

CHEMICAL APPROACHES TO SPHINGOLIPID RESEARCH

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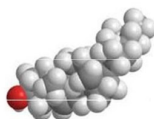
Lipids: structure and functions

Lipids are a large and diverse group of naturally occurring molecules that are related by their solubility in nonpolar organic solvents and general insolubility in water.

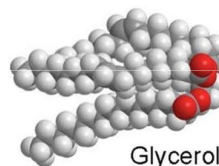
Lipid molecules contain a **hydrophilic region** or (**polar head**), and a hydrophobic region (nonpolar tail).



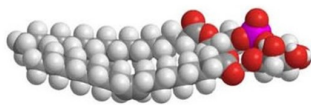
Fatty acyls



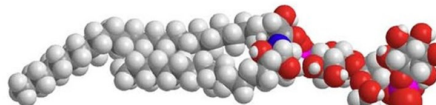
Sterol lipids



Glycerolipids



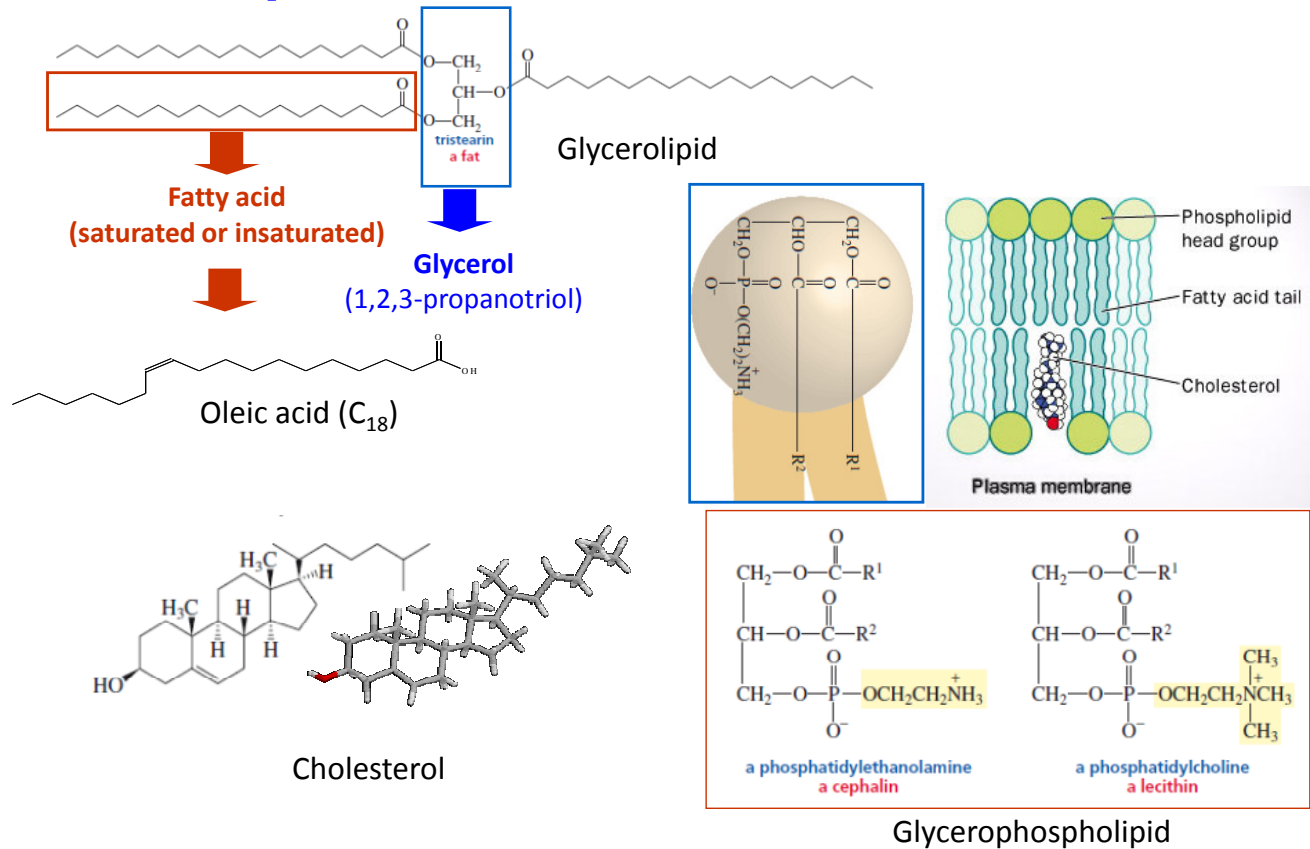
Glycerophospholipids



Sphingolipids

- Functions of lipids** include:
- Structure of biological membranes
 - Energy storage
 - Cell signalling

Lipids: structure and functions



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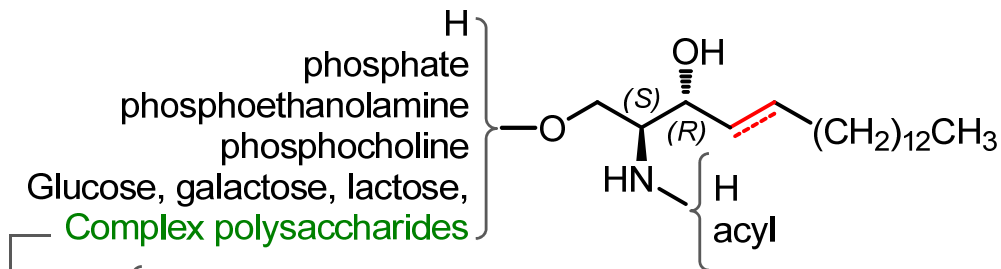
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Sphingolipids

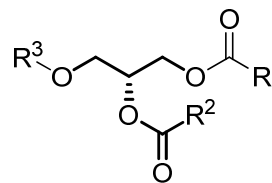
CSIC
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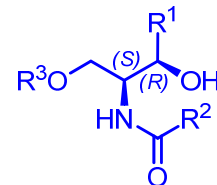
RUBAM



