SAND2009-0733C

# Whats up with algae?
## At Sandia

Sustainable Biodiesel Summit,
San Francisco CA, February 1, 2009

**Todd W. Lane Ph.D.**

*Sandia National Laboratories*

*Livermore, CA*

Sandia is a multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the United States Department of Energy. This work was sponsored by the U.S. DOE under contract DE-AC04-94AL85000.

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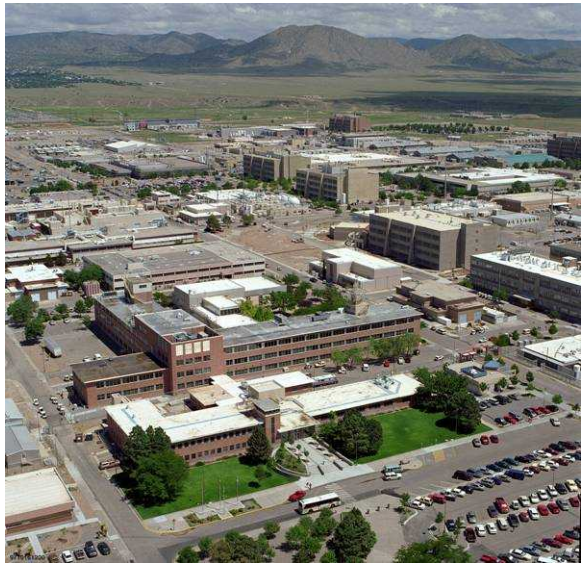
# Sandia National Laboratories

## … four main mission areas

- **Nuclear Weapons**
- **Homeland Security and Defense**
- **Defense Systems and Assessments**
- **Energy, Resources, and Nonproliferation**

## … two major facilities sites

**Albuquerque, New Mexico**



**Established 1949**

***Recent Statistics***

- **8,600 full-time employees**
- **1,500 PhDs and 2,700 MS/MAs**
- **2,200 on-site contractors**
- **$2.33 billion FY06 budget**

**Livermore, California**

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# Sandia Bioenergy-Related Activities

Monsanto CRADA

Microalgal Biodiesel Feedstock Improvement by Metabolic Engineering LDRD

Systems Dynamics Analyses

Minnesota Biobusiness Alliance

& Others

Interactive Water Quality Modeling to Assist Regional Water Planning

LDRD

NM TRC Dairy waste streams to energy

JP8 Biofuel DARPA BAA06-43 UOP Project Team

JP8 Biofuel DARPA BAA08-07 New Team Projects*

Joint Bioenergy Institute (JBEI)

