Next-generation liquid chromatography column technology for enabling highly sensitive profiling of amount-limited clinical samples

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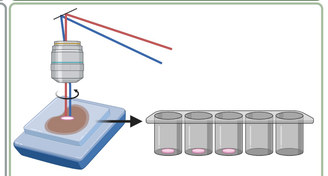
Introduction

- Deep and sensitive proteomic analysis of limited biological and clinical samples is of high significance and growing interest, as they often hold keys to solving long-standing puzzles in biomedical research
- Proteins and their biologically active modified states (post-translational modifications (PTMs) and proteoforms) better recapitulate phenotypic states of the cell and tissue in health and disease than RNAs and DNA
- State-of-the-art mass spectrometry (MS)-based proteomic techniques provide suboptimal separation performance, sensitivity, and profiling depth
- No amplification techniques for proteins, proteoforms, and PTMs
- Lack of early diagnostics for multiple deadly diseases, including cancer and Alzheimer's disease
- We propose **next-generation ultra-low flow (ULF) liquid chromatography (LC) columns** to enhance the sensitivity of MS-based proteomic and multiomic molecular profiling assays for diverse types of scarce biological and clinical samples

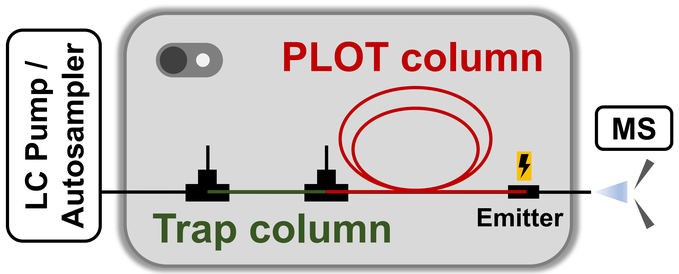
Application areas

Scarce, precious, and amount limited clinical and biological samples

Microbiopsy/fine needle aspirate	*Rare cell populations/liquid biopsy*	*Laser capture microdissection*
		
• minimally invasive sampling • amount-limited samples • neonatal/pediatric samples • animal disease models (tissues/fluids) • molecular species of low abundance - PTMs, IPs • patient-derived organoids	• minimally invasive sampling • circulating tumor cells • 1-100 cells per mL blood • blood derived isolates (e.g., extracellular vesicles) • cell transplantation therapies • adoptive cell therapies (ACT) • single-cell analysis • spatial multiomics/surfaceomics	• heterogeneous tissue sampling • spatial resolution • analysis of single cells and subcellular regions • closely localized small cell populations • FFPE tissue samples

