CE Pharm 2020
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Breakthroughs that change patients' lives

Development and Optimization of a LabChip® Method for Characterization of a Heavily Sialylated Protein and its Advantage in High-Throughput In-Process Development Support

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Pfizer

Outline

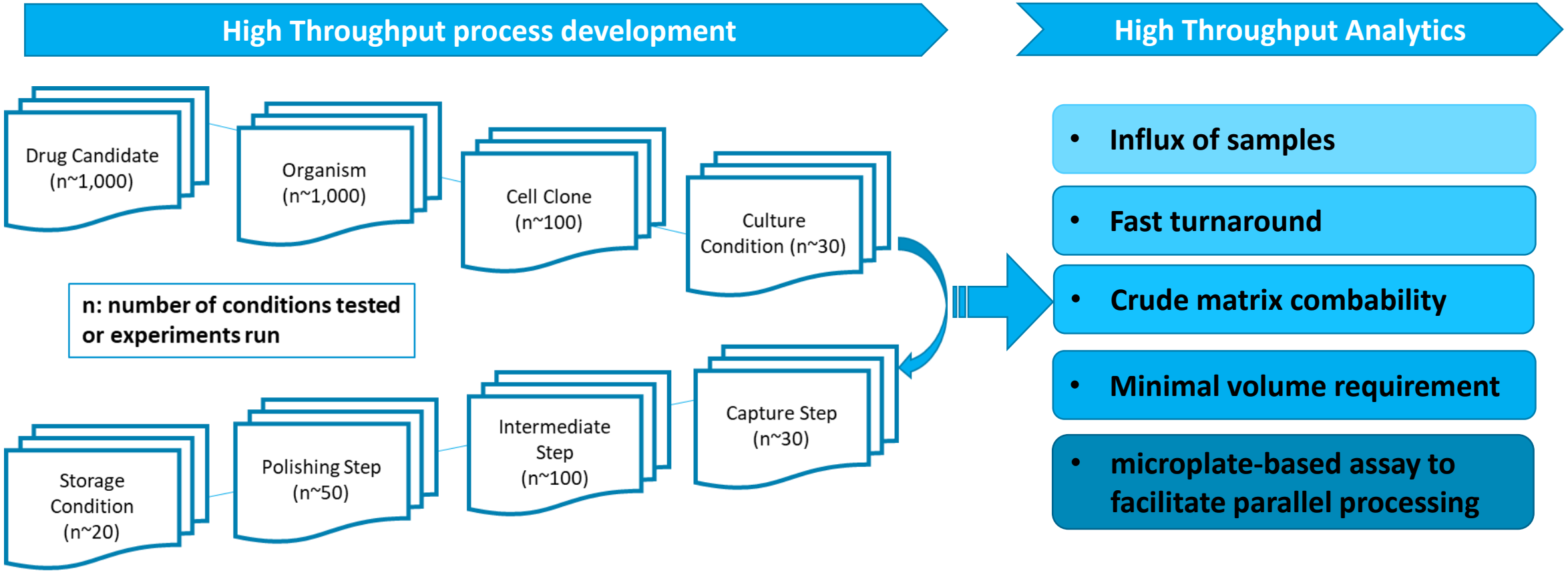
- Introduction
  - Overview of High-Throughput (HT) process development and process qualification
  - Analytics used in high-throughput studies
  - Challenges from conventional to HT technology

- Case Study
  - Current reducing CE-SDS method for protein X using PA800+
  - LabChip method development and troubleshooting
  - Results from LabChip and PA800+

- Discussion

HT Biomanufacturing Process Development

Goal: assess the impact on potential Critical Quality Attribute (CQAs) and generate data to identify and set control limits on Critical Process Parameter (CPPs).



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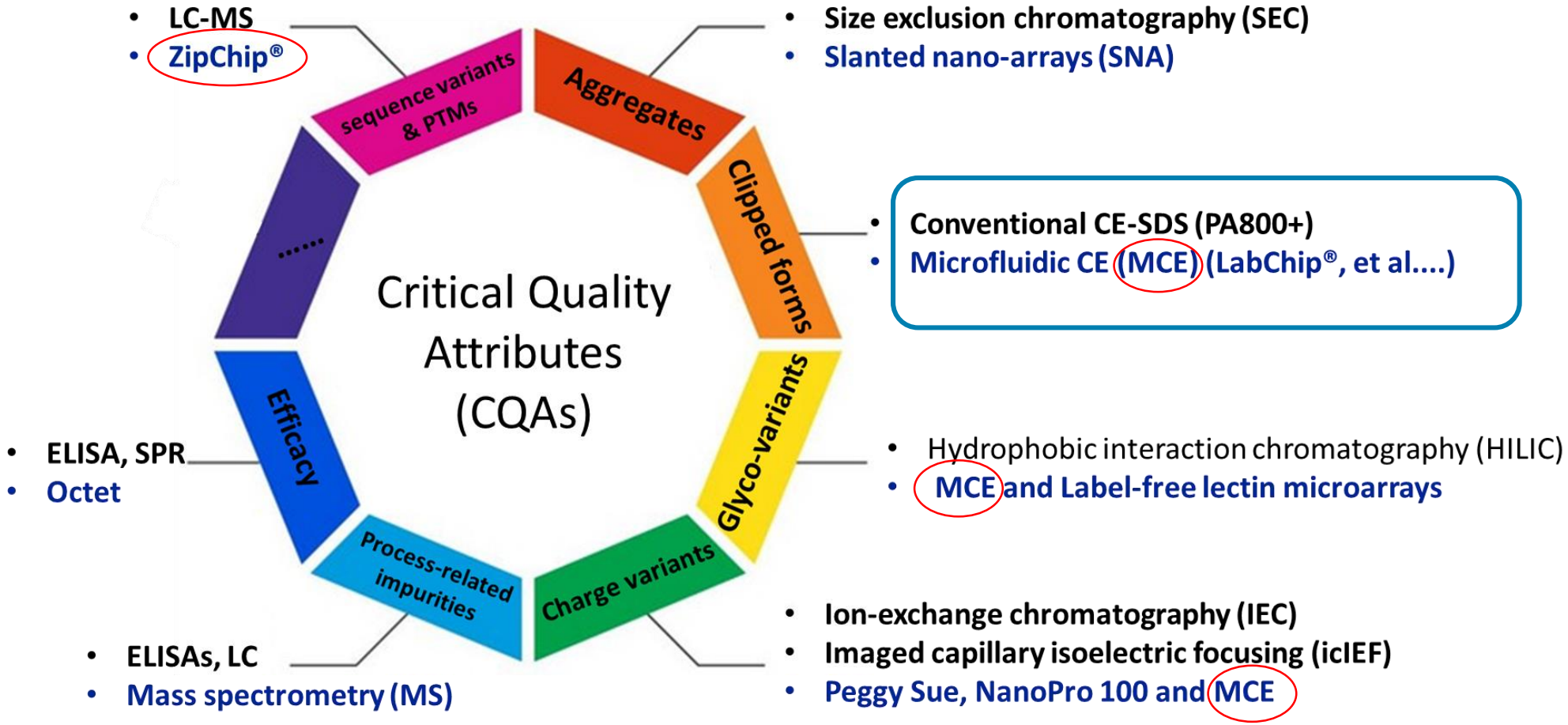
Current Opinion in Chemical Engineering 2014, 6:25–32

Analytics used in high throughput studies

- **Conventional** and **emerging HT technologies** available for measuring CQAs in upstream and downstream processing



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Biotechnol Bioeng. 2017 Nov;114(11):2445-2456.

Size Based CE Application in Pharmaceutical Development

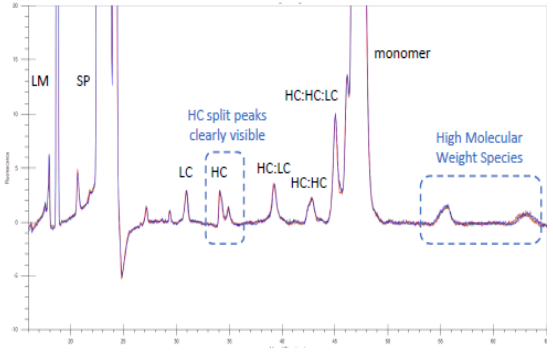
Gold-Standard
Conventional CE-SDS (PA800+)

HT Technology
MCE (LabChip® GXII Touch)

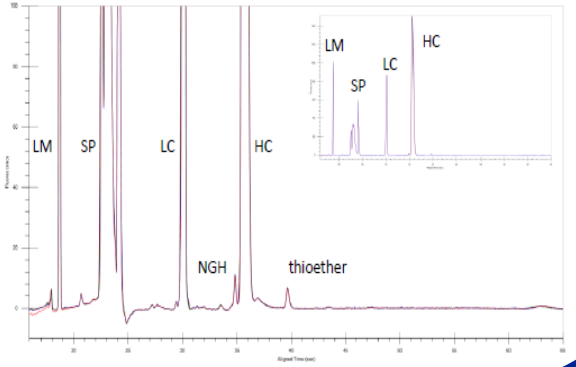
Meets HT Analytics requirement

- ✓ < 1 minute per sample; 96-well plate in < 1.25 hr
- ✓ Multiple assays in 96-well or 384-well format
- ✓ Wide variety of samples with limited sample preparation time
- ✓ Low detection limit: 5 ng/mL with minimal sample volume requirement (~ 5 µL)