Sulfatides
Lactoseries
Neolactoseries
Globoseries
Ganglioseries



Glycerolipids



Sphingolipids

J. Lipid Res. (2009) 50: S9-S14

J. Lipid Res. (2005) 46: 839-861

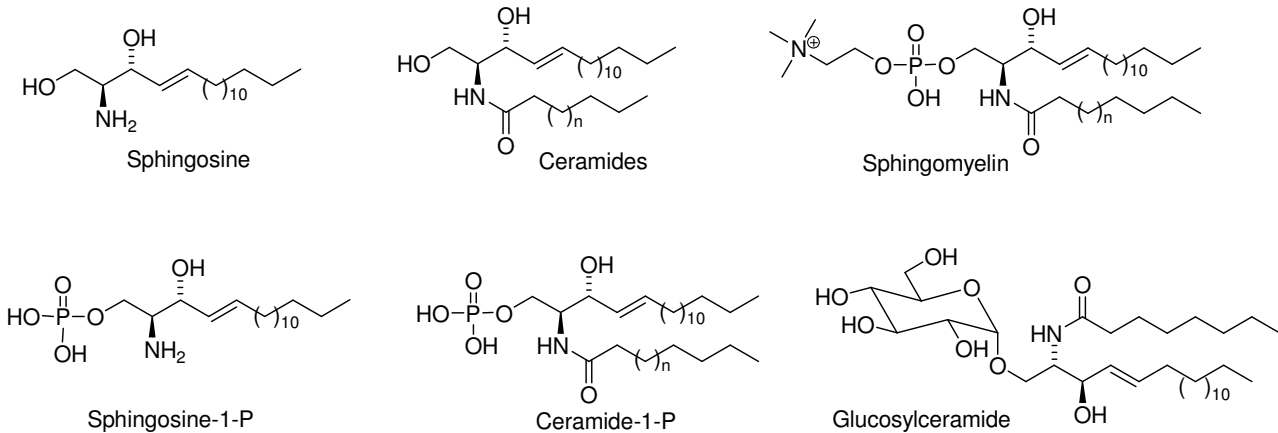
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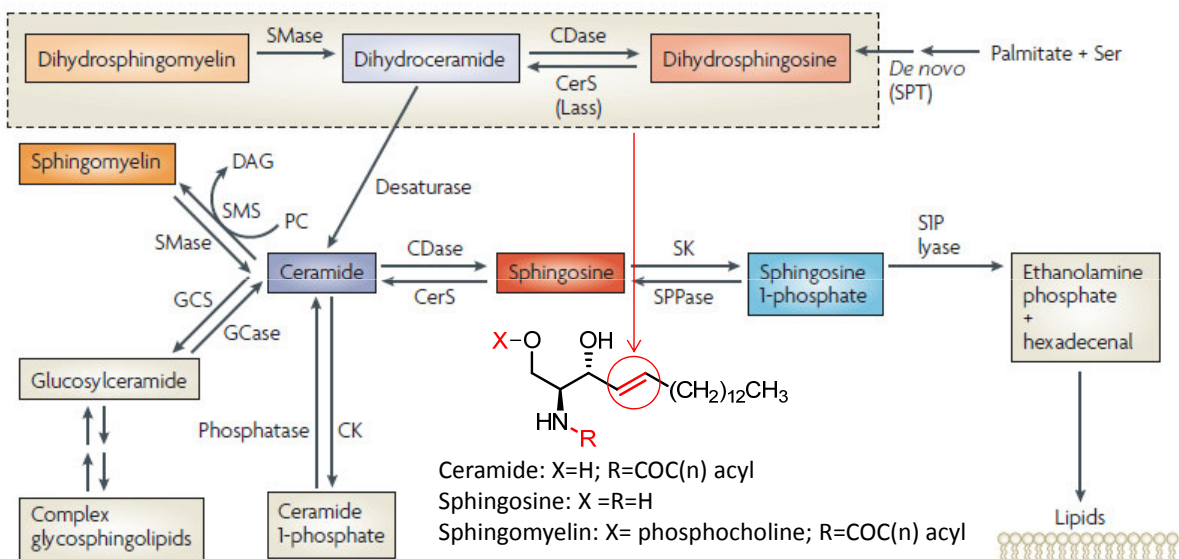
4

<http://www.lipidmaps.org>

Spingolipids are a family of **lipids** that play essential roles both as **structural cell membrane components** and in **cell signalling**. The cellular contents of the various spingolipid species are controlled by **enzymes** involved in their **metabolic pathways**

