Every superhero has an origin story, and **Bioseparations Product Manager**, Brian Rivera, is no different. He might not be your typical hero, and he might not have the typical origin story, but Mr. Rivera is our Super Bio-Man!

Brian started his biologics journey at the University of California, Davis, where he earned a B.S. in Genetics. Like many who enter the world of science, he had visions of being pre-med. Yet, that was not the path destined for our bio-hero.

He went on to work for a biotech company, with only 12 other employees. This is where Mr. Rivera gained his source of power. The small workplace gave him an opportunity that more schooling and a big corporate setting could never do, the ability to learn *everything*. Brian absorbed it all—from analysis and small-scale purification of large molecules, all the way to large-scale manufacturing. It is safe to say that this bio-man knows chromatography!

When our hero made his way to **Phenomenex**, he brought with him his knowledge of large molecule analytical method development.

When asked what has been the most exciting development in bioseparations, he said, “The easy to use LC-MS has be able to change host cell protein analysis. Instead of using the ELISA test, which will always have a place in a lab, LC-MS can be used by almost anyone in a lab. The user-friendly mass spec has showed incredible advancement and fast growth, while uncomplicating routine work-flow.”

Mr. Rivera went on to address the idea that LC-MS will allow for easier and faster testing and can help transition small molecule use to large molecules.

We decided to pick Super Bio-Man’s mind a little more, and ask him some of our customers biggest **bioseparations** inquires.

1.What is bioseparations chromatography and why do we care?
Bioseparations chromatography, for all intents and purposes, is the purification or characterization of large molecules by a variety of LC techniques. For Phenomenex, we are primarily focused with the characterization of large molecules by **HPLC/UHPLC**, using analytical techniques such as reversed phase, size exclusion, etc. However, for peptides and oligonucleotides, we do offer purification solutions, as these large molecules are amenable to reversed phase conditions.

Why do we care? Well proteins (specifically, **monoclonal antibodies and their fragments/conjugates**), oligonucleotides like siRNA/RNAi, peptides…these are all being used for a variety of different therapeutic platforms. Every pharmaceutical company wants to be, and stay on, the cutting edge of research and are thus exploring—or already fully vested in—large molecule work. Of course, this comes with the challenges of characterization of 150Kd proteins

2. Why is characterization of large molecules so difficult?
Small molecules are *relatively* easy to characterize—impurities are run by simple **RP-HPLC techniques**, further qualified/quantitated by mass spec, you know. This is what pharmaceutical companies have been doing for the past several decades.

Large molecules are different story. Think of a 1300+ amino acid monoclonal antibody. A myriad of different post-translational modifications can occur, some of which can have some huge implications (aka Critical Quality Attributes). These must be monitored by either peptide mapping, “middle-down” reversed phase techniques, **ion-exchange chromatography**, HILIC, HIC, etc. There are really a variety of techniques that must be used during the initial characterization. Further, these techniques are orthogonal. One technique can’t tell you everything about the biomolecule. And let’s not mention sample prep…peptide mapping was enough to get me out of the lab.

Another unique situation—large molecules can aggregate—and aggregation, from a therapeutic standpoint, can lead to immunogenicity. This is simply not an attribute that has an equivalent in the small molecule world. SEC is the typical method—and it isn’t the straightforward analysis that one might think it would be. Especially since many analysts are now looking at utilizing simultaneous size exclusion and hydrophobic interaction separations.

Finally, bioanalytical (i.e. pharmacokinetics) of large molecules is complicated. This is no simple protein crash (well, duh) and rudimentary MS. This combines immunocapture techniques with paramagnetic beads and LC-MS/MS that pushes detection limits.

3. So, sample preparation for biomolecules, pretty different, yeah?
Sample prep is quite different in the large molecule world. Even rudimentary techniques like the use of high molecular weight spin filters to concentrate protein samples are unique to the protein world. Of course, there are the enzymatic digests and the slight nuances that go into, say, an IdeS digestion, make sample prep for proteins very different.

Another thing to consider is that proteins and peptides stick to everything. Autosampler vials and inserts must be critically looked at. Needle washes must be optimized. Adsorption and recovery from syringe filters must be considered. These are all things that must be experimentally determined because let's face it—proteins and peptides never behave the way you want them to. They are like unruly children that won't go to bed or eat their veggies.

4. What are your top tips for the novice bio-chromatographer?
Core-shell has a lot of benefits for large molecule separations. Proteins have slow diffusion rates, and this goes directly against mass transfer; i.e. band broadening is significant with large molecules. As such, particle morphology is huge for improvements in chromatography. Larger pore size, sure, but thin porous shell and a solid core? This is what is necessary for good intact reversed phase analysis.

Temperature isn't a method development parameter in the small molecule world. Nominal gains in efficiency with small molecule might be obtained. Large molecule? Selectivity can be altered significantly with a change in temperature. Also, in the world of large molecules, we run hot! So, 70, 80, even 90° is not uncommon.

Size Exclusion Chromatography is not plug and play. You need to do some method development! Mobile phase composition, flow-rate and even temperature here might affect the results.

Finally, proteins don't behave the way you expect. Isoelectric points, molecular weight, hydrophobic index…this might not have any bearing to the analysis you are intending to

perform. So, reach out to your **Phenomenex Technical Consultant** to steer you in the right direction. Especially if you are new to the world of **bioseparations**– you're not alone, and Phenomenex is here to help!

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Summary

Article Name

Sitting with Scientists: The Super Bio-Man - Brian Rivera

Description

Bioseparations Product Manager, Brian Rivera doesn't have the typical origin story that many in his field have, but that is what makes him Super Bio-Man!