Implementation of Online Amino Acid Analysis for Medium and Feed Optimization in Mammalian Cell Culture

IFPAC Annual Meeting, 2008

01/29/2007

Polina Rapoport, Szu-Han Wang, Deborah Pascoe

Late Stage Cell Culture BioProcess Development,
Genentech, Inc.

Genentech
IN BUSINESS FOR LIFE

Outline

- Introduction
- Bioreactor Test Runs
- Feedback Control
  - Case Study #1: Glutamine Control
  - Case Study #2: Asparagine Control
- Conclusions
- Acknowledgments

Genentech
IN BUSINESS FOR LIFE

Introduction

Traditional amino acid analysis:

- Used to study the nutrient requirements of mammalian cell cultures
- Applicable for development of improved cell culture media and feeds for the production of biopharmaceuticals
- Performed in an off-line iterative manner, samples are investigated after the experiment is over

Project Goals:

Implement Dionex DX-800 as an on-line amino acid measurement technique

- Provide a real-time assessment of the nutritional requirements and metabolic behavior of CHO cell lines in culture
- Allow the implementation of feedback control to maintain the desired concentrations of amino acids
- Perform controlled amino acid feeding studies with model cell lines

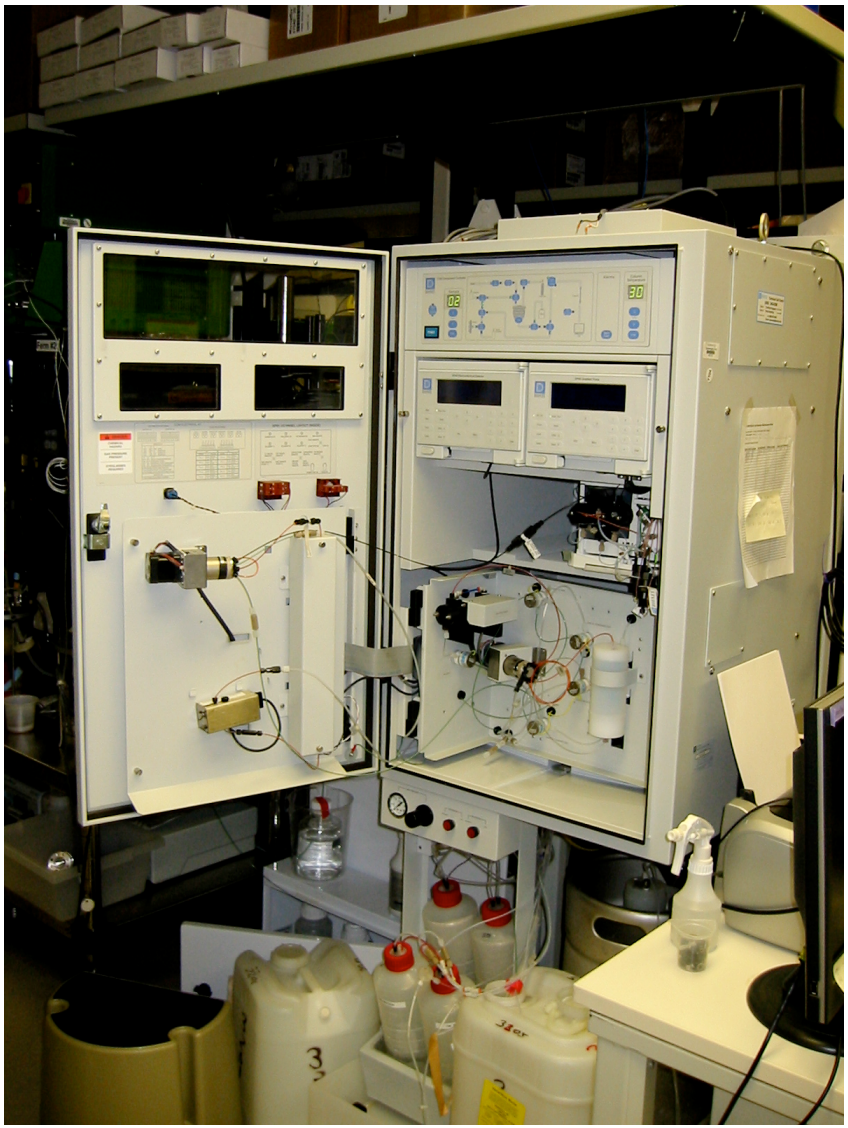
Genentech
IN BUSINESS FOR LIFE

Introduction: What is the DX-800?

- The DX-800 is an on-line HPLC system capable of sampling up to 21 bioreactors
- An electrochemical detector measures the change in redox potential at the electrode, no amino acid derivatization is required
- Cell-free sampling is required: samples are taken using a Flownamics FISP® probe (0.2µm ceramic filter)
- The system is capable of measuring all 20 amino acids and glucose. Each sample takes 65-80 minutes to analyze
- The DX-800 is capable of basic on/off feedback control to add feeds

