Characterizing Exosomes Using Next-Gen Protein Sequencing™

Fred Kweh, PhD
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"Next-Gen Protein Sequencing™ (NGPS™) has the potential to be even more impactful than DNA sequencing. Proteins are the true effectors in biology because they drive biochemical processes and determine cellular function. When you consider the vast investment in DNA sequencing and compare it to the number of actual cures that have resulted, the impact has been limited. Understanding proteins at a deeper level is what really matters, and this technology has the potential to drive far greater advancements in the future than next-generation DNA sequencing."

– Fred Kweh, PhD

Challenge: Traditional tools struggle to detect subtle protein variants and post-translational modifications, making it challenging to understand the biogenesis, cargo composition, and therapeutic effects of exosomes

Innovation: Leveraging perinatal-derived exosomes from amniotic fluid, cord blood, umbilical cord, and placenta to understand and treat rare diseases like Prader-Willi syndrome

NGPS Integration: Used to sequence proteins in exosomes to understand production, compare variants in diseased vs healthy individuals, and monitor therapeutic impact by tracking shifts in protein profiles post-treatment

Advantages: Affordable and accessible single-molecule variant-level resolution across exosome batches, providing orthogonal insights to existing tools and accelerating therapeutic development

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Exosomes, small extracellular vesicles (30–150 nm in diameter) secreted by cells, play a central role in intercellular communication by transporting molecular cargo such as proteins, lipids, and nucleic acids between cells. They are formed within endosomal compartments and are released into the extracellular environment when these compartments fuse with the cell membrane. Exosomes are secreted by various cell types under both normal and pathological conditions and can be taken up by nearby or distant cells to influence their function.

With the ability to transport cargo in a highly targeted manner, exosomes have emerged as a highly promising vehicle for therapeutic applications. KWEHEALTH is leveraging the regenerative potential of perinatal-derived (PnD) exosomes from amniotic fluid, cord blood, umbilical cord, and placenta to treat intractable diseases. Advanced cell-free exosome therapeutics have the potential to retain the healing properties of their parent cells while minimizing the risk of adverse post-transplant events associated with cell therapies.

We spoke with Dr. Kweh about how the KWEHEALTH team plans to leverage Next-Gen Protein Sequencing (NGPS™) on the Quantum-Si Platinum® instrument to identify protein variants associated with various diseases and to analyze both the processes involved in exosome production and the exosomes themselves.

Q. How are you planning to leverage NGPS on the Quantum-Si Platinum® instrument in your development of exosome-based therapeutics?

A: At KWEHEALTH, our focus isn't just on advancing analytics. We're looking for technologies that will drive significant progress in the field of exosome-based therapeutics. I believe that NGPS is a game-changer for several reasons.

The Quantum-Si Platinum® instrument offers a cost-effective benchtop alternative to mass spectrometry, making NGPS more accessible. Its small footprint, ease of use, and ability to generate deep insights into protein variants make it ideal for our work with exosomes which are a complex and emerging modality.

The exosome production process is very complex, and we need to understand the biology of exosomes. With the Quantum-Si Platinum® instrument, we plan to sequence the proteins contained within exosomes to more fully understand both the process and product. This technology allows us to do that efficiently. We can generate a great deal of data to really characterize our process and product and understand batch-to-batch variability.

We can characterize our product while also considering the patient's background, allowing us to say, "This patient has a disorder where certain proteins are dysregulated. We're treating with our exosomes, and we'll monitor these proteins to see if our therapy has an impact."

However, it's not just about detecting the presence of protein A or protein Y; it's about identifying which variant is present. It could be an inactivated form or a slightly modified version linked to the disease. A protein may be present in the patient without a significant change in overall concentration, but its variants could shift. For example, in a disease state, variant B might be dominant. After treatment, it could transition to variant A, even if total protein levels remain stable. This level of detail is crucial for understanding therapeutic effects, and with this technology, we can explore these minor but critical differences.

Q: What diseases are you currently focused on?

A: I've dedicated more than a decade to studying the rare genetic condition Prader-Willi syndrome (PWS), uncovering key biochemical differences

in affected individuals. However, critical gaps remain in understanding the molecular underpinnings of the disease – gaps that NGPS could help fill.

One major finding was elevated insulin levels in PWS patients compared to normal controls. But the question remains as to whether it is the same form of insulin, or if there are subtle variations that could influence disease progression. Traditional methods haven't addressed this, but NGPS offers the potential to compare insulin variants at a deeper level.

Another key focus is ghrelin, a hormone that regulates hunger. PWS patients experience an insatiable appetite and have abnormally high ghrelin levels. Previous attempts to lower ghrelin with biochemical compounds like somatostatin have had little effect. Research suggests that post-translational modifications, such as acetylation, could be altering ghrelin's function. Our team aims to sequence ghrelin and its receptors in PWS patients, comparing them to healthy controls to uncover meaningful differences.

By applying NGPS to these proteins, our goal is to reveal novel insights into PWS, potentially paving the way for more targeted and effective exosome-based treatments.

In addition to PWS, we are applying NGPS to other critical areas of research. For Parkinson's disease, our focus is on understanding protein variants, particularly alpha-synuclein, and using exosomes to target inflammation. We plan to conduct *in vitro* studies with cellular models of Parkinson's, treating them with exosomes designed to address these specific issues, and then sequencing the proteins to track changes.

Another exciting application is in wound healing, particularly with our perinatal-derived exosomes. These exosomes show promise in aiding recovery for burn victims.

Q. How will NGPS help you demonstrate to regulators that you have a consistent process?

A: The use of NGPS will be crucial in demonstrating the consistency of exosome production. Exosomes are complex and can contain over a thousand peptides, making them difficult to analyze. By sequencing the proteins within exosomes, we hope to understand how variability in manufacturing impacts protein variants. NGPS will be used to develop a more controlled process for producing protein variants consistently across batches. This consistency is essential, as it demonstrates that the manufacturing process is well-standardized and that no unintended protein variants are introduced during production.

Q: Overall, how has your experience been with Quantum-Si's Platinum® instrument?

A: My experience with the Quantum-Si Platinum® instrument has been fantastic. It's a novel, orthogonal tool that provides unique insights into our work, all while being small, cost-effective, and easy to operate. The system has been performing well, and I'm excited about the potential it brings, especially in developing new therapeutic modalities like exosomes. While developing novel therapies can be challenging, this technology opens up incredible opportunities for those working on non-traditional approaches. It's a great reminder for anyone working on a novel modality that new analytical tools like this can really help to move the field forward and sustain that momentum.