

Analysis of the “Starvation Trigger” for Algal oil Synthesis.

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Outline of talk

1. Introduction

- A. Why microalgae?
- B. Microalgal systems for biodiesel
- C. Microalgal Genomes

2. TAG accumulation in model diatoms

3. Preliminary results from proteomic analysis of “starvation trigger”

Transcription profiling of “starvation trigger”



Why use algae for fuel?

- **Greater photosynthetic efficiency**
 - 5-10%, compared with typically <0.5% in higher plants
 - Less land “footprint” required relative to biomass production with higher plants
- **Can use impaired waters: brackish, saline, nutrient-rich**
 - Reduced impacts on fresh water supplies
 - Productive use of impaired waters and waste streams
- **Can utilize marginal or non-agricultural land**
 - Reduced biofuels production impacts on high-value & high-productivity land
 - Reduced impacts on competing agricultural commodity markets
- **Can extract/use CO₂ from Flue gas emissions for growth**
 - >90% efficiency demonstrated in pilot projects
 - Can potentially provide net GHG Abatement “service” and “green credit” value
- **Can extract/use nutrients from contaminated water for growth**
 - Provides wastewater treatment “service” while creating biomass feedstocks
- **Can produce feedstocks for biofuel & other valuable coproducts**
 - Lipid content in algae species can exceed 50%... Input for biodiesel production
 - Coproducts include animal feed, fertilizer, biopolymers, industrial chemicals, etc.
 - Can provide GHG abatement

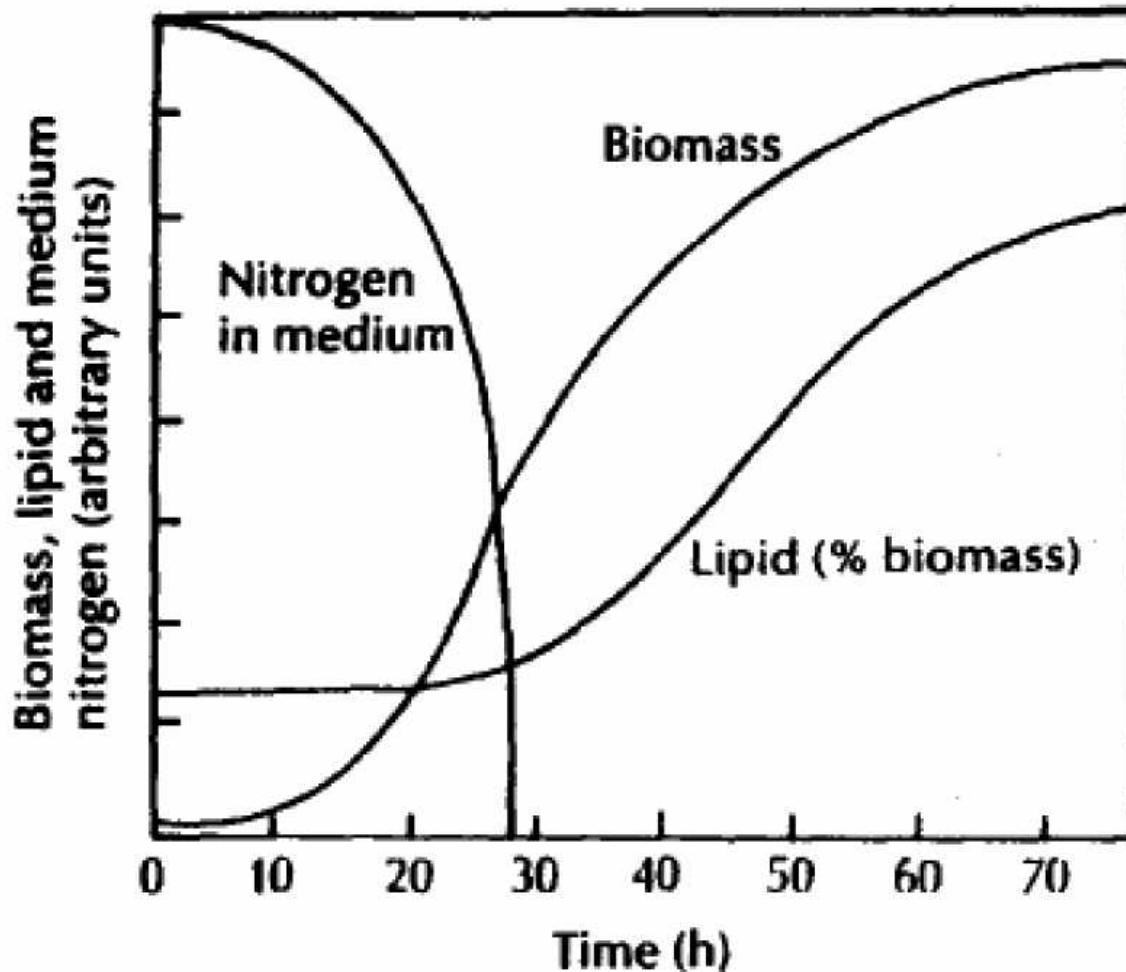


Microalgae have production advantages

Crop	Oil Yield (L/acre)	Land Area Needed (M acre)	% of existing US crop area
Soybeans	180	480	652
Corn	70	1246	1692
Oil palm	2408	36	48
Microalgae [‡]	23,755	3.64	5

- Cell culture density: *P. tricornutum*, 4×10^7 cells/mL
- Triacylglyceride (TAG) weight per cell: 46.0 pg/cell (assumes 60% TAG of total dry weight per cell)
- Assume 1L cell culture: $(1000\text{mL})(4 \times 10^7 \text{cells/mL})(46.0 \text{ pg/cell}) = 1.84 \times 10^{12} \text{ pg}$
- Transesterification efficiency, assume 80% conversion $\rightarrow 1.47 \times 10^{12} \text{ pg}$ biodiesel output per 1L = 1.47g of biodiesel.
- **Enough biodiesel to replace all petroleum transportation fuels could be grown by algae farms in 15,000 square miles, or roughly 12.5 percent of the area of the Sonoran desert.**

Starvation triggers TAG accumulation



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



There is a wide choice of model systems.