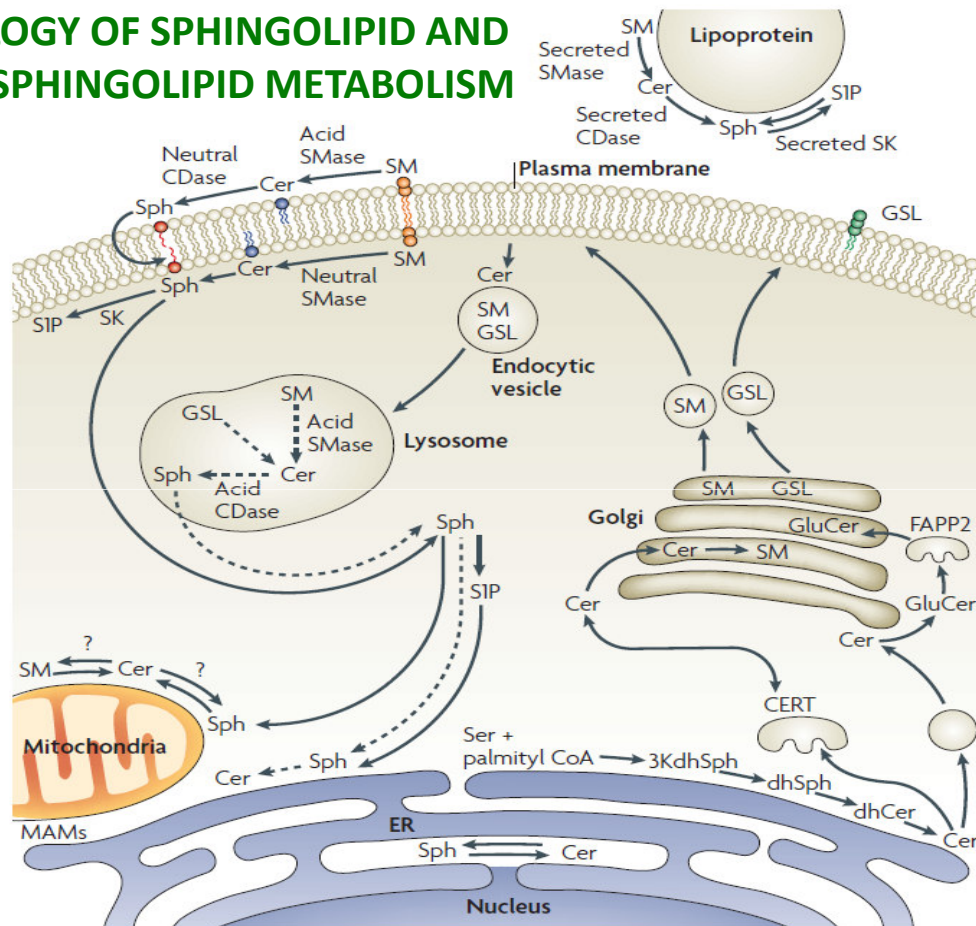


Spingolipid metabolism

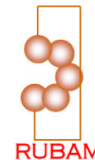


Hannun, Y. A.; Obeid, L. M. *Nat Rev Mol Cell Biol* **2008**, *9*, 139–150.

TOPOLOGY OF SPHINGOLIPID AND GLYCOSPHINGOLIPID METABOLISM



Hannun, Y. A. *et al Nat Rev Mol Cell Biol* 2008, 9, 139-50.



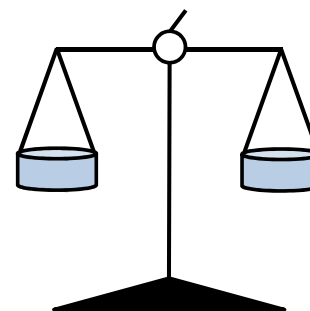
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Sphingolipid and glycosphingolipid functions

Structural role

- | | |
|--------------------------------|--|
| CERAMIDE | Growth inhibition |
| SPHINGOSINE | Cell cycle arrest |
| DIHYDROSPHINGANINE | Cell differentiation |
| | Cell senescence |
| | Apoptosis |
| | Autophagy |
| SPHINGOSINE-1-PHOSPHATE | Cell growth stimulation |
| CERAMIDE-1-PHOSPHATE | Cell proliferation |
| | Angiogenesis |
| | Cell migration |
| | Calcium mobilization |
| | Autophagy |
| GLYCOSPHINGOLIPIDS | Signal transduction |
| | Cell proliferation and differentiation |
| | Cell-cell recognition and adhesion |



SPHINGOLIPIDS AND GLYCOSPHINGOLIPIDS IN DISEASE

- SPHINGOLIPIDOSES
- LIPOTOXIC DISEASES: DIABETES 2
- ATHEROSCLEROSIS
- CANCER
 - MULTIDRUG RESISTANCE
 - METASTASIS
- AIDS
- NEURODEGENERATIVE DISEASES
- SYSTEMIC MYCOSES: FUNGAL METABOLISM

Chemical tools for the study and modulation of sphingolipid metabolism

Sphingolipid analogues
Enzyme inhibitors

Probes to study SL metabolism
Fluorogenic substrates for enzyme activity
Azidosphingolipids (click chemistry)

Chemical tools for the study and modulation of sphingolipid metabolism

Sphingolipid analogues Enzyme inhibitors

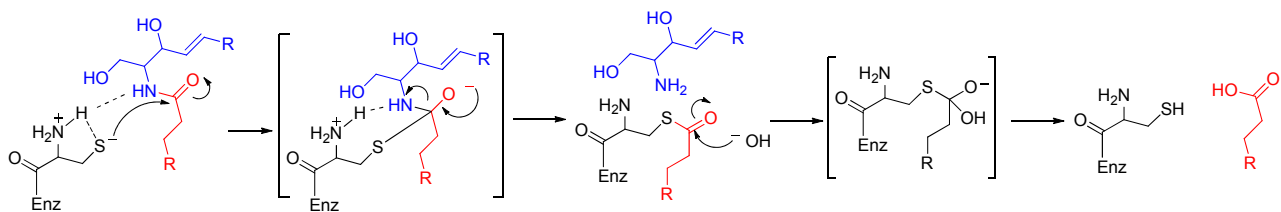
Selected targets

Dihydroceramide desaturase (Des1)

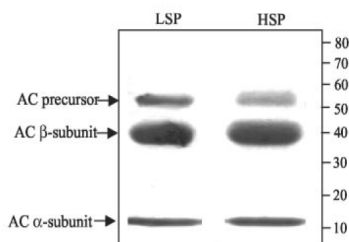
Sphingosine 1P-lyase (S1PL)

Acid ceramidase (ASAH1)

Acid ceramidase is a Cys protease



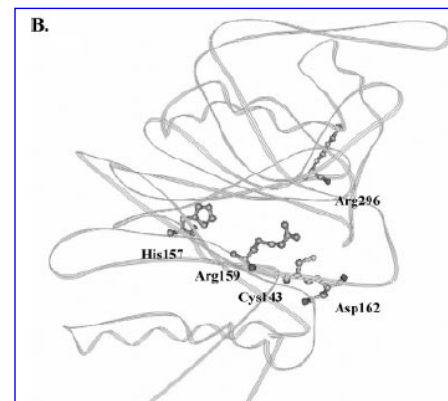
Shtraizent, N. *et al.* Autoproteolytic Cleavage and Activation of Human Acid Ceramidase. *J Biol Chem* **2008**, *283*, 11253–11259.



