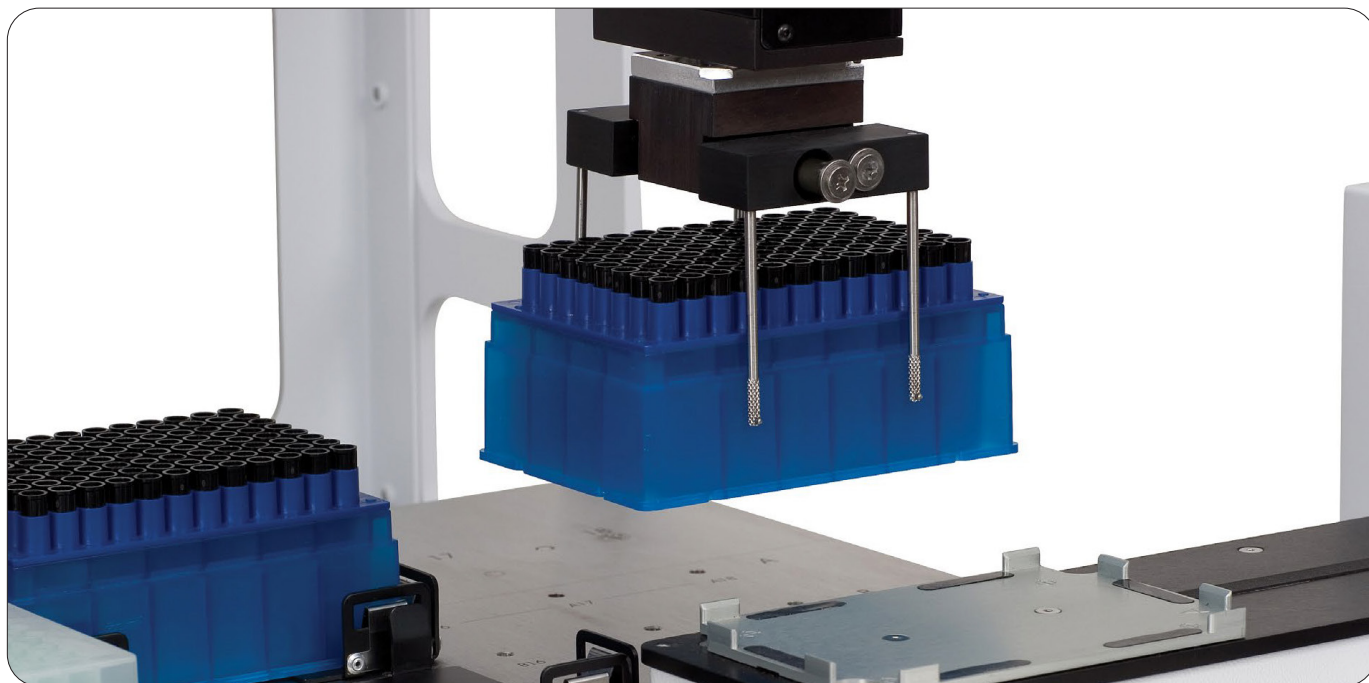
The analytical assay – AlphaLISA

The AlphaLISA homogeneous, bead-based proximity assay technology has long been used in high-throughput screening associated with small molecule drug discovery, but, recently, its use has moved to the large molecule arena. The AlphaLISA assay provides an alternative to traditional ELISAs and enables detection of molecules of interest in buffer, cell culture media, serum and plasma in a way that is highly sensitive, quantitative, reproducible and user-friendly.

Using the JANUS and LapChip systems, and the AlphaLISA assays allows researchers to purify and analyze samples in 1 day, as opposed to weeks with other systems (see Table 1).

Table 1: Comparing the different steps and features of manual and automated workflow

Feature	Preparative system (manual)	JANUS BioTx platform (automated)
Chromatography runs in parallel (no.)	1	8
Column volume	5 mL	600 µL
Protein consumption (mg) (e.g. standard capture step)	175-325	21-39
Time to perform 8 runs (h)	~32	~1
Time to perform 96 runs (d)	~16	~1



Close-up of the Revvity JANUS BioTx Pro Plus workstation used in the study

Methods and results

To demonstrate the efficiency of the purification process using the JANUS workstation, the AlphaLISA assay and the LabChip platform, we collected, washed and purified a clarified feedstock that had a biosimilar mAb.

The JANUS BioTx Pro Plus workstation was used for all chromatographic experiments. Eight different resins were packed by Atoll into 200 or 600 μ L RoboColumn units.