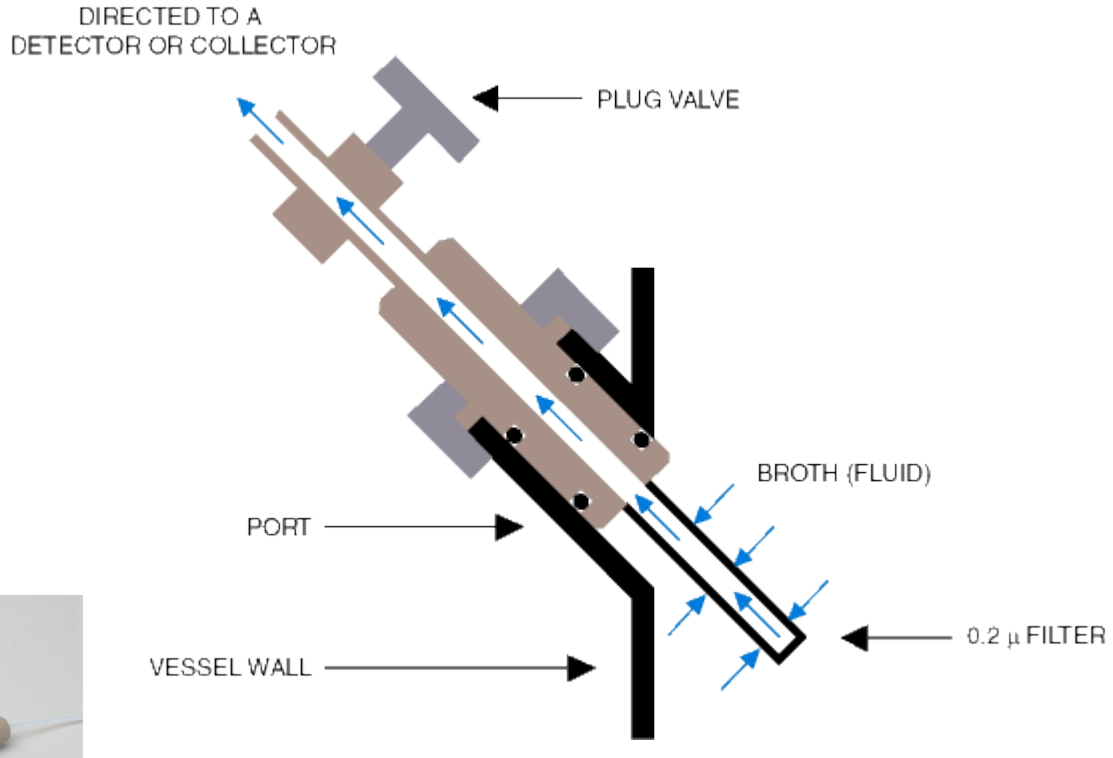
Genentech
IN BUSINESS FOR LIFE

Introduction: What is the DX-800?



Flownamics FISP Probes

Sterile In-situ Sampling System for Fermentation and Cell Culture



- Available for 12 and 19 mm ports
- Fits into the bioreactor's top port

Genentech
IN BUSINESS FOR LIFE

Courtesy of Flownamics Analytical Instruments, Inc

Typical Sample Chromatogram

Genentech
IN BUSINESS FOR LIFE

Bioreactor Test Runs

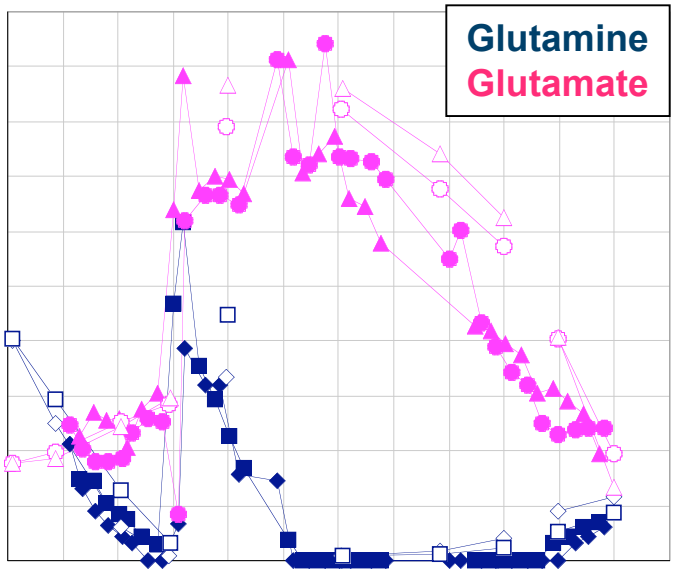
The DX-800 was set up to sample two 2L bioreactors.

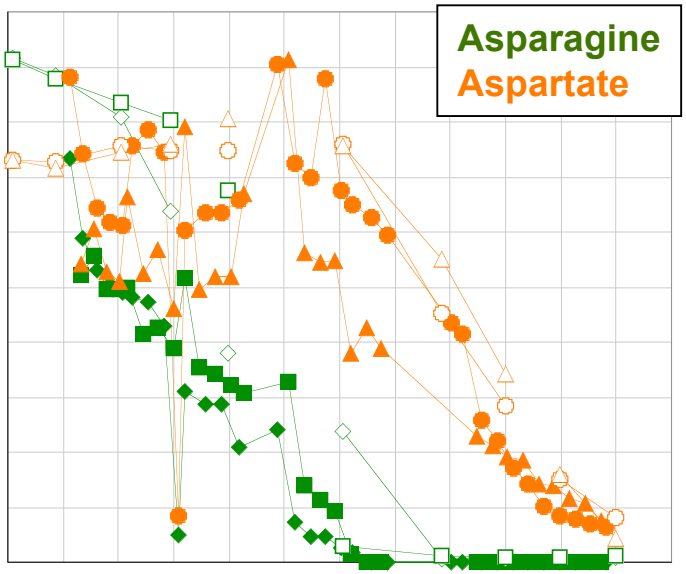
- For each test run, two 2L bioreactors were sampled by the DX-800
- Sampling frequency was set to ~5 hour intervals
- Bioreactors were run using a standard fed-batch cell culture process

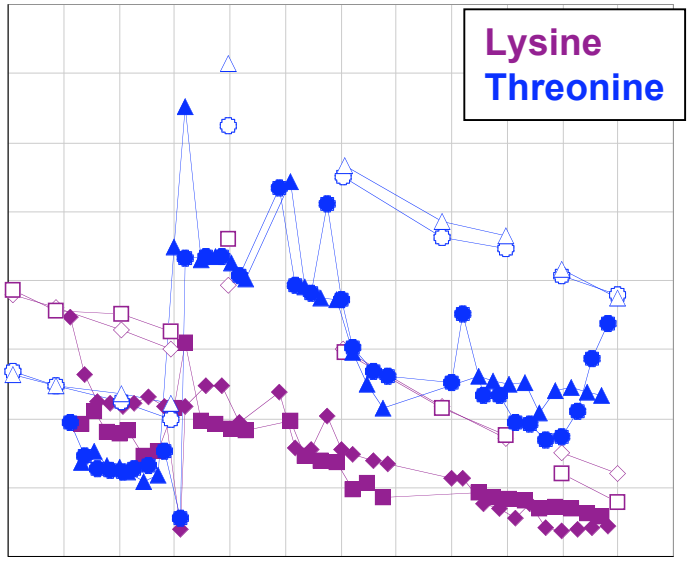
A standard Dionex AAA gradient method with a single point calibration curve was used.