DOE-Funded Team LBNL,LLNL,SNL,others

Biomass to Bioenergy: Protein & Metabolic Engineering

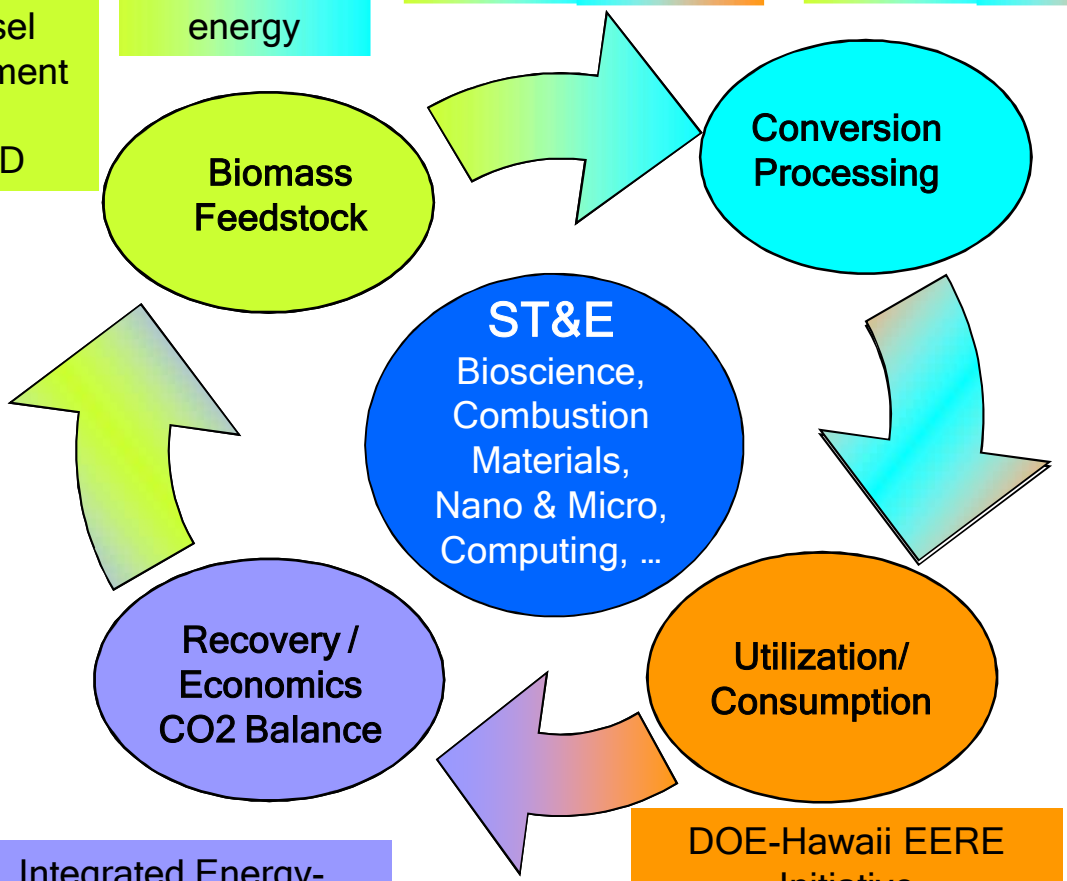
LDRD

Breakdown of Lignocellulose Via Mixed-Microbes

LDRD

Metabolic Engineering of a Thermophile for C5/C6 Fermentation

LDRD




Integrated Energy-Water Assessment and Planning

LDRD

DOE-Hawaii EERE Initiative

Alternate Fuels

DOE FreedomCar

Advanced Engine & Combustion R&D and Testing

* New DARPA-funded algae-to-JP8 team projects expected to begin 4QFY08 or 1QFY09

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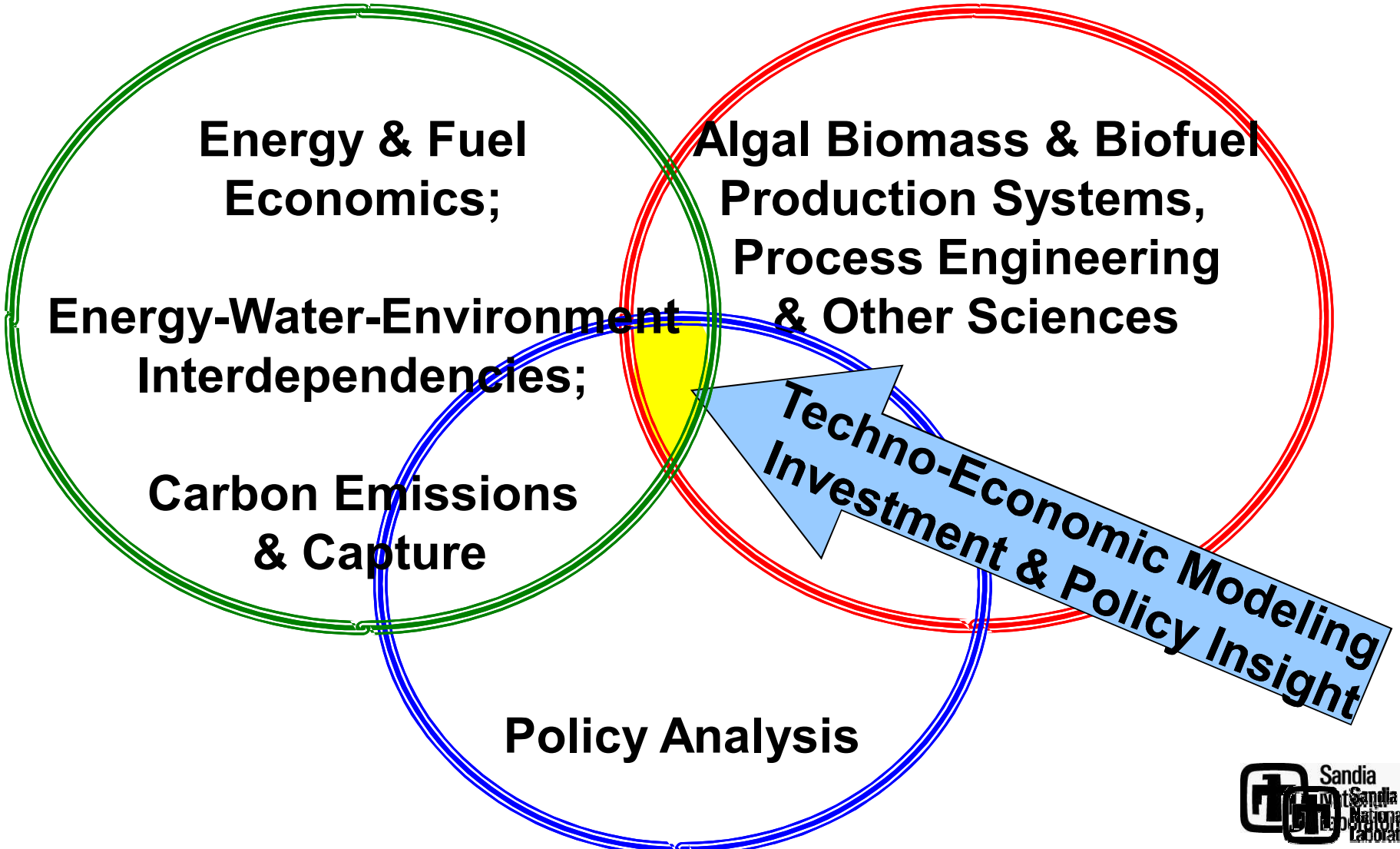
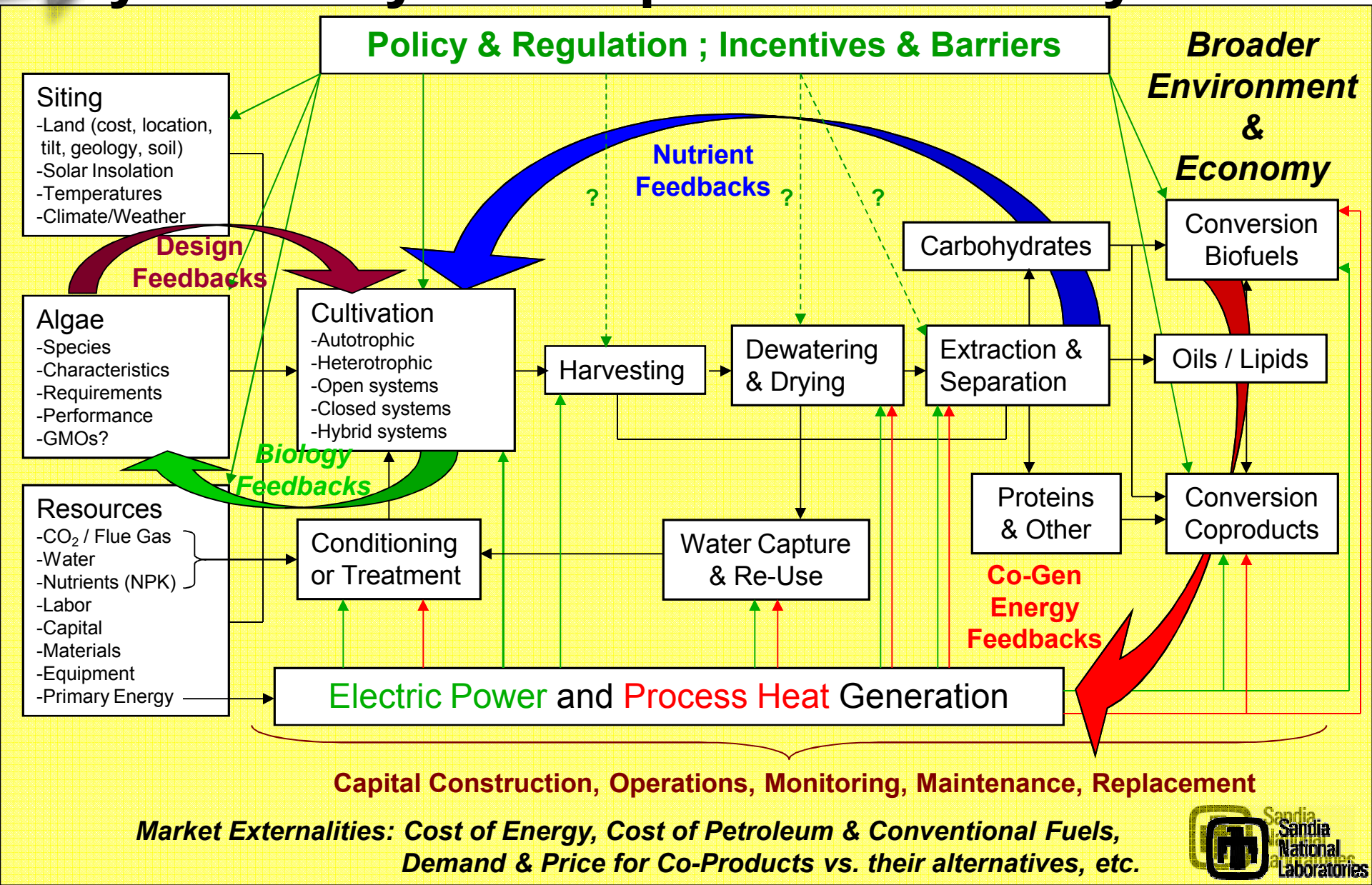
# Technical and Economic Modeling:

