

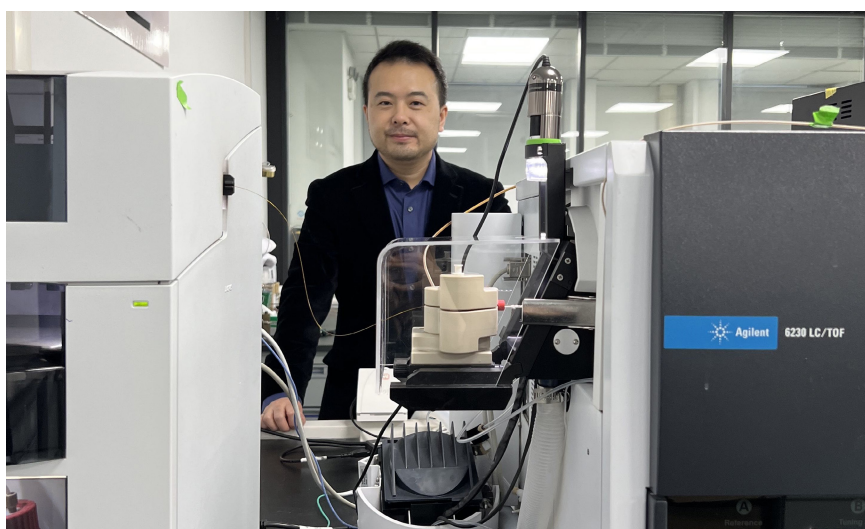
# Building an Efficient Bridge Between Capillary Electrophoresis and Mass Spectrometry

## A CE/MS-based approach for characterization of biotherapeutics and identification of critical attributes

"The weakest point was in the middle," explained Dr. James Xia, a biochemist with a PhD in mass spectrometry-based proteomics. Having worked in the biopharmaceutical industry, Dr. Xia identified capillary electrophoresis/mass spectrometry (CE/MS) as a promising technique for protein characterization. However, he noted, "Experts in the field have been using CE and MS for more than a decade yet have faced challenges in terms of sensitivity and overall performance."

"Imaged capillary isoelectric focusing (CIEF) was commonly employed, which provides a consistent and reproducible separation. This method is well established and has been used extensively for the analysis and quantification of charge variants." Xia continued, "But, imaged CIEF is limited by its use of UV absorbance detection, which cannot identify the specific species present in each peak."

Xia had a strong desire to address the challenges of charge variant identification, recognizing that MS detection would be crucial to provide answers. The crux of the issue was transferring molecules between the CE and MS stages—specifically, the difficulty in mobilizing the molecules once they were focused inside the capillary, as this process could broaden peaks and decrease sensitivity. So, the question remained: How can the analytes be effectively transported from CE to MS, ionized efficiently, and introduced to the MS without loss of resolution or sensitivity?



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This led Xia to start CMP Scientific Corp, where he currently serves as CEO and principal scientist. CMP Scientific began as a single-product company, selling the CMP Scientific EMASS-II CE-MS ion source. Since its inception, CMP Scientific has expanded its product offerings to include the ECE-001 CE system, separation capillaries, electrospray emitters, the CR3520 CIEF-MS reagent kit, standards, and peripheral devices.

The result? CMP Scientific developed an efficient, effective bridge between the CE and MS through their ion source and separation capillaries, enabling the successful transfer of separated components to an MS for identification without band broadening—yielding a 10-fold sensitivity improvement compared to previous solutions. Together, these products systematically improved CE/MS protein characterization analyses. As such, CMP Scientific now offers a solution that delivers the first successful, streamlined CIEF/MS workflow for charge variant separation and identification.

"I am thrilled to take CE/MS capabilities to the next level," said Xia. "Our cutting-edge technology is used daily by some of the world's foremost biopharmaceutical companies to develop superior biotherapeutics. This is incredibly fulfilling, knowing that we are making a significant impact in the analytical instrumentation industry, in research labs, and in the quality assessment of these potentially life-saving biotherapeutics."

Inspired by his customers' success, Xia decided to expand CMP Scientific's offerings to include analytical services. "At first, we provided capillary zone electrophoresis/mass spectrometry (CZE/MS) services to evaluate the quality of the ADC conjugation process, with a focus on intact mass analysis and calculation of the drug-to-antibody ratio," said Xia. He went on, "We then experienced a rapid growth in demand for both CZE/MS and CIEF/MS sample analysis."

Currently, CMP provides CZE/MS analysis to identify peaks in CE/SDS assays of antibody therapeutics, as well as CIEF/MS for charge variant analysis. Previous techniques were effective for quantitation but did not provide peak identification. "The added identification information provided by our approach makes the CMP Scientific CE/MS workflows highly valuable," Xia added, "I believe that our CE/MS workflows will one day be a staple in the biopharmaceutical industry."

To facilitate these new services, Xia needed a cost-effective mass spectrometer with a wide mass range to analyze large, intact biomolecules. When faced with the question of which MS to purchase, he said, "There was no question—the Agilent 6230B time-of-flight (TOF) LC/MS." His early biopharmaceutical customers were already successful with the Agilent TOF. "Once we saw how smoothly our EMASS-II CE-MS ion source worked on the Agilent 6230B LC/TOF, we were inspired to use the same setup in our facilities," explained Xia.

After using the TOF extensively for years, Xia noted, "The 6230B LC/TOF is straightforward to use. Our team members have varying levels of experience, yet we've found that new hires typically require less than a week of training on this system. It's easy to set up a method and sequence, then hit go." He continued, "The 6230B LC/TOF is also robust. We often encounter tight deadlines on customer projects, which require us to run the TOF continuously, day and night. It's our workhorse for our CE/MS services. The stable performance of the Agilent TOF was vital for us to accomplish so much in the past few years."

Xia also emphasized the cost-effectiveness of the 6230B LC/TOF. "It greatly enhances our productivity. The ability to provide timely answers to our customers is essential to our success, and the low cost of ownership, minimal maintenance, and little downtime are critical factors in reaching our business goals," said Xia. He added, "The overall operation costs for the 6230B LC/TOF are significantly lower when compared to other mass spectrometers in our lab."

In addition, Xia attributes an extra productivity boost to the Agilent MassHunter Workstation software. "We have found MassHunter with BioConfirm offers a streamlined workflow. I can't imagine surviving our data analysis needs without MassHunter—all work reports and summary documents are prepared with it. To us, this software has become as important, and as fundamental, as Microsoft Office software for our daily business operations," explained Xia.

Xia summarized his thoughts, "What we've found amazing is that this workflow of the CMP Scientific EMAS-II CE/MS technology with the Agilent 6230B LC/TOF works with a wide range of biomolecules, including monoclonal antibodies, bispecifics, antibody drug conjugates, and fusion proteins. This solution enables us to consistently obtain high-quality data for both intact mass and post-translational modification analysis—providing valuable insights for our clients."

The future looks bright for Xia and CMP Scientific. He said, "We've been getting more samples than we can handle, which is a great problem to have." The biopharmaceutical market is rapidly growing and presents an enormous opportunity for CMP Scientific's services and products. "We are seeing a flood of new modalities in the pipeline, namely bispecifics and antibody drug conjugates. At the preclinical stage, the scene is crowded," explained Xia.

Xia is eager to continue exploring deeper into protein characterization via CE/MS. He's looking forward to future collaborations with Agilent and is excited about the potential a new Agilent 6545XT AdvanceBio LC/Q-TOF can add to CMP Scientific's specialized CE/MS analytical services, further advancing biotherapeutic drug development on a global scale.

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