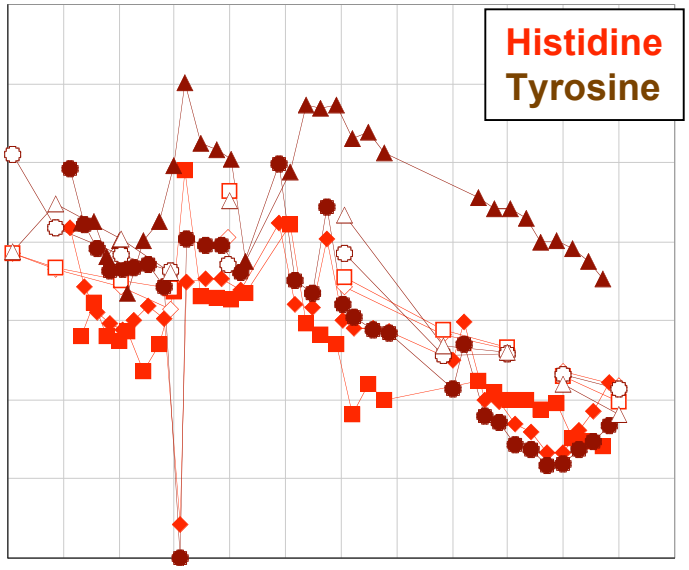
Genentech
IN BUSINESS FOR LIFE

Bioreactor Test Runs: Results









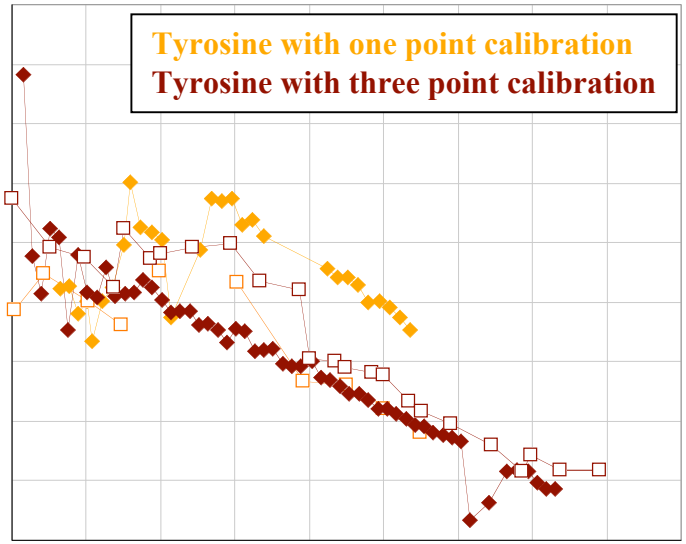
Closed Symbols =
DX-800 Measurements

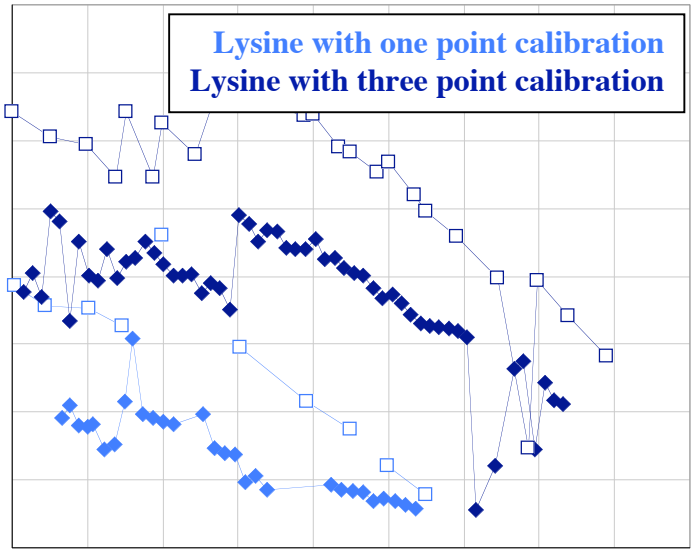
Open Symbols =
Off-Line RP-HPLC
Measurements

Assay Development

Offset between off-line and on-line measurements was observed for some amino acids. A three-point calibration curve was implemented.

- ❑ Average concentration measurements, using a three-point calibration curve, were more accurate than those using a single calibration point
- ❑ However, for some amino acids a consistent off-set from the off-line measurements remained





Closed Symbols = DX-800 Measurements

Open Symbols = Off-Line RP-HPLC Measurements

Genentech
IN BUSINESS FOR LIFE

Bioreactor Test Runs: Conclusions

The DX-800 was capable of continuously sampling two 2L bioreactors for the duration of a 12-day cell culture run.

- Sterility was maintained with continuous sampling
- No impact on cell culture performance was observed