## *Science & Technology-based Insight for Investment and Policy Decision-Support*



# Systems Dynamics Applied to Complex and Dynamically Interdependent "MetaSystem"

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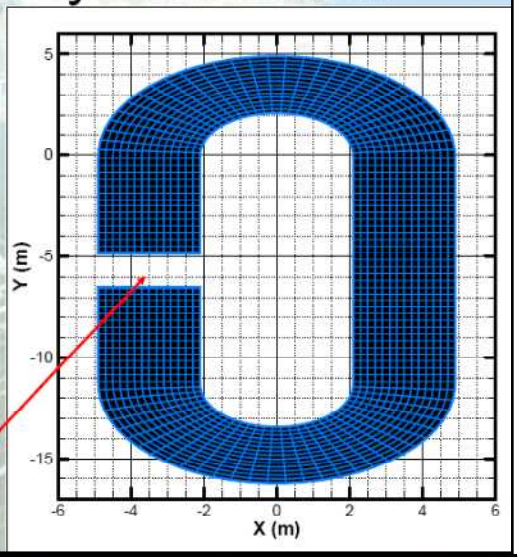
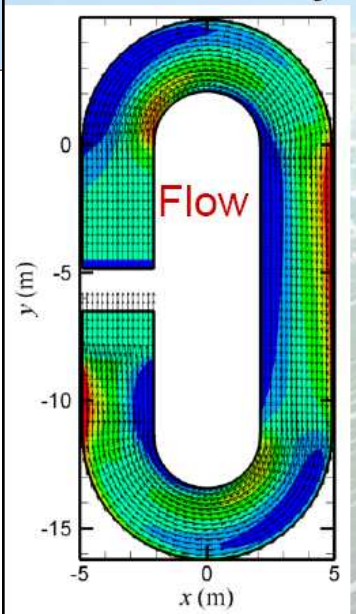
# Numerical Example: Algal Raceway Simulation

- Orthogonal grid
- 130 m$^2$ (0.013 ha)
  - Width: 3 m
  - Length: ~43 m
- 1860 active cells
  - 15 cells across
  - 124 cells around
- Uniform 0.3 m depth
- Withdrawal/return BC added (paddlewheel)



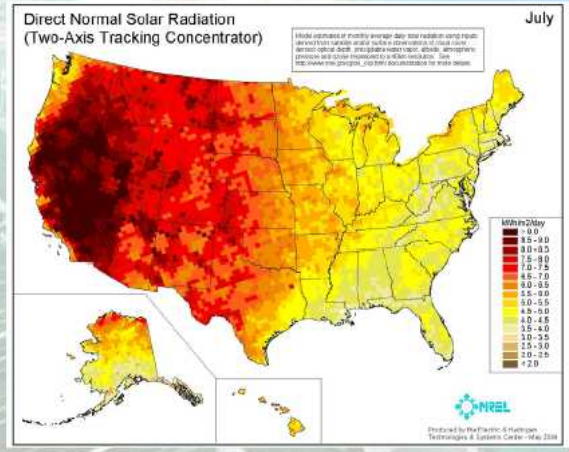

# Hydrodynamics




- EFDC solves vertically hydrostatic momentum and continuity equations for turbulent flow
- Withdraw/return BC simulates a paddlewheel
- When properly configured, solution stabilizes quickly
- Explicit numerical solution yields problems amenable for solution on Sandia's large computing clusters

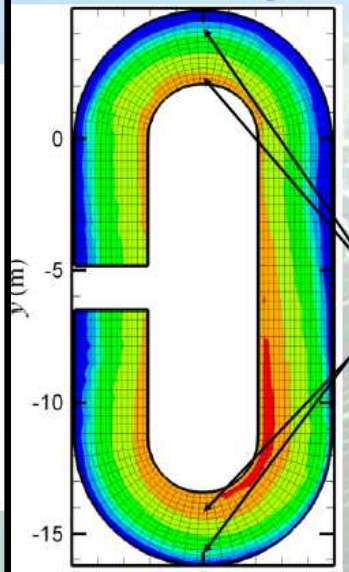
# Atmospheric Forcing

- Compiled one year of input data for Palm Springs, CA, 2005
  - January
  - July
- Hourly observations of the following:
  - **Incident radiation**
  - **Temperature**
  - Cloud cover
  - Evaporation
  - Precipitation
  - Relative humidity
  - Atmospheric pressure
  - Wind-driven shear
  - Coriolis forces




*National Solar Radiation Data Base and National Climatic Data Center

# Algal Growth Model




Algal growth is affected by helical flow patterns that mix the water column

- Algal growth kinetics are based on US Army Corp of Engineers' CE-QUAL model
- Includes solar radiation, nutrient availability, predation, temperature, respiration, etc.