Non-reducing



Reducing



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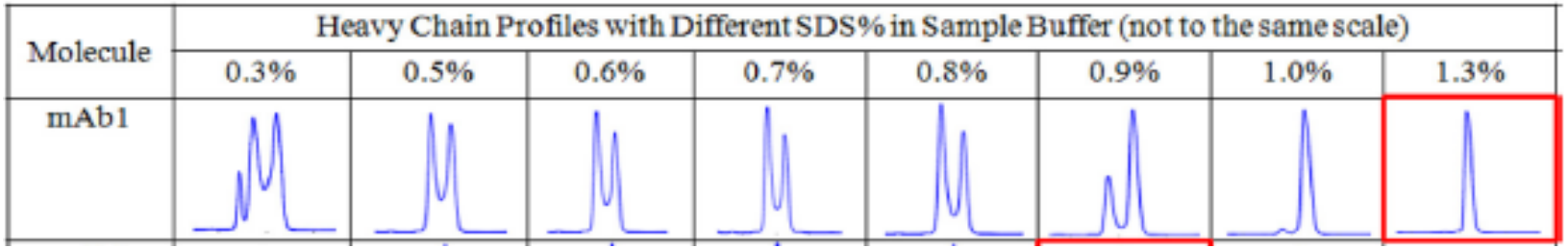
Challenges from Conventional to HT

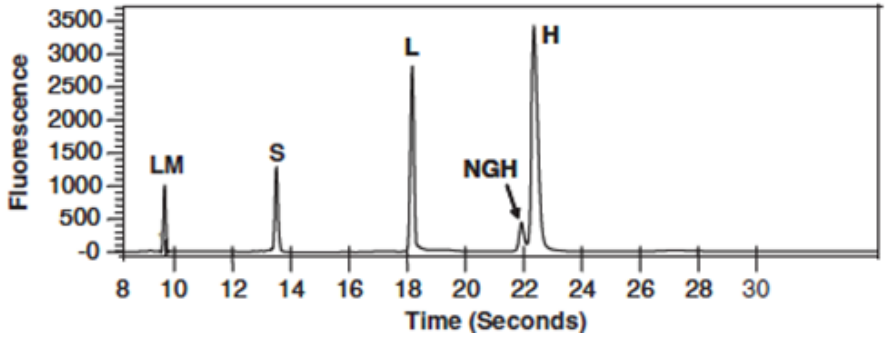
Atypical profile attributed to molecule-specific heterogeneity

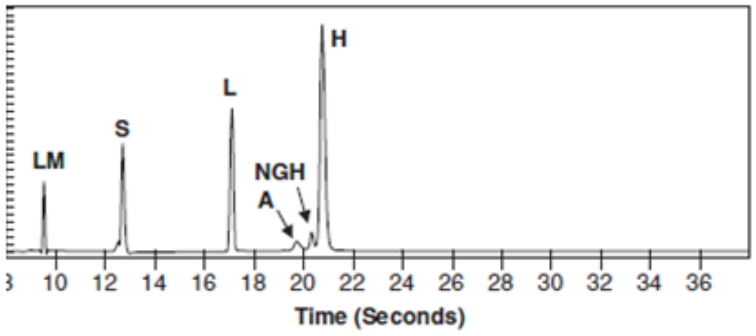
- **Atypical profile**: heavy chain was split into two isomers
- **Fix**: Customize SDS/LDS concentration in sample buffer



Journal of Pharmaceutical and Biomedical Analysis 120 (2016) 46–56

- **Atypical profile**: Artificial peak observed prior to NGH
- **Fix**: Double the denaturation solution to sample ratio



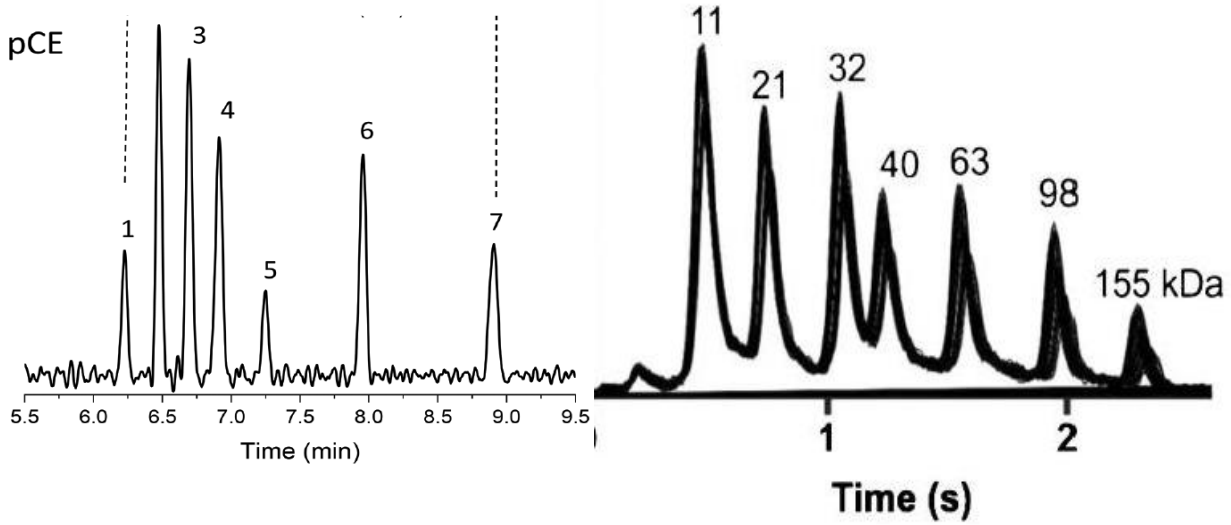


Future enhancements to instrumentation, protocols, and separation chemistries are required

Electrophoresis 2008, 29, 4993–5002

Challenges from Conventional to HT

➢Higher resolution is needed to separate product variants of similar sizes without sacrificing signal response.



Anal. Chem. 2020, 92, 49−66

➢Higher sensitivity is desired to accurately quantify low-expressing proteins and proteins from HT purification.

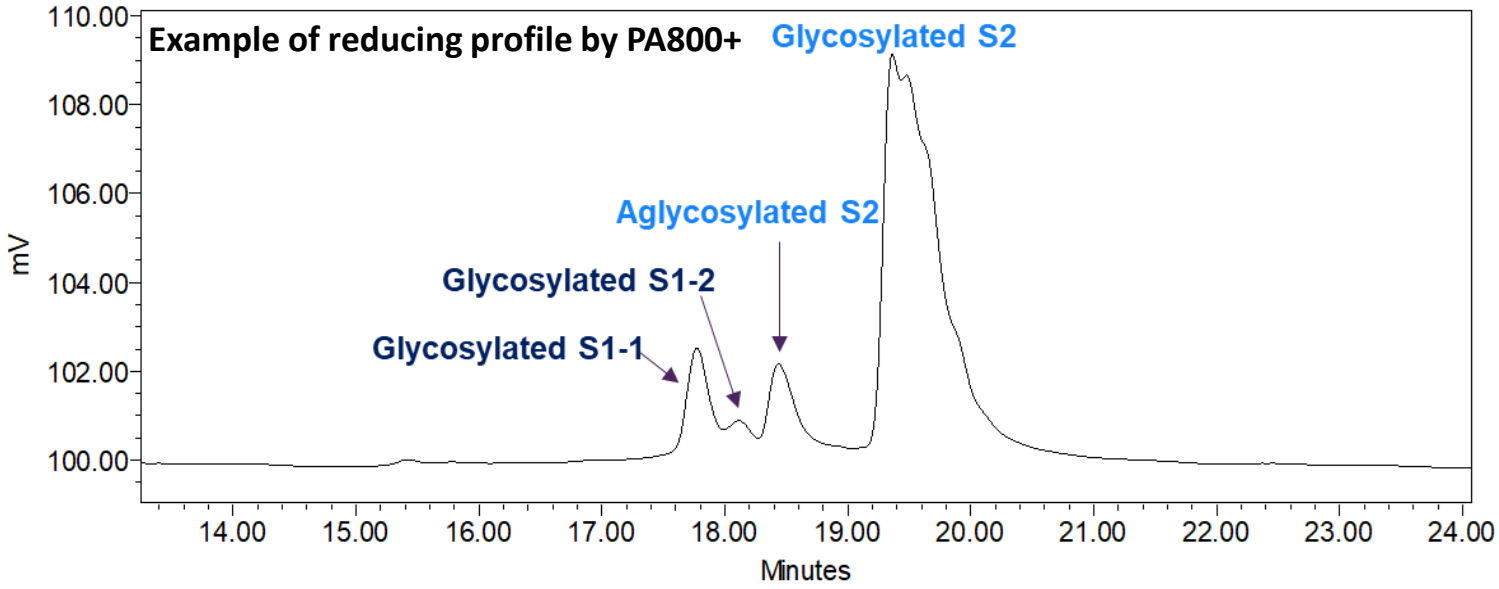
- Sample pre-concentrating (ultra-filtration, et al)