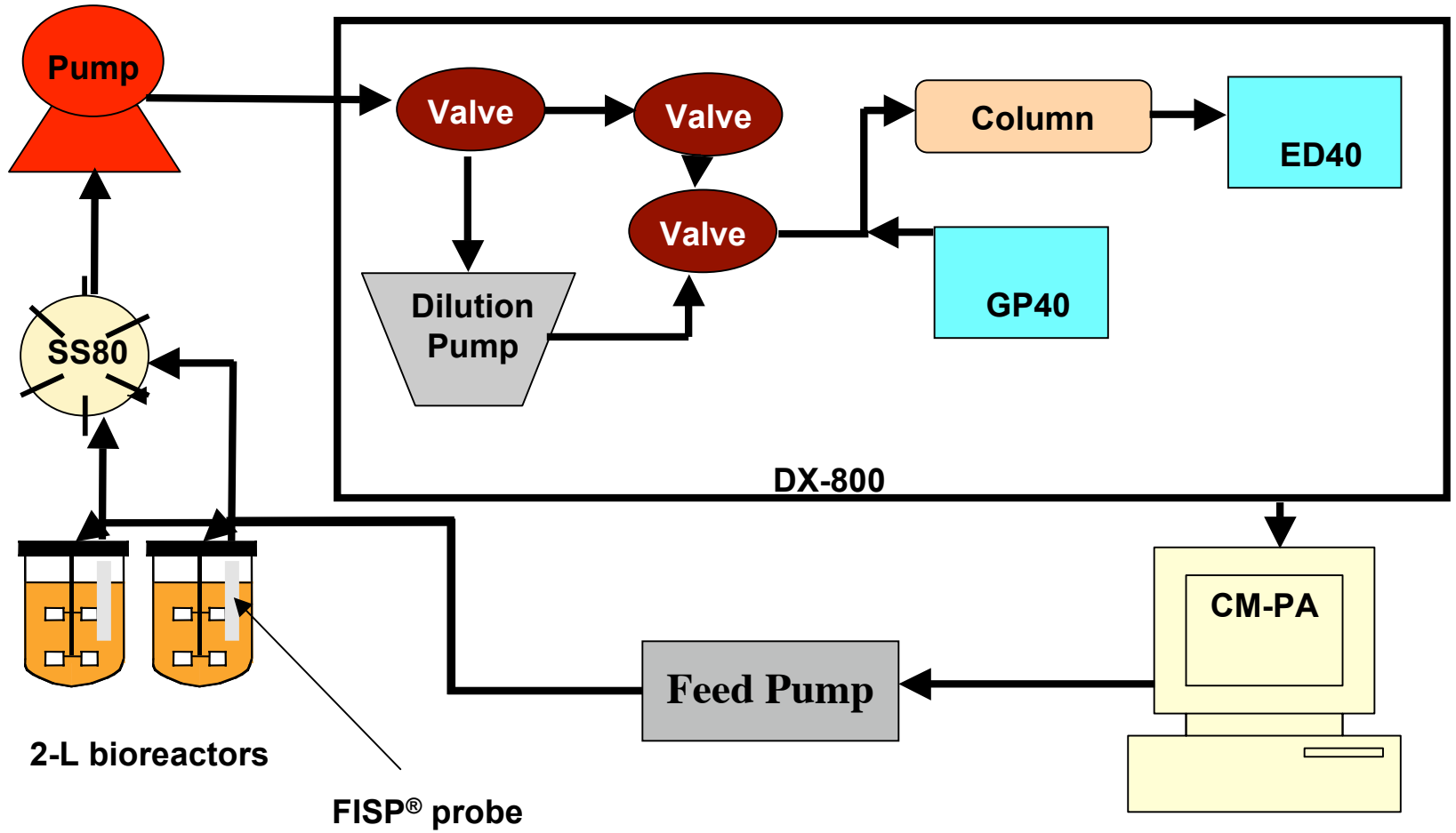
Concentrations for some amino acids measured by DX-800 were lower when compared to an off-line amino acid assay method.

- The off-line method used pre-column derivatization and reversed-phase high performance liquid chromatography (RP-HPLC)

Further assay development with DX-800 including an implementation of a three point standard curve, improved reproducibility and comparability to the off-line assay.

Genentech
IN BUSINESS FOR LIFE

Feedback Control via DX-800



Genentech
IN BUSINESS FOR LIFE

Amino Acid Feedback Control

- Two amino acids were chosen for feedback control: glutamine and asparagine
  - Both major sources of carbon and nitrogen in cell culture
  - Both typically consumed at high enough rates in culture to be good candidates for feedback control
- Feedback control studies were set-up to control one of these amino acids at a time because of CM-PA control limitations
  - Alarms in CM-PA are set up in the order of priorities. For example:

    Alarm 1: Glucose < 1g/L
    Alarm 2: Glutamine < 1g/L

  - If both metabolites are below specified limits, only Alarm 1 will be acted upon (i.e., only the glucose feed pump will be activated)

Genentech
IN BUSINESS FOR LIFE

Case Study #1: Glutamine Control

- Glutamine feedback control studies were performed with two model cell lines in two separate experiments

- For each cell line, two bioreactors were set up and controlled at low glutamine concentration

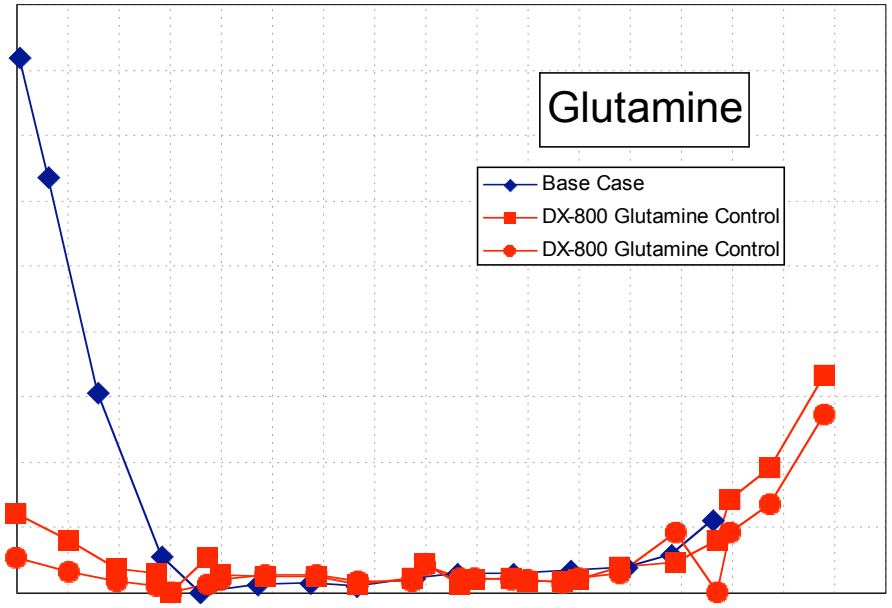
- The DX-800 took samples at ~5 hour intervals