Four parameters of the eight resulting purified proteins were assessed:

- Protein A leakage was determined using an AlphaLISA residual protein A kit.
- Residual HCP was measured using an AlphaLISA residual CHO HCP broad reactivity kit.
- Residual host cell DNA levels were measured using an AlphaLISA residual DNA kit.
- Purity was determined by capillary electrophoresis on a microcapillary LabChip GXII Touch platform, using a LabChip Protein Express assay. Results were analyzed using LabChip GX Reviewer software.

Standard curves were generated for the residual protein A, CHO-P (broad range), and residual DNA AlphaLISA detection assays. Figure 2 indicates that each assay has a broad dynamic range with a large signal-to-background. All AlphaLISA assays exhibited excellent performance for detection of these bioprocess contaminants.

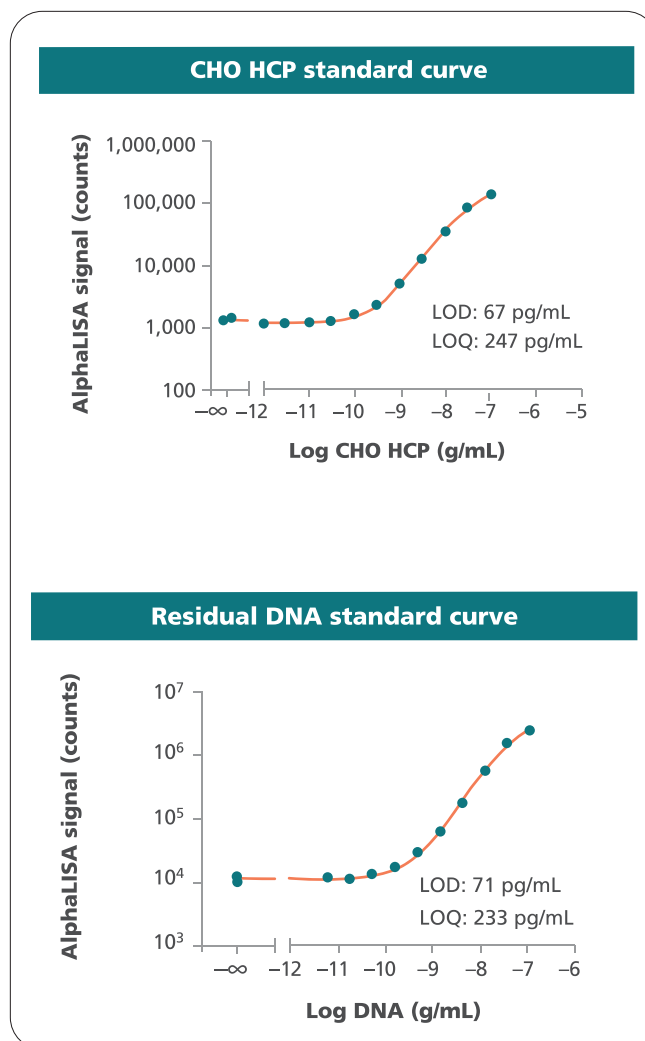
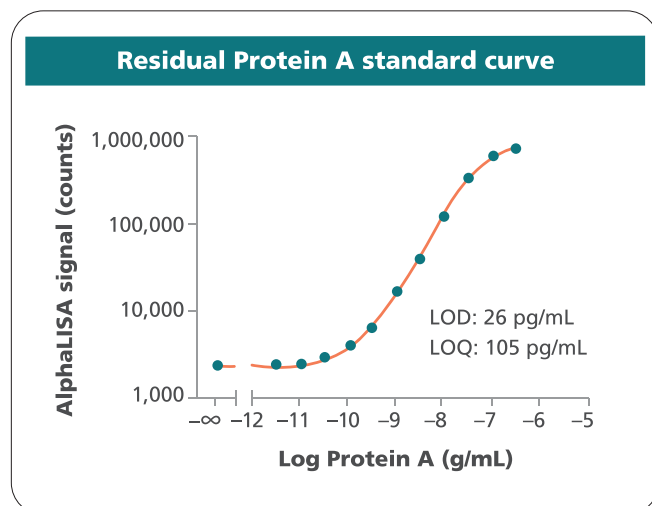


Figure 2: LOD and LOQ scores, and residual protein A standard curves after using the AlphaLISA assay kits.

