CE IN DRUG DEVELOPMENT: WHERE HAVE WE BEEN AND WHERE ARE WE GOING?

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CASSS CE-PHARM
SAN FRANCISCO

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OUTLINE

• CE --- Early Days, CASSS CE-Pharm
• Human Genome Project
• Current Status of CE
• Opportunities for CE, Analytical
• Opportunities for CE, Emerging Biotech
• Analytical – Clinical Design
• Conclusions

What have I learned over 60 years?
• 1967 – Stellan Hjerten – narrow tube electrophoresis

• 1982 – Jim Jorgenson – HPCE

• 1984 – Shigeru Terabe – MEKC

• 1987 – Barry Karger – DNA Separation

• 1988/89 – Bob Brownlee – Microphoretic Systems
HPCE '89
First International Symposium on
High Performance Capillary Electrophoresis

April 10-12, 1989
Boston, Massachusetts
CE vs. LC, CE vs. SLAB GEL

• LC $2B market in early ‘90s
• 10% for CE would be $200M!
• BUT – why was LC $2B?
• Lesson – to replace need significant reason

• Slab Gel – slow, manual, not very quant.
• Column – rapid, automate, quant.
Rapid separation and purification of oligonucleotides by high-performance capillary gel electrophoresis

(nucleic acid analysis/high-resolution isolation)

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Communicated by Fred W. McLafferty, August 15, 1988 (received for review July 1, 1988)

Fig. 2. HPCE separation of polydeoxyadenylc acid mixture, (dA)₂₅₀. Capillary size was 270 × 0.075 mm i.d.; running buffer was 0.1 M Tris/0.25 M borate/7 M urea, pH 8.3, and the gel contained 7.5% T and 3.3% C. The applied field was 400 V/cm.

Ed Yeung
Norm Dovichi
Figure 2. Read length of 1300 bases using the separation matrix LPA 2.0% (w/w) 17 MDa/0.5% (w/w) 270 kDa at 125 V/cm and 70 °C. Sample was prepared as in Figure 1.
The Critical Need for Efficiency – Read Length

\[ R_s = \frac{1}{4} (\alpha - 1) N^{\frac{1}{2}} \]

\( (\alpha - 1) = \frac{\Delta \mu}{\bar{\mu}} \quad \text{charge/hydrodynamic volume} \)

\[ R_s = 0.7, \ N = 10 \times 10^6 \quad \text{plates/meter} \]

\( \alpha = 1.001 \)

But efficiency alone did not solve the problem!!
INTEGRATION

- Labelling strategy – ET fluorescent dyes
- 96 capillary array instrumentation
- Automated linear polymer replacement
- Separation – CE migration, efficiency
- Data processing and storage
- Robustness!!

1. Understand and define goal clearly
2. Holistic view of workflow
CASSS CE Pharm

- Mid 1990s – Beyond DNA, CE slow advance
- HPCE meeting – pushing the frontiers
- Need to increase applications of CE
- Idea – form a separate meeting to focus on applications, later merge with HPCE
- CASSS CE formed in 1998
- So successful, decided to stay independent
- 20 years and still going strong – meets a need
CE ADVANTAGES

• High resolution
• Reproducible, robust (validated methods)
• Open tube – larger molecule (intact protein)
• Fast separations
• Charge-based, subtle size separations
CE - ISSUES

• Coating
• Sample size – inject nL, prep. µL
• Dynamic range – overload column
• Coupling to mass spectrometry
• Why not use LC???
GLYCAN ANALYSIS BY CE

- Glycan profiling
  - High resolving power
  - Excellent sensitivity
  - Rapid Separations
  - Automation
  - Simplicity
  - Large-scale / High throughput

- Single and multi-capillary modes

Flexibility – fast screening or detailed analysis of structural isomers

- Applications
  - Qualitative and quantitative characterization of reducing glycans
  - Glycosylation studies of biopharmaceuticals, mAB’s, etc.
  - Carbohydrate sequencing
  - Structural elucidation by CE-MS
CAPILLARY ELECTROPHORESIS

HILIC vs CE – Mannose-6-Phosphate Analysis


- Mannose-6-phosphorylated glycans important on lysosomal enzymes
- Peaks appear as broad on HILIC due to additional anion exchange interaction
- Peaks elute with high efficiency in CE due to additional charge from the phosphate group
150 µm OD, 30 µm ID Separation capillary

Porous tip

405 nm laser

Detector

Plume

MS inlet

Subnanomolar sensitivity

Andras Guttman
LabChip System

Workflow:
- Integrates the entire SDS-PAGE process onto a microfluidic chip
- Determine size, quantification, and purity
- Automatic sampling from 96- or 384-well plate