  Electrophoresis 2011, 32, 1129–1132

Case Study

Protein X Configuration and Glycosylation Occupancy

- Protein X:
  - Two subunits, S1 and S2, covalently linked by disulfide bonds
  - Fully occupied glycosylation sites on S1. One partially occupied glycosylation site on S2
  - Heavily sialylated with high heterogeneity
  - The size of S2 is three times larger than S1



- For release, purity was monitored by reducing CE-SDS
- For lab-scale process qualification, purity and aglycosylated S2 need to be monitored

First Look from PA800+ to LabChip

	Protein Express (PE) Assay (14-200 kD)	Low Molecular Weight (LMW) Assay (5-80 kD)
Designed for	Size, concentration, % purity of proteins	Size, concentration, % purity of proteins
MW range	14 – 200 kD	5 – 80 kD
Run time per sample	42 seconds	60 seconds
Samples per reusable chip	400	400

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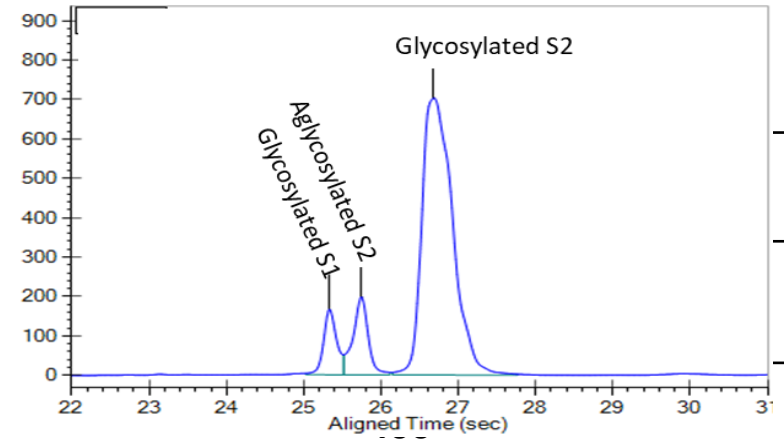
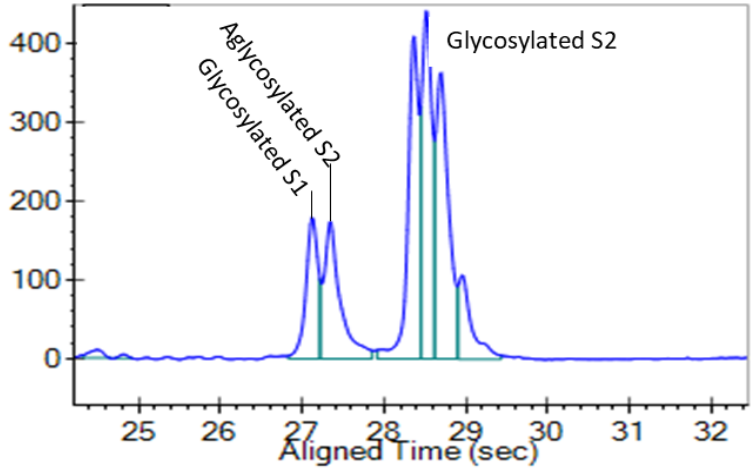
Designed for		
MW range		
Run time per sample		
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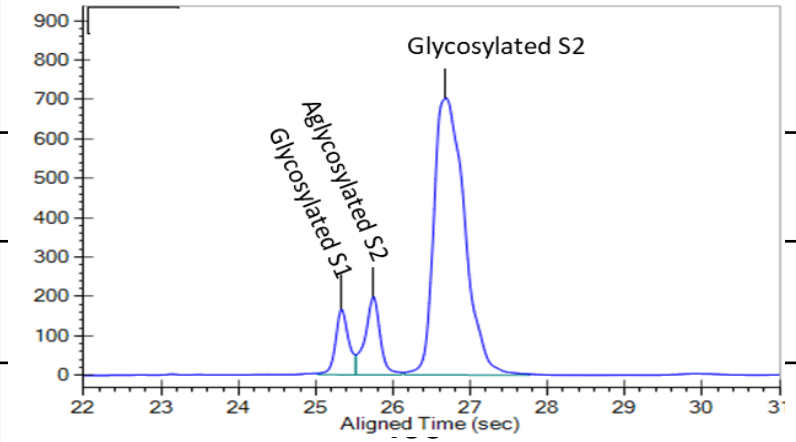
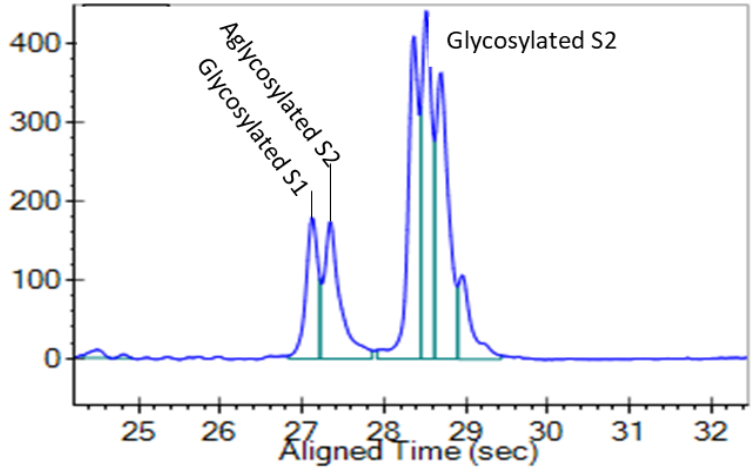
Designed for		
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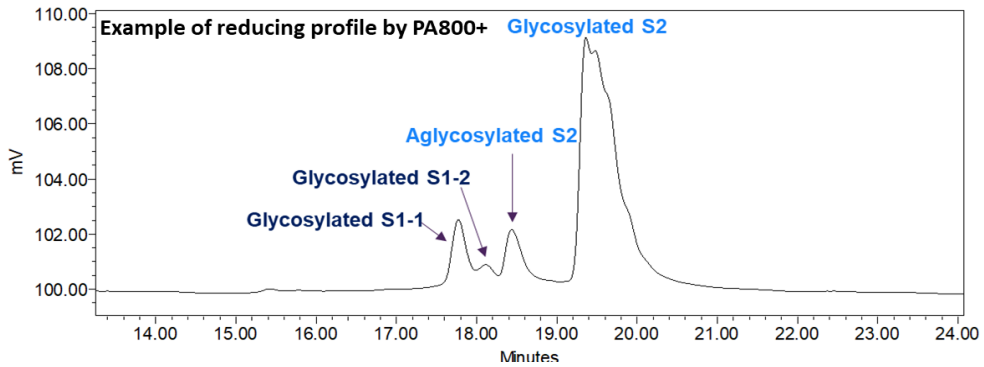


Challenges:

- Atypical profile: glycosylated S1-2 peak was lost
- Inaccurate reporting of aglycosylated S2

Journey from PA800+ to LabChip

Get the S1-2 peak back

Optimized gel concentration:
Ferguson plot
(log_{10}M~gel concentration)
M: Mobility

- Using PE gel matrix running on a LWM Chip;
- PE gel is less concentrated than LMW gel

Ensure Reproducibility

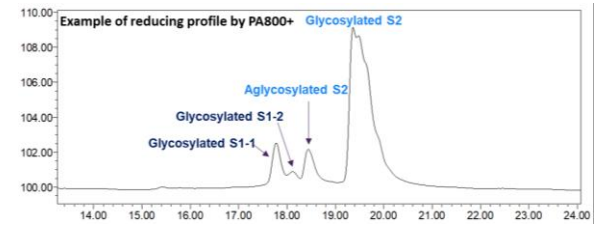
Separation channel regeneration/conditioning

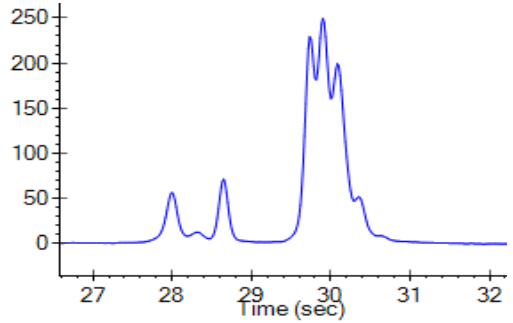
Approach 1 (mimic PA800+): Base/Acid/Water (not work)
Approach 2: Acid/Water (moving forward)

