



# **BTEC: Analytical Services and Capabilities**

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# What is BTEC?

- Simulated cGMP facility
- Education and training
- Process and analytical services
- First and largest in the world



# Advisory board



# Unique educational programs

## Minimize training done by industry

- Hands-on experiences
- Simulated cGMP
- Regulatory compliance
- Industry focused curriculum
- Industry experienced instructors

## Multi-disciplinary

- Undergraduate, graduate, industry
- Upstream, downstream, analytical
- Small, intermediate, large scales



# BTEC strategic plan: Economic development



- University courses, programs
- Industry courses & events
- State-of-art facility
- Collaborate with industry
- National, international leadership in biomanufacturing
- Outreach to K-12
- Support community colleges

# Facility features

- 63,000 gsf labs
- 9,000 gsf classrooms
- \$39 MM infrastructure
- \$15 MM equipment
- \$6 MM/y operating



# EDUCATION AND TRAINING



# Academic programs

Chemical &  
Biomolecular Engr.  
Biomanufacturing  
Concentration

Food, Bioprocessing &  
Nutrition Sciences  
Bioprocessing Degree  
(BBS)



Microbiology  
Professional Master in Microbial  
Biotechnology (MMB)

Biotechnology (BIT)

# Professional development courses

## Tracks

Biomanufacturing

Bioprocess Development

Bioprocess Engineering

Analytical Technologies

Customized courses also available



# FDA training

- Contract (\$455,000 over 5 years)
  - 4 hybrid online/hands-on courses
    - Upstream bioprocessing
    - Downstream bioprocessing
    - QC/Analytical
    - Aseptic processing
  - Cohorts of 16 inspectors trained annually
  - FDA approved execution of Option Year #4 (2011-2012)



# BARDA (DHHS) training

- Contract (\$860,000 Year 1, renewable over 5 years)
  - cGMP Influenza Vaccine Manufacturing
    - Regulatory/quality systems
    - Facilities and utilities
    - Upstream bioprocessing
    - Downstream bioprocessing
    - QC/Analytical
    - Aseptic processes
  - Three cohorts of 12 students – 11 countries; 3-week sessions



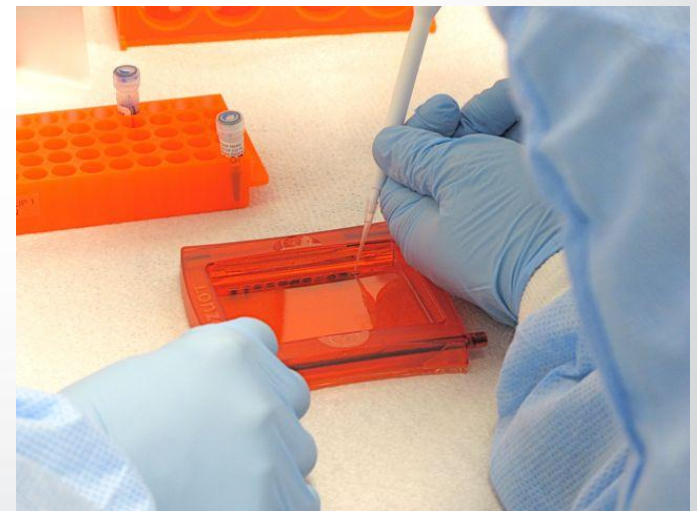
# BIOPROCESS AND ANALYTICAL SERVICES



# Bioprocess and analytical services capabilities

BTEC offers:

- **Faculty with expertise** in biomanufacturing topics
- Staff with **hands-on biomanufacturing experience**
- **Facilities and equipment ideal** for this type of work
- **Students eager** to learn
- **Flexibility** in the types of projects
  - “Risk-free environment”
  - Smaller projects that contract organizations not likely to take on



# Analytical Services

- Analysis and Testing
  - Amino acid analysis on spent media (UPLC)
  - Small molecule concentration in chicken plasma (HPLC)
  - Isoflavone concentration in soybean extract (HPLC)
  - Antiviral activity (qPCR)
  - Environmental monitoring (bioburden, coliform, LAL)
- Assay Development
  - Protein expression (Western Blot)
- Process Development Support
  - SDS-PAGE
  - Bioburden
  - Endotoxin (gel-clot)
  - Microbial ID

# BTEC Analytical Capabilities

## Quantitation

- HPLC
  - Reverse phase
  - SEC
  - Cation exchange
- ELISA
- Capillary electrophoresis

## Characterization

- MALDI-TOF
- LC-MS
- MALLS
- Circular dichroism

## Purity

- Electrophoresis
  - Microchip
  - SDS-PAGE
  - Capillary gel electrophoresis
- HPLC