# Genomic information is key to understanding TAG formation

## Biodiesel strains

- ***Botryococcus braunii***
- ***Cyclotella cryptica***
- ***Chlorella prothecoides***
- ***Chlorella vulgaris***
- ***Dunaliella bardawil***
- ***Monallanthus salina***
- ***Nannochloris sp.***
- ***Nannochloropsis sp***
- ***Navicuala sp***
- ***Pleurochrysis carterae***
- ***Phaeodactylum tricornutum***
- ***Thalassiosira pseudonana***

## Genome projects

- ***Aureococcus anophagefferens***
- ***Chlamydomonas reinhardtii*** **(complete)**
- ***Cyanodishycon merolae***
- ***Chlorella vulgaris***
- ***Dunaliella salina***
- ***Emiliania huxelyi (complete)***
- ***Fragillariopsis cylindrus***
- ***Micromonas pusillus***
- ***Micromonas sp***
- ***Monosiga brevicollis***
- ***Ostreococcus tauri***
- ***Ostreococcus lucimarinarus***
- ***Phaeodactylum tricornutum*** **(complete)**
- ***Thalassiosira pseudonana*** **(complete)**

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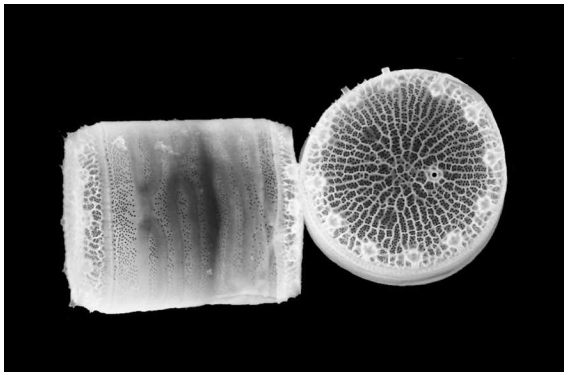
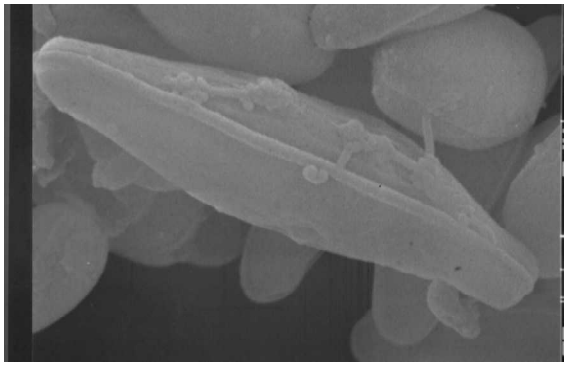
# Two model diatoms provide genetic systems and TAG metabolism



*Thalassiosira pseudonana*

Centric, Marine
31.3 Mbp
11390 gene models
3000 additional transcripts
Biolistic transfection
North American model



*Phaeodactylum tricornutum*

Pennate, Euryhaline
Lightly silicified
High lipid strains
26.1 Mbp
10010 gene models
Biolistic transfection
European model

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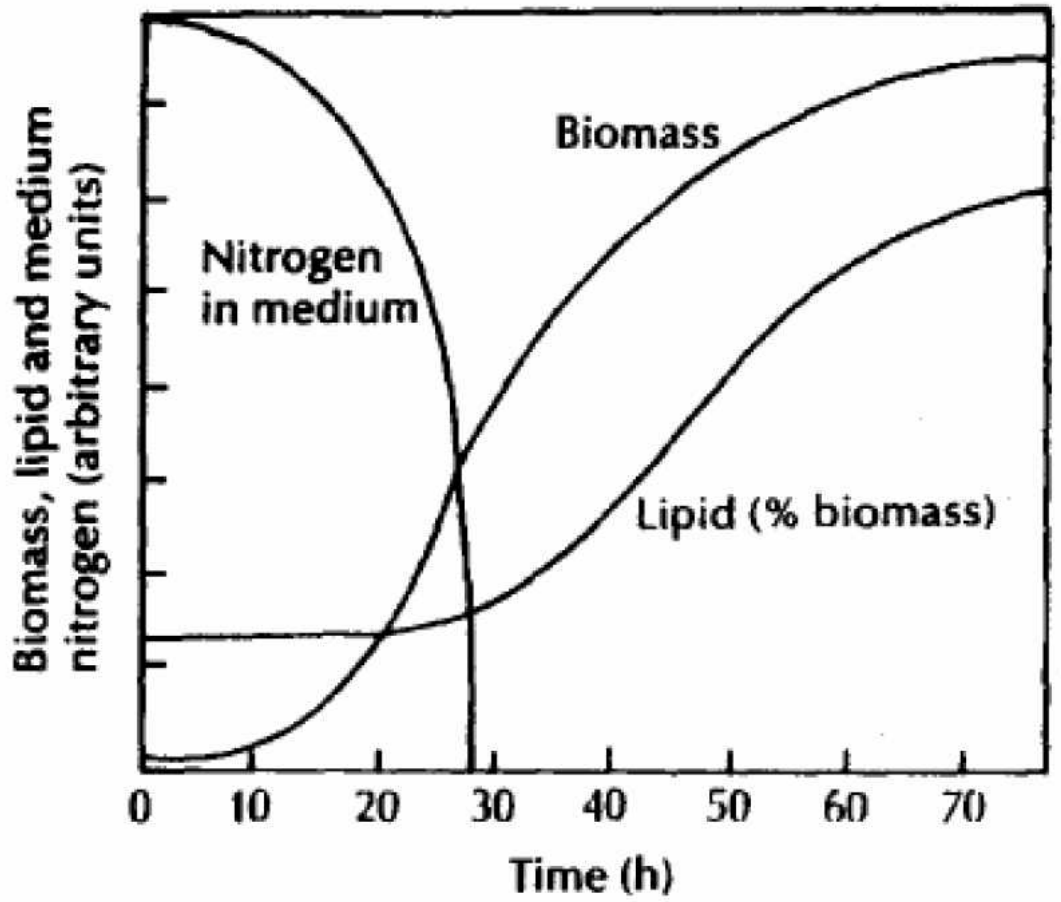
# Nutrient starvation induces Lipid production: the "Lipid Trigger"