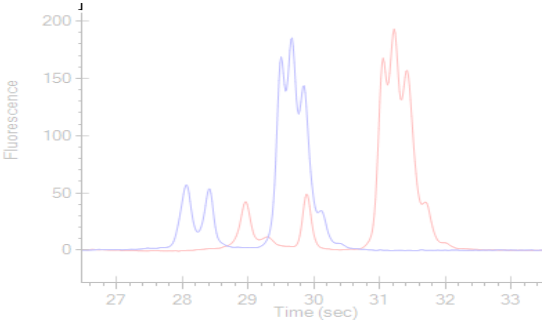
Ensure Comparability

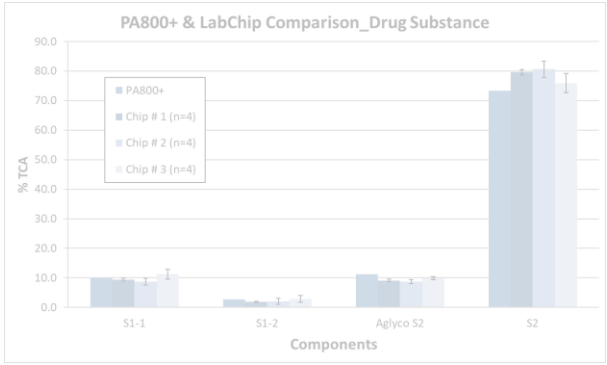
Samples from process qualification were tested side by side by both PA800+ and LabChip

Comparable data between PA800+ and LabChip with HT capability









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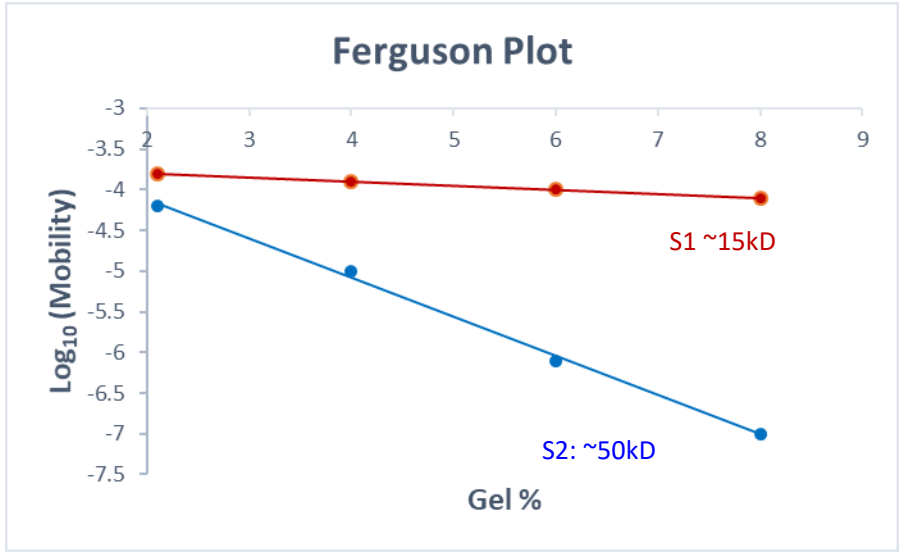
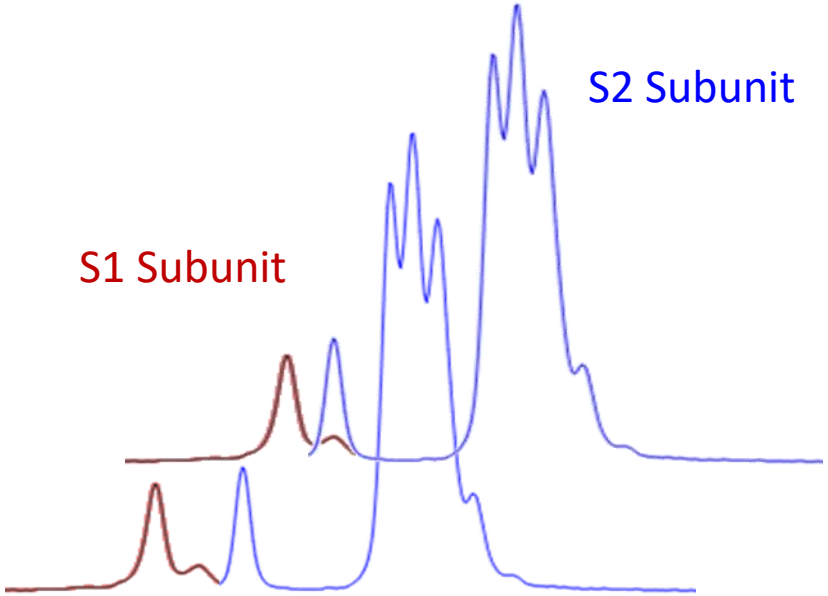
More Description about Ferguson plots





- **<u>Ferguson plot (explained why PE gel gives better separation)</u>**
- Log_{10} (mobility) is inversely proportional to gel concentration
- The slope of the plot is proportional to the MW of the molecule
- With lower % gel, mobility of both S1 and S2 increases
- At certain range, S1 linear mobility change is greater than S2 with lower gel %
- Mobility of S1 and S2 changes differently due to significant size difference

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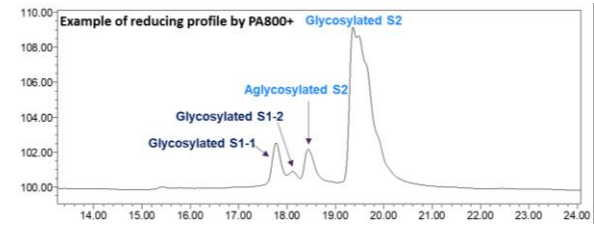