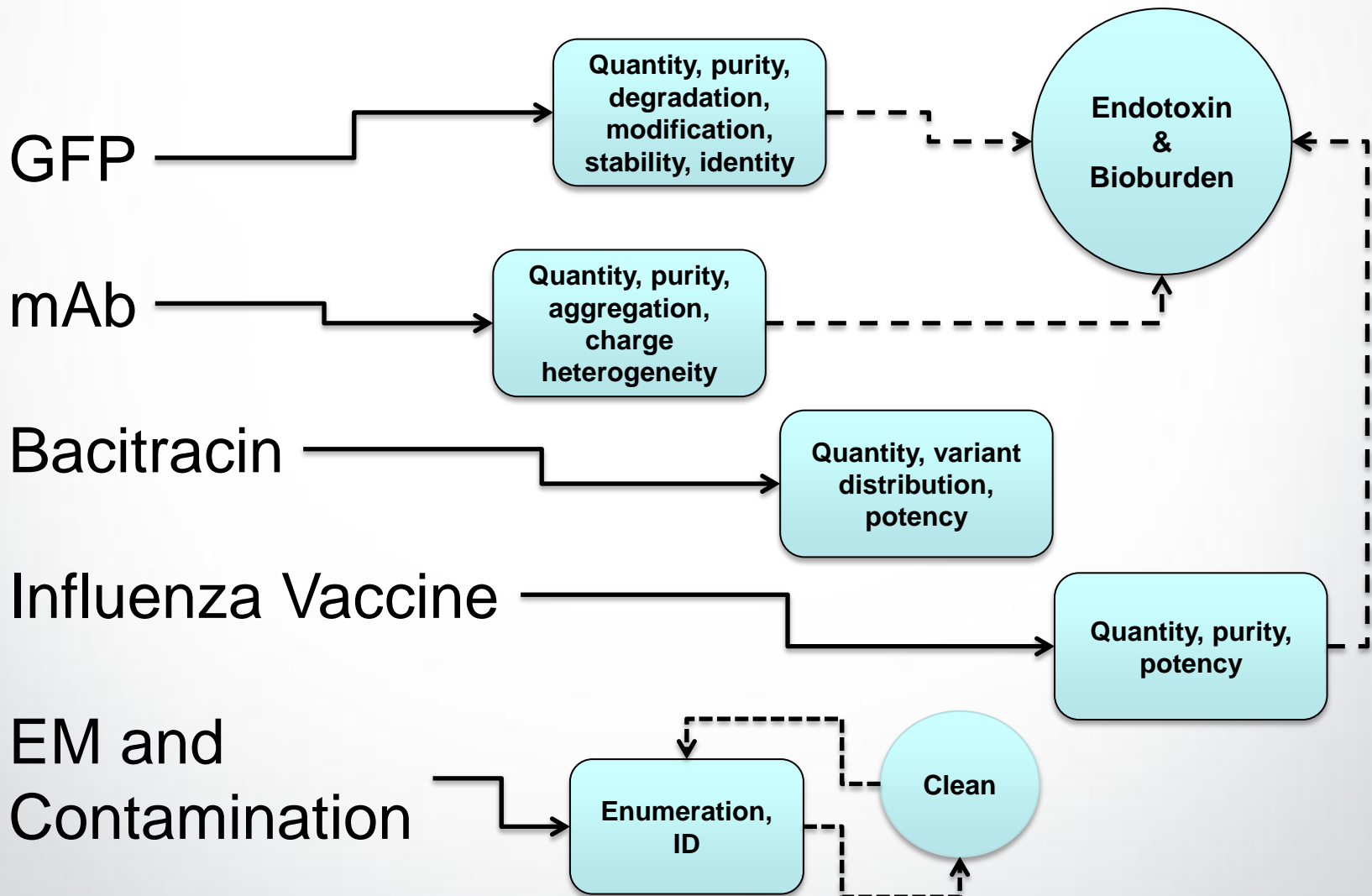
## Microbial

- Environmental monitoring
- Bioburden
- Endotoxin
- Identification

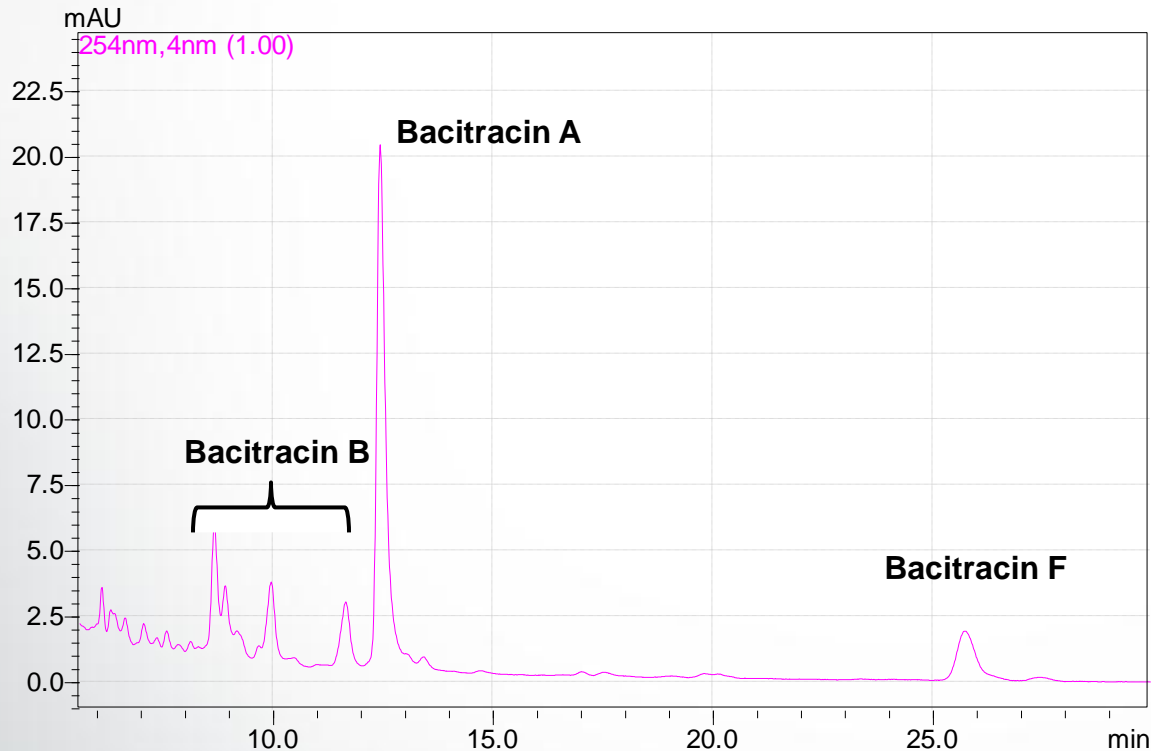
## Optimization and Validation

- Amino acid analysis (UPLC)
- Total organic carbon (TOC)

# BTEC Analytical Case Studies



# Method Development: Bacitracin



Column: Phenomenex Kinetix C18, 2.6  $\mu\text{m}$ ,  
150 x 4.6 mm

Mobile Phase: 20 mM phosphate, pH 6.0 in  
MeOH (53%) and MeCN (6%)