Idealized pattern of lipid accumulation in an oleaginous microorganims grown in batch culture (Ratledge, 1989)

Nitrate
Phosphate
Silicate

Low temperature

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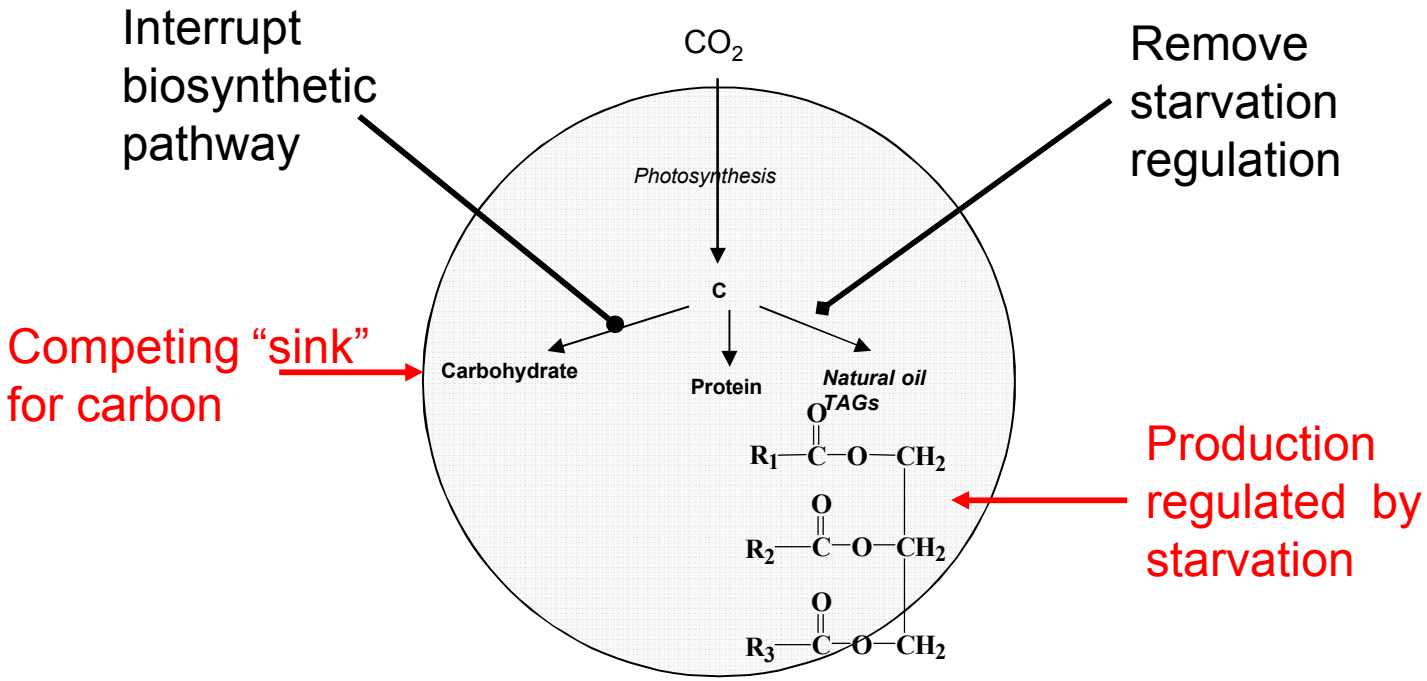
# The Genome: Key to the lipid trigger and controlling the flow of carbon




Initial Target:
UDPglucose:β-(1→3)-glucan-β-glucosyltransferase (also known as chrysolaminarin synthase),

Initial Target:
ATP citrate lyase
Acetyl coA carboxylase (ACCase)

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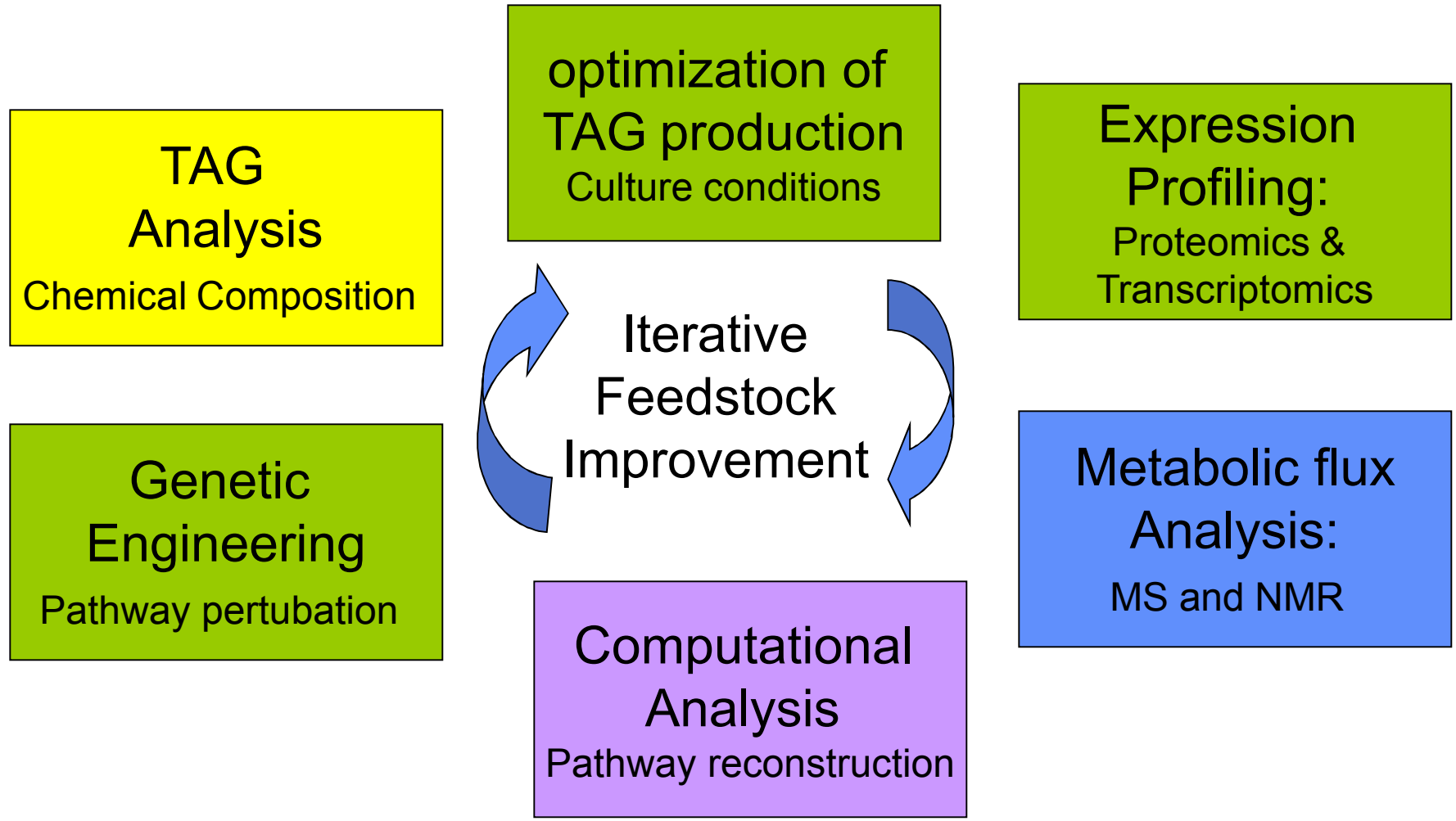
# Our Technical Approach Integrates Several Disciplines

