Radioisotopes in Plant Science: Meeting Global Challenges in Sustainability...

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“Nuclear science is entering a new era of discovery in understanding how nature works at the most basic level and in applying that knowledge in useful ways.” (RISAC, 2007)

Major Science Drivers for Rare Isotope Beams

1. nuclear structure
2. astrophysics
3. fundamental symmetries
4. other applications

- stockpile stewardship
- material science
- medical research
- reactor research
"To avoid catastrophic environmental change, humanity must stay within defined planetary boundaries for a range of Earth-system processes." Johan Rockström (Director Stockholm Resilience Center), *Nature* 2009.

We must redefine science drivers to be relevant to society!
Outline

1. Radioisotopes in plant science: then and now...

2. A look into the future: the role rare isotopes can play in plant science...
Plants are model organisms for studying life processes using tracer technology...

- CO₂
- H₂O
- Nitrogen
- Micronutrients
- Organics
- Water transport through xylem and phloem
- Source tissue (leaf cell) and sink tissue (root cell)
Radiotracers in plant biology--then and now...

Martin Kamen at the LBL 37” cyclotron in 1938 — 1995 Fermi Award.

Melvin Calvin—1961 Nobel Prize in Chemistry

Martin Gibbs — BNL 1949-1957
Beyond Genomics...

- genomics reveals that plant metabolism and its control are complex and poorly understood
- ~50% of genes encode unknown enzymes
- understanding basic metabolism and its control is crucial to efforts in biotechnology to ‘improve’ energy crop growth via metabolic engineering

Arabidopsis thaliana

Black Cottonwood (Populus trichocarpa)
DOE Joint Genome Institute
U.S. Department of Energy Office of Science Genomics:GTl Program

Multiscale explorations for systems understanding

Gain a predictive understanding of how cells work in communities, tissues, and plants and, ultimately in global ecosystems.

Explore the functioning and regulation of pathways and dynamic networks in cells.

Understand how proteins function individually or in interactions with other cellular components.

The genome determines dynamic biological structure and function at all scales, from genes to ecosystems.

Understanding fundamental life processes requires investigations that reach across multiple levels, from the information encoded in individual genomes to the functioning of cells as communities and plants in an ecosystem.
Energy sustainability through plant biomass...

- Yearly net CO₂ fixation by land plants is 56 B tons amounting to roughly 170 B tons of plant biomass world-wide.

- 70% of all biomass is estimated to be comprised of cell-wall macromolecules.
Major Challenges

Cellulosic Biomass

Develop crops dedicated to biofuel production

Deconstruction microbes

Improve enzymes and microbes that break down biomass into sugars

Deconstruction enzymes

Sugars

Fermentation microbes

Optimize microbes that convert sugars into biofuels

Biofuels
Making Energy Costs Energy…

Fossil Energy Ratio = \frac{\text{Energy Delivered to Customer}}{\text{Fossil Energy Used}}

Projected

10.3

Current

1.36

0.54

0.34

0.81

0.45

Cellulosic Ethanol

Corn Ethanol

Soybean Biodiesel

Microalgal Biodiesel

Fossil Fuel Gasoline

Fossil Fuel Electricity

Brookhaven Science Associates
Burning Questions…

1. How do we minimize biomass nitrogen content?

2. Can plants be induced to grow in a shorter life-cycle?

Miscanthus – a hybrid grass that can grow 13 feet high in a season – each 1200 lb. bale potentially can yield 48 gal. of ethanol.
Positron emitting radioisotopes

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Half-life</th>
</tr>
</thead>
<tbody>
<tr>
<td>carbon-11</td>
<td>20.4 min</td>
</tr>
<tr>
<td>nitrogen-13</td>
<td>10 min</td>
</tr>
<tr>
<td>oxygen-15</td>
<td>2 min</td>
</tr>
<tr>
<td>fluorine-18</td>
<td>110 min</td>
</tr>
</tbody>
</table>

Using elements of life...
BNL Plant Radiotracer Program

- Plant sugars: $^{11}\text{CO}_2$
- Nitrogen input: $(^{13}\text{NO}_3^-, ^{13}\text{NH}_3, ^{13}\text{N}_2)$
- Plant hydraulics: $\text{H}_2^{15}\text{O}, ^{18}\text{F}^-$

Radiolabel Biomolecules

Radiolabeled Inorganic Substrates

- Plant sugars: $^{11}\text{CO}_2$
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Imaging Instrumentation

from Shoots-to-Roots

from Roots-to-Rhizosphere

from Roots-to-Shoots
BNL Plant Radiotracer Laboratory

Standardization of technology lends itself to reproducibility of data...
New strategies for using PET imaging in plants...