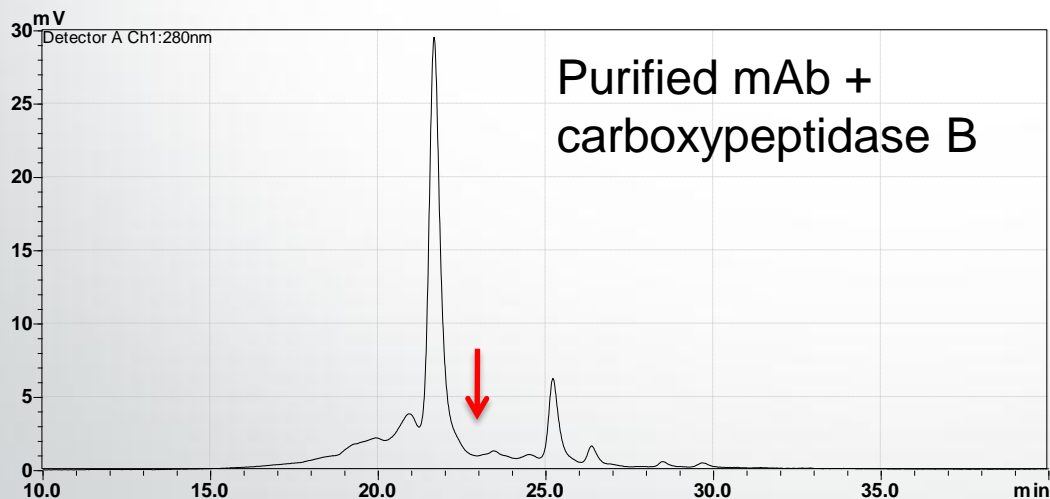
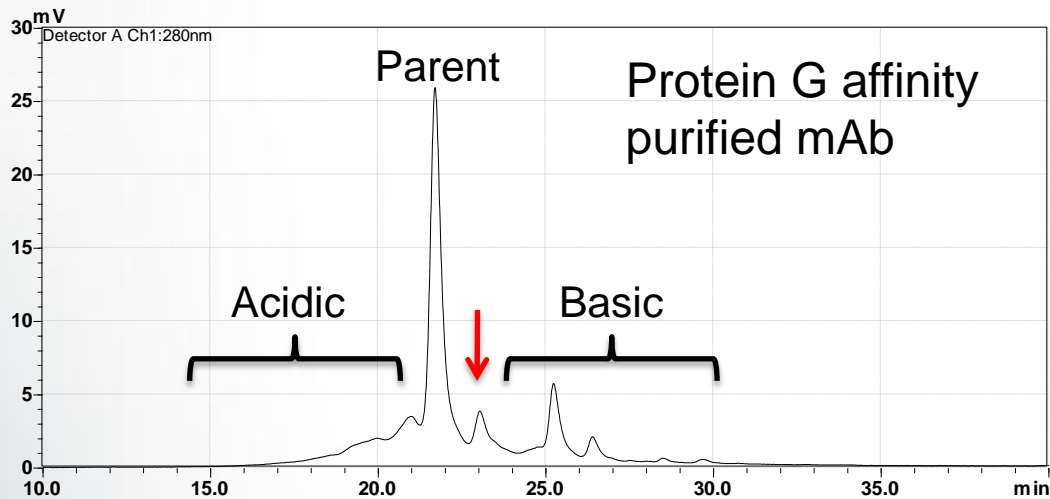
Isocratic: 0.6mL/min

Sample Temp: 4°C

Column Temp: 40°C

*HPLC method fully validated per ICH Q2(R1)*

# Characterization: Charge Heterogeneity



Column: Dionex MAbPac SCX-10, 250 x 4.6

Mobile Phase A: 20 mM MES, 60 mM NaCl, pH 5.6

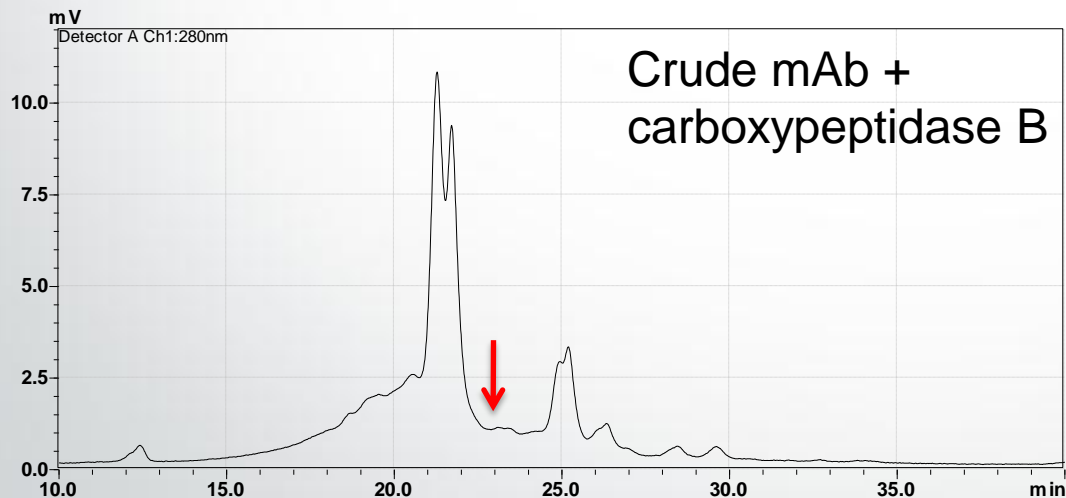
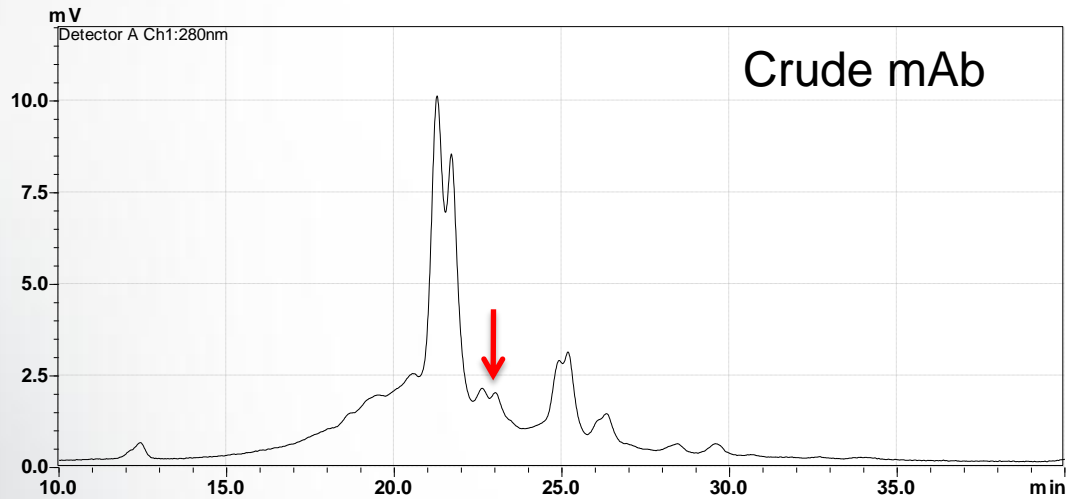
Mobile Phase B: 20 mM MES, 300 mM NaCl, pH 5.6