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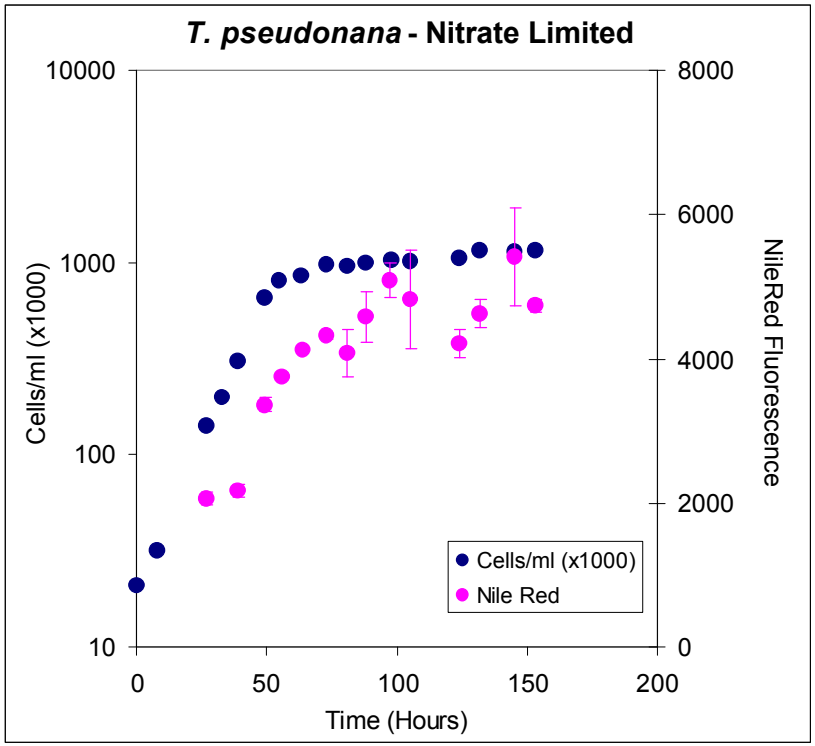
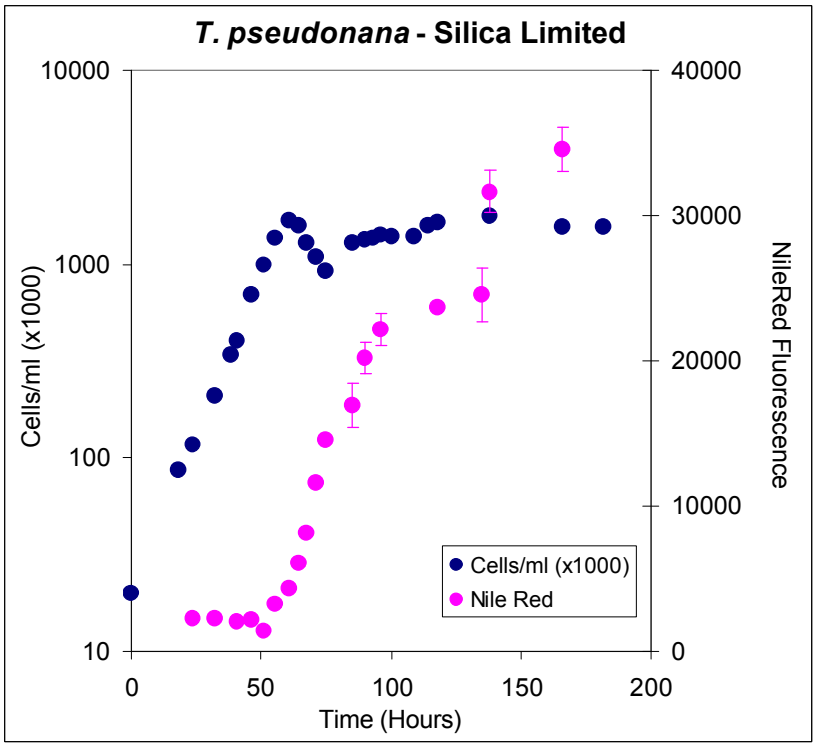
# TAG accumulation is induced by nutrient starvation in model diatoms.




