Biomass and Bioproducts from Nannochloropsis sp.
Nannochloropsis as a candidate first-generation biofuel production organism

- *Nannochloropsis salina*
  - CCMP Strain 1776
- Lipid Biochemistry:
  - Assay development,
  - Biomass and algal oil content management
  - Polar lipid identification
  - Single cell imaging (SNL)
Deep Divergence Between Various Phytoplankton

- Green plants (green algae, including prasinophytes, and land plants)
- Rhodophyta (red algae)
- Chlorophyta (microalgae with uniquely cyanobacterium chloroplasts, e.g., Cyanophora)
- Animals (Metazoa)
  - Cnidaria (coelenterates, e.g., sea anemones, jellyfish)
  - Placozoa (stem of metazoan phylogeny)
- Ichthyosporea
- Fungi (mushrooms, sac fungi, yeasts, molds, rusts, smuts, etc.)
- Nucleomycetes (filose aneaeoba, e.g., Nuclearia)
- Eumycota (ascomycetes and basidiomycetes)
- Archancocyla (e.g., Entamoeba)
- Lobosea (obese aneaeoba, e.g., Amoeba, Chaos and Diffugia)
- Cercozoa (amoebabellates, e.g., Euglyphida, chlorarachniophytes)
- Foraminifera (complex cells with reticulopodia and a test shell)
- Radiolarians (polyistines and acantharia)
- Alveolata (ciliates, e.g., paramecia and dinoflagellates)
- Stramenopiles (stramenopiles, chlorophytes, brown algae, chrysophytes and relatives)
- Haptophyta (haptophytes, haptosphaerophytes, e.g., coccolithophores)
- Psuedomonadales (e.g., Prototrophs, e.g., Eukaryotes)
- Tetrads (a clade of heterotrophic flagellates)
- Cryptophyta (microalgae with a plastid, e.g., Cryptomonas)
- Katablepharids (heterotrophic, biflagellate, e.g., Tatalapharids)
- Nanoyzooa (euglenids, dinoflagellates, and kinetoplastids, e.g., Euglena and Trypanosomes)
- Heterolobosea (amoeboflagellates with microsporidian kinetoplast)
- Jakobida (free-living, heterotrophic flagellates)
- Parabasaloidea (trichomonads and hypermastigotes, e.g., Trichomonads and Trichonympha)
- Fornicta (dpinoflagellates and retortamonads, e.g., Giardia and Chilomastix)
- Preaxecytlya (oxymonads + Trinastix)

± Protists of uncertain placement
How Will Production Strains Compete with Predators / Pathogens?

Ciliates, Rotifers
Heterotrophic flagellates
Chytrid fungi
Bacteria, Viruses
Other algae: green, yellow, diatoms, cyanobacteria
Two frequently quoted conclusions from the DOE Aquatic Species Report are:

i) local strains will overtake introduced strains and

ii) Southeast New Mexico is Too Cold for Growing Algae

Not necessarily so…
Nitrate and Bicarbonate Levels Alter Lipid Content in Nannochloropsis

Marijn de Jong
Jan Winkler
Isaac Rhodes
Excitation and Emission Data for Nile Red in Triacylglycerol and Phospholipids.
Nile Red Assay

1. Samples counted on Flow Cytometer and diluted to 200 cells per microliter
2. Frozen at -80C and stored for assay when convenient
3. Thawed and incubated with Nile Red reagent for 30 minutes
4. Count >5,000 events and capture the mean yellow-channel fluorescence
Lipid accumulation is a stationary phase response in *Nannochloropsis* sp.

Growth of *Nannochloropsis* in high/low nitrate and bicarbonate (top panel)

Lipid content determination by Nile Red Fluorescence in the same cultures as above (bottom panel)

New Mexico State University
Linear increase in total lipid determined gravimetrically after growth in 1 mM nitrate + 5 mM NaCO₃

\[ R^2 = 0.9977 \]
Correlation of gravimetric total lipid and NR dependent fluorescent shift plotted semi-log.

\[ R^2 = 0.9828 \]
Total extractable Lipids were Fractionated on Silica Column

**Eluent Series**

1. Chloroform  
2. Acetone  
3. and Methanol

Increasing Polarity

- Carotenoids
- TAGs
- Chlorophyll and Polar lipids
TAG and Carotenoid Levels Peak at 21 Days

1 mM nitrate, 5 mM bicarbonate treatment;

<table>
<thead>
<tr>
<th>Day</th>
<th>CHCl3</th>
<th>Acetone</th>
<th>Methanol</th>
</tr>
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<tbody>
<tr>
<td>14</td>
<td>3.1</td>
<td>6.8</td>
<td>7.3</td>
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<tr>
<td></td>
<td>18.0%</td>
<td>39.5%</td>
<td>42.4%</td>
</tr>
<tr>
<td>21</td>
<td>28.8</td>
<td>17.6</td>
<td>8.9</td>
</tr>
<tr>
<td></td>
<td>52.1%</td>
<td>31.8%</td>
<td>16.1%</td>
</tr>
<tr>
<td>35</td>
<td>20.7</td>
<td>19.8</td>
<td>10.9</td>
</tr>
<tr>
<td></td>
<td>40.3%</td>
<td>38.5%</td>
<td>21.2%</td>
</tr>
</tbody>
</table>
# Polar Lipid FA Composition

Field – grown *Nannochloropsis*

<table>
<thead>
<tr>
<th>Fatty Acid as Methyl Ester</th>
<th>Percent composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>6%</td>
</tr>
<tr>
<td>C16:0</td>
<td>28%</td>
</tr>
<tr>
<td>C16:1</td>
<td>26%</td>
</tr>
<tr>
<td>C18:1</td>
<td>8%</td>
</tr>
<tr>
<td>C20:5</td>
<td>32%</td>
</tr>
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</table>
Neutral Lipid FA Composition Field – grown *Nannochloropsis*

<table>
<thead>
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<th>Fatty Acid as Methyl Ester</th>
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</tr>
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<tr>
<td>C16:0</td>
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</tr>
<tr>
<td>C18:0</td>
<td>6%</td>
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<tr>
<td>C18:1</td>
<td>21%</td>
</tr>
<tr>
<td>C20:5</td>
<td>8%</td>
</tr>
</tbody>
</table>
Ion Cyclotron Resonance Mass Spectrometry
N1O7 = probable betaine lipids
O6 = Triacylglycerols

Hetero-atom composition of major peaks in positive ion mode Ion-Cyclotron-Resonance (ICR) Mass Spectrometry
Betaine Lipids

Phosphatidyl Choline versus DiacylGlycerylTrimethylhomoSerine

O6 Type Distribution

Relative Abundance

Double Bond Equivalents

Day 1
Day 7
Day 14
Day 21

New Mexico State University
Next Stop: SOLIX Biofuels
Two Testbeds:

Fort Collins – 6,000 Liter system and ~1.5 Kg/day dry weight algae biomass with CO2 enrichment

Coyote Gulch – 120,000 Liters in three basins, CO2 from amine plant

Test Strain: *Nannochloropsis*
Acknowledgements

- NMSU P.I.s
  - Lammers - Chemistry & Biochemistry
  - Van Voorhies - Molecular Biology
  - Boeing - Fisheries and Wildlife
  - Tanner Schaub, ICR-MS

- Center of Excellence for Hazardous Materials Management – Carlsbad, NM

- Sandia National Lab
  - Grant Heffelfinger
  - Howland Jones
  - Seema Singh

- Funding: State of New Mexico Energy Innovation Fund, Sandia National Lab (LDRD), Gift from Sapphire Energy