Anton Paar

– INTERVIEW –

kernalbio

Interview: Thomas Colace,
Associate Director, LNP Formulation

Life sciences startup uses Anton Paar's Litesizer to develop next-generation medicines

Kernal Biologics is a venture capital-backed startup developing cell-specific mRNA therapies, founded by scientists and entrepreneurs with a broad range of experience in synthetic biology, immuno-oncology, and deep learning. Their vision is to develop curative immunotherapies for cancers and COVID-19 by using deep learning and synthetic biology.

With mRNA 2.0 technology, Kernal's proprietary platform decreases immunogenicity upon existing mRNA technologies and enables cell-specific therapeutic protein expression allowing the design and development of more efficacious, tolerable, and affordable medicine for the global market. To do this, Kernal uses Anton Paar's Litesizer particle size analyzer.

We spoke to Thomas Colace, Associate Director, LNP Formulation about research in the time of COVID, next-generation mRNA therapy, and his company's use of Anton Paar instruments. Colace operates out of Kernal's base at LabCentral in Boston, a shared lab facility for high-potential life sciences startups spread over 100,000 sq. feet in Cambridge and on the campus of Harvard University.

Thomas Colace
Associate Director

Q: What exactly does your company do?

A: Kernal Biologics is developing the next generation of mRNA therapies. Everyone's familiar with mRNA 1.0 and the fantastic success it's had with the COVID vaccines, but there are a lot of improvements we think can be made to mRNA to enable them to be used for other therapeutic use cases such as for cancer immunotherapy.

Kernal's approach is two-fold: First, Kernal is able to make changes to the mRNA molecule or to the mRNA sequence that allows the molecule to be more stealth and to evade the host immune system. One challenge with mRNA 1.0 is that the mRNA is recognized as an invader by a lot of cells and this can shut down protein translation. In the case of a vaccine this might be helpful because it could be somewhat of an adjuvant, it might help stimulate the immune system, but in a scenario where you're trying to produce as much protein as possible, it's best to have that mRNA molecule either be stealth or to not activate innate immune receptors.

The other approach that Kernal is taking to next-generation mRNA therapies is to make mRNA onco-selective. Kernal has used convolutional neural networks to find a pattern that can help mRNA be selectively translated by cancer cells as opposed to healthy tissue. And this might allow us to send potentially very toxic protein payloads which could then be expressed by cancer cells, rendering them to commit programmed cell death vs. healthy cells that may be spared.

Q: What's your role?

A: My role is delivery. The mRNA is all upstream from what I do. I encapsulate the mRNA into lipid nanoparticles, and those lipid nanoparticles are then delivered either systemically or locally, via all injection routes, into pre-clinical mouse models. And so where [Anton Paar's] Litesizer comes in for our functional area is in characterization of those lipid nanoparticles. The characterization both by size and by zeta potential gives us an idea about the quality of the particles, so about, potentially, their function. The size of the lipid nanoparticle may be related to its potency.

So, it's very important for us to understand both the size and the zeta potential of our particles in order to help us optimize and iterate on our formulation designs as we seek to find more targeted LNP formulations, formulations that maybe go to tissues that may be cancer-specific, or organ-specific, as well as just overall potency, in creating lipid nanoparticles that result in the highest level of mRNA expression in the cells.

Q: How does Anton Paar's Litesizer instrument fit into your work?

A: It's twofold: It plays a role in discovery, and it plays a role in quality assurance. Not to say that we're at a stage where we have strict quality assurance in place but it's in making sure that we're able to replicate things that we've done in the past. Being able to characterize the LNPs both by size and zeta potential gives us confidence that we've created the same thing twice.

In terms of discovery both size and zeta potential are most likely variables, whether dependent or independent, that affect the potency of our LNPs in our model systems. And so being able to have those data points to use as parameters in model development is very helpful to us. In general, you could in theory work without characterizing your particles, but I think you'd be missing a dynamic. You'd be missing really important information that might help you make better decisions in the future about the quality of your LNPs and their potency.

Litesizer in the Kernal Biologics Laboratory

Q: Why is it so important to perform DLS in-house with Litesizer?

A: It's super-important in terms of lipid nanoparticle formulation. If you're not seeing this information, you're formulating blind. You have no idea if maybe you made a small mistake in your protocol and you're about to send these particles to a downstream assay that's costly and uses a lot of time, and not being confident that what you're sending into those downstream assays is what you think it is, it's just not worth it.

To be honest, I can't tell you how often DLS has caught my mistakes. I make a formulation, it looks fine, I measure it, something's off. I go back to my notes. Something's off. With that quality control step that literally takes 2 min, that's all you need, that's why it's so useful, we've probably saved countless mistakes from going further down the experimental pipeline. And it's also not worth it to store the LNPs for days or weeks at 4°C and then go measure them. Changes happen.

And this is another reason why we use the dynamic light scattering, is to evaluate the stability of our particles. If there is a problem with stability, size and zeta potential will be your first warning signal. And sometimes you can see that just by your eye because these are colloidal suspensions so they appear cloudy. And if it appears even cloudier, then you have a problem. But if you really want to be able to quantitate how severe that problem is then you need something like DLS to do that.