Gradient: 10% B (0-2 min), 10-55 %B (2-32 min), 55% B (32-37 min), 55-100 %B (37-42 min), 100% B (42-47 min), 100-10% B (47-48 min), 10%B (48-63 min)

Column Temp: 30°C

*Carboxypeptidase B cleaves C-terminal Lys and Arg*

# Characterization: Charge Heterogeneity



**Column:** Dionex MAbPac SCX-10, 250 x 4.6

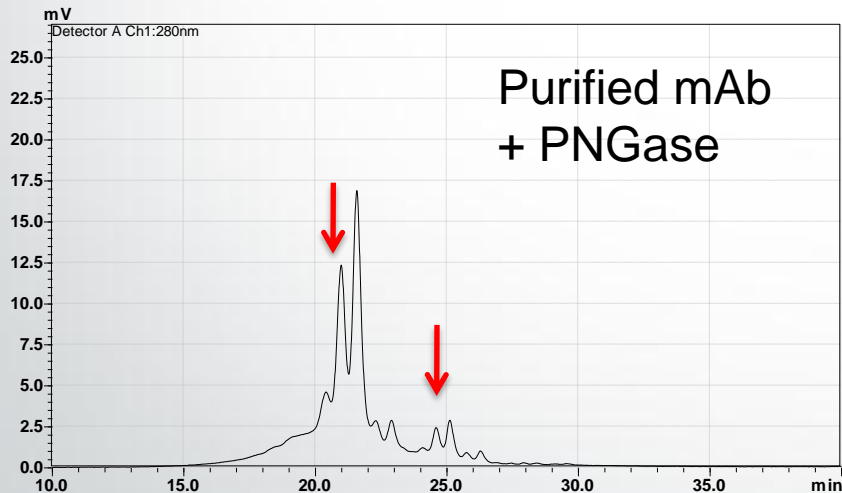
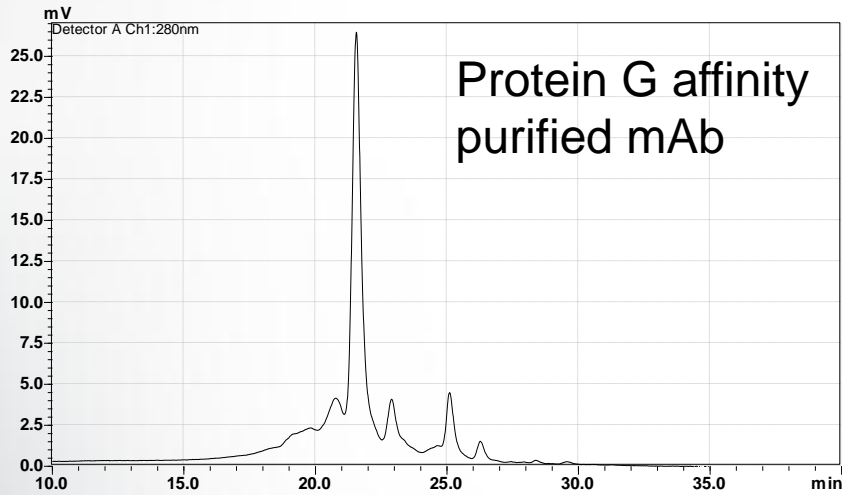
**Mobile Phase A:** 20 mM MES, 60 mM NaCl, pH 5.6

**Mobile Phase B:** 20 mM MES, 300 mM NaCl, pH 5.6

**Gradient:** 10% B (0-2 min), 10-55 %B (2-32 min), 55% B (32-37 min), 55-100 %B (37-42 min), 100% B (42-47 min), 100-10% B (47-48 min), 10%B (48-63 min)