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

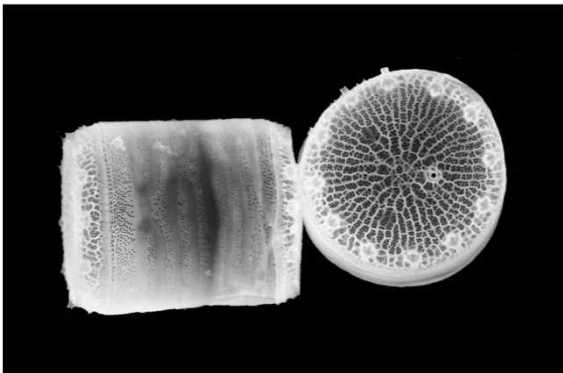
There is a great deal of species and strain to strain variation in growth characteristics and yield of triacylglycerides.



There are a variety of microalgal genome projects But few of direct relevance to biodiesel

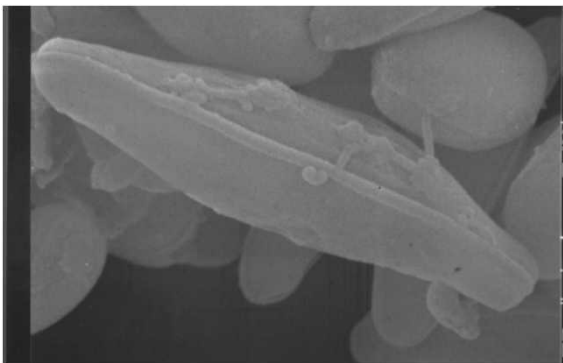
- **Aureococcus anophagefferens**
- **Chlamydomonas reinhardtii (complete)**
- **Cyanodishycon merolae**
- **Dunaliella salina**
- **Emiliana huxelyi (complete)**
- **Fragillariopsis cylindrus**
- **Micromonas pusillus**
- **Micromonas sp**
- **Monosiga brevicollis**
- **Ostreococcus tauri**
- **Ostreococcus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

Model diatoms



Thalassiosira pseudonana

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

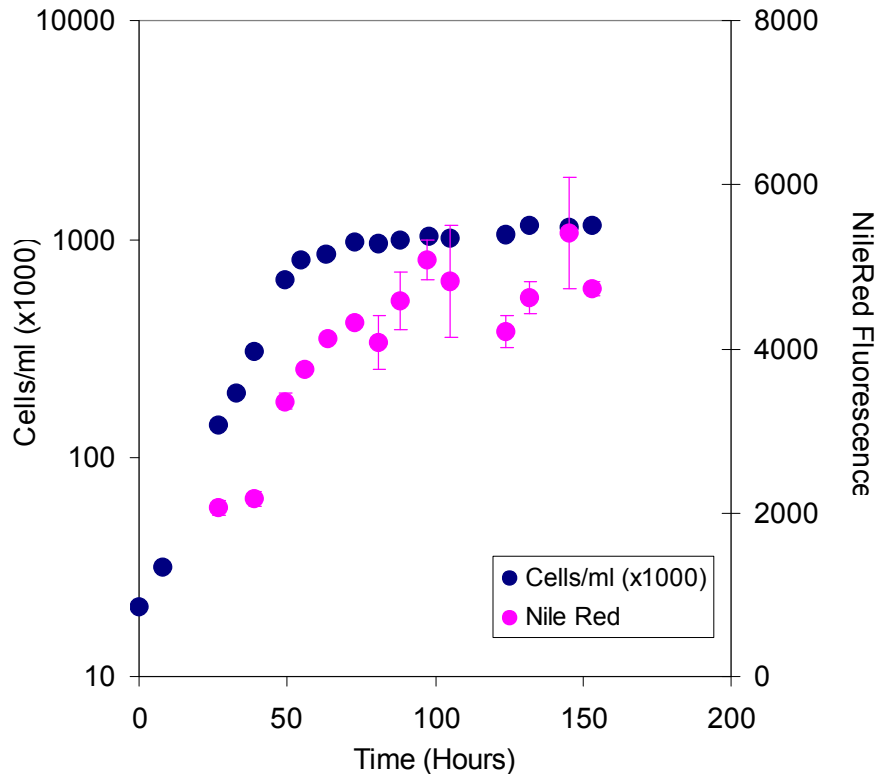


Phaeodactylum tricornutum

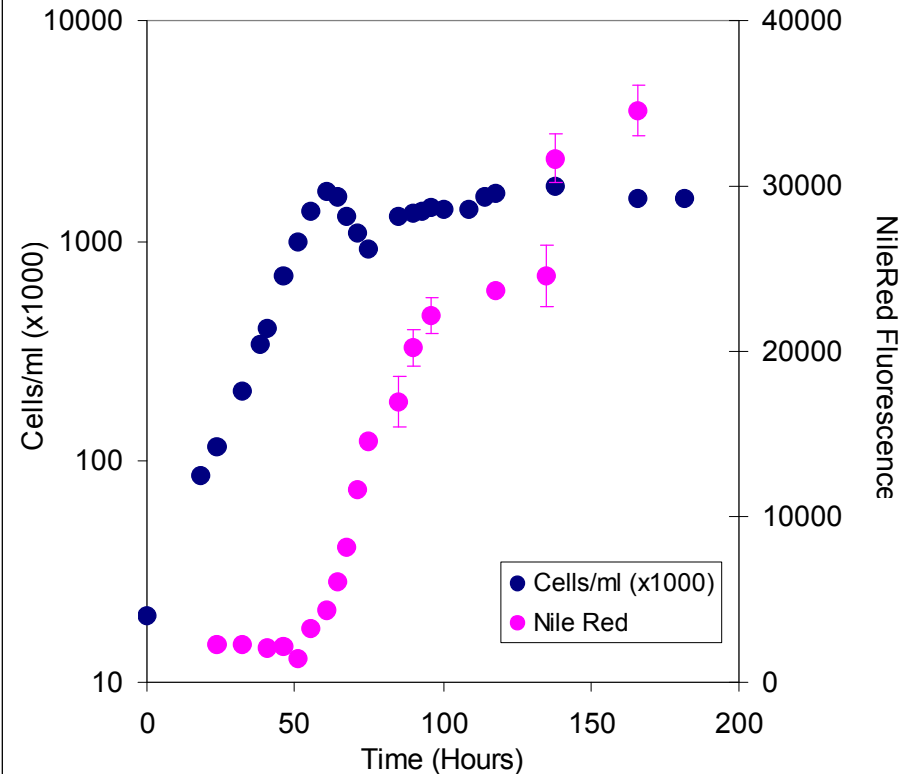
Pennate, Euryhaline
Lightly silicified
High lipid strains
26.1 Mbp
10010 gene models
Biolistic transfection
European model