A.

Cleavage site

Human AC (Q13510)	...YELFTICTSI...
Monkey AC (Q60HH4)	...YEFFTLCTSI...
Mouse AC (Q9WV54)	...YELFTMCTSI...
Human AC -like protein (Q02083)	...YESSVFCTSI...
Mouse AC -like protein (Q9D7V9)	...YEASAFCTSI...
Rat AC -like protein (Q5KTC7)	...YEASAFCTSI...
C elegans AC (O45686)	...YEIFTVCTSV...
C elegans uncharact. prot. (Q09551)	...YEMSRFCTSI...



Acid ceramidase in disease

Table 3. Cancer Types in which a Role for AC has been Reported

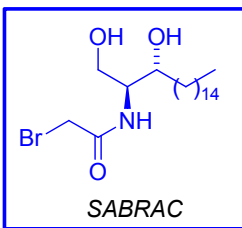
Cancer Type	Cell Lines	References
Prostate	DU145, LnCAP, PC3, PPC1	[52a, 52e, 53]
Breast	Samples from patients	[52c, 58]
Fibrosarcoma (murine)	L929 (parental and sublines)	[59]
Head and neck squamous cell	SCC1	[29]
Liver	HepG2, Hep-3B, SK-Hep, Hepal c1 c7	[56]
T-cell large granular lymphocyte leukemia	Samples from patients	[52b]
Glioblastoma	U-87 MG, U87-W E6	[55]
Melanoma	Samples from patients	[60]

Table 2. Diseases in which a Role for Ceramidases has been Reported

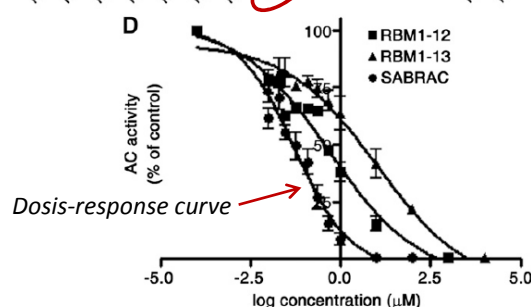
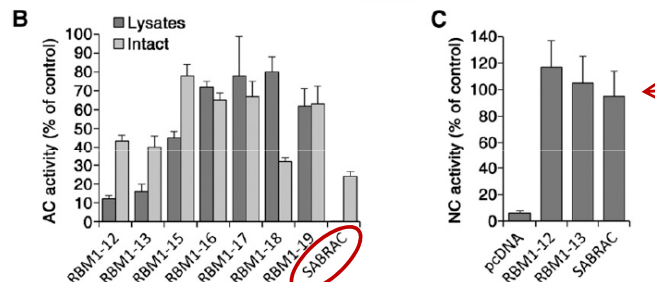
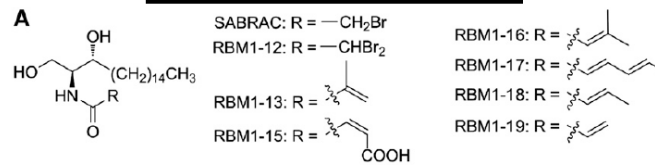
Disease	Ceramidase	Reference
Farber disease	AC	[38]
Atopic dermatitis	bacterial	[39]
Harlequin ichthyosis	n.s.	[40]
Alzheimer's disease	AC	[42]
Cystic fibrosis	AC	[43]
Myocardial ischemia-reperfusion	n.s.	[44]
Diabetic nephropathy	NC	[45]
Type 2 diabetes, insulin resistance and metabolic syndrome	AC	[46b, 46e]
	NC	[46a]
	ACER	[46c]
	n.s.	[46f]
Pulmonary fibrosis	AC	[48]
Retinal degeneration	ACER Drosophila	[49] [12b]

Anticancer Agents Med Chem **2011**, *11*, 830-843
Prog Lipid Res **2010**, *49*, 316-34

Camacho, L. *et al.* Acid Ceramidase as a Therapeutic Target in Metastatic Prostate Cancer. *J. Lipid Res.* **2013**, *54*, 1207–1220.



Irreversible inhibitor
IC₅₀ 52 nM



Chemical tools for the study and modulation of sphingolipid metabolism

Sphingolipid analogues
Enzyme inhibitors

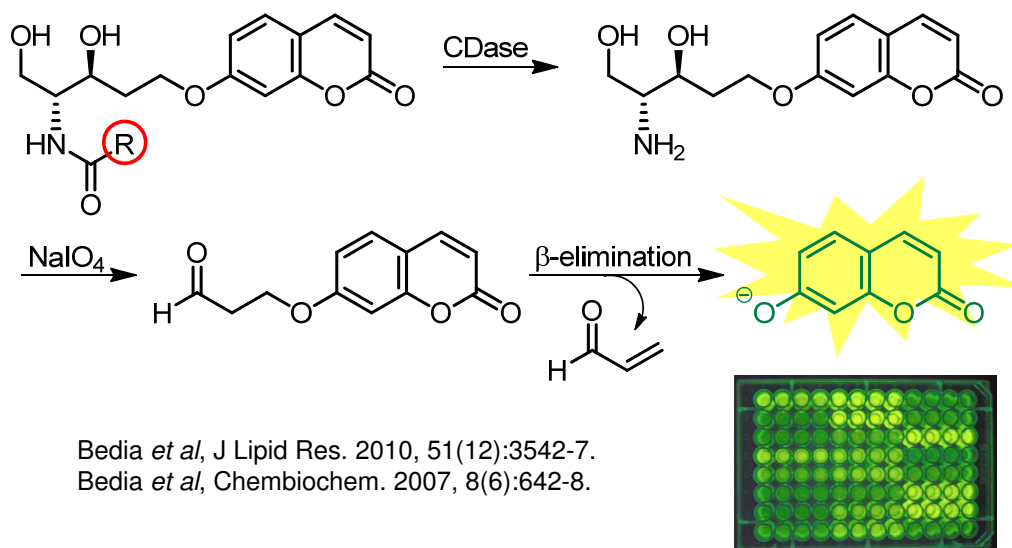
Probes to study SL metabolism

Fluorogenic substrates for enzyme activity

Azidosphingolipids (click chemistry)