**Column Temp:** 30°C

# Characterization: Charge Heterogeneity



**Column:** Dionex MAbPac SCX-10, 250 x 4.6

**Mobile Phase A:** 20 mM MES, 60 mM NaCl, pH 5.6

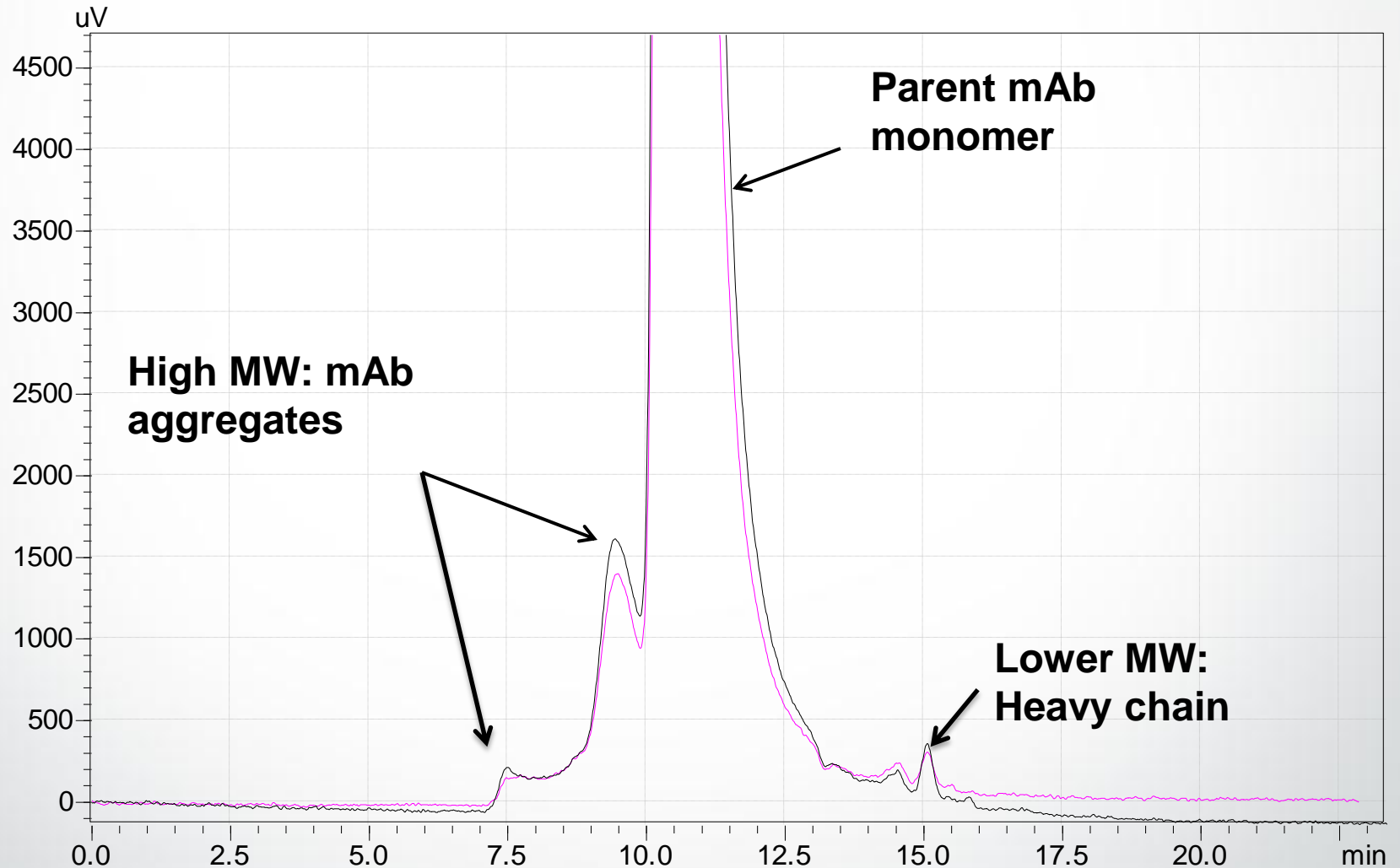
**Mobile Phase B:** 20 mM MES, 300 mM NaCl, pH 5.6

**Gradient:** 10% B (0-2 min), 10-55 %B (2-32 min), 55% B (32-37 min), 55-100 %B (37-42 min), 100% B (42-47 min), 100-10% B (47-48 min), 10%B (48-63 min)

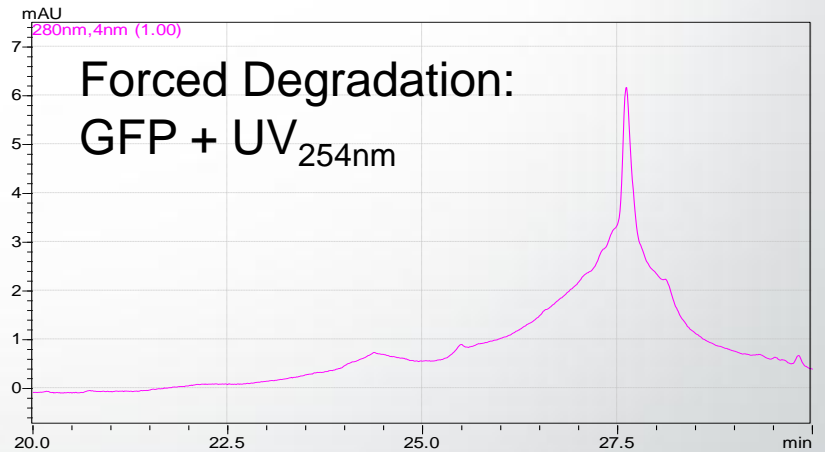
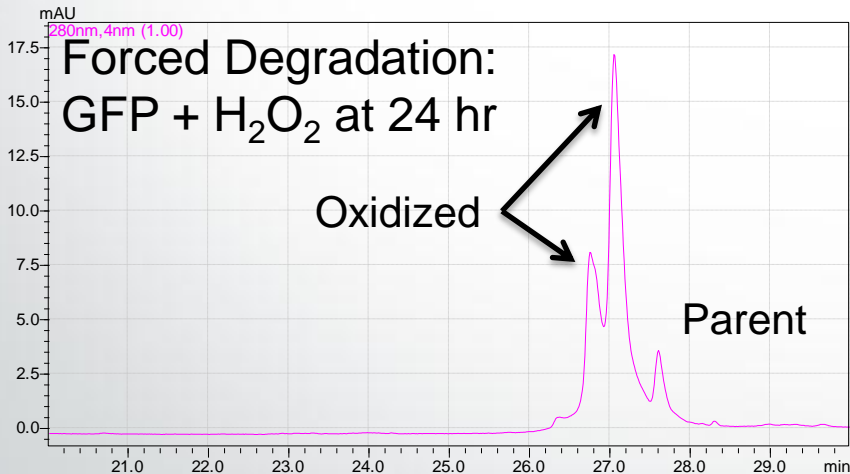
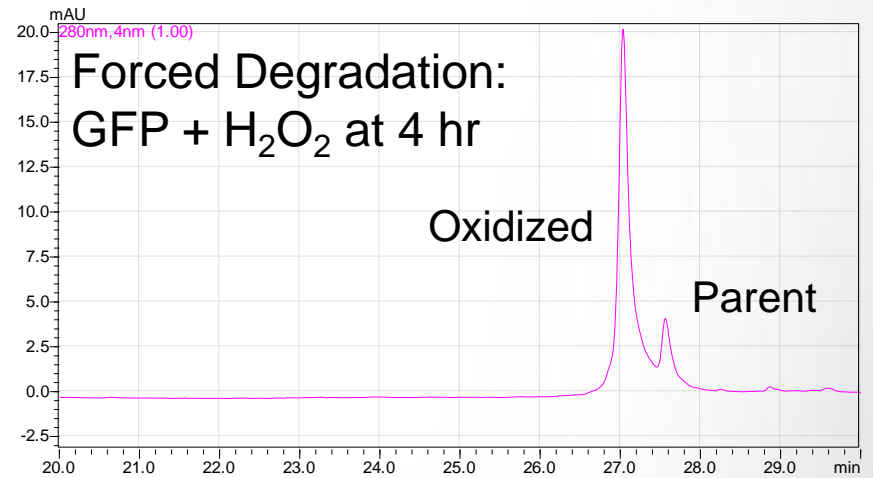
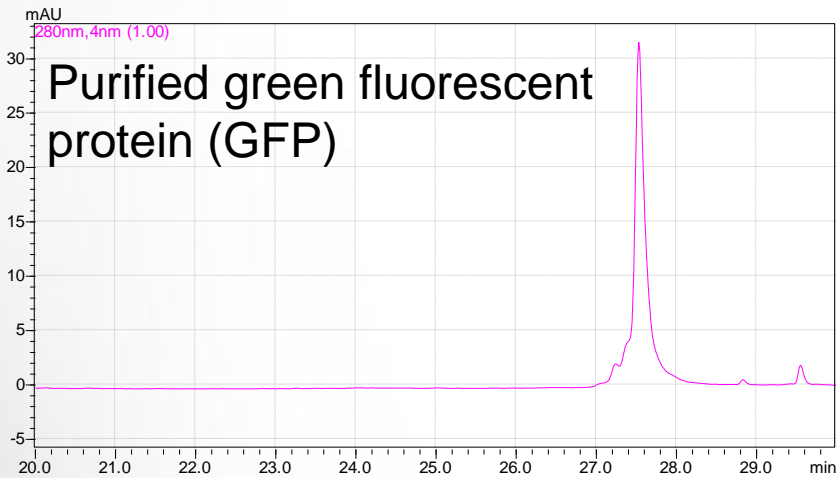
**Column Temp:** 30°C