TAG accumulation induced by nutrient starvation

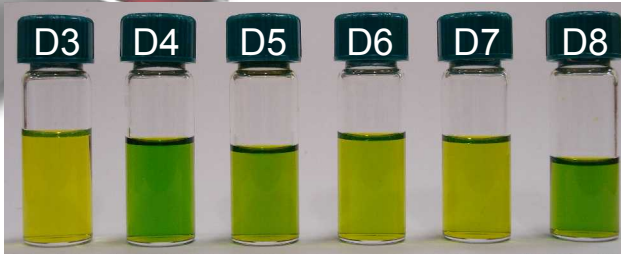
T. pseudonana - Nitrate Limited



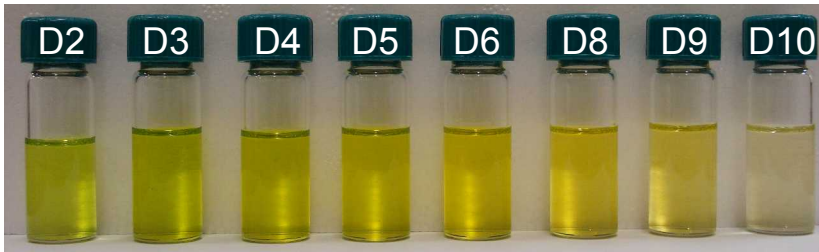
T. pseudonana - Silica Limited



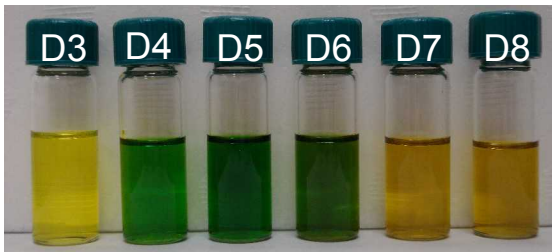
Hexane extractions



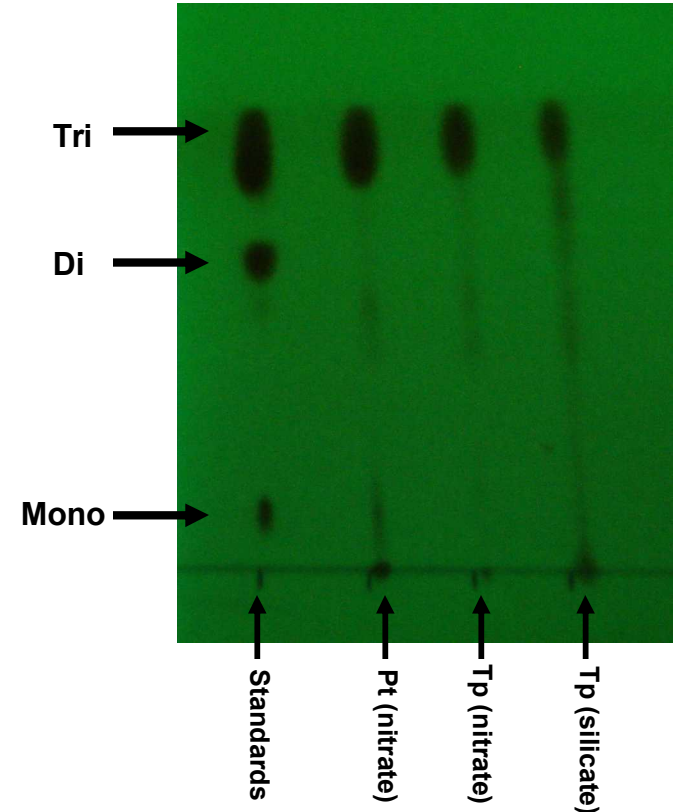
Phaeodactylum tricornutum
(nitrate limited)



Thalassiosira pseudonana
(nitrate limited)



Thalassiosira pseudonana
(silicate limited)

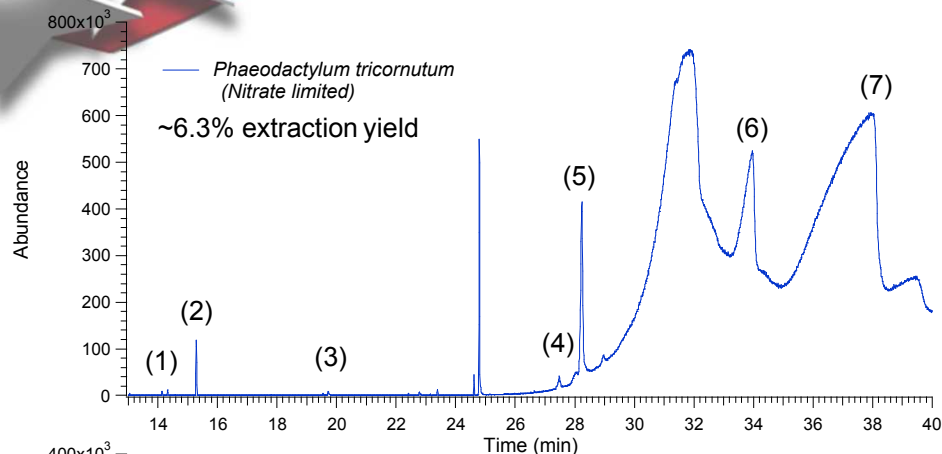


	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
<i>Phaeodactylum tricornutum</i> (nitrate limited)	NH	4.8148	1.6720	4.2974	5.7164	7.4515	6.3088	NH	NH
<i>Thalassiosira pseudonana</i> (nitrate limited)	1.5957	4.9057	6.2706	10.0671	8.8785	NH	13.0435	15.1042	14.9171
<i>Thalassiosira pseudonana</i> (silicate limited)	NH	9.1398	5.6231	10.9938	16.5485	15.4915	16.193	NH	NH

