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PMedIC SEMINAR SERIES

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Mass Spectrometry-based Single Cell Proteomics for Characterization of Inner-Ear Sensory Hair Cells

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Sensory hair cells of the inner ear detect sound—within the auditory system—and movements of the head—within the vestibular system. Hair cell transduces these mechanical stimuli using its hair bundle, a cluster of ~100 actin-based stereocilia. By the year 2005, protein mass spectrometry sensitivity became sufficiently high that the Barr-Gillespie lab could begin to define the proteome of the hair bundle; continual subsequent sensitivity improvement allowed a better and better view of the proteome. Sample preparation limitations ultimately have slowed progress, forcing us to look for better approaches.

The last five years have brought revolutionary advances in protein mass spectrometry that increase overall sensitivity by many orders of magnitude. Two of these advances, derived from the Zhu lab, were nanoPOTS sample preparation and FAIMS separation. NanoPOTS significantly improved the sample recovery by performing the protein extraction and digestion in nanoliter-scale droplet microarray. To enhance the sensitivity of MS system, we leveraged the newly-released FAIMS interface and developed an advanced data acquisition method, TIFF (transferring identification based on FAIMS filtering). With these advances, we have been routinely quantifying >1000 proteins from single cells.

We have exploited these advances to address new questions important for hair cell functions. For example, we measured single-cell proteomes from developing vestibular hair cells in the chick utricle and revealed the role of the actin-binding protein TMSB4X. We then turned to the mouse auditory system, and first used FACS with Fgf8flox-GFP;Atoh1-Cre mice to isolate single inner hair cells from the cochlea and determine their proteomes. Finally, we used these techniques to study small pools of individually-isolated inner and outer hair cells (IHCs and OHCs), revealing many known and unknown differences between these two cell types.

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