Library synthesis and screening

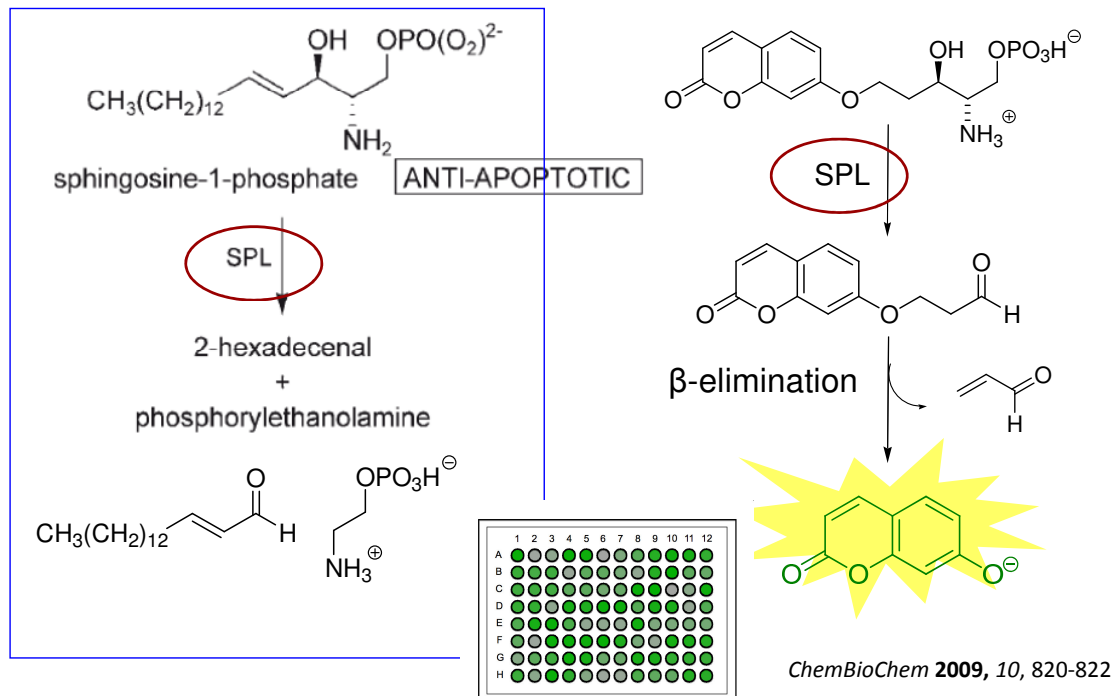
Fluorogenic assay for ceramidase activity



Bedia *et al*, J Lipid Res. 2010, 51(12):3542-7.
Bedia *et al*, Chembiochem. 2007, 8(6):642-8.

Excellent performance in both intact cells & cell lysates

Synthesis of a Fluorogenic Analogue of Sphingosine-1-Phosphate and Its Use to Determine Sphingosine-1-Phosphate Lyase Activity



Chemical tools for the study and modulation of sphingolipid metabolism

Sphingolipid analogues
Enzyme inhibitors

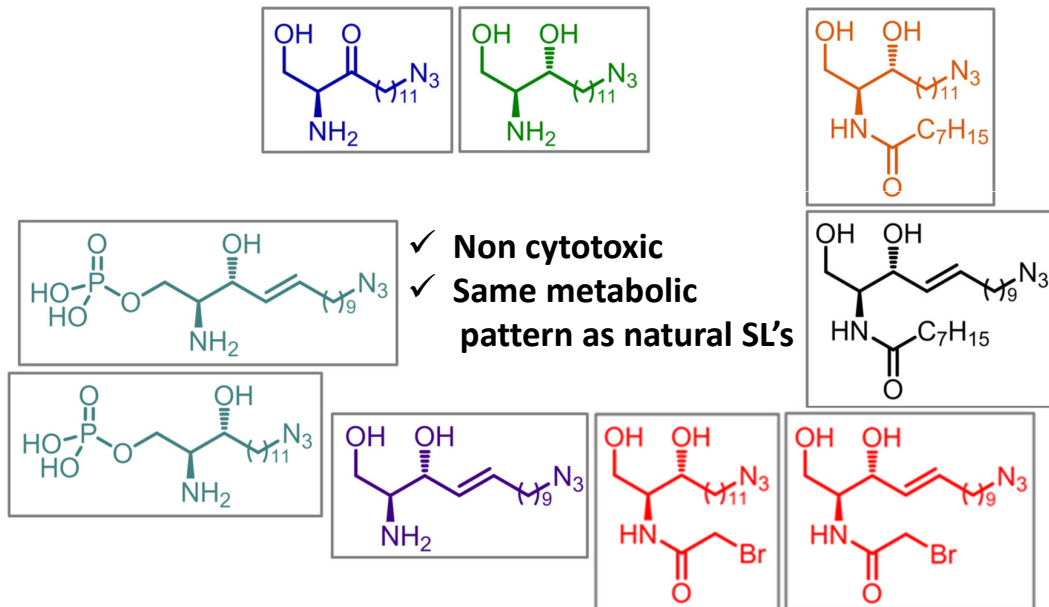
Probes to study SL metabolism

Fluorogenic substrates for enzyme activity

Azidosphingolipids (click chemistry)

ω -Azidosphingolipids

Probes for sphingolipid metabolism, sphingolipid targets, sphingolipid trafficking and cell population tagging for LC/MS analysis.