PNGase (Peptide N-Glycosidase F) cleaves between the innermost GlcNAc and Asn residues of N-linked glycoproteins.

# Characterization: Aggregation

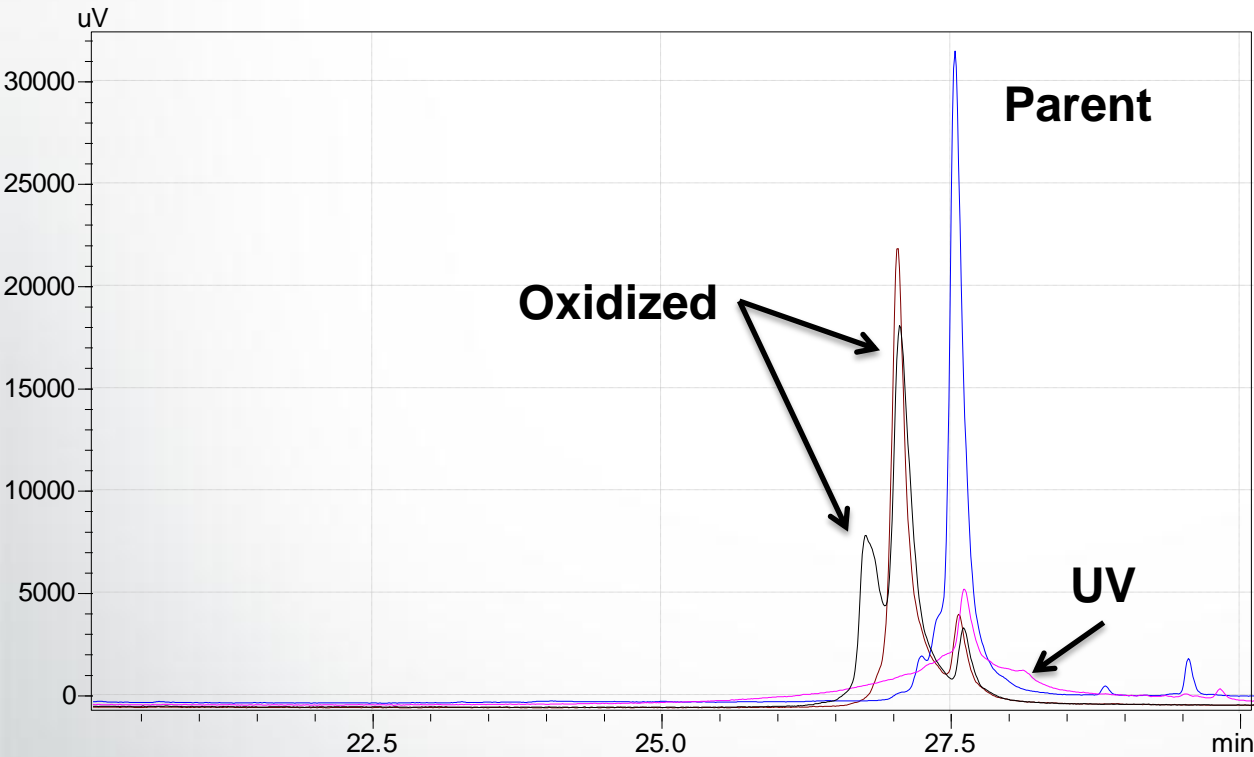


# Protein Stability



# Protein Stability

## Forced Degradation Overlay



Column: Kinetex C18, 150 x 4.6, 2.6- $\mu\text{m}$ , 100 $\text{\AA}$

Mobile Phase A: 0.5% TFA in H<sub>2</sub>O

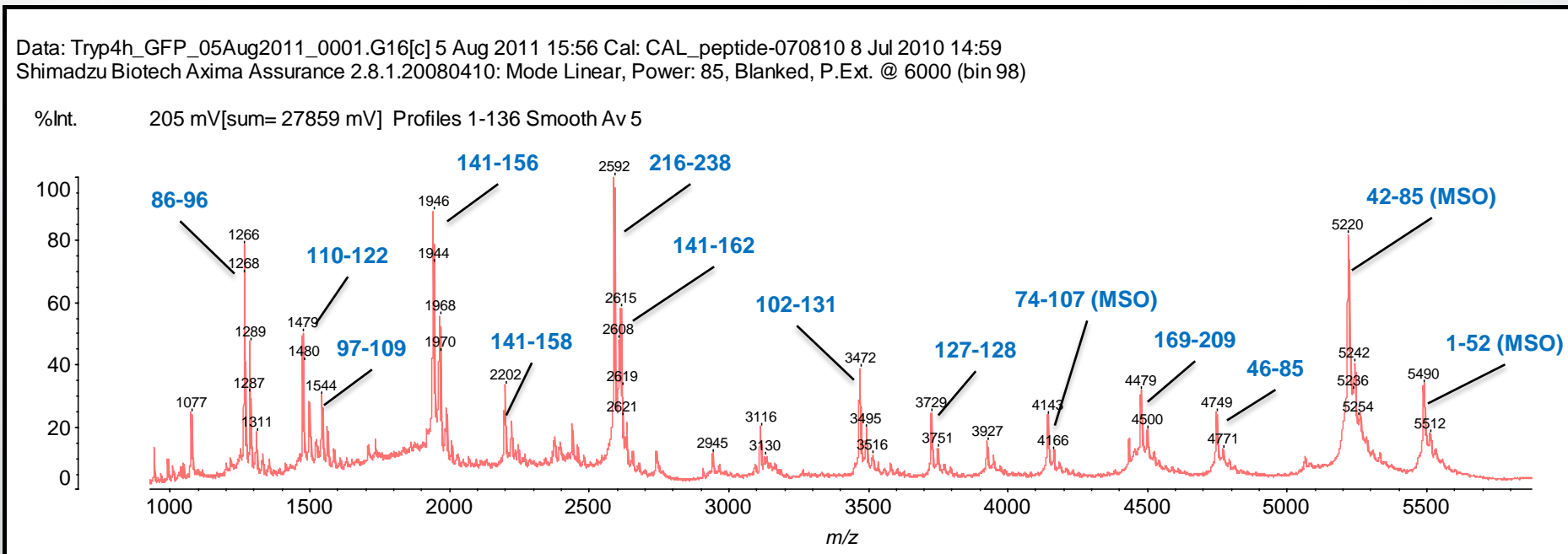
Mobile Phase B: 0.5% TFA in MeCN

Gradient: 5% B (0-0.2 min), 5-50% B (0.5-29 min), 50-80% B (29-31 min), 80% B (31-33 min), 80-5% B (33-34.5 min), 5% B (34.5-39 min)