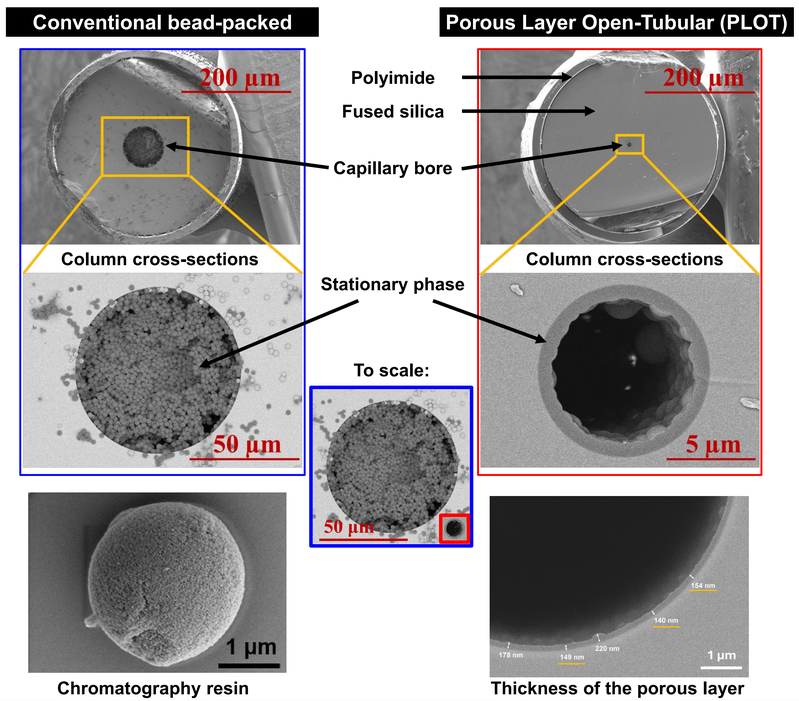
Product vision

Cell phone-size plug-and-play product



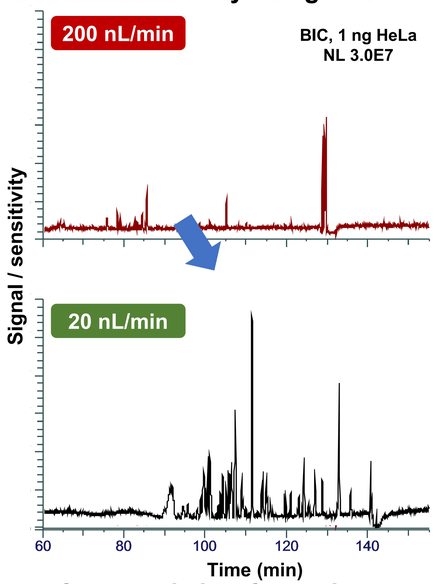
- Patent pending
- Manuscripts published (Gregus & Ivanov, *Anal. Chem.*, 2025)
- PLOT columns can be prepared in bulk quantities (>10 meters)
- LC and MS vendor neutrality
- Estimated platform price: ~$2K
- Estimated usage per site / per month: 2+ platforms
- $0.25 - $1K for consumables

Technology – novel ULF LC columns



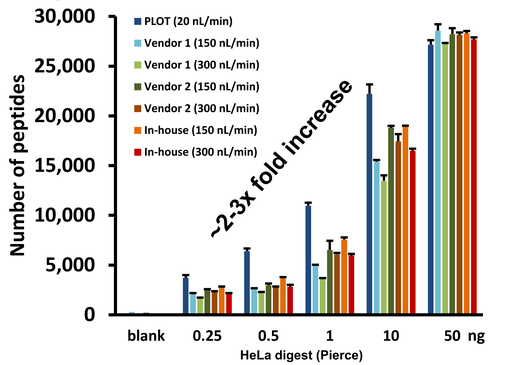
Benchmark experiments

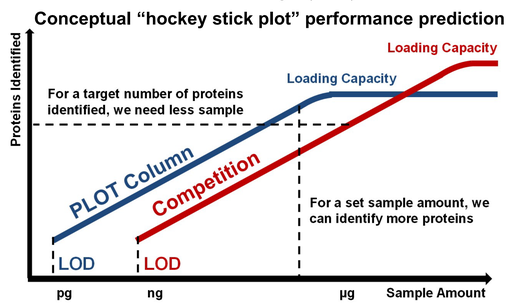
Gains in sensitivity using PLOT vs. bead-packed columns



⇒ **Improved signal-to-noise**

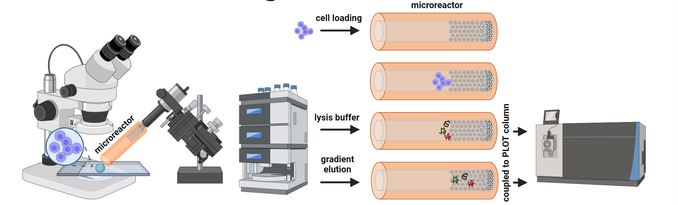
⇒ **More identifications, more information about the sample**



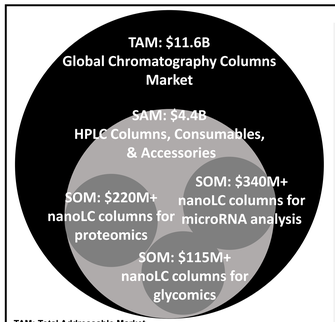


Analysis of small populations of cells

Schematic representation of on-microreactor analysis of selected cells using the ULF PLOT LC-MS



Market sizing



TAM: Total Addressable Market
SAM: Serviceable Available Market
SOM: Serviceable Obtainable Market

- CAGR Projected growth 5.9% - 12% from 2023 to 2030
- Drivers: technological advancements (new therapeutics, vaccines, and reliable diagnostic methods for disease research, academic laboratories, and clinical trials) and increased needs for improved proteomic analysis
- Possible expansions to metabolomic, lipidomic, glycomic, and oligonucleotide applications