Throughput and sample requirements:
- Fast sample analysis - 41 seconds per sample
- Can run crude samples
- 96-well plate analyzed in < 1.25 h
- Can do >400 samples per day per instrument
- 2 µl of sample
- Minimal sample preparation
- 21 CFR Part 11 support
Microfluidic CE-MS
Fast separations

M. Ramsey
908 Devices
In-Process Monitoring of mAb Production

Time-course of cell culture

Excellent correlation between CE-MS and LC-MS data
NIST mAb N3

0.5μg/μL NIST mAb N3

A. Paulus, Thermo
Microfabricated HPLC Column

Pharmfluidics
HPLC 2012 Anaheim
GRAND CHALLENGE

• High resolution multidimensional separation of intact proteins – chromatography, electrophoresis, affinity


• PTMs (phosphorylation, glycosylation, proteolysis, alternative splicing ..........

• High Rs LC columns, IEF, gel free SDS PAGE, CE, ..... 

• High Rs MS (FT, Orbi), ion mobility, fragmentation
TOP DOWN MS

- Intact protein
- Fragmentation in MS for structure
- Proteoforms (PTM)
- Separations challenging
  - small changes (PTM) in large species
  - again many species
- Partially/fully denatured, native
- MS advances in m/z range and resolution
Intact/Top-Down for Biopharmaceuticals vs. Traditional Bottom-Up Techniques

Information on correlated modifications is lost with proteolysis.

The molecule is functional, not the peptide.
Avonex (rh\(\text{INF-}\beta 1\)) (Biogen)

- Single glycan (N80)
- Deamidation, succinimide (N25)
- Disulfide bond (C31-C141)
- Oxidation

Multiple sclerosis
$2\text{B+ sales}

23 kDA

Karger et al.
N = 400,000 plates
CE-MS of Intact Avonex

PEI coating, BGE – 2.6 pH
138 proteoforms, 55 quantitated
2% hydrodynamic volume difference
CVA-MS of Cetuximab

Initial CVA-MS focused on Cetuximab, however data analysis was extremely complicated. In-depth annotation of Cetuximab charge variants completed, 104 proteoforms across 9 chromatographic peaks identified.

K = C-terminal lysine
NGNA = N-Glycolylneuraminic acid

Competition for CE
24 proteoforms annotated in CVA Peak 5 using relative abundance data from offline N-glycan analysis and LC-MS peptide mapping
ION MOBILITY - 1D-CE or -LC plus IM the future?

Resolution approx. 300

Figure 1. (A) Schematic diagram of the multipass SLIM SUPER IM-MS instrument used in this work; (B) photo of one of the two SLIM module surfaces; and (C) illustration of an ion switch (switch on. ion cyclone; switch off. transmit ion to MS).

SIGNIFICANT CE OPPORTUNITIES

• Limited sample/single cell

• Native MS

• DNA/RNA therapeutics
Fine Needle Biopsy

- Needle aspirates
- Less invasive compared to core biopsies
- 0.14-0.50 mm i.d. needle
Low Flow Improves MS Signal

- Decreased ion suppression
- More uniform response

Increased:
- ionization efficiency
- ion transfer efficiency
- overall ion utilization efficiency
Quantitative “Assays”

Metabolites

- Limit of Detection: ~25 amol
- Quant. repeatability: ~10%

Proteins (Peptides)

- Limit of Detection: 210 zmol = 126,000 copies
- Complimentary to LC-MS (1-20 ng by CE vs. 1-10 µg by LC)

S. B. Choi, P. Nemes*, et al., JASMS 2017, 28, 597
Transformative Opportunities for Single-Cell Proteomics

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Abstract

Many pressing medical challenges, such as diagnosing disease, enhancing directed stem-cell differentiation, and classifying cancers, have long been hindered by limitations in our ability to quantify proteins in single cells. Mass spectrometry (MS) is poised to transcend these limitations by developing powerful methods to routinely quantify thousands of proteins and proteoforms across many thousands of single cells. We outline specific technological developments and ideas that can increase the sensitivity and throughput of single-cell MS by orders of magnitude and usher in this new age. These advances will transform medicine and ultimately contribute to understanding biological systems on an entirely new level.

![Counting Molecules](image)

- Sample mRNA with 20% efficiency \( \Rightarrow \) Error \( = \frac{\sigma_X}{X} = \frac{1}{\sqrt{4}} = 50\% \)
- Sample protein with 2% efficiency \( \Rightarrow \) Error \( = \frac{\sigma_X}{X} = \frac{1}{\sqrt{1000}} \approx 3\% \)

1500+ proteins
Separating single cells by cell-type and cell-cycle phase based on their proteomics.

Specht et al., 2018, doi: 10.1101/399774
NATIVE CE-MS

• Separation under native (or close to) native conditions
• Higher charge state spacing of high MWTs
• Lower # of charged states (high sens.)
• Non-covalent association (antigen-mAB)
• Protein complexes
• CE – open tube, minimal (or no) surface interaction.
Figure 1. Comparison of normal and native ESI of a 148 kDa antibody.

