# TECHNOLOGIES AND TOOLS TO SUPPORT MRNA DEVELOPMENT AND MANUFACTURING

A conversation with Philip Probert, Centre for Process Innovation

## ABOUT

Philip Probert is technology leader for CPI biologics in the northeast of England in the UK He manages a team of scientists that deliver biologics process development work across upstream, downstream and analytical functions. Susan Dana Jones is a recognized leader in bioprocessing, She has over 30 years' experience in managing complex biopharmaceutical development programs from discovery through late-stage clinical trials and commercialization.

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*Susan Dana Jones: Where are mRNAs being used what are the applications? I think we obviously all know about the COVID vaccine which is a wonderful success story but where else do you see mRNA being used?*

PP: mRNA has been around for a while and when we talk about it we talk about not just the payload but the packaging system – the lipid nanoparticles and other types of encapsulation systems. Obviously there's a lot of interest in mRNA vaccines for COVID at the moment, but mRNA-based therapies are also being trialled for a range of other diseases. So things such as cystic fibrosis or replacement of enzymes in the liver

What is interesting with mRNA as a modality - as opposed to AAVs or gene therapies - is that mRNA is transiently expressed, so it takes away some of this safely concerns associated with DNA-based therapeutics. That said, because mRNA is transient it potentially needs to be administered a bit more frequently and at higher doses. However, in general, there is a lot of interest in therapeutic mRNA and there are a huge number of organizations trying to improve the technology and apply it to a certain indications.

*SDS: What are some of the areas that need to be improved in terms of the technology used to manufacture mRNA based vaccines and therapeutics?*

PP: Probably the key challenge for mRNA right now is how you get it to the right tissue. So most of the mRNA and LNP that's injected with typically will end up in the liver due to selection of reasons, and that's not really a bad thing. The liver does a huge amount for the body: it produces a lot of enzymes a lot of proteins there's a lot of diseases that are due to issues with the liver

But if you want to target other things you've got to bypass the liver. And that then ties into how you vector your mRNA to get to the kidneys or the lungs and that is one of the real challenges - getting enough product to the right tissue. You can't necessarily just dose more of it because then you might you end up potentially with side effects due to the lipids.

*SDS: It's fascinating. So I'm sure the audience will be really interested to understand how you go about making mRNA vaccines and therapeutics – how does it compare with other types of biomanufacturing?*

PP: So I think it's one of the interesting parts of the organization I work at is because we do work with the breadth of different molecules is that you see a lot there are a lot of parallels.

So in mRNA, although the initial production is all done enzymatically - so there's no need for cells - the actual process still fits with traditional manufacturing models. There's an upstream component, there's a downstream component and there's associated analytics. A lot of the approaches, the unit operations, are similar. We're still using chromatography, there's still tangential flow filtration steps, there's still the application of bioreactors. And many for analytics are also quite similar. So we're still using high performance liquid chromatography, still using capillary electrophoresis, we're still using enzyme-based antibody analytics.

But there are also differences. So, for example, upstream steps in mRNA production are shorter. Also, raw material costs can be quite significant because a lot of them are high value and complex. Then when we go into downstream we're still considering things such cut off sizes and filters, resin chemistries within chromatography it's just there are distinctions in terms of specifically what we're trying to produce, what we're trying to remove. And again analytically there's kind of a slightly different perspective in terms of how we approach those assays.

So it's kind of what keeps things interesting. It means that while a lot of learning that can be applied from one product to another, there are also distinctions that need to be made in terms of how you approach development of our of RNA processes.

*SDS: Can you talk a little bit about the analytics you use to characterize and assess the purity of an mRNA-based product?*

PP: Like any kind of chemical synthesis process, mRNA production it's a very well defined. We know what it is we're starting with - so we're buying enzymes we're buying DNA and therefore we know what we're adding in at the start and as we go through the process. So that means we can develop much more specific assays against impurities than for cell derived products. For example, when we are looking at all host cell proteins we measure protein and that's it. For mRNAs we can use enzyme-linked immunosorbent assay (ELISAs) against specific enzymes we know we've added, so that's very powerful in terms of designing those specific analytics.

But there are also analytical challenges. For example, because mRNA vaccines and therapeutics are new products there's not much data on good quality. For vaccines, we know what the specifications should be but for RNA we don't. What integrity do we really need? Or for double stranded RNA products - which is a hot topic - how much is too much? Also the availability of reference material is really difficult. You cannot go off the shelf and buy a high quality RNA that would be suitable for say human use.

So there are some benefits - you know what you're trying to measure particularly from a purity perspective but it's difficult to define. What is high quality for your product and what to sort of assays may come down the line that we may end up needing to use?

The other consideration is that because the RNA itself needs to be expressed, we need to be able to measure that expression level. There's a lot of work going into things like in vitro cell based assays but those assays can be quite limited in terms of predicting how a product will perform in an animal or a patient. And therefore a lot of the customers we're working with still use animalized models to show if the product is successful or functional.

There's also a question of how we improve the predictability of in vitro assays so we don't have to have as much animal-based testing. If nothing else it takes quite a long time and adds a lot of cost to projects.

There's a lot of interest in analytics and reference material, but until the first therapeutic mRNA comes to market, it will continue to be hard to identify what is required to make an effective product.