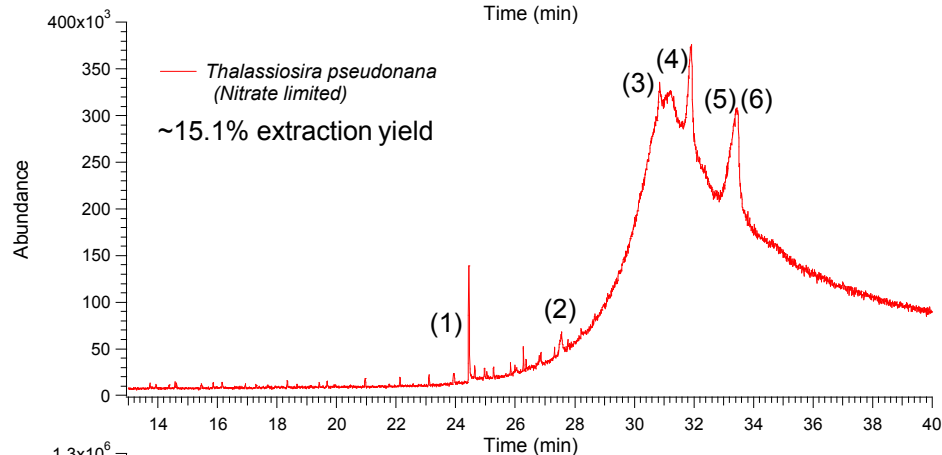
- Highlighted boxes indicate samples compared in TLC plate above and will be compared by GC-MS
- Extraction values include chlorophyll

- Extraction percentages based on dry weight
- NH: Not harvested

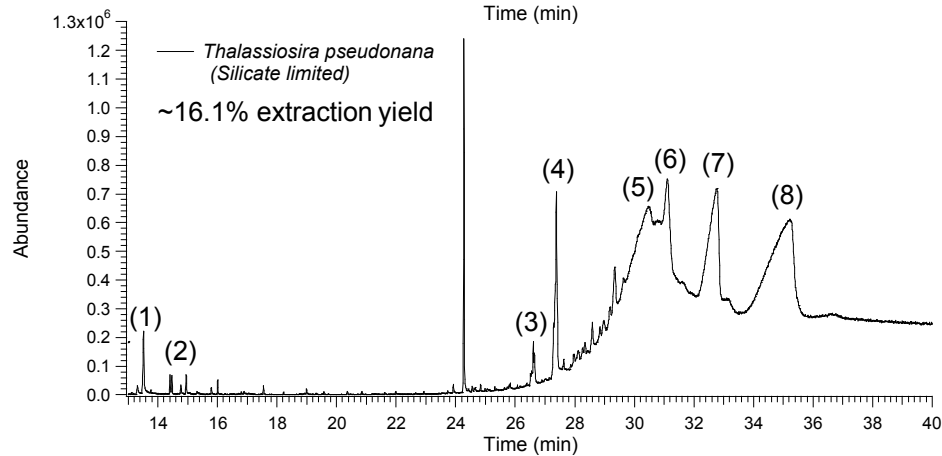
TAG identification by GC-MS



(1)	n-Hexadecanoic acid
(2)	4,7,10,13,16,19-Docosahexaenoic acid
(3)	Hexadecanoic acid, 2-hydroxy-1 -(hydroxy methyl) ethyl ester
(4)	Octadecanoic acid, hexadecylester
(5)	Hexadecanoic acid, 2-hydroxy-1, 3-propanediyl ester (TAG)
(6)	Hexadecanoic acid (TAG)
(7)	Tripalmitin (TAG)