Figure 3 shows the amount of each contaminant detected by AlphaLISA assays for samples using each of the eight resins. Data is shown for three cycles of purification. Samples A-C (the Praesto resins) show very low levels of contaminating residual protein A, DNA and CHO HCPs in the eluates. The other five resins had varying levels of the different contaminants.

Each purification step (load, intermediate wash, and elution) was run on the LabChip GXII Touch to evaluate the molecular weight and concentration of the proteins detected at each step. Figure 4 shows the nearly identical elution electropherogram profiles after analysis, demonstrating minimal residual contaminating protein carryover after the wash steps. The small peaks at ~29 kDa and ~54 kDa represent degraded mAb and not contaminating HCPs. The peak at ~138 kDa represents the nonglycosylated variant of the mAb tested.



Figure 3: Quantification of protein A leakage, HCP content and host cell DNA from three purification cycles using AlphaLISA assays.

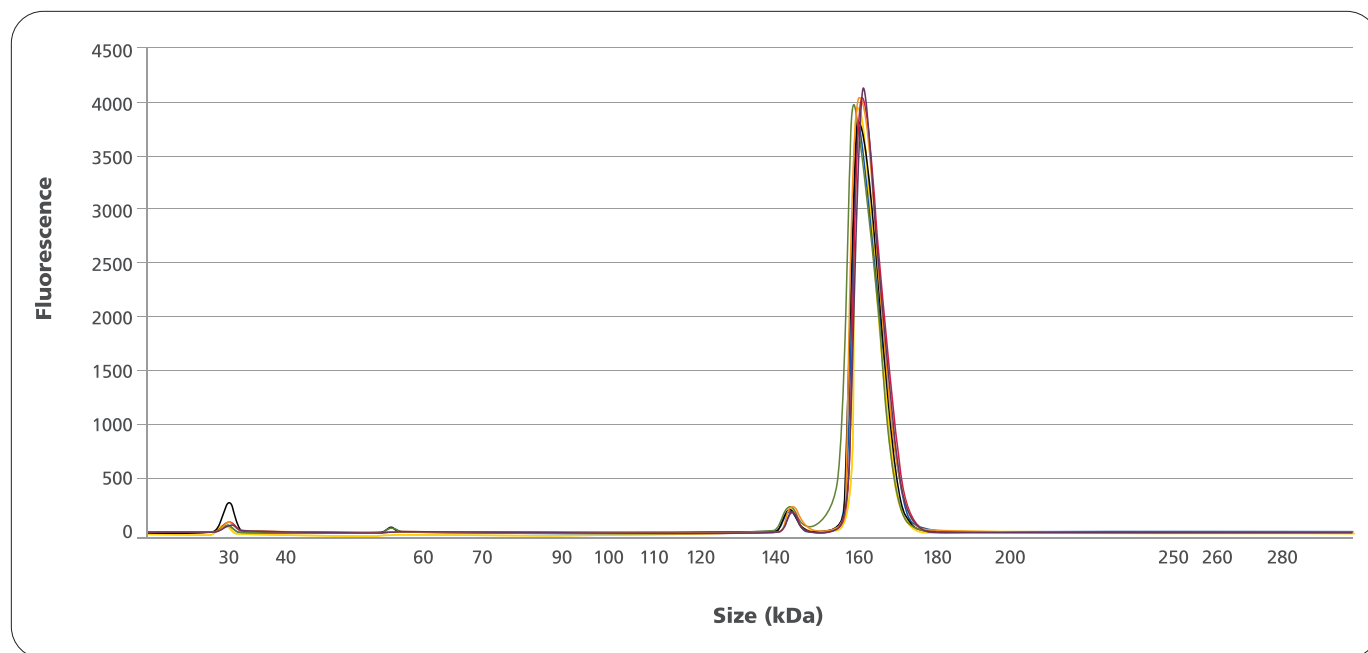


Figure 4: Overlay of elution electropherogram profiles generated by the LabChip GXII system from the eight resins.

Conclusion

Under the study conditions, we observed significant variation in the HCP levels in the eluate and in the intermediate wash fractions. It is likely that individual resin optimization of wash conditions would improve the removal of HCP.

The automated JANUS BioTx process development platform, the microcapillary LabChip GXII Touch platform and the AlphaLISA technology enabled high-throughput characterization of mAb samples. In combination, the technologies speed up analysis, thus providing maximum efficiency within high-throughput process development workflows. These approaches help to eliminate bottlenecks in sample preparation and characterization, **resulting in shorter development cycles and increased efficiency of purifying and characterizing proteins compared with traditional methods.**



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