*SDS: Can you talk about designing the mRNA production process? What do you do to ensure scalability?*

PP: The main challenge we see in standard biologics process development is that you've got a culture of live cells. So when you scale things up you've got to worry about things like agitation, oxygen transfer rates and the fact you've got quite unpredictable systems and you're trying to maintain behaviour from an amp 15 all the way up to say a 1000 litre single-use bioreactor.

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That's challenging and that necessitates working at a certain scale to ensure what you do is predictive of larger scale whilst not trying to spend too much money.

For RNA we don't have to worry about mixing. We're not particularly fussed over oxygen transfer because we don't need it. And therefore we can do a lot more at smaller, micro-plate scale. You're not limited to, say, a 24 amp 250 or a 40 amp 15, you can use 96-well plates. In fact the challenge is how you actually do all that pipetting and making sure your screen is not to ambitious.

As a result we end up applying quite a different DoE approach for RNA-based processes to cell based processes because we can do a lot more. Doing massive screens isn't necessarily value-adding if you're not doing it well and it's still worthwhile doing relatively iterative designs.

Nevertheless in general with mRNA, if it works a small scale then you can expect a fairly linear scale up to large scale. But there are still considerations because of the complex supply chain, the cost of materials and the sensitivity or the labile nature of a lot of components you're using. In essence scale up becomes more about logistics. How do you get things to the right place at the right time at the right temperature? You don't want to spoil a batch because you left something out for too long because these raw materials can be exceptionally expensive. And because the process isn't going to adapt to a bad raw material, if the inputs are bad the outputs will be bad. So things like template DNA quality and some of these raw materials have a critical impact to your product quality.

But again because of that question of the analytics and what defines good quality it can be quite difficult to know that until you go into your potency assay and discover actually you've got a problem because what looks okay chemically actually hasn't performed biologically.

*SDS: So, given all that, how does a developer go about determining the functionality of an mRNA based product?*

PP: It is similar to how we would do with a biological screen. You would run a screen and then identify your smaller set of samples that you might then apply to a cell based assay. That will show you broadly if it is functional. But as soon as you move into encapsulation you need to be working with in a live model because it's very difficult to predict LNP performance in vitro because of the complexity of the biology.

Again it highlights the importance of doing potency work because you really cannot tell how something's going to target a tissue until you inject it into an animal and that limits utility of running big DoEs.

*SDS: So how much flexibility do you have in your production platform? How can you enable a quality-by-design space flexible approach in mRNA?*

PP: I think you know there's various kind of modalities or processes that people talk about being platform processes. For mAbs, for example, there's basically platform that different organizations use. They will modify the variable region and then they will follow a process and there's some need for tweaking perhaps depending on the particular antibody but broadly they are following a platform approach.

Similarly, RNA has a primary sequence you can change. You can create a radically different protein by just changing the combination of nucleosides in the sequence, so it ties into being a kind of a platform type approach in the same way. That said RNA can still form quite complex secondary structures or can vary in length and if you incorporate different modified nucleotides or enzymes, you may get different process performance.

Likewise for encapsulation you're effectively just changing the raw material, the lipid mixes going in to create a different type of product. So the whole process can be considered to be reasonably platform.

So the approach we take uses a standard selections of nucleotides and very standard lipid mixes. The main distinction then comes in terms of size. So for an mRNA of seven kilobases – you need to select the appropriate downstream processing steps - what cut off you apply to your TFF membranes, how do you run your chromatography steps? If you're doing a reverse phase, whether you fractionate.

So there are some distinctions you make for different types of products. I think as the technology develops while specific unit operations are unlikely to change, what you do within each may change. At the moment there are a lot of technology providers coming out with new types of process solutions, new types of enzymes, new types of lipids and it's interesting to see whether we will stay where we are now – with everyone trying different things - or will we converge on a very standard type of approach.

*SDS: What are the best technologies available to improve your chances of success if you're developing a new mRNA therapeutic?*

PP: We spoke about supply chain about raw materials quality that's recognizing that it's really important because actually the process is relatively simple compared to the complexity for cell based process. However what cells are good at is taking quite simple materials and make them really expensive products.

With RNA we're starting with expensive materials and we're making an expensive product. Those materials are expensive not just because they are often effectively biologics themselves, but because they're coming from cells or are made using complex chemical synthesis steps that have the IP attached to those methods.

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With RNA we're starting with expensive materials and we're making an expensive product. Those materials are expensive not just because they are often effectively biologics themselves, but because they're coming from cells or are made using complex chemical synthesis steps that have the IP attached to those methods. And there's a lot of IP in this space because it's quite a novel field and there's quite a few organizations which have really led the charge on the technology and understandably they hold a lot of the IP. So for new companies coming in, accessing technology can be quite difficult because you need to liaise with limited number of suppliers. Whether that is the nucleotides you're using, the CAP you're using or particularly the lipids, the need for such interactions can be a barrier to entry.

However with the interest in RNA - and the recognition that actually there's money to be made - a lot of companies - big companies like Thermo Fisher or Roche as well as smaller SMEs – are trying to overcome existing monopolies by developing new technologies and solutions So for us at CPI we try to stay engaged with different providers. We keep an eye on the field because we hit some of the same issues with licensing and ability to get access to technology.

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This case study was originally presented at Evaluating Biopharma's Biomanufacturing Optimization online and interactive event. You can watch Philip's presentation in full and on demand here. Details of future Evaluating Biopharma events can be found here.

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