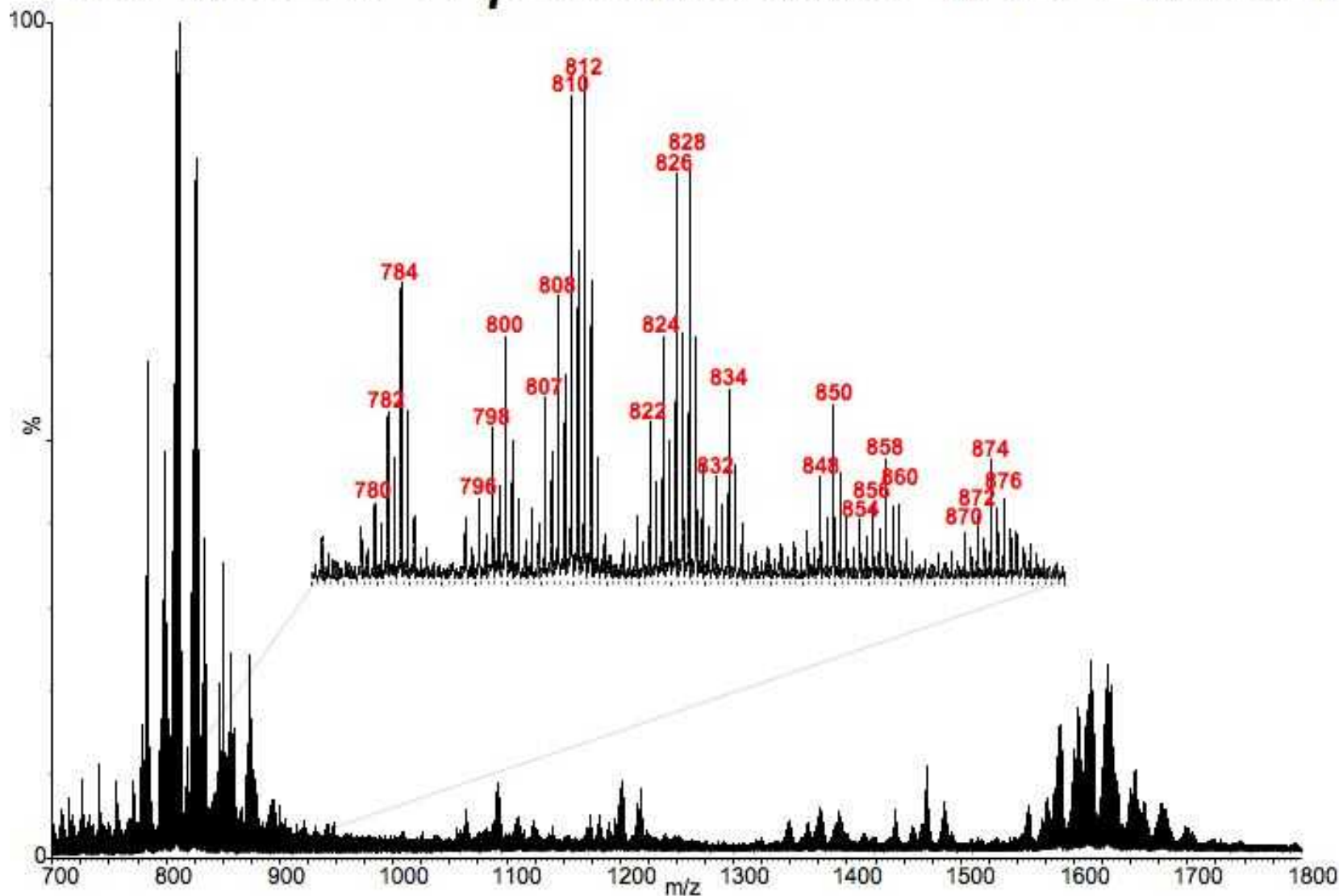


(1)	26,26-Dimethyl-5, 24(28)-ergostadien-3.beta
(2)	Oxalic acid
(3)	Trimyristin (TAG)
(4)	Hexadecanoic acid, 2-[(1-oxotetradecyl)-1, 3-propanediyl ester (TAG)
(5)	Hexadecanoic acid (TAG)
(6)	Tripalmitin (TAG)

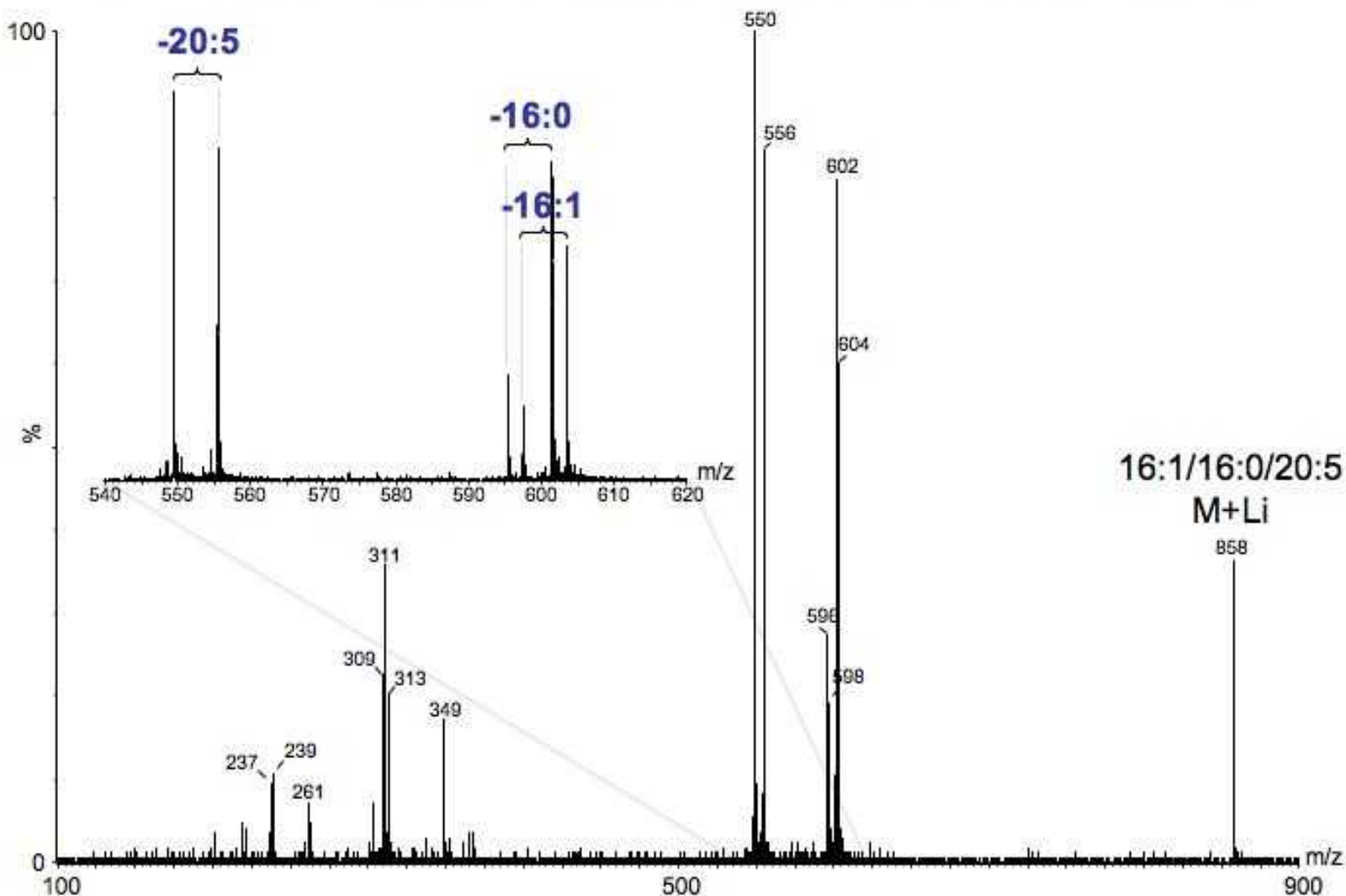


(1)	n-Hexadecanoic acid
(2)	4,7,10,13,16,19-Docosahexaenoic acid
(3)	Octadecanoic acid
(4)	Dodecanoic acid
(5)	Hexadecanoic acid (TAG)
(6)	Trimyristin (TAG)
(7)	Hexadecanoic acid (TAG)
(8)	Tripalmitin (TAG)