From another perspective, if you're trying to evaluate the stability of your particles upon freezing, which is very important for when you want to transport the particles, you need DLS to make certain you're not making changes to those particles through the process of freezing and thawing them possibly through several repeats.

Q: How did you decide on Anton Paar's Litesizer? What made the difference?

A: We did contact other companies with similar equipment. For me, there were two drawbacks: One, the equipment was significantly more expensive and two, the lead time was excruciatingly long with no offer to provide a demo or a loaner or anything like that. So I thought, well, there are other people doing this, why don't we take a look? The reason why we were able to pull it off was because we were able to get the demo machine in here.

The team behind the equipment and the support that we get is almost as important as the equipment itself and [Anton Paar Technical Sales Representative Luis Botelho] was so helpful in getting the demo machine in when we did place the order he knew there was going to be a lead time and he secured us demo equipment so we would be able to bridge the gap and this wasn't offered by any other vendor. And we had basically a whole bunch of lipid nanoparticles that had been tested on a similar instrument, and so we had 100s of data points, and tons of samples and we read them on the Litesizer and we were pleasantly surprised with how tight the measurements were between the two instruments.

In fact I was somewhat shocked because I was in my mind allowing some leeway because those particles were in some cases greater than 6 months or a year old sitting at 4°C and they're not really, at those temperatures, supposed to be stable for that long but these ones turned out to be remarkably stable and we were able to create those comparisons and de-risk using the Litesizer in place of the competitor instrument and I think that's a win for us.

Q: What do you especially like about Litesizer?

A: One cool thing that I hadn't seen before is the univette, the ability to use incredibly small sample sizes is really a big benefit for people working with LNPs. While the detection technology may not be super-sensitive to concentration, LNPs can be sensitive to concentration below a certain level and if you're in a scenario where you have to be very resource-sparing sometimes you can't give up enough of your sample to make a DLS measurement especially when it comes to zeta potential where you need slightly higher concentrations to get consistent measurements.

The univette really allows us to make sure that we're operating in a concentration range that will get consistent results and not to use too much sample. That was a great thing to have. With the Litesizer software you have a little bit more access to the data that underlies your samples and that allows you to replot it if you want. I think that's pretty valuable. In terms of making the switch, going from another instrument to Litesizer, there wasn't anything I had to do or learn or prepare myself for.

It was basically like, "Go." You learn the software a little bit, it's a little bit different but in terms of setting up the assays it's all the same. In terms of ease of use, they're very similar, in terms of setting up an experiment, running an experiment, saving a method. And then the reporting feature I do like the reporting feature in Kalliope™ I think that's really nicely built, they look good.

Q: Would you buy another instrument from Anton Paar?

A: Oh [definitely]. The next time I need a piece of equipment I'm going to go to [Anton Paar Technical Sales Representative Luis Botelho] and ask him if he has it and if I can see it. There's no doubt about it. Of course, we're going to shop around and make sure we get the best deal and all of that. But like I said having that type of support from Lu is just invaluable, you can't put a number on how much that sways the opinion on what equipment you're going to go with.

I was talking to some past colleagues about my decision to not use a competitor instrument, just to see if it would pass the red-face test, that kind of thing. And I said, "Oh we're thinking about using Anton Paar and my ex-colleague said, 'Oh I didn't know they made DLS instruments but that's a great company.'" So as soon as I had that from her that she in her past had worked with Anton Paar equipment that was another huge thing. The reputation of the company is so important as well.

Q: So your Anton Paar contact point has been helpful …

A: Oh yeah, [Anton Paar Technical Sales Representative Luis Botelho] must have come to LabCentral like six times, he dropped the machine off, set it up. When we got the machine, our actual instrument, I guess it had been left out in the cold, unshipping, for way too long. And so he set it up and it gave a warning: The instrument's way too cold to be used right now.

And he said, "I've dealt with this before, I'm going to have to come back tomorrow. It's going to take 24 hours for this thing to come back up to temperature." He didn't say, "I'm going to have to come back next week, he said, "I will be here tomorrow, and you will be up and running tomorrow," and that was great.

Looking ahead, Anton Paar's Litesizer is well-anchored in Kernal's flagship project: oncoselective mRNAs and delivery of mRNA to extra-hepatic tissues – the design of lipid nanoparticles that help target different kinds of cancers. When Kernal has developed a formulation they feel confident moving forward to patients, then comes GMP manufacturing, which the company wants to do in-house if feasible, using Litesizer for two GMP release assays: particle size and sub-visible particles. The Kalliope™ software is compliant for GMP manufacture, in terms of having the correct audit trails etc. "That's a super-important thing as well," says Colace. "It would be a lot less valuable if we had to find a new technology when we go GMP."

Infobox: Litesizer 500

INSTRUMENT: Litesizer 500

SAMPLES: particles in dispersion, peptides or macromolecules in solution

MEASUREMENT PRINCIPLE: dynamic light scattering (DLS). electrophoretic light scattering (ELS), static light scattering (SLS)

MEASURED PARAMETERS: particle size (0.3 nm to 10 µm), zeta potential >= +/-1000 mV (for particles diameters of 3.8 nm – 100 µm), molecular mass (980 Da to 20 MDa), transmittance and refractive index