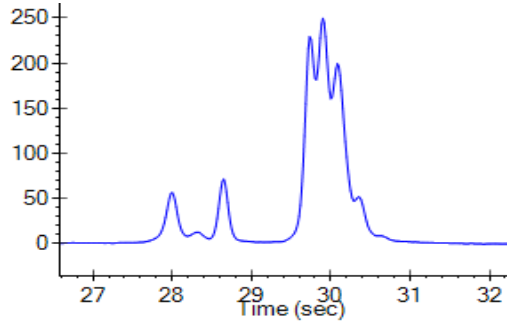

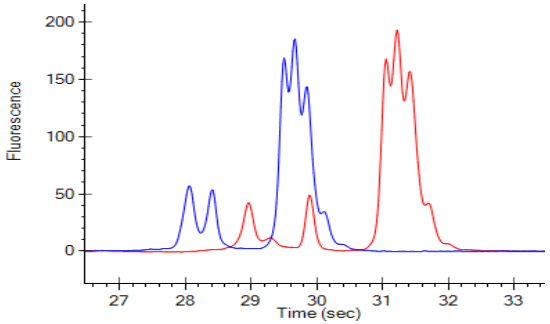

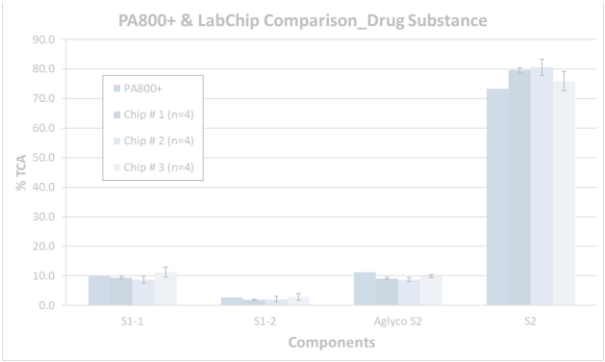

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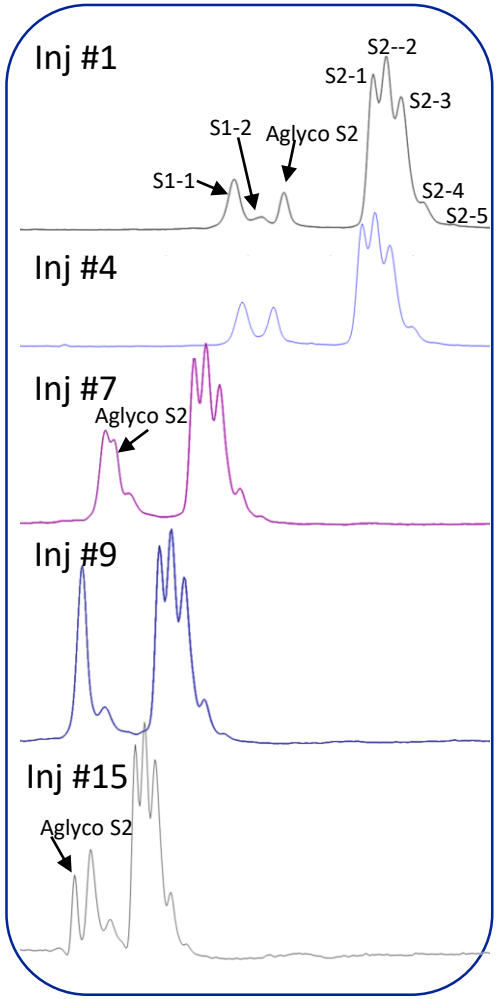
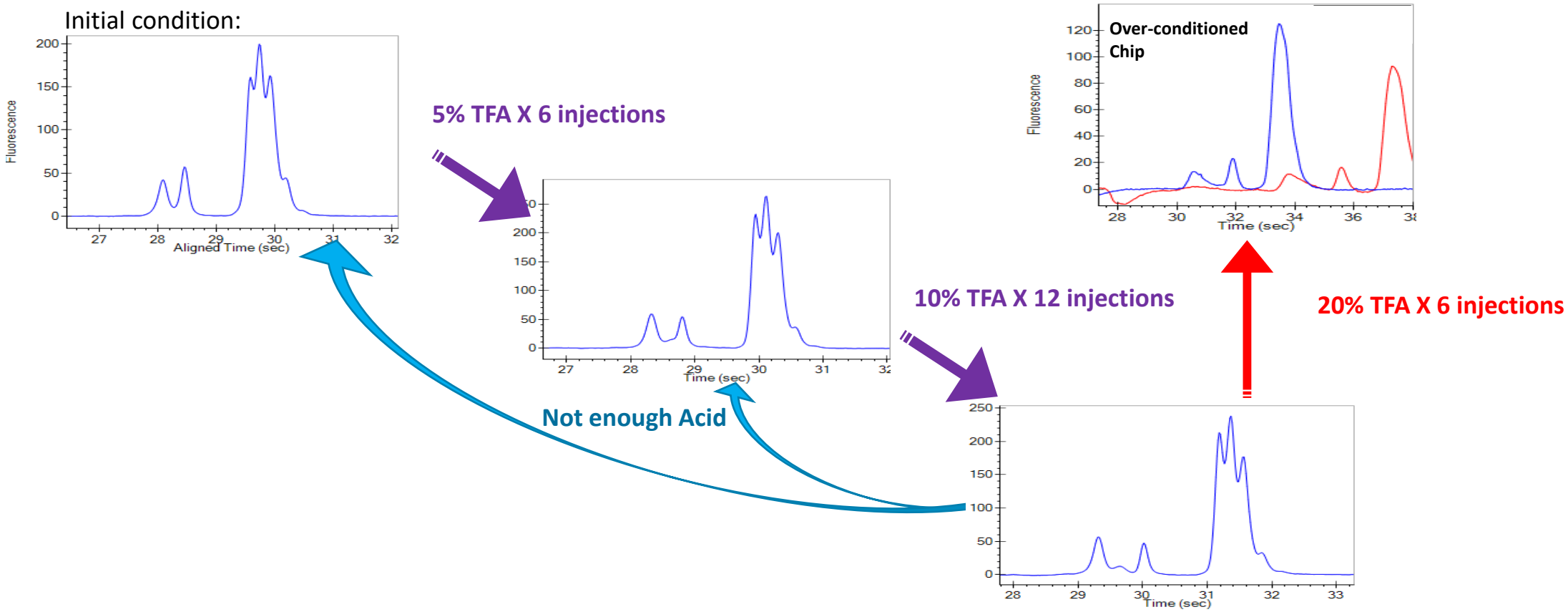
Progressively deteriorating Profile



- A progressively deteriorating electropherogram was observed with the accumulation of replicate injections.
- Something may be building up inside the separation channel gradually after multiple injections and can change the mobility of reduced species.

Capillary Conditioning with Acid



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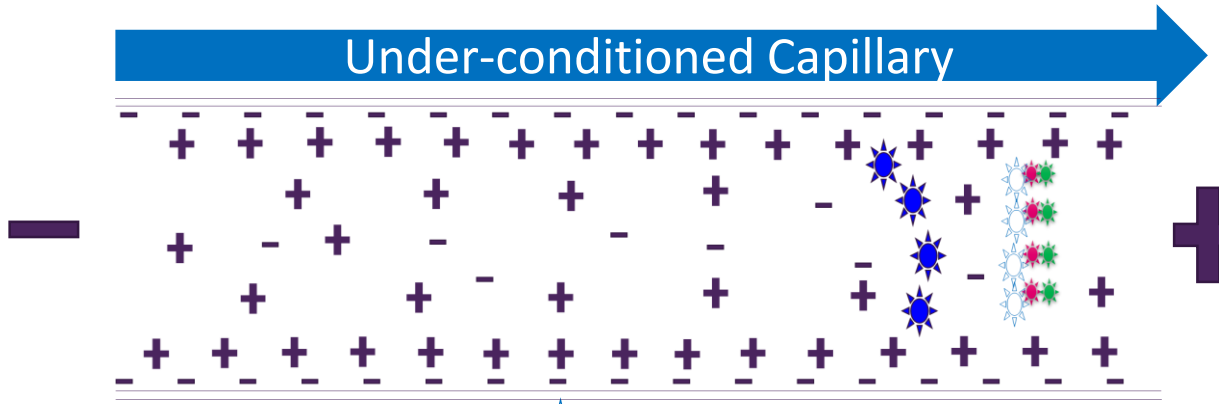
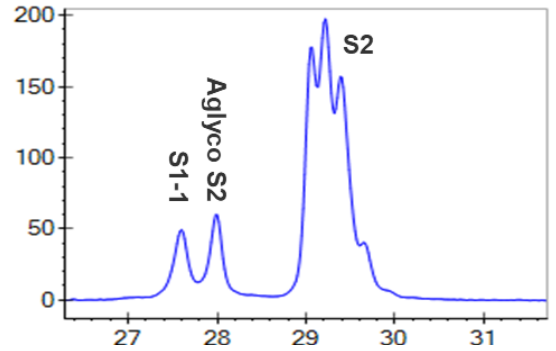
Capillary Conditioning Window Hypothesis





S1-1 (), S1-2() and Aglyco-S2() species compared to S2 species ()

$$V_{ep} = \mu_{ep} E = \frac{q}{6\pi\eta r} * \frac{V}{L}$$

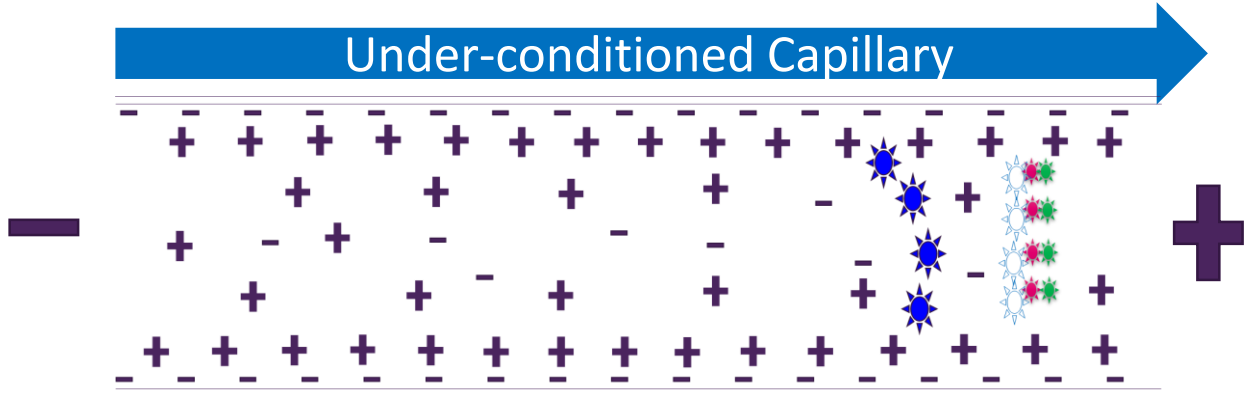
Symbol	Description
V_{ep}	Electrophoretic Velocity
μ_{ep}	Electrophoretic mobility
E	Electric Field
V	Voltage
L	Length of the capillary
q	Total charge of the analyte
r	Hydrodynamic radius of the solute

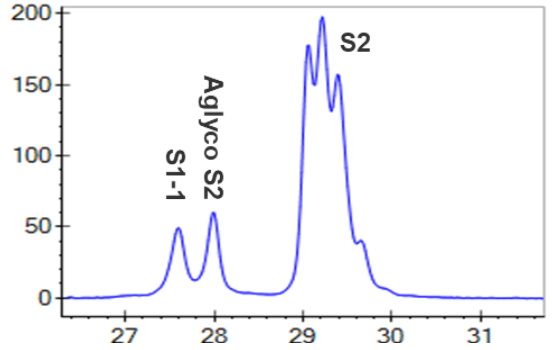
- Fused silica capillary has a net negative surface charge under basic pH due to silanol groups (SiO^-) (pKa 6.0 to 9.0)
- Water soluble silica adsorbing polymer (pH 7 to 9) coats the capillary with net charge as that of inner capillary surface (0.01% to 2.0%) (*US patent 5948227*)
- Sample buffer contains LDS at pH 8.4 which coats the protein with overall negative charge
- Separation is governed by electrophoretic mobility