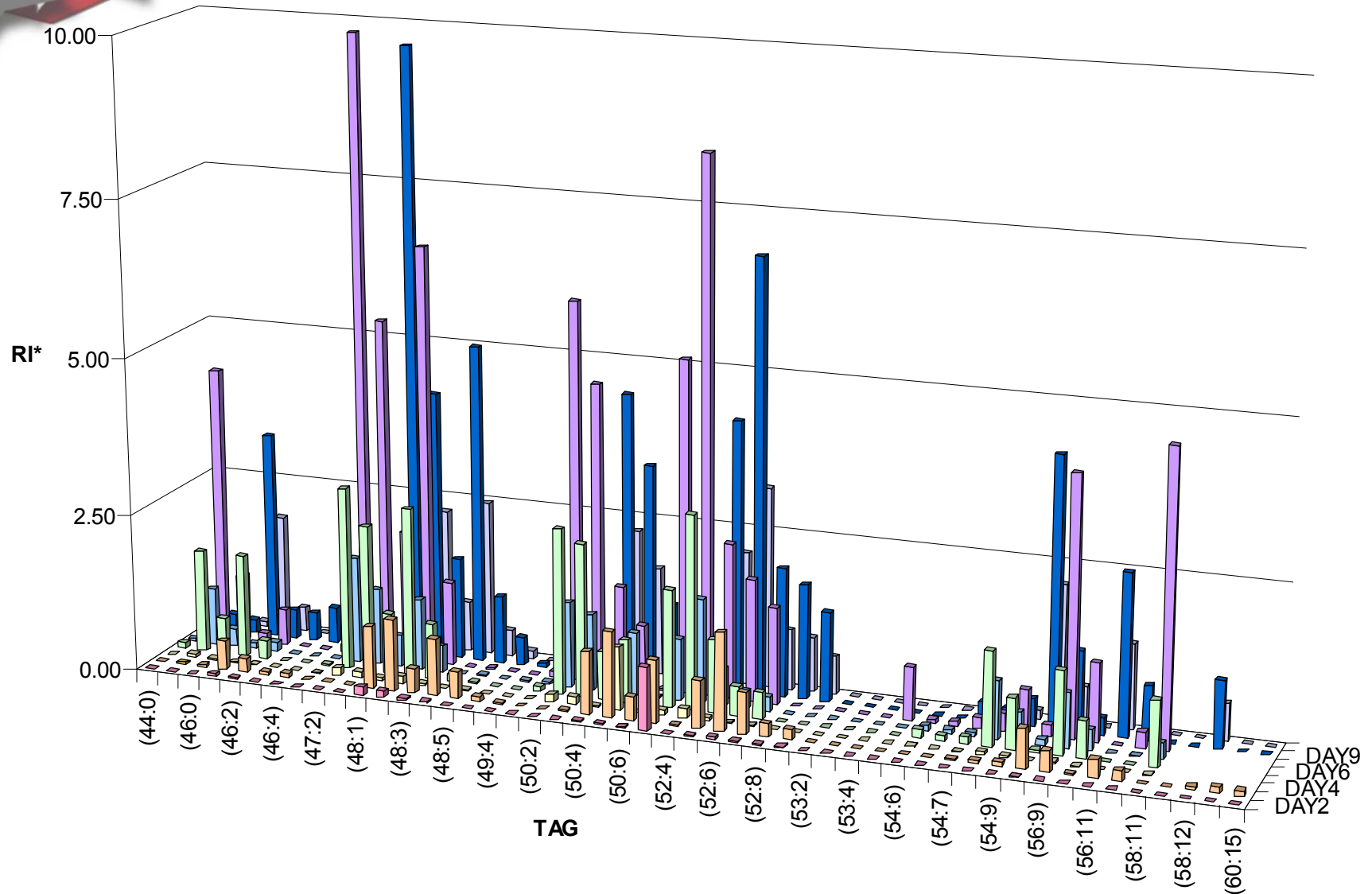
ESI-MS of *T. pseudonana* TAG Extract



MS/MS Determines TAG Structure

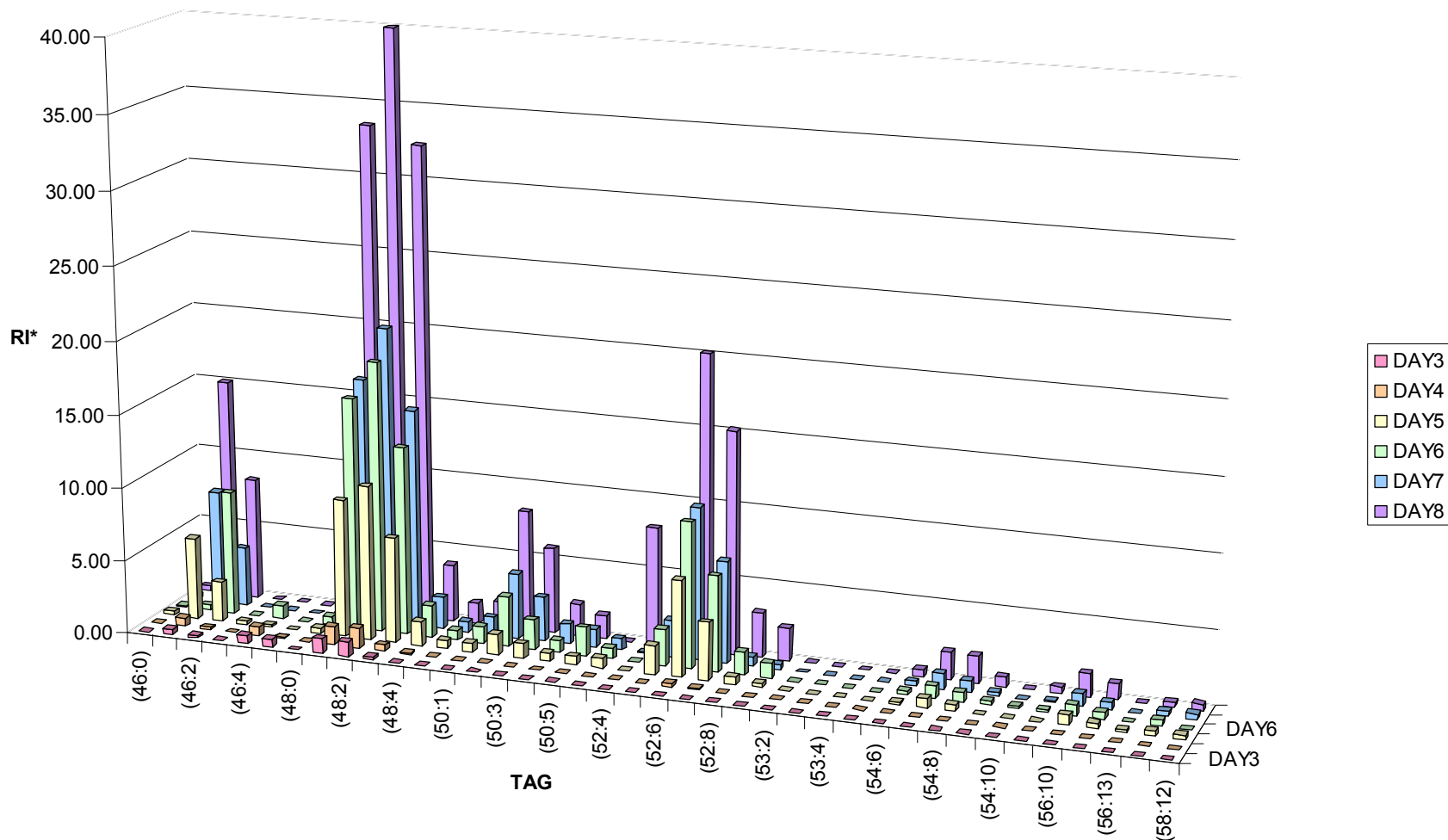


TAG profile of nitrate starved *T. pseudonana*



- * TAG intensity ratios relative to internal TAG (51:3) standard
- time points taken from mid-log (032708) till 040408