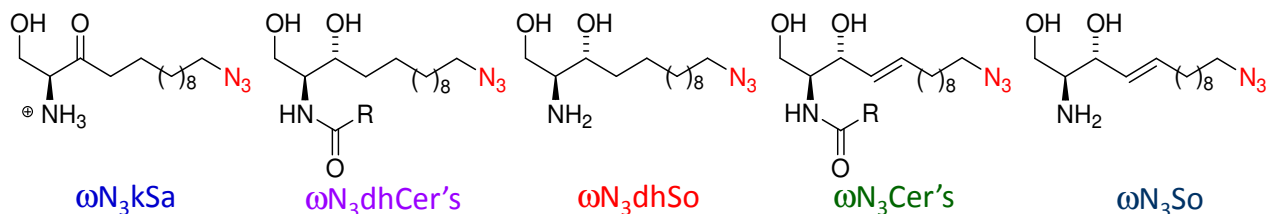
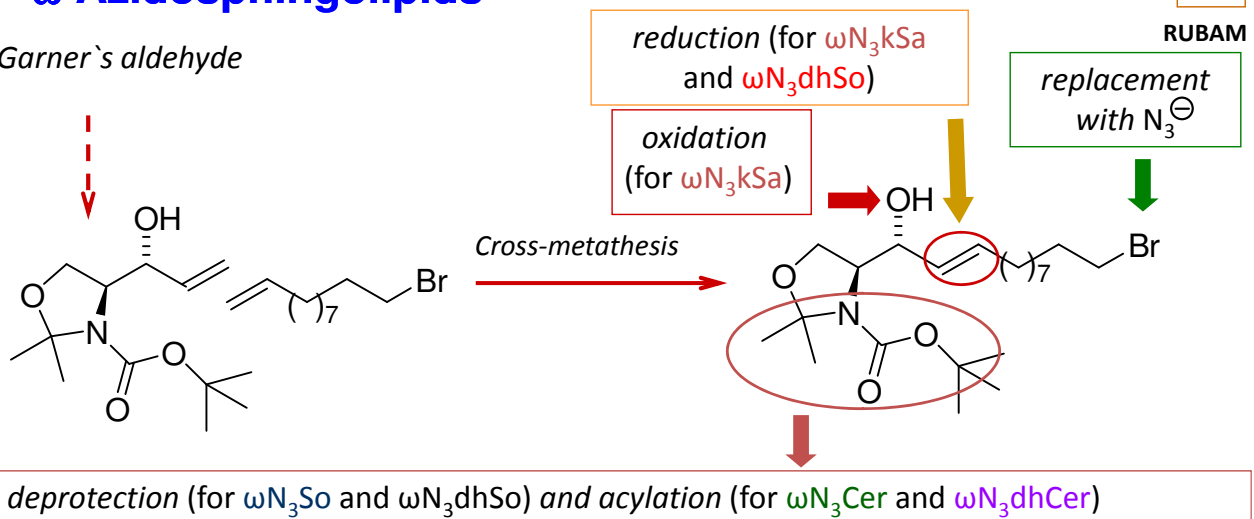
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General approach to "clickable" azidosphingolipids

Garner's aldehyde



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Click Chemistry Reactions

“Click chemistry” concept: Sharpless y col.

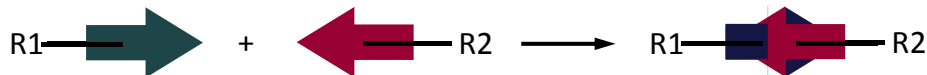
Kolb HC, Finn MG, Sharpless KB, *Angew Chem Int Ed.* **2001**, 40, 2004-21

“A click chemistry reaction must be **modular**, wide in scope, give very **high yields**, generate only **inoffensive byproducts** that are easily separated and be stereospecific. The process must include **simple reaction** conditions, readily available starting materials and reagents, the use of **no solvent** or a solvent that is benign or easily removed, and **simple product isolation**”.



C=C Additions
Nucleophilic opening of strained rings
Cycloadditions
'Special' carbonyl chemistry

Click Chemistry Reactions



Efficient and selective **bioorthogonal reaction** to link molecules rapidly and in high yield.

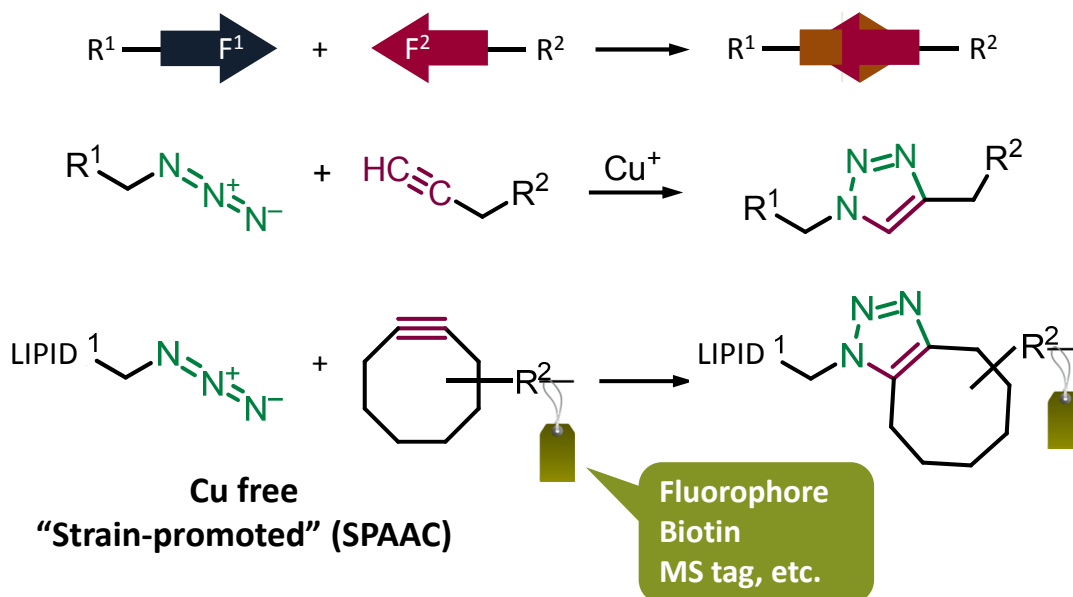
Bioorthogonal reaction: any chemical reaction that can occur inside of living systems without interfering with native biochemical processes.

