Why small molecules can change the world in big ways?

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March 7th, 2015
Unlocking the chemical mysteries in life
http://wenglab.wi.mit.edu/
A single organism contains tens to hundreds of thousands of compounds in its metabolome!
What are small molecules?

- Low molecular weight
Ethanol
Molecular Formula: $\text{C}_2\text{H}_6\text{O}$
Molecular Weight: 46.06844 g/mol

Polytheonamide A
Molecular Formula: $\text{C}_{219}\text{H}_{376}\text{N}_{60}\text{O}_{72}\text{S}$
Molecular Weight: 5033.75254 g/mol

What are small molecules?

- Low molecular weight
- Regulate biological processes
Acetylcholine
MF: C$_7$H$_{16}$NO$_2$\(^+\)
MW: 146.20744 g/mol

D-glucose
MF: C$_6$H$_{12}$O$_6$
MW: 180.155880 g/mol

Terpineol
MF: C$_{10}$H$_{18}$O
MW: 154.24932 g/mol
What are small molecules?

- Low molecular weight
- Regulate biological processes
- Small molecules are important to us.
The uses of natural products by humans
How small are small molecules?

Cut in half

10 cm
(1 x 10^{-2} m)
Cells

Cut in half 8 times

100 µm (1 x 10^-4 m)
Chromosomes

Cut in half 11 times
Ribosome

Cut in half 20 times
Protein

Cut in half 22 times

60 Å
(6 x 10^{-9} m)
syn-Propanethial-S-oxide

Cut in half 24 times

6 Å
(6 x 10^{-10} m)
Goat’s rue: a successful story from medicinal plant to first-line anti-diabetic drug

- Used in medieval Europe as an herbal medicine

\[
\begin{align*}
\text{Goat’s rue (Galega officinalis)} \\
\text{Nat. size} \\
\text{PL. 66}
\end{align*}
\]

- Galagaine identified by Tanret in 1914

- Galagaine failed in clinical trials during 1920s and 1930s

- Intensive studies on goat’s rue due to its toxicity to livestocks

\[
\begin{align*}
\text{Phenformin (1957-1978)} \\
\text{Metformin (1958-present)}
\end{align*}
\]

Tim Fallon and Fu-Shuang Li
Liquid Chromatography Mass Spectrometry (LC/MS) analysis of *Galega officinalis* leaf extract

Galegine (0.3% dry weight)

*Galega officinalis*  
*Schoenus nigricans*  
*Verbensina enceledioies*  
*Biebersteinia heterostemon*
Liquid Chromatography Mass Spectrometry (LC/MS) analysis of *Galega officinalis* leaf extract

- **Galegine** (0.3% dry weight)
- **Peganine** (0.1% dry weight)

*Adhatoda vasica*  
*Peganum harmala*

*Galega officinalis*
Small molecules: Biology's 'dark matter'
How do plants make their pigments?
### Mendel’s genetic experiments on plant flavonoid biosynthesis

<table>
<thead>
<tr>
<th>Character</th>
<th>Dominant Trait</th>
<th>×</th>
<th>Recessive Trait</th>
<th>F&lt;sub&gt;2&lt;/sub&gt; Generation</th>
<th>Ratio</th>
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</thead>
<tbody>
<tr>
<td>Flower color</td>
<td>Purple</td>
<td>×</td>
<td>White</td>
<td>705:224</td>
<td>3.15:1</td>
</tr>
<tr>
<td>Flower position</td>
<td>Axial</td>
<td>×</td>
<td>Terminal</td>
<td>651:207</td>
<td>3.14:1</td>
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<tr>
<td>Seed color</td>
<td>Yellow</td>
<td>×</td>
<td>Green</td>
<td>6022:2001</td>
<td>3.01:1</td>
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<tr>
<td>Seed shape</td>
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<td>×</td>
<td>Wrinkled</td>
<td>5474:1850</td>
<td>2.96:1</td>
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<tr>
<td>Pod shape</td>
<td>Inflated</td>
<td>×</td>
<td>Constricted</td>
<td>882:299</td>
<td>2.95:1</td>
</tr>
<tr>
<td>Pod color</td>
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<td>×</td>
<td>Yellow</td>
<td>428:152</td>
<td>2.82:1</td>
</tr>
<tr>
<td>Stem length</td>
<td>Tall</td>
<td>×</td>
<td>Dwarf</td>
<td>787:277</td>
<td>2.84:1</td>
</tr>
</tbody>
</table>

- **Anthocyanins**: Glc-O-[O-Glc]-O-Glc-O-Rha
- **Stilbene**: HO-\(\text{CH}_2\)-HO
- **Isoflavone**: HO-\(\text{CH}_2\)-\(\text{CH}_2\)-HO
- **Flavone**: HO-\(\text{CH}_2\)-\(\text{CH}_2\)-\(\text{CH}_2\)-OH
- **Condensed tannins**: HO-\(\text{CH}_2\)-\(\text{CH}_2\)-\(\text{CH}_2\)-OH

**Gregor Mendel** (1822–1884)
Molecular genetics studies using the model plant
Arabidopsis thaliana
How did complex metabolic pathways evolve?