TAG profile of nitrate starved *P. tricornutum*



- * TAG intensity ratios relative to internal TAG (51:3) standard
- time points taken from mid-log (DAY3) till DAY8

Determination of acyl chains

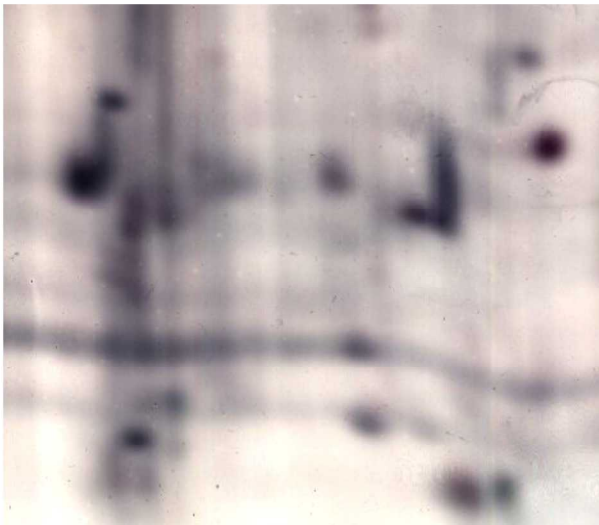
TAG/FA	14:0	14:1	16:0	16:1	16:2	18:2	18:3	18:4	20:4	20:5	22:6
(46:4)											
(46:3)											
(46:2)											
(48:5)											
(48:3)											
(48:2)											
(50:6)											
(52:7)											
(52:6)											
(54:10)											
(56:12)											
(56:11)											
(56:10)											
(58:12)											
(64:17)											

Partial TAG profile of *T.pseudonana*. Fatty acid composition of TAGs were confirmed by tandem mass spectrometry.

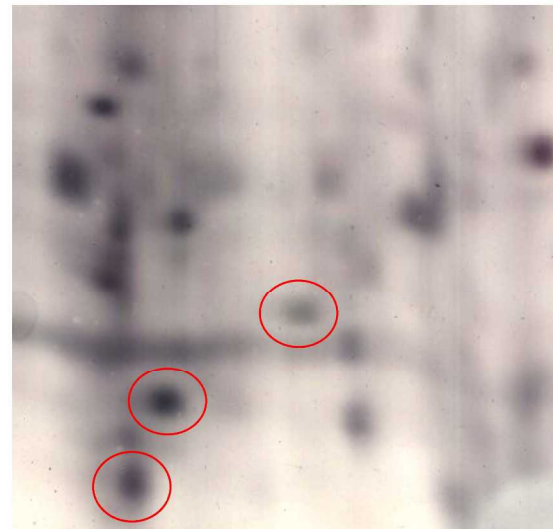
* Order of branching is unknown, position of unsaturation is unknown.

Proteomic analysis of the Si trigger in *T. pseudonana*

Nutrient replete



Si starved

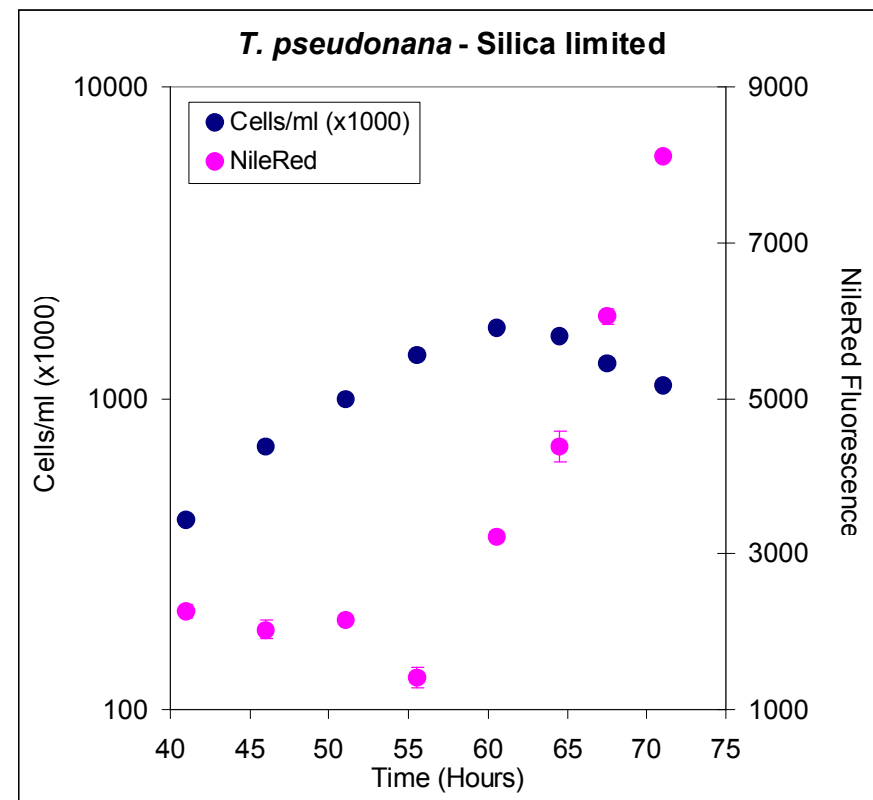
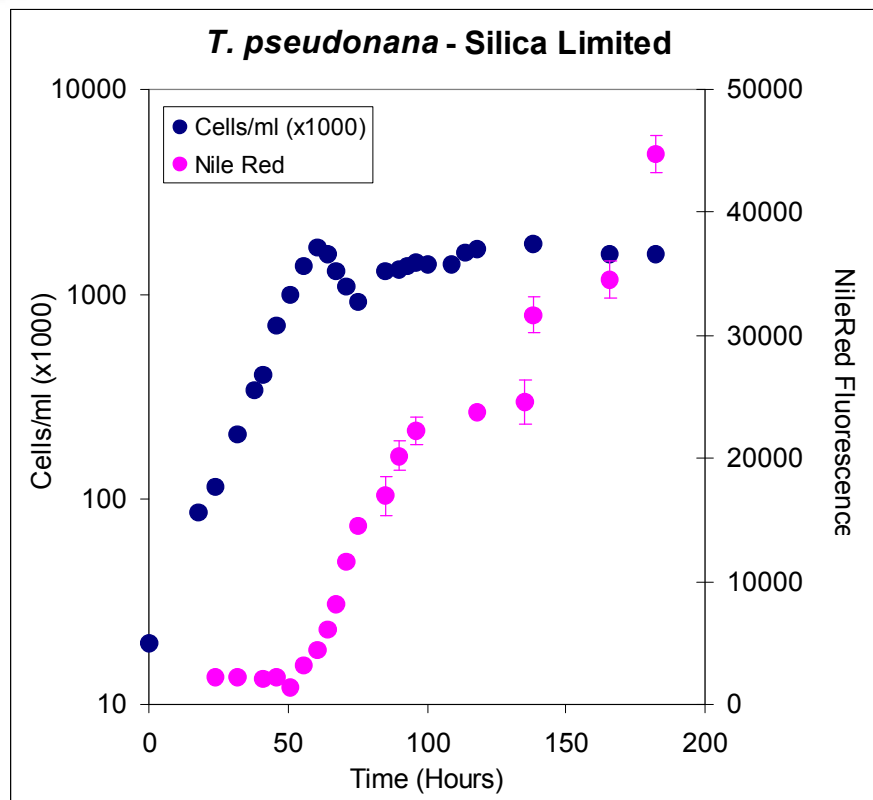