Yu *et al* 2009

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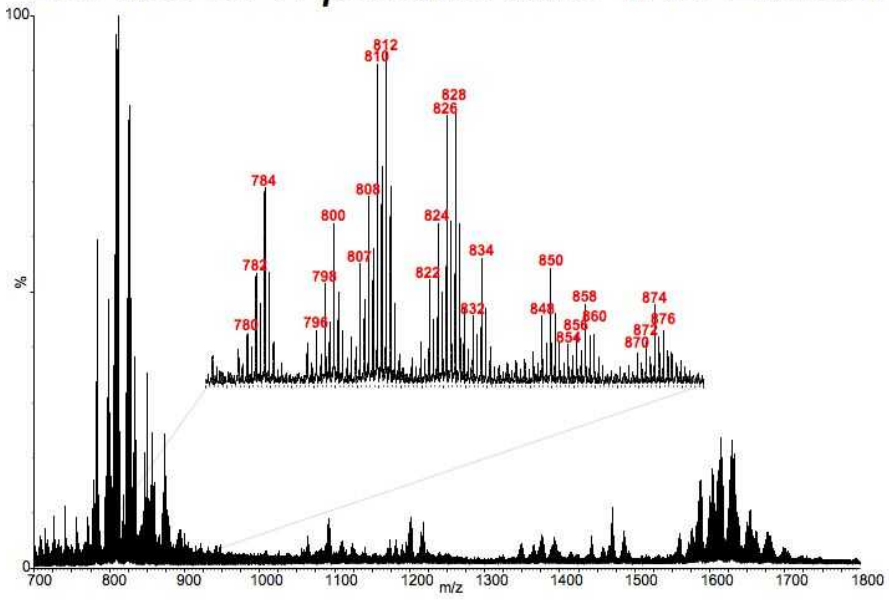
# Characterization of wt TAG production by mass spectrometry

ESI-MS of *T. pseudonana* TAG Extract

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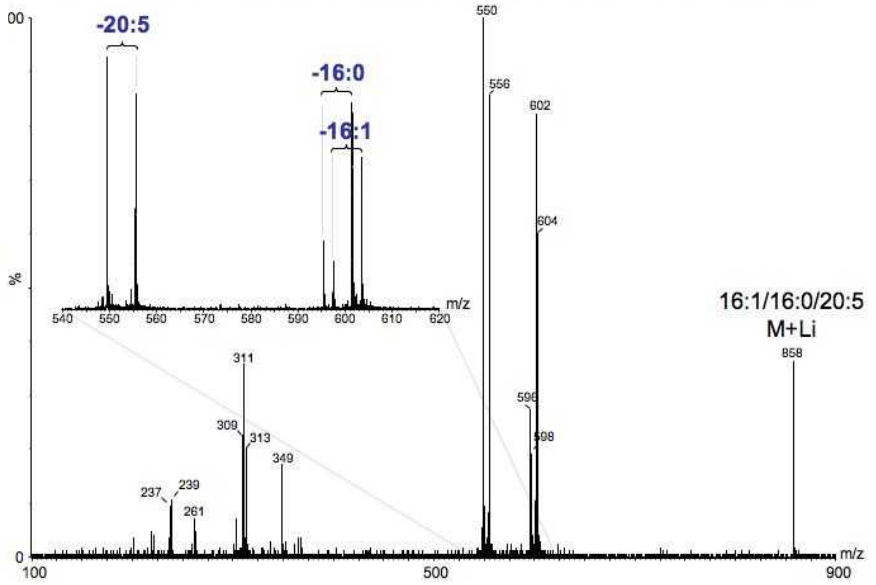
MS/MS Determines TAG Structure

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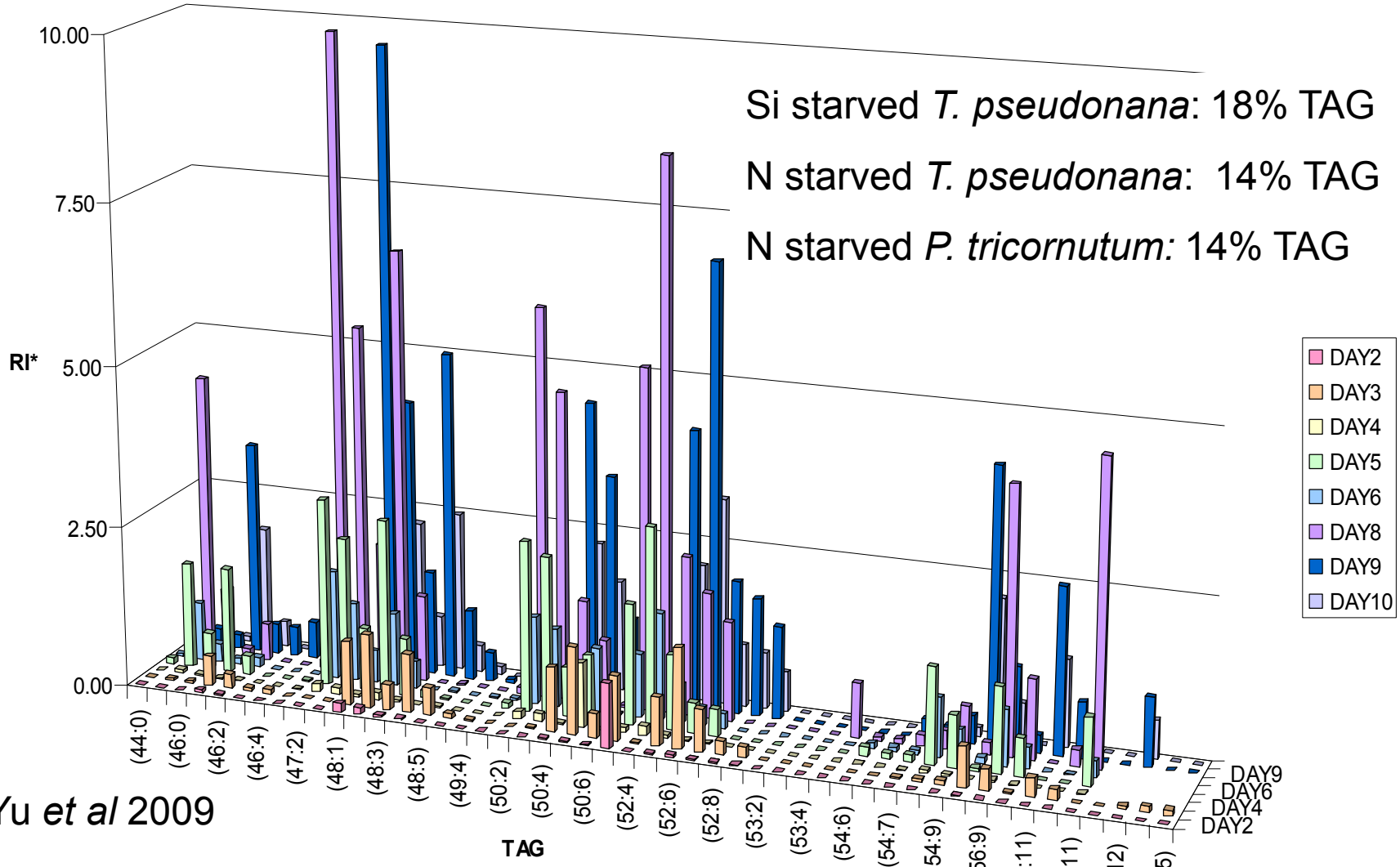
# TAG profile of nitrate starved *T. pseudonana*