- Alarms were set up in Chromeleon-PA (CM-PA) to control glutamine feeds

Genentech
IN BUSINESS FOR LIFE

Case Study #1: Glutamine Control

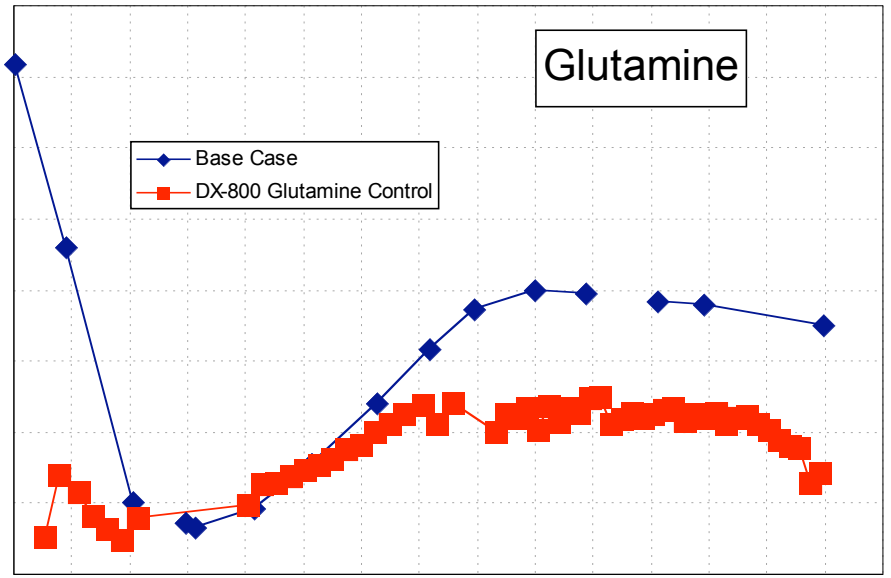
Cell Line A



Cell Line A consumed glutamine.

Cell Line B started producing glutamine independent of the initial level in culture, so glutamine control was not possible in the later part of the run.

Cell Line B

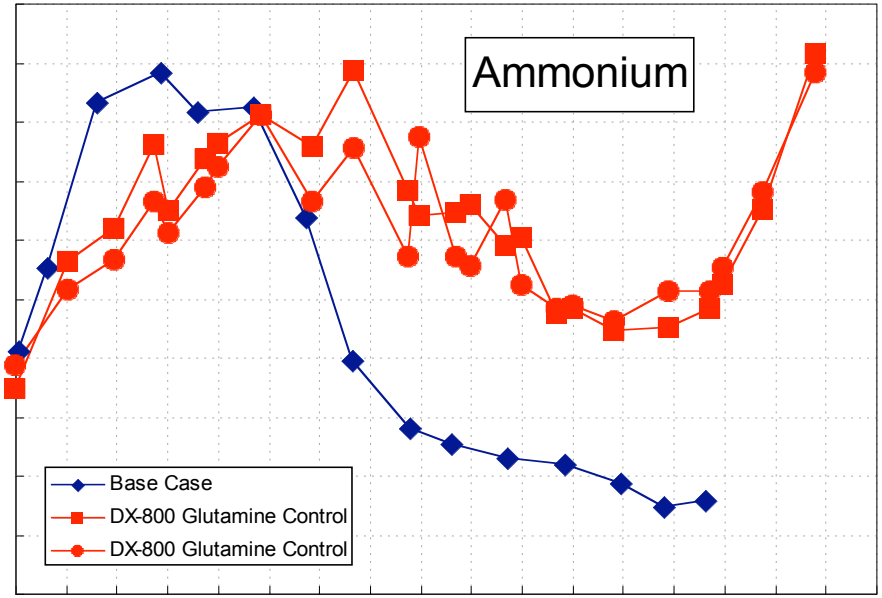


Cell growth and productivity for both cell lines were comparable between the Base Case and the Glutamine Control cultures.

Genentech
IN BUSINESS FOR LIFE

Case Study #1: Glutamine Control

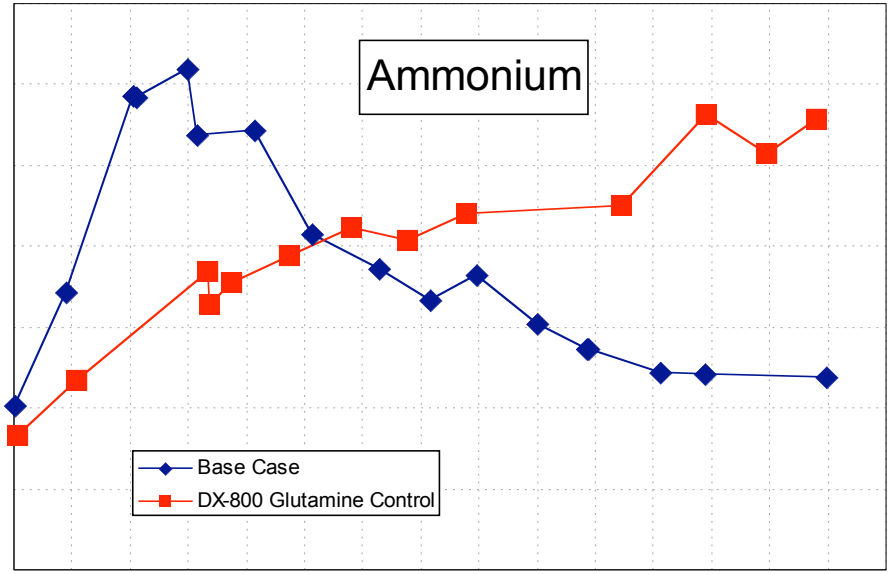
Cell Line A



For Cell Line A, ammonium concentration stabilized when glutamine was controlled.

Cell Line B produced ammonium continuously when glutamine was controlled.