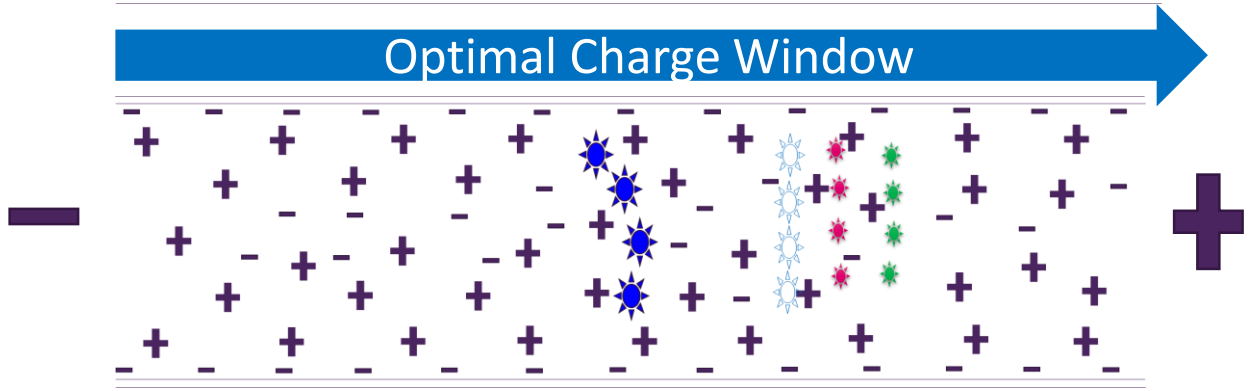
What is hydrodynamic radius?

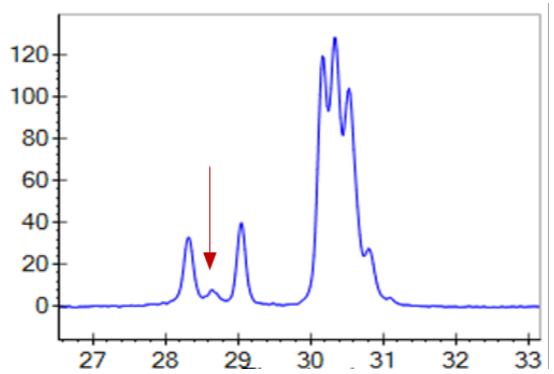
Electrophoresis, 2013, 34, 1812–1819.

Capillary Conditioning Window Hypothesis



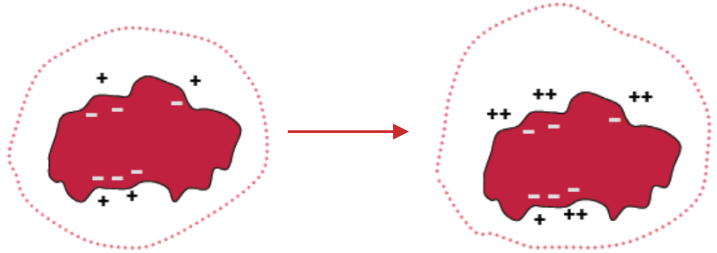






- Acid injections increases H^+ ions in the matrix;
- Ionic interactions increases hydrodynamic radius (r), thus reducing electrophoretic mobility and providing ideal separation

$$V_{ep} = \mu_{ep} E = \frac{q}{6\pi\eta r} * \frac{V}{L}$$



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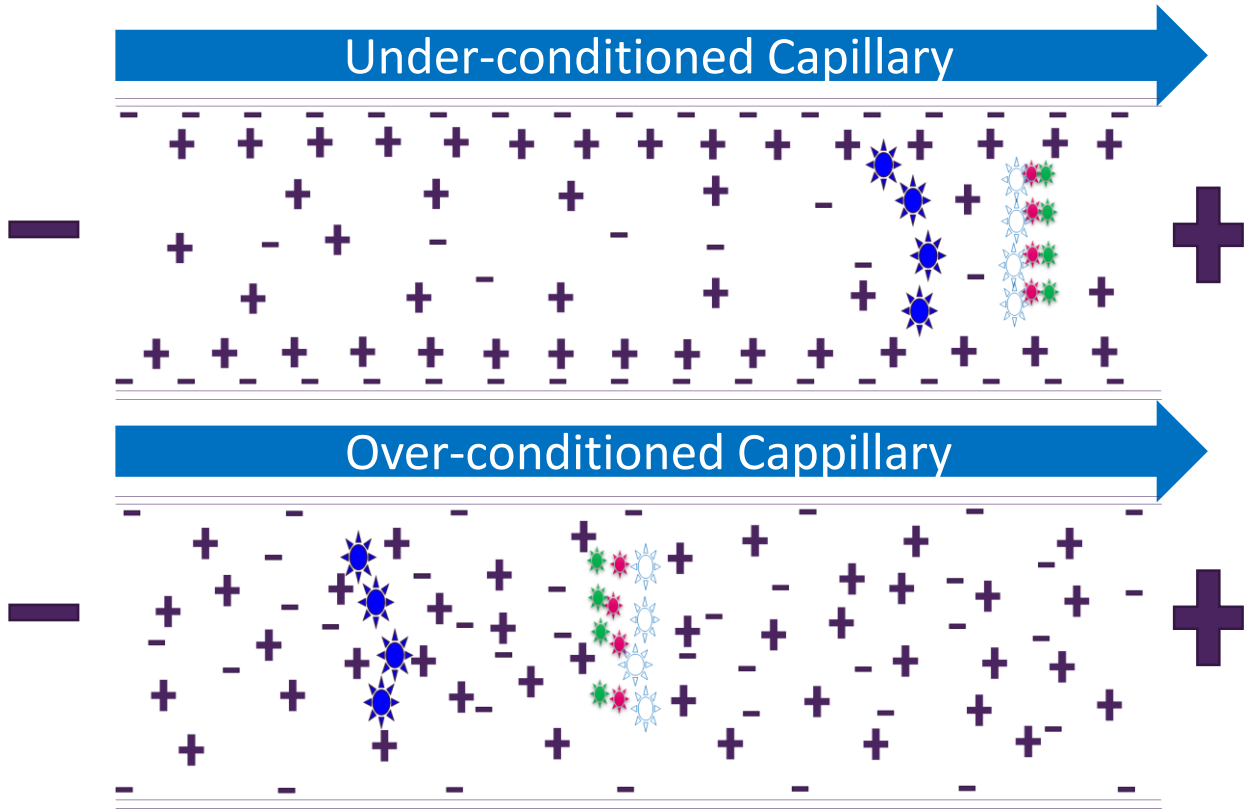

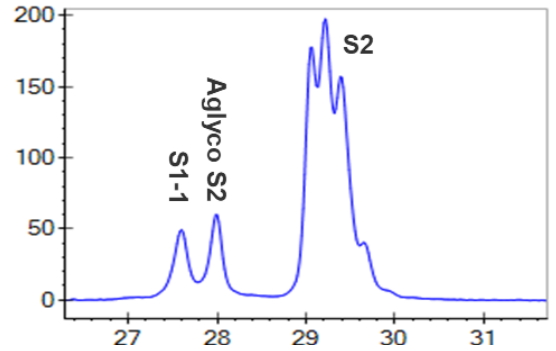

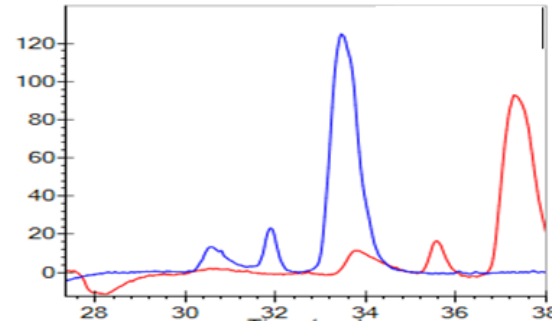

- Excess H^+ ions in the bulk layer; very slow or changed mobility
- Overall less charge on sialylated species micelles

$$V_{ep} = \mu_{ep} E = \frac{q}{6\pi\eta r} * \frac{V}{L}$$

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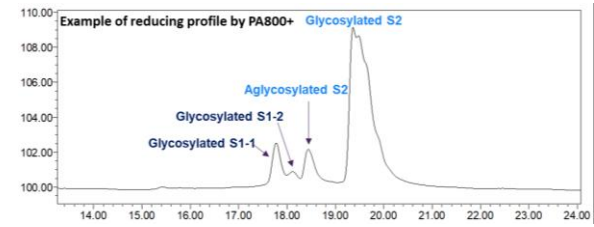