Column Temp: 40°C

# Protein Identification: Peptide Mass Fingerprinting

## 1. Cleave protein with trypsin for >4hr at 37 °C



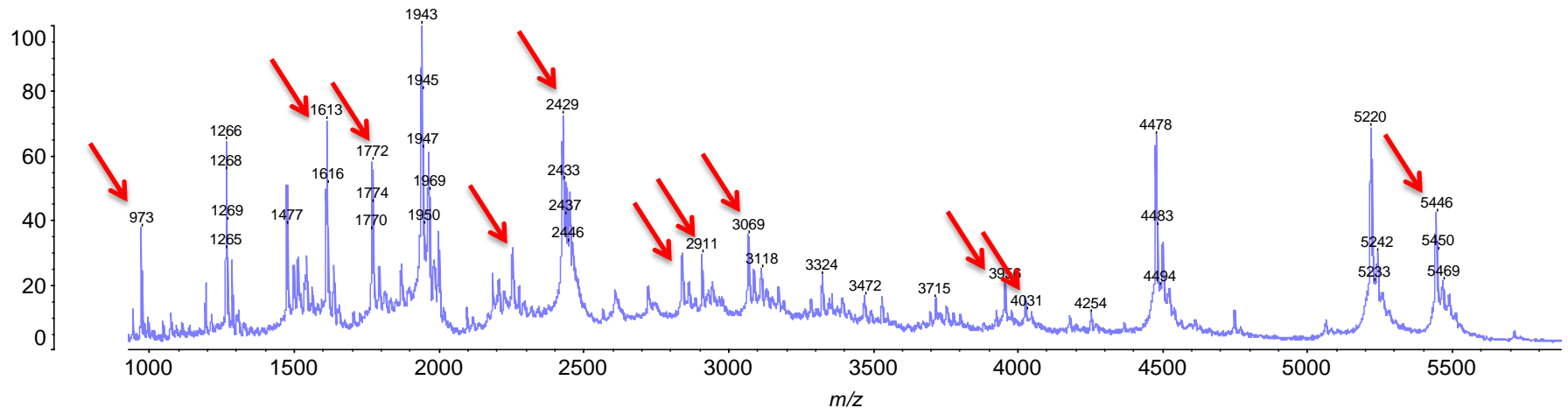
Sequence Coverage: 93.2%

# Characterization: Protein biotinylation

1. Biotinylate GFP by coupling to 1° amines;
2. Cleave with trypsin

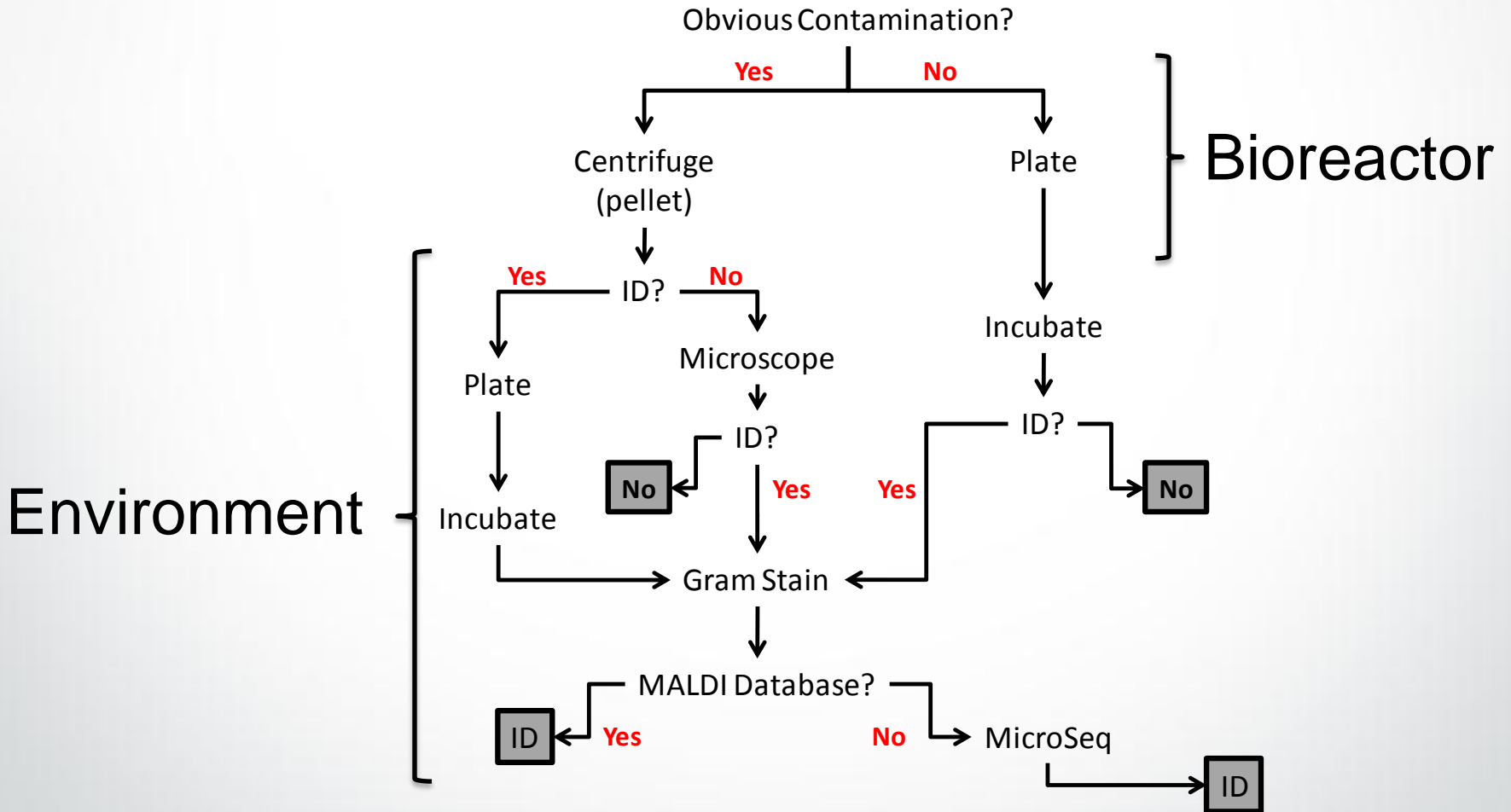
Data: Tryp4h\_GFPb\_05Aug2011\_0001.G15[c] 5 Aug 2011 15:54 Cal: CAL\_peptide-070810 8 Jul 2010 14:59  