Most popular click reactions in chemical biology

Thiol-ene Reaction
Staudinger Ligation
Huisgen 1,3-Dipolar Cycloaddition

Click Chemistry Reactions

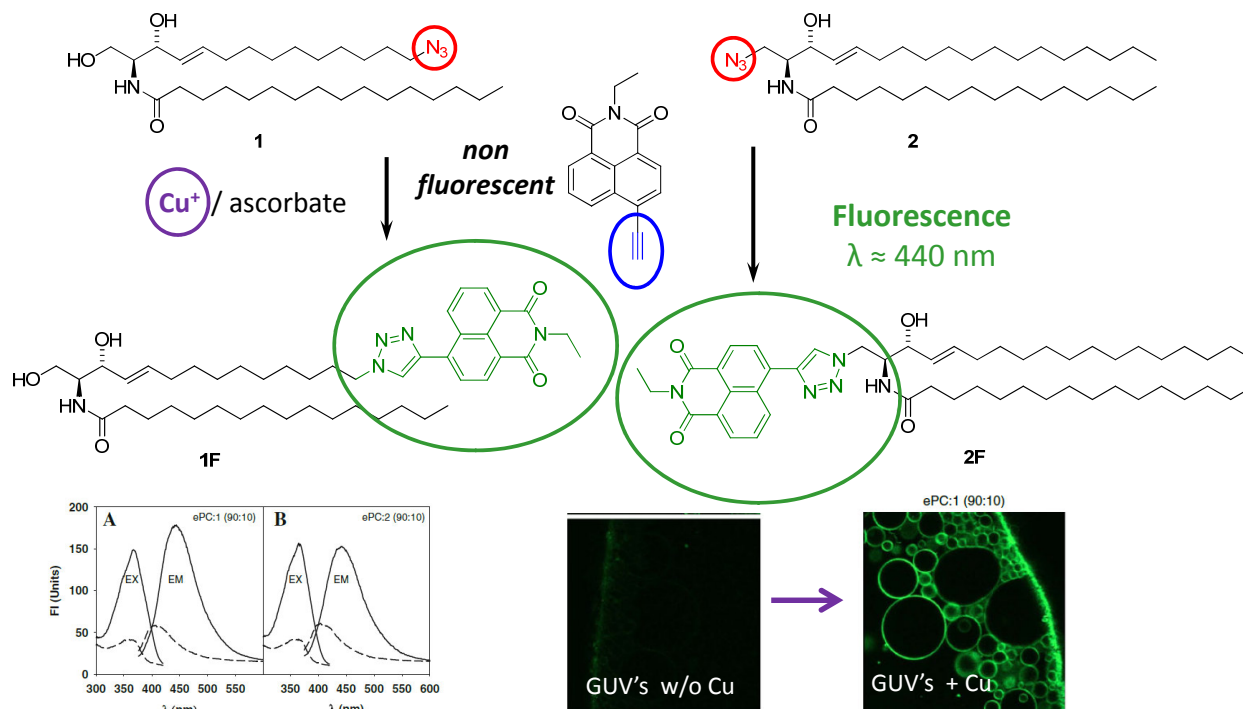
Huisgen 1,3-Dipolar Cycloaddition



APPLICATIONS OF AZIDOSPHINGOLIPIDS

- **Fluorescent membrane lipids**
- **Cell trafficking and localization studies**
- **Quantitative cell metabolism**

Fluorescent membrane lipids ("in situ" fluorogenic click chemistry)