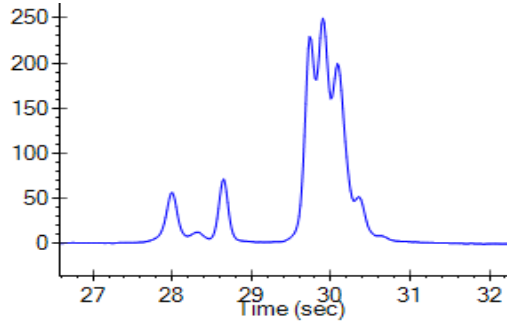

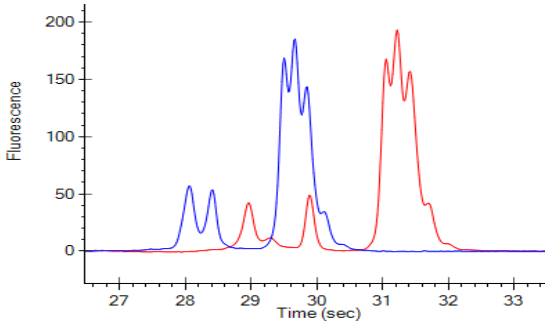

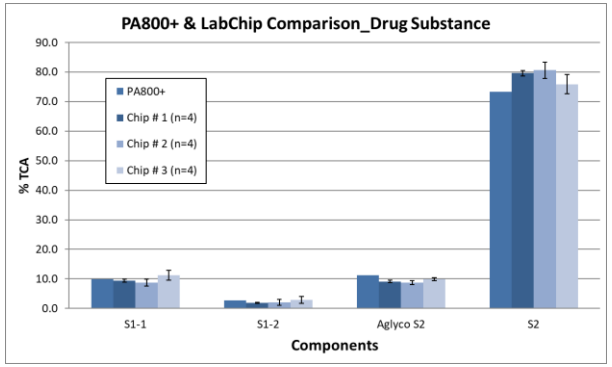

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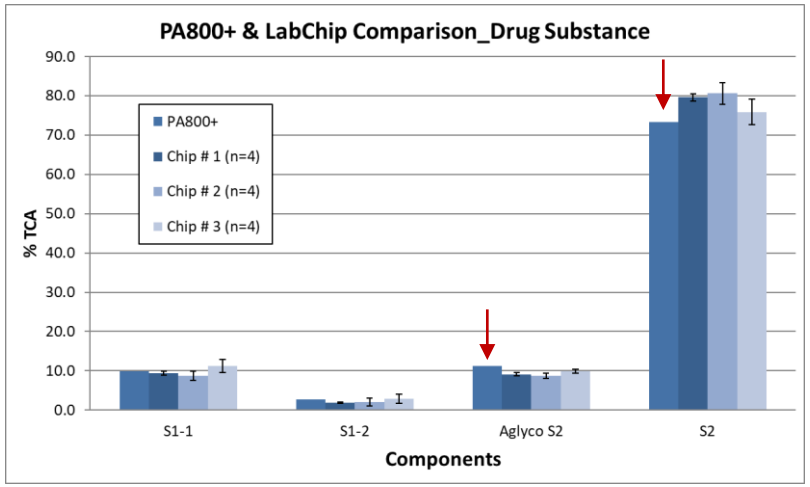
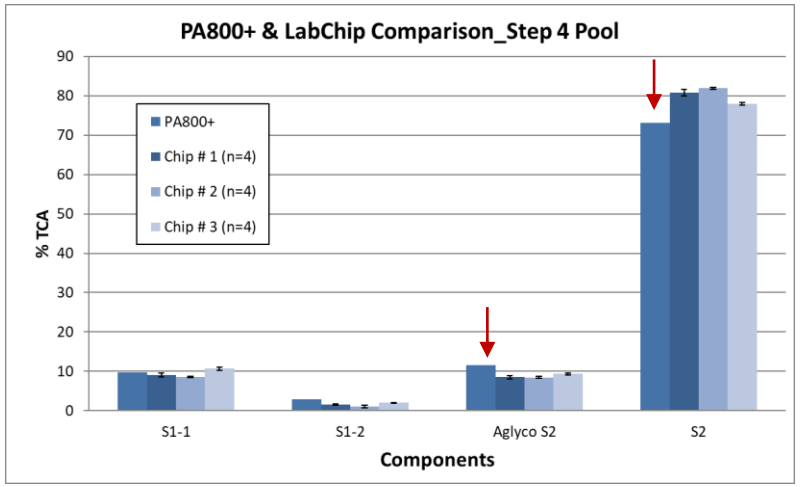
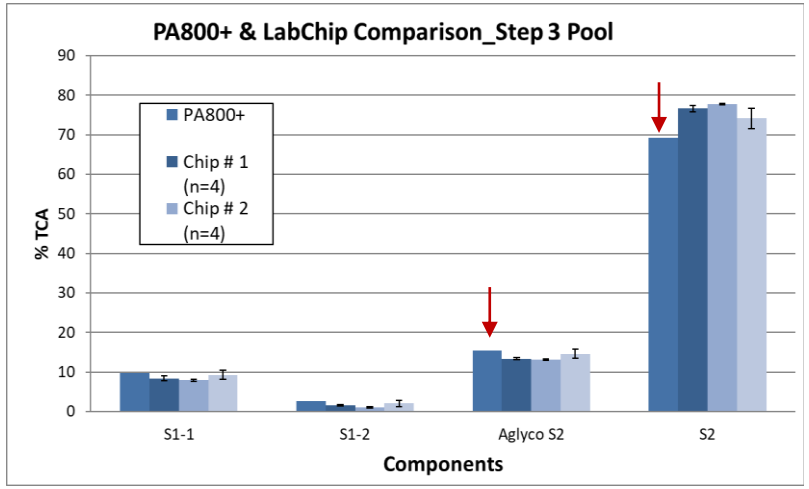
Results Comparison of LabChip and PA800+

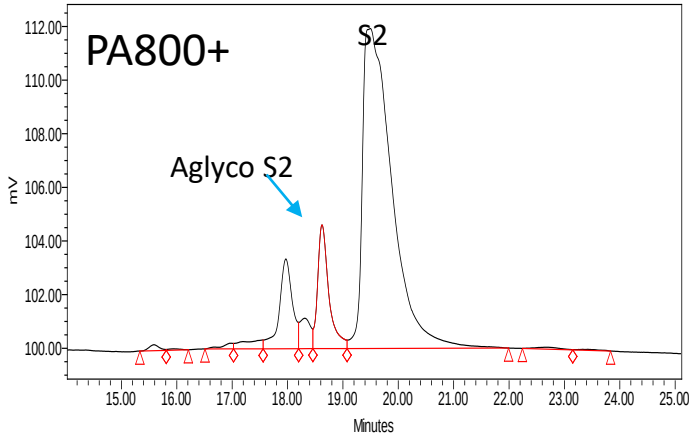
- **Samples:** Drug substance, upstream and downstream samples (step 1-4)
- **LabChip:** 3 chips were used with four replicates for each sample on every chip

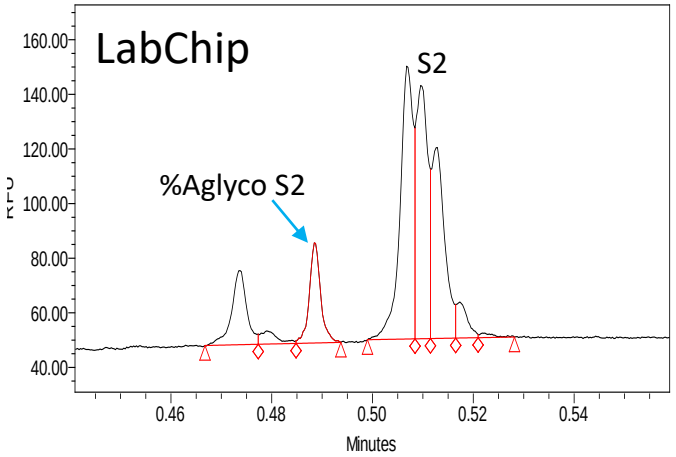






- **Results:**
- Comparable data for PA800+ and LabChip
- LabChip: Higher %S2 + Lower %Aglyco S2