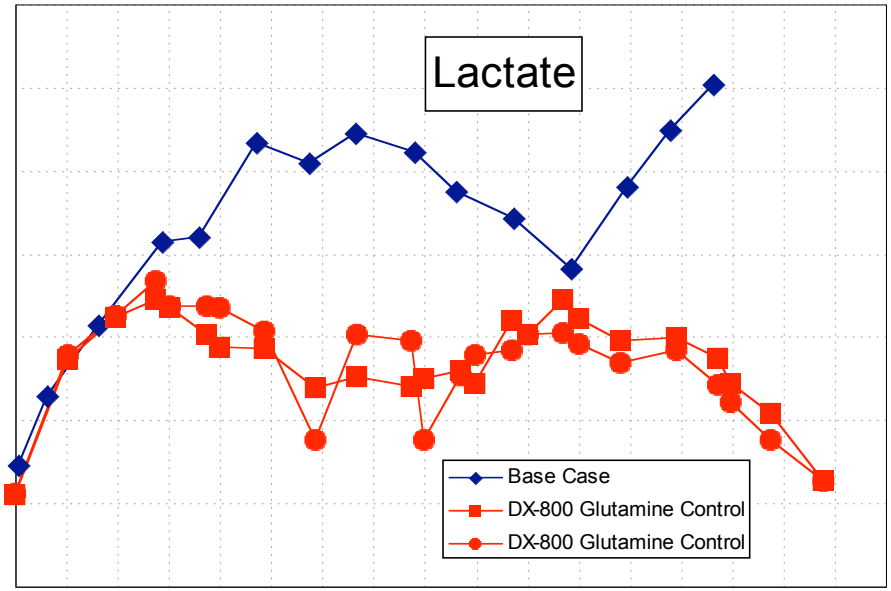
Cell Line B



Genentech
IN BUSINESS FOR LIFE

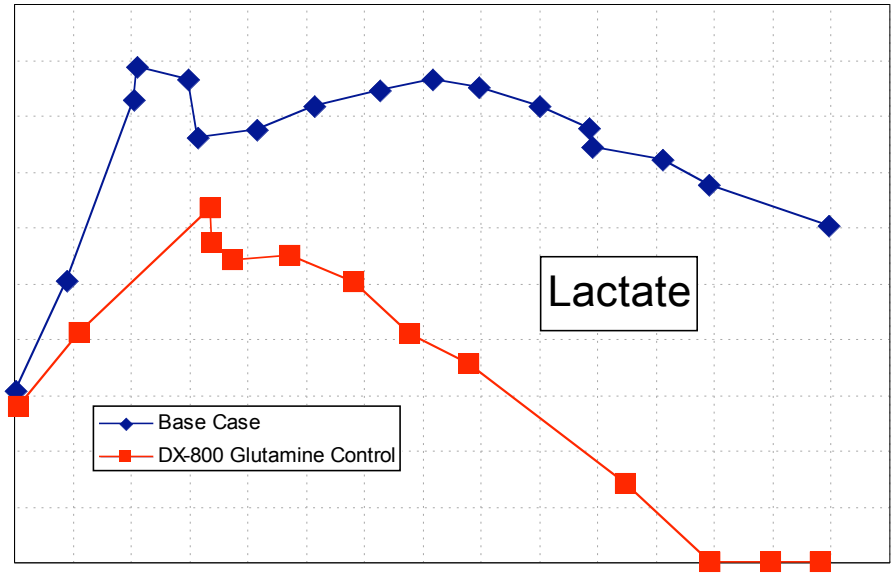
Case Study #1: Glutamine Control

Cell Line A



Both cell lines generated lower lactate concentrations when glutamine was controlled at a low level in culture.

Cell Line B



Genentech
IN BUSINESS FOR LIFE

Case Study #1: Findings

- Cell growth was similar under different glutamine control strategies for these cell lines in these process conditions

- The two cell lines had different responses to glutamine in culture
  - Cell Line A consumed glutamine
  - Cell Line B produced glutamine

- Differences in ammonium and lactate profiles were induced under the different glutamine conditions
  - Lower initial ammonium with glutamine control
  - Lower lactate levels with glutamine control

Genentech
IN BUSINESS FOR LIFE

Case Study #2: Asparagine Control

- Asparagine feedback control studies were performed with Cell Line B only
  - Asparagine was controlled at two different levels in two separate experiments

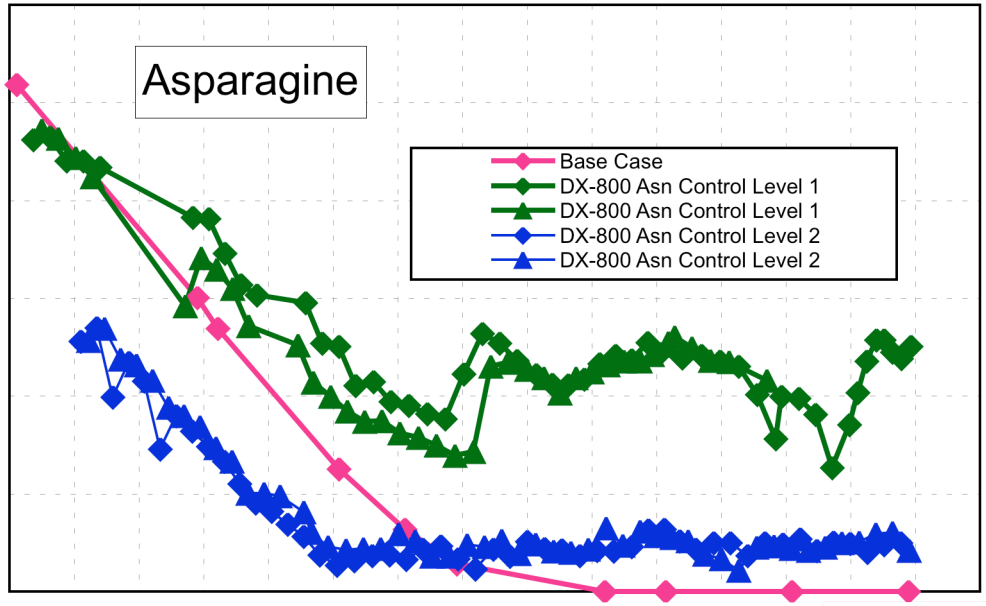
- Two bioreactors were set up and control was initiated once asparagine reached a specified level in culture