Si starved *T. pseudonana*: 18% TAG

N starved *T. pseudonana*: 14% TAG

N starved *P. tricornutum:* 14% TAG

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* TAG intensity ratios relative to internal TAG (51:3) standard

- time points taken from mid-log (032708) till 040408

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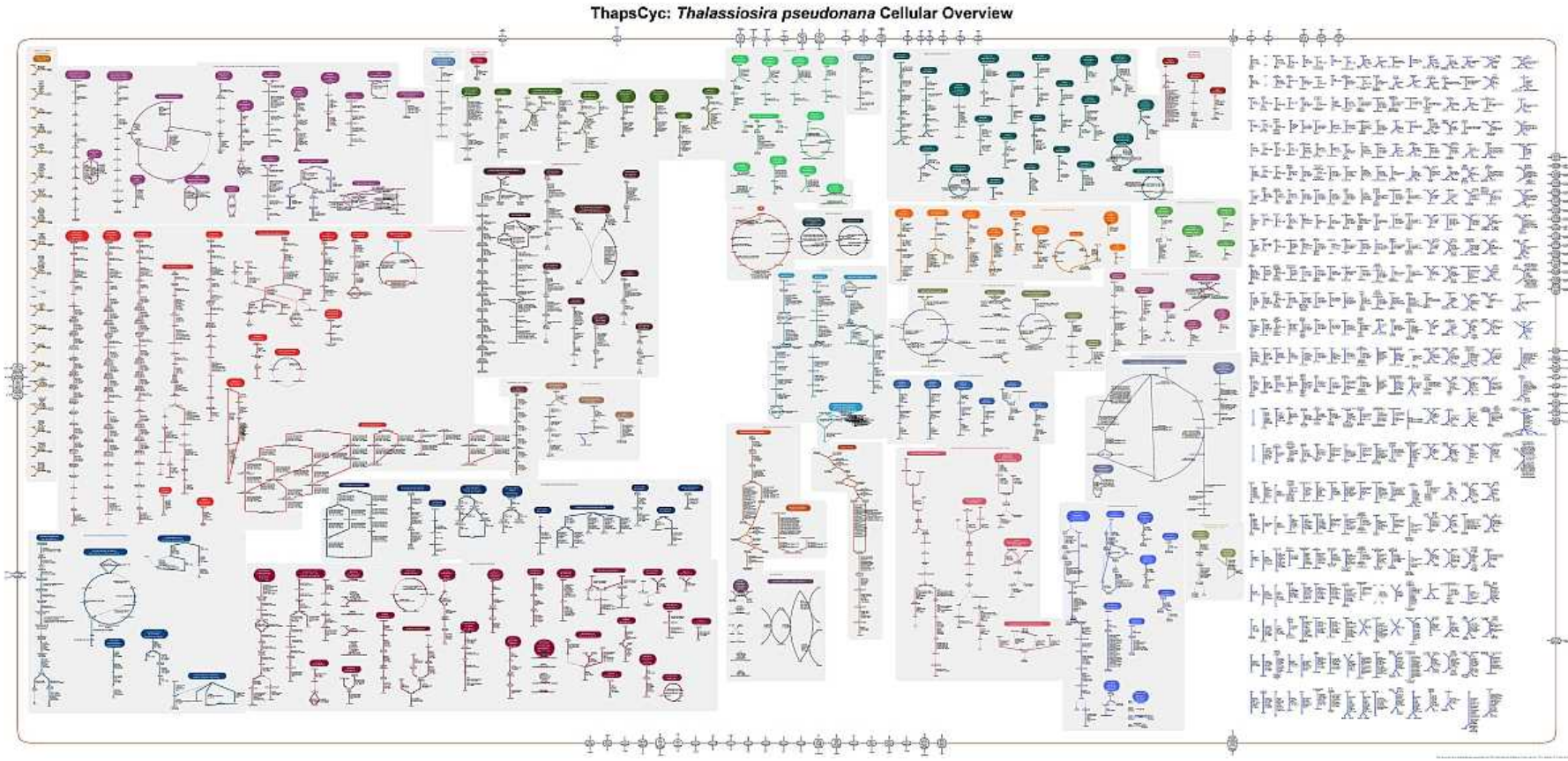
# We have developed a metabolic pathway model from genomic annotation.

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# Digital Transcriptomics: cDNA sequencing and EST compilation

49.8 million reads total -- ~12.5 M reads per library

RNA transcript abundance variation as cells enter stationary phase and TAG synthesis

- 4 timepoints
- Transcript abundance for 21,000+ genes (median depth per base)
- 13390 gene models (including Mock et al 2008)
- 8000 additional EST clusters – many appear to be real genes

Merging of EST clustering results and gene models currently underway.

Transcript abundance data will feed directly into computational models: Transcriptional regulation of specific pathways will be inferred

# Acknowledgements

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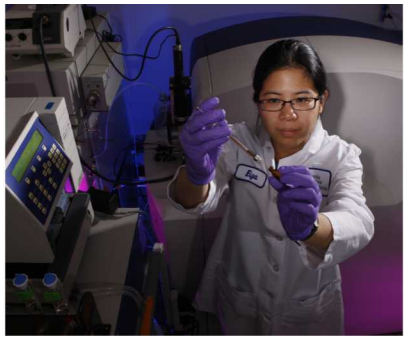

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