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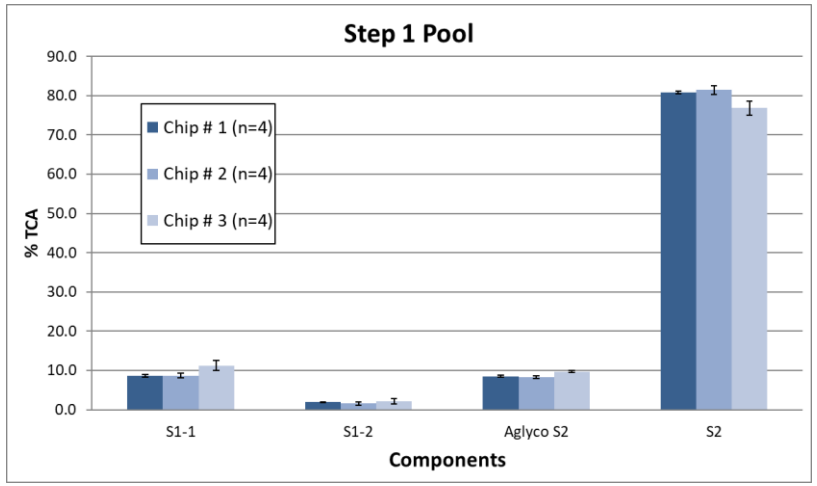
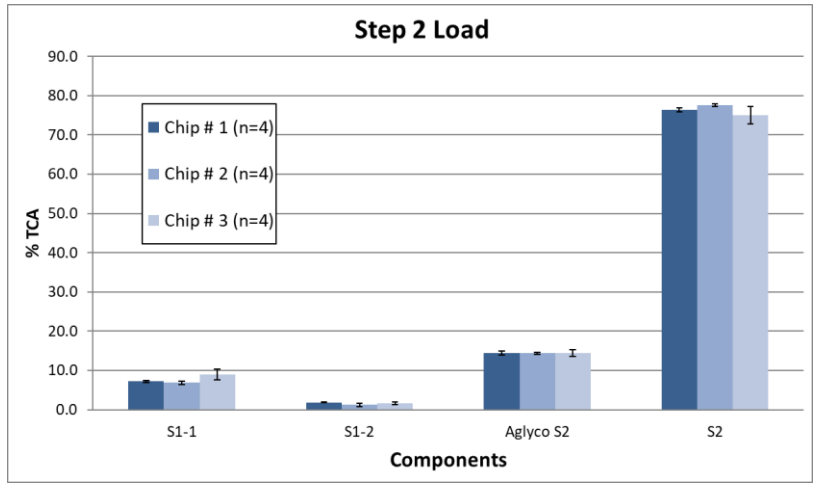
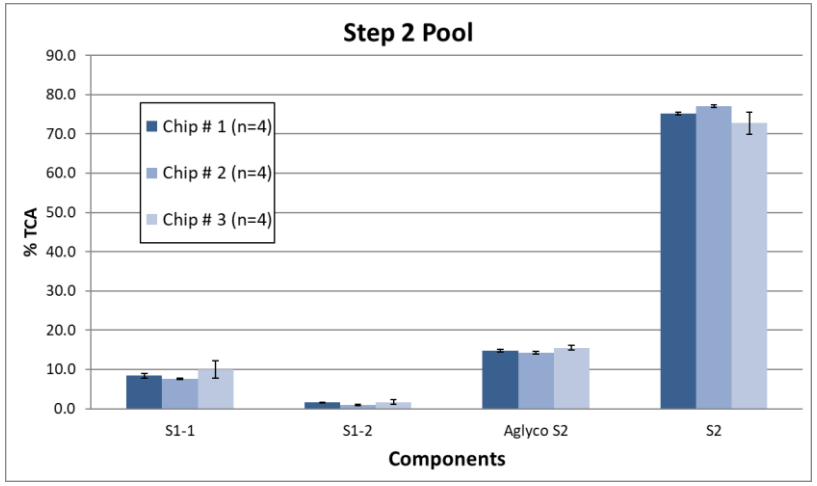
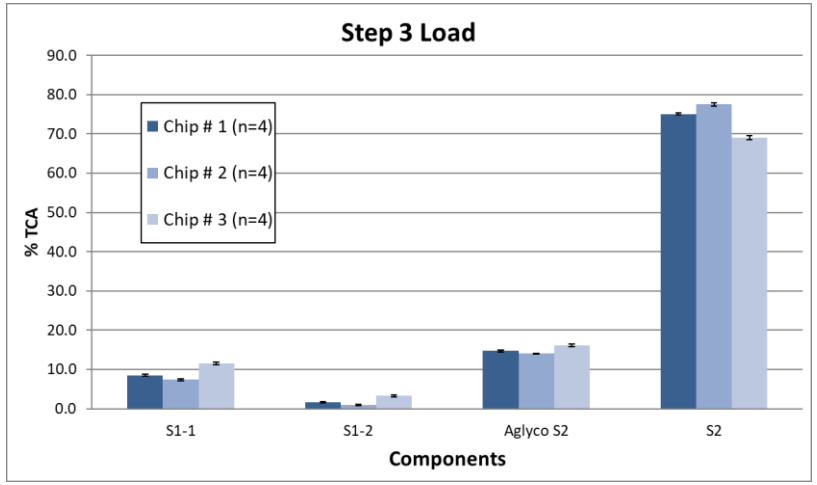
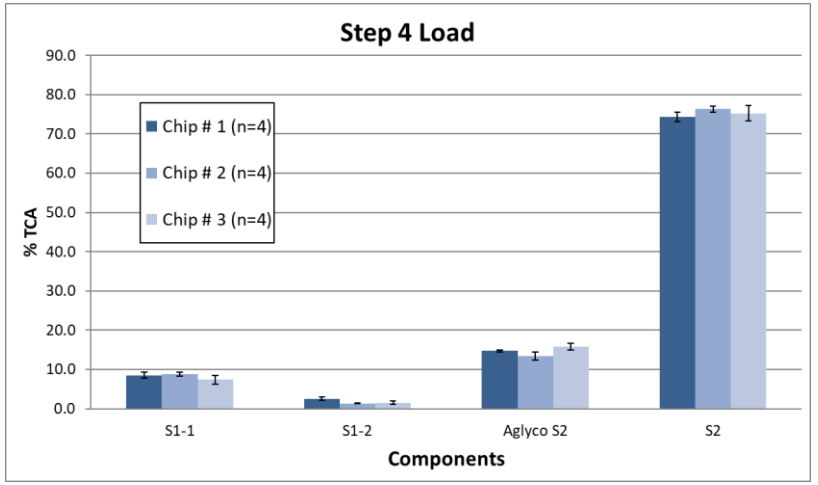
Comparison of Data: LabChip(s)











- Compatible with variety of sample matrix
- Comparable data from three chips

Summary

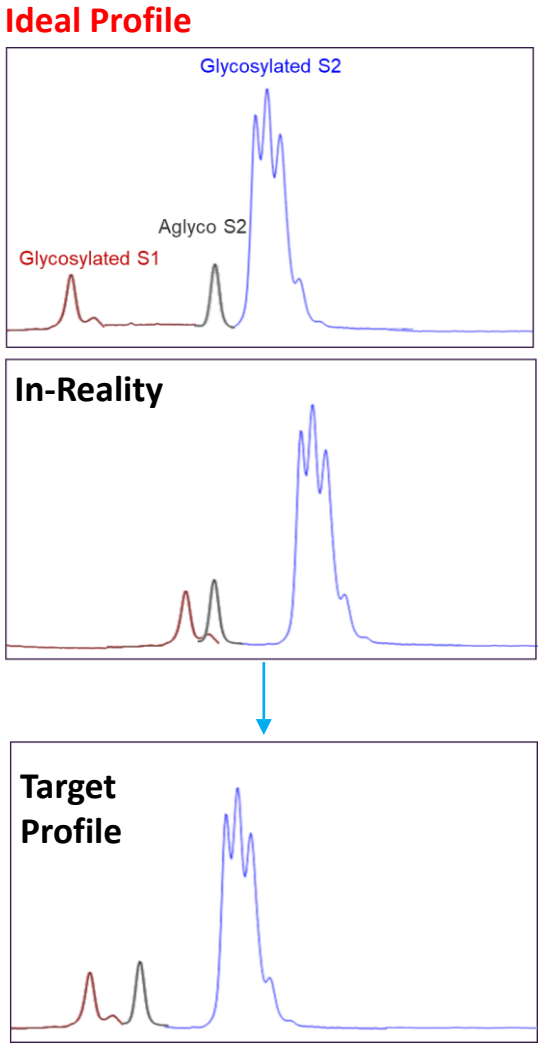
- **Basics for CE-SDS**
  - SDS / protein = 1.4
  - The electrophoretic mobility of the protein – SDS complex is inversely proportional to the log (Mw).

$$V_{ep} = \mu_{ep} E = \frac{q}{6\pi\eta r} * \frac{V}{L}$$

  - Lower charge / size ratio for glycoproteins and lower mobility for glycosylated S1 and S2.

- **To obtain the target profile**
  1) Decrease the % gel → mobility increased for all species; but S1 increased more due to significant size difference between S1 and S2 subunits (Ferguson plot)
  2) Acid conditioning → mobility decreased for all species

- **LabChip® offers comparable results compared to PA800+ with significant advantage in high-throughput and extensive buffer compatibility**



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Thank you all for your attention!