- The DX-800 took samples at ~5 hour intervals

- Alarms were set up in Chromeleon-PA to control asparagine feeds

Genentech
IN BUSINESS FOR LIFE

Case Study #2: Asparagine Control

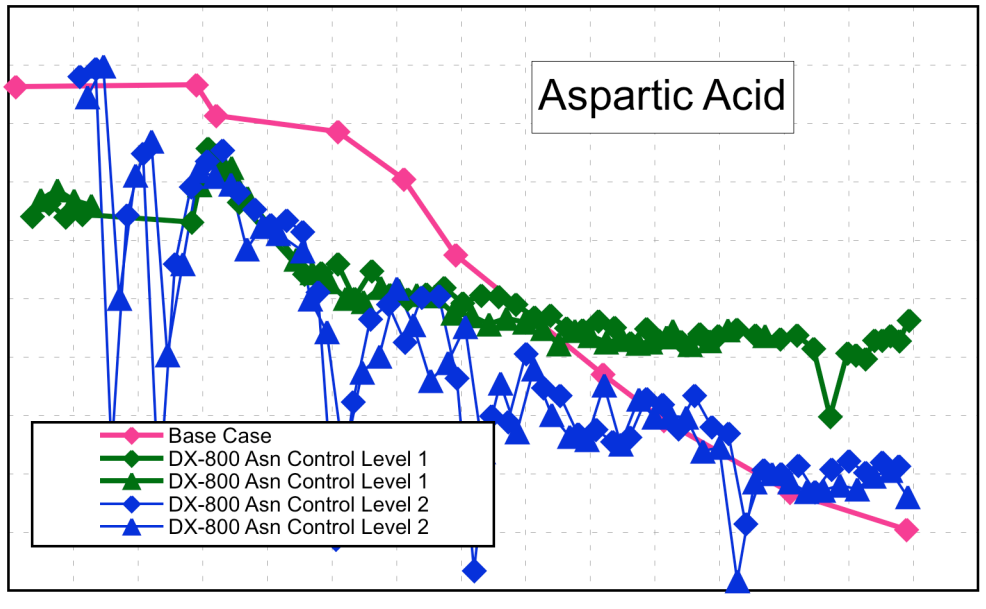


Cell Line B only:

Aspartic acid consumption was reduced when asparagine was fed back into the culture.

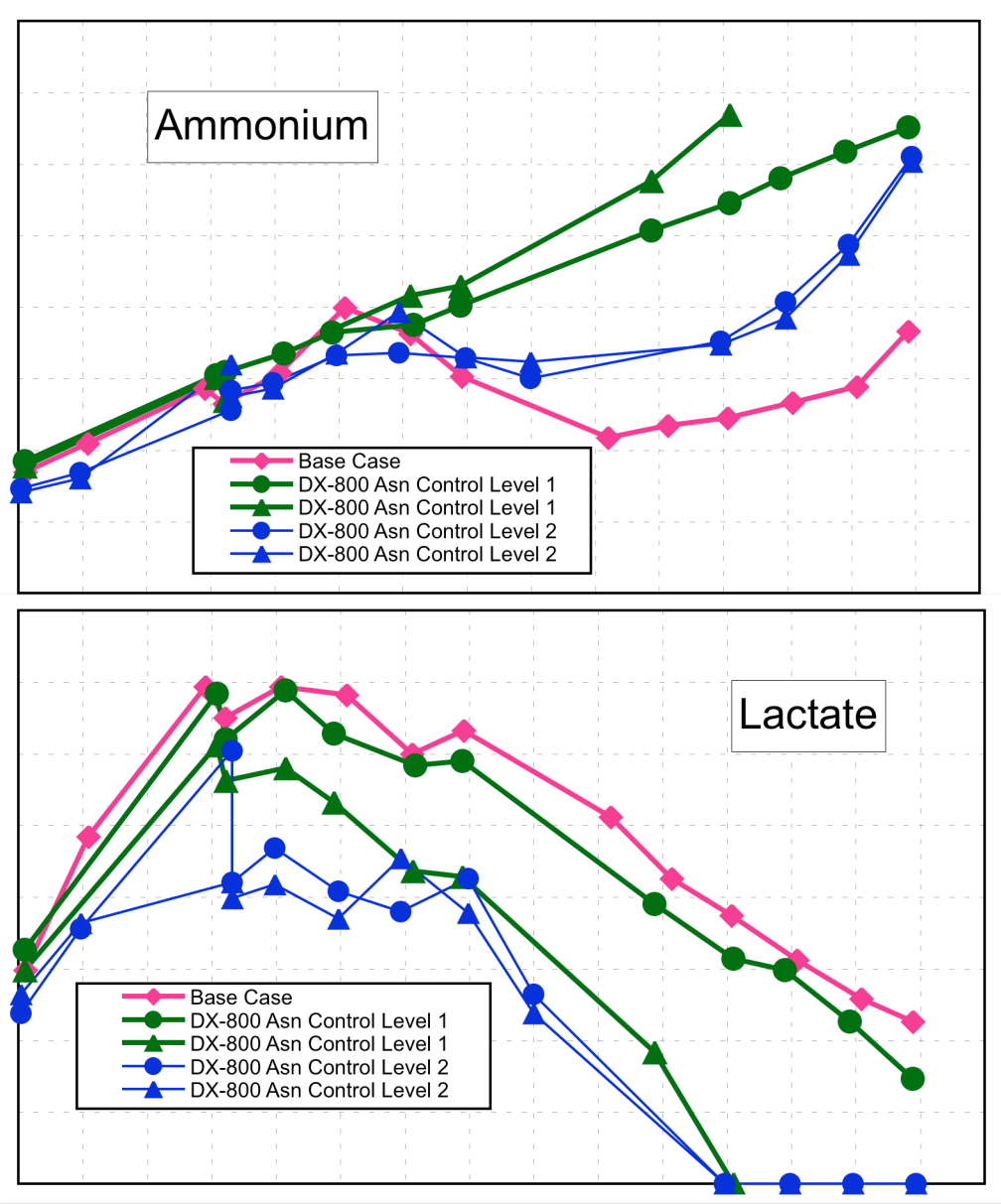
Consumption of of aspartic acid increased with lower levels of asparagine control.

Cell growth and productivity were comparable between the Base Case case and the Asparagine Control cultures.