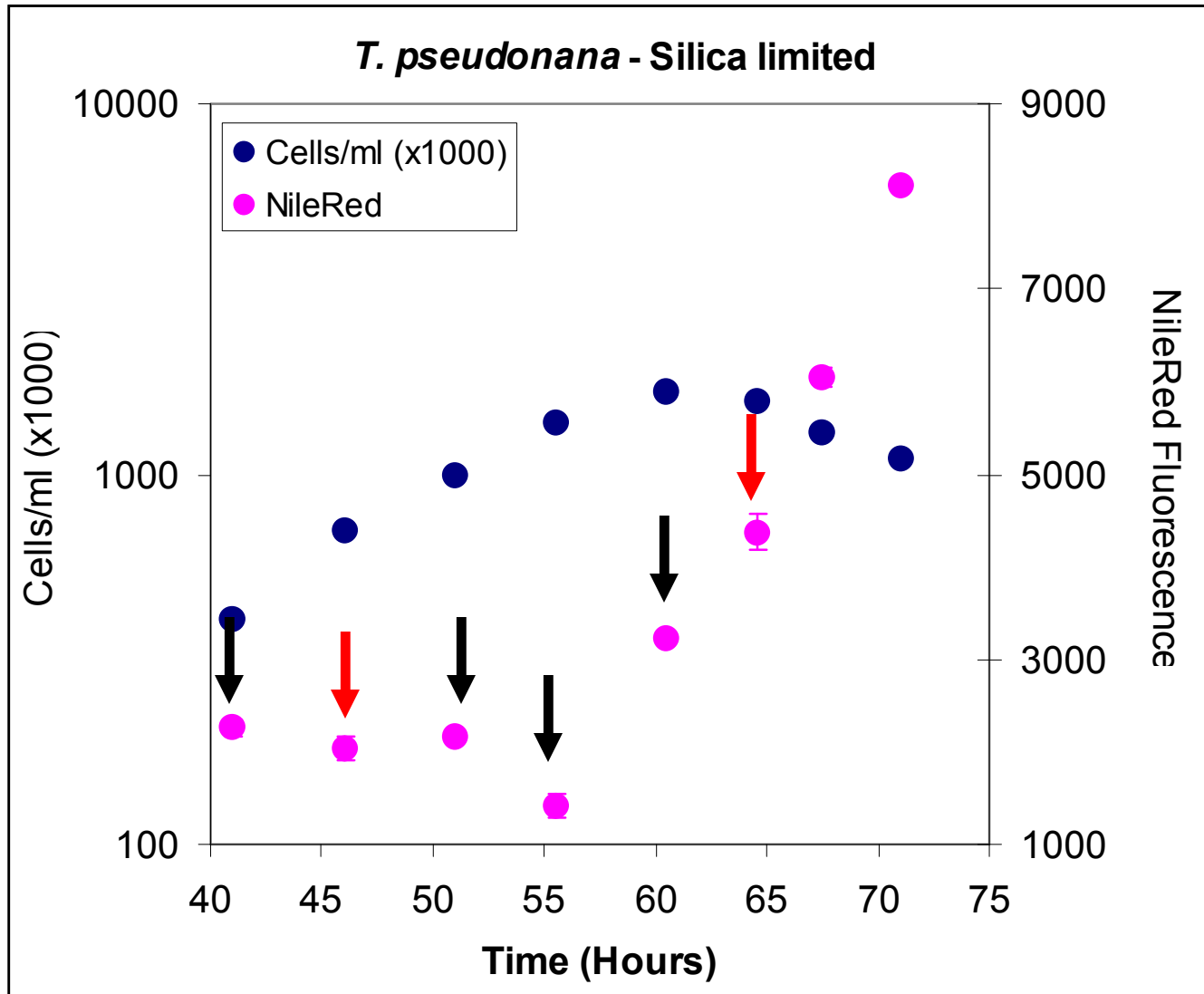
Summary of Proteomic analysis

- **Compared nutrient replete cells silicate and nitrate starved.**
- **2D-PAGE followed by global analysis**
- **12 upregulated in nitrate starvation only**
- **15 up regulated in silicate starvation only**
- **56 up regulated under both silica and nitrate starvation.**
- **Excised spots from gels sequenced by MS**

Increase in TAG accumulation occurs early in stationary phase



Timecourse of transcriptional profile.





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