High-Throughput Screening: targeting quantitative PET imaging of roots. Opportunities to couple with GFP root array measuring gene expression.
UPBEAT1 gene
• negatively controls meristem size
• controls cell number of elongation zone
• differentially expressed at distal meristem region

Brady et al. Science 2007
Metabolomic Tools
in the perfect world...
in the not so perfect world...

Arabidopsis thaliana metabolic network

Clearly influx and efflux are not constants in the real world of a living plant...

**Challenge**: Can radioisotopes be used to trace metabolic flux under non steady-state conditions?

**Question**: How important is isotopic fractionation?
Use of short-lived $^{11}\text{C}$ reveals new insights into non steady-state metabolic reprogramming of aromatic amino acids during defense induction.

Evidence for post-transcriptional regulation of the phenylpropanoid pathway and cell-wall lignin biosynthesis.

Geoscientists have been using kinetic isotope effects involving nontraditional stable isotopes for decades in the research of:

- hydrology
- sedimentology
- geochemistry
- oceanography

Deciphering causes for isotopic fractionation in nature is difficult enough when one has to factor in for just mass-dependent kinetic isotope effects. However, with biological processes mass-independent kinetic isotope effects can manifest due to differences in nuclear spins and magnetic moments of nuclei which can impact biological processes.
Carbon isotope fractionation in biological systems

Why are heterotrophic tissues generally $^{13}$C-enriched compared with leaves?

- Nier and Gulbransen (1939) JACS 61: 697-698.

More specifically, pine exhibited less isotopic fractionation between tissues than aspen.
Why are heterotrophic tissues generally $^{13}$C-enriched compared with leaves?

One theory suggests that cell respiration at night favors the heavier isotope.

In the data shown on the previous slide pine needles will have a lower stomatal density than the broad leaf aspen, and therefore, isotopic fractionation will be less pronounced between tissues of pine than aspen.
Tracer studies in Maize reveal that respiration favors the lighter isotope...

A faster rate of CO₂ emission of the lighter isotope may be a reflection of the mass-dependent kinetic isotope effect from stomatal conductance. We all see delays in the onset of the emission of the heavier isotopes after the plant is placed into darkness which may be a reflection of mass-independent magnetic isotope effects involving enzyme actions. (Ferrieri, unpublished).
Use of $^{13}$N has lead to the discovery of a non-photorespiratory pathway that enables nitrogen mobilization when plants become stressed.

Isotopes of Carbon and Nitrogen

Extending the isotope portfolio of carbon and nitrogen can provide new insight into how enzymes function in biological systems?
Micronutrients also play essential roles in plant growth...

Function of some biologically relevant metals

- Ca$^{+2}$ stabilizes cell walls and acts as a counterion ($^{44}\text{Ca}/^{40}\text{Ca}$)
- Zn$^{+2}$ is an important cofactor of enzymes ($^{66}\text{Zn}/^{64}\text{Zn}$)
- Mg$^{+2}$ is important in enzyme activation ($^{26}\text{Mg}/^{25}\text{Mg}$)
- Fe$^{+2/+3}$ is essential for Fe-Sulfur & heme protein synthesis ($^{56}\text{Fe}/^{54}\text{Fe}$)
Fractionation of Metal Isotopes by Higher Plants

The use of rare isotopes can push the frontier of biological science to a new level of understanding enzyme function and control that can have major implications to facing today's global challenges in sustainability.

“Plants are our future...”

Roger Beachy--NIFA Director

(ACS 2009 Press Conference)