Shimadzu Biotech Axima Assurance 2.8.1.20080410: Mode Linear, Power: 85, Blanked, P.Ext. @ 6000 (bin 98)

%Int. 148 mV[sum= 17121 mV] Profiles 1-116 Smooth Av 5

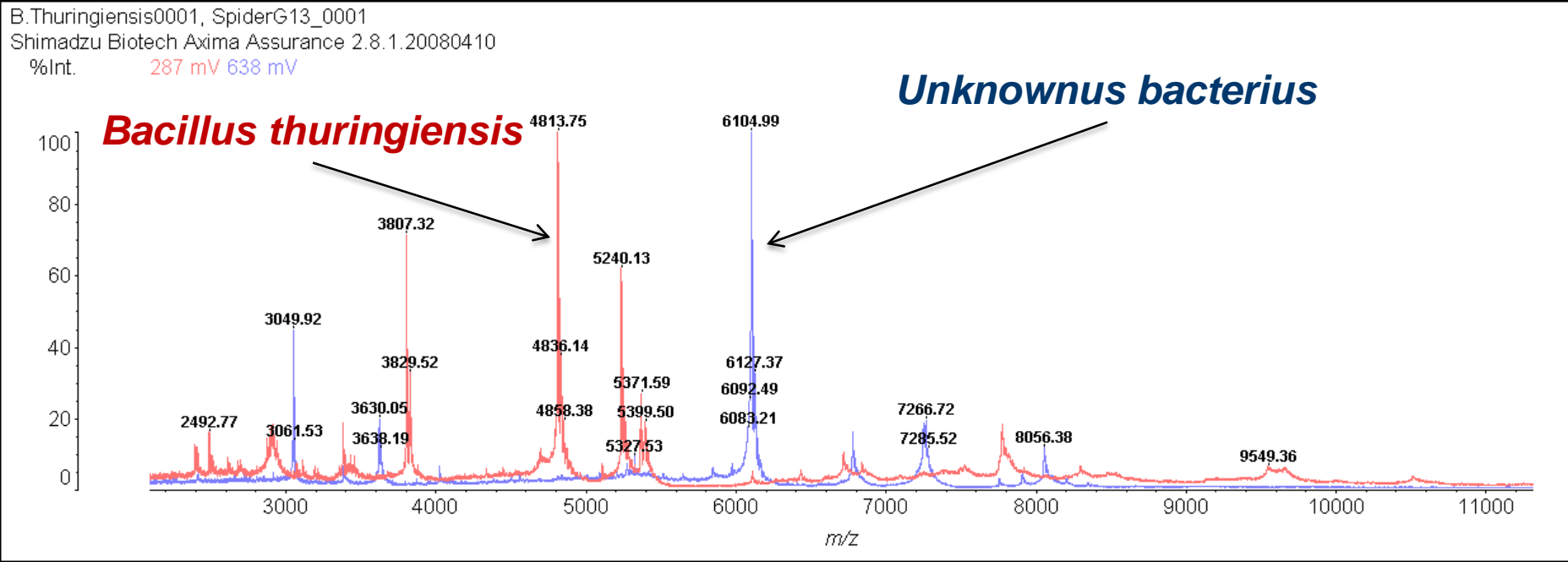


Biotinylation Sites: Lys 41, 101, 107, 113, 156, 158

# Microbial ID Decision Tree



# Microbial Identification by MALDI-TOF



Status: isolating microbial flora, identifying, banking and building MALDI database

# **o The Life Force of Tomorrow's Industry**