Genentech
IN BUSINESS FOR LIFE

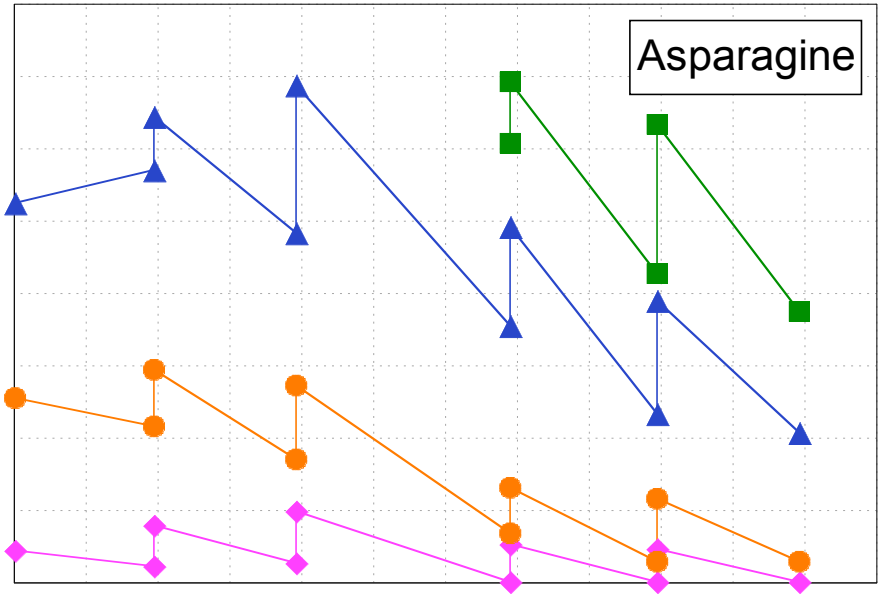
Case Study #2: Asparagine Control

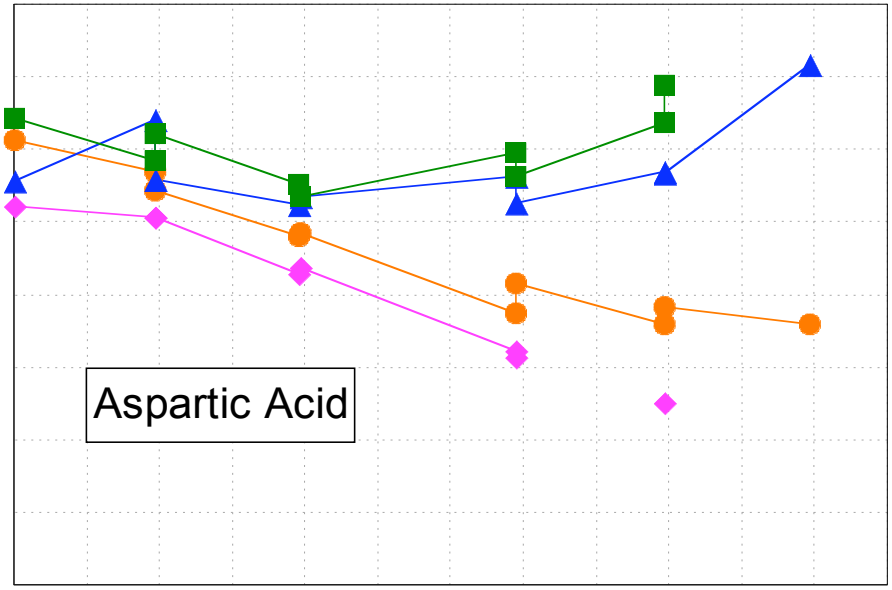


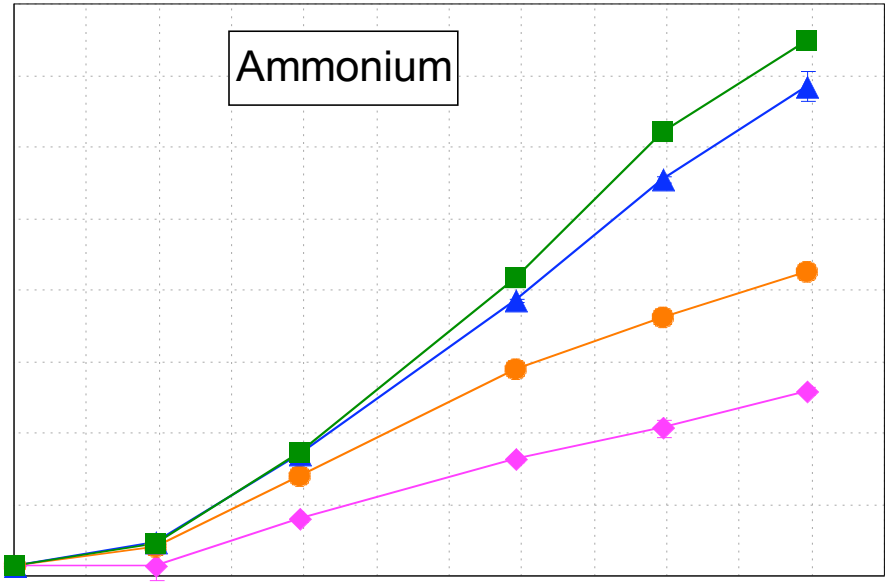
Cultures with Asparagine Control had higher final ammonia, but lactate levels were similar to the Base Case.

Reducing the level of asparagine control resulted in reduced ammonia levels and ammonia profile was more similar to the base case.

Case Study #2: Asparagine Control







Asparagine control in a shake flask model shows that controlling at increasing levels of asparagine leads to reduced aspartic acid consumption and increased ammonia production.

Genentech
IN BUSINESS FOR LIFE

Case Study #2: Findings

- Cell growth was similar under different asparagine control strategies for this cell line in these process conditions.

- Feeding asparagine increased the ammonia concentration and reduced the aspartic acid consumption rate in the bioreactors and in a shake flask model.

- Unlike the glutamine case, controlling asparagine did not induce a change in lactate metabolism.

Genentech
IN BUSINESS FOR LIFE

Conclusions

- Online HPLC measurement was used to successfully maintain set concentrations of two model amino acids in cell culture using CM-PA feedback control

- Effective simultaneous control of multiple amino acids is not possible with the current CM-PA setup and assay duration

- Use of HPLC to measure and control culture nutrients can be useful for media optimization

- Metabolic impact of controlled feeding differed by cell line, showing the need for broad analysis of multiple cell lines before implementing a platform strategy

Genentech
IN BUSINESS FOR LIFE

Acknowledgments

Genentech

Melinda Krebs
Kirin Jamison
Mike Arulgnanendran
Christina Bevilacqua
Shun Luo
Robb Shawley

Tina Larson
Heather Evans

Suzanne Kuo
Bob Kiss
Dana Andersen

Late Stage Cell Culture
BioProcess Development

Dionex

Rick Cooley
Christine Wieser-Punty
Mike Doyle
John Mazzanti
Petr Jandik
Valoran Hanko

Flownamics

Michael Biksacky

Genentech
IN BUSINESS FOR LIFE