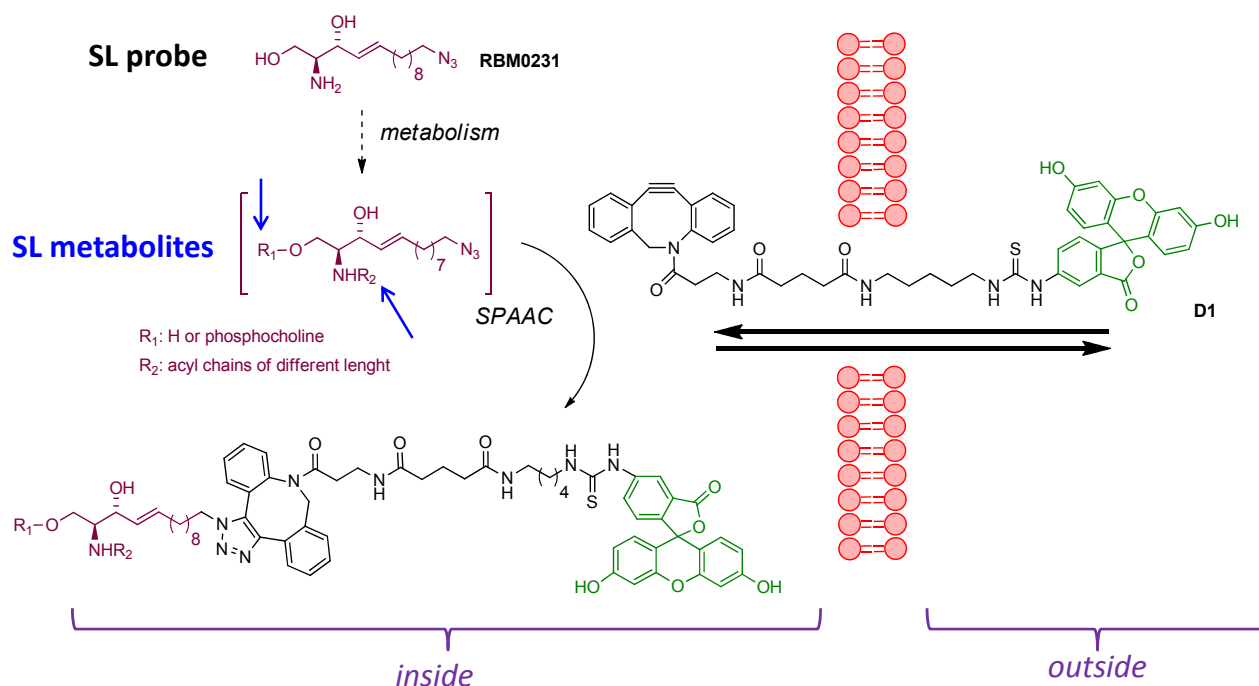


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Cell trafficking and localization studies "strain promoted" click chemistry ("SPAAC")

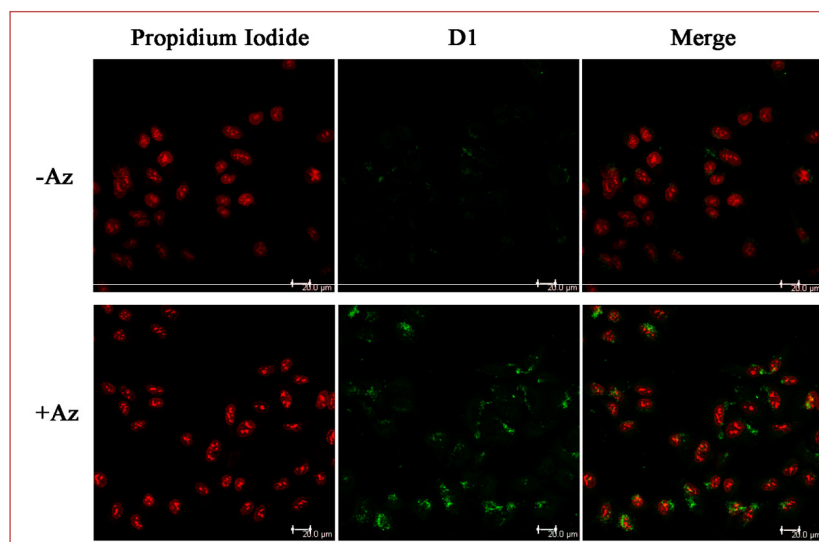


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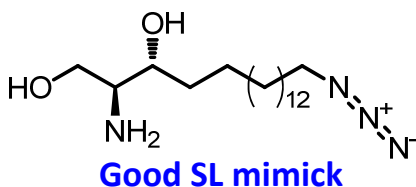
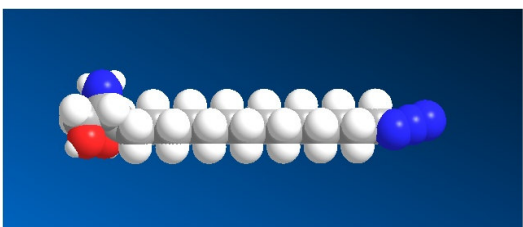
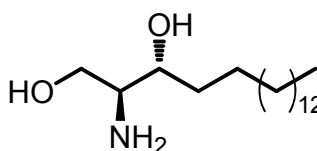
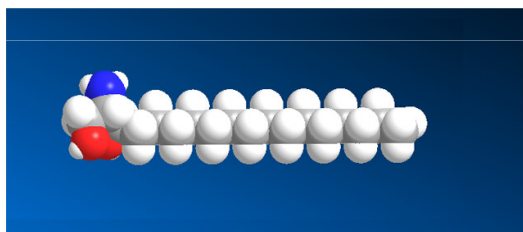
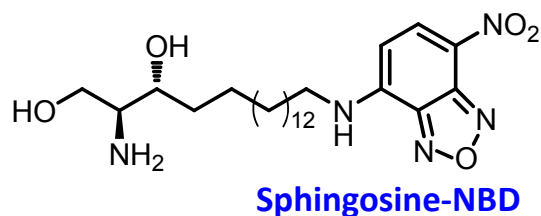
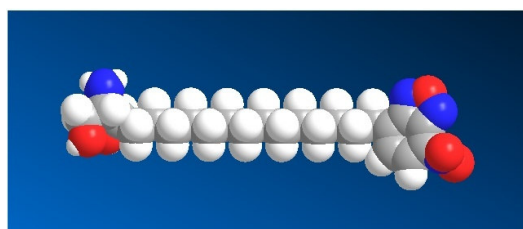
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Cell trafficking and localization studies “strain promoted” click chemistry (“SPAAC”)



Fluorescence analysis by confocal laser scanning microscopy. A549 cells without (Az-) or with (Az+) the azide probe. Propidium iodide (in red) was used for nuclei staining.

“Azide + click” vs fluorophores for sphingolipid labeling

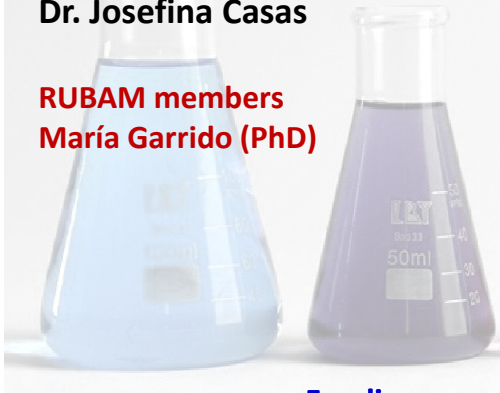


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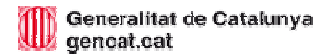
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