Washington University School of Medicine
Ribosomal proteins

Native

Denatured

Ivanov, Karger, JASMS, 28, 2614 (2017)
DNA/RNA Therapeutics

- Anti-sense DNA
- RNAi (siRNA)
- Long chain, modified RNA
- Gene therapy
- Cell therapy

There is a need for in depth analytical characterization (e.g. raw materials, manufacturing, delivery, formulation…..)

Opportunity for CE. Get in early!
BioMarin: Hemophilia A

- Cell and Gene Therapy Products – CASSS
- Robert Baffi presentation
- Single Treatment – CURE!!
- AAV vector – viral protein (VP) analysis
- Deamination of VPs decreased potency
- Capsid Analysis for Comparability
Highly Challenging Program Success (Failure) Rates

- ~90% failure from Phase I; 50% from Phase III!

MODIFICATIONS OF PRODUCT IN HUMANS

- In vivo CQA
- Modifications may differ from cell lines and animal studies
- Effect on efficacy and safety
- Structure - function
- Clearance rate
- Analytical challenges – limited sample (?)
- Could have significant impact on clinical design
Assessing in vivo dynamics of multiple quality attributes from a therapeutic IgG4 monoclonal antibody circulating in cynomolgus monkey.

Yinyin Li, Yu Huang, Janine Ferrant, Yelena Lyubarskaya, Yue (Emma) Zhang, Siyang (Peter) Li & Shiaw-Lin (Billy) Wu

*mAbs*, Volume 8, 2016 - Issue 5
How does this deamindation affect function?
Quantitation and pharmacokinetic modeling of therapeutic antibody quality attributes in human studies

Yinyin Li, Michael Monine, Yu Huang, Patrick Swann, Ivan Nestorov, and Yelena Lyubarskaya
Biogen, Cambridge, MA, USA

ABSTRACT
A thorough understanding of drug metabolism and disposition can aid in the assessment of efficacy and safety. However, analytical methods used in pharmacokinetics (PK) studies of protein therapeutics are usually based on ELISA, and therefore can provide a limited perspective on the quality of the drug in concentration measurements. Individual post-translational modifications (PTMs) of protein therapeutics are rarely considered for PK analysis, partly because it is technically difficult to recover and quantify individual protein variants from biological fluids. Meanwhile, PTMs may be directly linked to variations in drug efficacy and safety, and therefore understanding of clearance and metabolism of biopharmaceutical protein variants during clinical studies is an important consideration. To address such challenges, we developed an affinity-purification procedure followed by peptide mapping with mass spectrometric detection, which can profile multiple quality attributes of therapeutic antibodies recovered from patient sera. The obtained data enable quantitative modeling, which allows for simulation of the PK of different individual PTMs or attribute levels in vivo and thus facilitate the assessment of quality attributes impact in vivo. Such information can contribute to the product quality attribute risk assessment during manufacturing process development and inform appropriate process control strategy.
STRUCTURE-FUNCTION

• Example - Core fucosylation: affect binding to Fc receptor and ADCC
• Critical quality attribute????
• Structure ahead of function
• How best to study function – combination of measures – bioassay, animal, cell line, etc.

Key to take advantage of analytical advances
Opportunities in De-risking and Advancing Biologic Development—Integrating Characterization, CMC and Clinical Program Data and Analysis

DATE
October 9, 2018
TIME
2:00pm-6:00pm
LOCATION
Northeastern University Interdisciplinary Science and Engineering Complex (ISEC)
805 Columbus Ave.
Boston, MA 02118
Summit Keynote Speakers & Panelists

**STEVE KOZLOWSKI**
Keynote Speaker
Director, Office of Biotechnology Products, FDA

**WASSIM NASHABEH**
Keynote Speaker
Vice President, Regulatory Policy and International Operations, Roche Pharmaceutical Group

**KATY STEIN**
Panelist
Principal, Kathryn Stein Consulting; formerly Director of the Division of Monoclonal Antibodies FDA and SVP Product Development and Regulatory Affairs, Macrogenics

**PAMELA M. KLEIN, M.D.**
Panelist
Principal of PMK BioResearch; former CMO of Intellikine (acquired by Millennium/Takeda) and Vice President of Development at Genentech

**PATRICK SWANN**
Panelist
Vice President, Quality Science and Technology, Amgen
CONCLUSIONS

• CE --- a well accepted tool.
• Coupling to MS will continue to improve.
• Microfluidics will grow in the years ahead.
• Significant opportunities in limited sample analysis.
• Large mass analysis under native conditions.
• CE will impact DNA/RNA therapeutics.
• SIEZE THE OPPORTUNITY!!