Charles Darwin (1809 –1882)

Phenylpropanoids

4-coumaroyl-CoA

3x Malonyl-CoA

CHS

tt4

Chalcone

CHI

tt5

Flavanone

F3H

tt6

F3′H

tt7

Dihydroflavonols

FLS

Flavonols

GT

FLAVONOL GLYCOSIDES

(yellow)

DFR

tt3

Leucoanthocyanidins

(tt2, tt8, ttg1)

LDOX

tt18/tds4

Anthocyanidins

(tt16, tt1)

ANR

ban

Epicatechin

CE ?

condensation

PROCyanIDINS (condensed tannins)

(colorless)

Oxidized Procyanidins

(brown)

Oxidation

tt10 ?

PPO?

POD ?
Chalcone synthase (CHS) evolved from ancestral fatty acid biosynthetic enzymes.
The assembly of specialized metabolic pathways by recruitment of decedents of radiating enzyme families

boundary between primary and specialized metabolism

Weng et al, Science (2012)
Metabolic engineering of natural products: the next green revolution

GM purple tomato

Graph showing lifespan comparison:
- Standard diet: 142.0 ± 8.7 d average lifespan, 211 maximum (n = 24)
- Red tomato: 145.9 ± 12.6 d average lifespan, 213 maximum (n = 15)
- Purple tomato: 182.2 ± 8.6 d average lifespan, 260 maximum (n = 20)
Firefly luciferin biosynthesis: towards an autoluminescent system

Photinus pyralis

Adult male

Larva

Fu-Shuang Li

Tim Fallon

Firefly larvae hunting in Concord, MA last October

Okayama, Japan, 2008
A brief history of the firefly luciferin-luciferase system

- Firefly luciferin was purified from ~15,000 firefly lanterns in 1949, and crystalized in 1957, and its structure resolved in 1961 by William D. McElroy.

  ![D-(-)-Luciferin](image1.png)

- The gene encoding firefly luciferase was cloned from *Photinus pyralis* by Marlene DeLuca in 1985. Widely used as a reporter gene ever since.

- The first crystal structure of firefly luciferase resolved in 1996. Photochemical mechanism heavily studied.
Glowing Plants: Natural Lighting with no Electricity

by Antony Evans

Create GLOWING PLANTS using synthetic biology and Genome Compiler's software - the first step in creating sustainable natural lighting

8,433 backers
$484,013 pledged of $65,000 goal
0 seconds to go

Funded!
This project was successfully funded on June 7, 2013.

Antony Evans
First created | 28 backed
glowingplant.com
See full bio Contact me

San Francisco, CA Technology
Firefly luciferase

\[ \text{Oxyluciferin} + \text{ATP}, \text{Mg}^{2+} \rightarrow \text{Oxyluciferin} \text{[excited state]} \rightarrow \text{Oxyluciferin} \text{[ground state]} + \text{CO}_2 + \text{AMP} \]

Primary metabolism

Fatty acid breakdown

Fatty acyl-CoA synthetase

4-coumaroyl-CoA ligase

\[ \text{p-coumaroyl-CoA} \rightarrow \text{Adenylate intermediate} \]

Lignin biosynthesis

bioluminescence

Plant metabolism

Specialized metabolism

\[ \text{Primary metabolism} \quad \rightarrow \quad \text{Fatty acid breakdown} \]

\[ \text{fatty acyl-CoA synthetase} \rightarrow \text{Adenylate intermediate} \rightarrow \text{4-coumaroyl-CoA ligase} \rightarrow \text{Adenylate intermediate} \rightarrow \text{firefly luciferase} \rightarrow \text{bioluminescence} \]
Elucidating firefly luciferin biosynthesis

Early radioactive tracing experiments suggest that luciferin is made from benzoquinone and cysteine in fireflies.
High resolution metabolomics as a tool for deciphering luciferin biosynthesis

Minimal sample prep from lantern

LC/MS analysis
Taming messy chemistry?

Schultz and Wolfram, 1988

\[
\begin{align*}
\text{benzoquinone} & \quad \text{cysteine} \\
\begin{array}{c}
\text{HO} \\
\text{S} \\
\text{N} \\
\text{HO}
\end{array} & \quad \begin{array}{c}
\text{NH}_2 \\
\text{C} \quad \text{O} \\
\text{H}
\end{array}
\end{align*}
\]

Luciferin detected!
Lucibufagins and the peculiar predatory behaviors of *Photuris* fireflies

*Photuris* exuding a fluid that contains lucibufagins

A *Photuris* female feeding on a *Photinus* male

- lucibufagins

+ lucibufagins

Eisner et al., 1997
How to nurture creativity?
I. Follow true interests
II. Intuition and thought experiments