Name:__________**KEY**____________
Student ID:______________________

Be sure to write your name and student ID above. Read the two questions carefully, think, then write your answers in the lined space (front and back of this page). When finished, please hand your answer in.

Question One.

Design two bilayer membranes using the structures provided. One membrane should be resistant to high pressure. The other membrane should be resistant to cold temperatures. Use no more than 3–5 lipid species per membrane. Explain from a physico-chemical perspective why the pressure-resistant and cold-resistant membranes are different.

General Considerations:

• As is common in most biological membranes, a major requirement is for a zwitterionic headgroup (with both –ve and +ve groups and net charge of zero). This will maximize hydrophilicity of the head group and encourage spontaneous bilayer formation.

• Diacyl lipids will optimize the molecular spacing in the hydrophobic interior of the membrane (monoacyl lipids disrupt membrane integrity). At high pressure, monoacyl lipids may be useful, but it is unlikely.

*The only diacyl lipid with a net charge is **ceramide-1-phosphate (18:1 & 8:0, 1 –ve charge)**, so this could be a major component of both pressure-resistant and cold-resistant membranes. Other diacyl lipids have 3 hydroxyl (OH) groups that provide dipole to interact with water: the **N-16:0 and N-24:0 phytosphingosines** have the best acyl chain lengths.*

At high pressure lateral and transverse compression will occur. The lateral compression will cause membrane rigidity (and a higher Tm), which can be offset by unsaturated kinks in the acyl groups. The transverse compression would cause the membrane to be thinner, and can be offset by the use of long chain acyl groups.

*In addition to **ceramide-1-phosphate (18:1 & 8:0)** and **N-24:0 phytosphingosine**, shorter acyl chains with unsaturated kinks, are **ceramide (18:1 & 12:0)** and **ceramide (chicken) (16:1 & 16:0)**.*

At cold temperatures, the fluidity of the membrane will be seriously impaired. Unsaturated acyl groups can offset this. The acyl chain lengths should be similar to biological membranes at normal temperatures (about 18 carbons).

*In addition to **ceramide-1-phosphate (18:1 & 8:0)** and **N-16:0 phytosphingosine**, **ceramide (18:1 & 12:0)** and **ceramide (chicken) (16:1 & 16:0)** provide the best mix of unsaturation and acyl chain length.*

Scoring (___/100):
effort (minimal effort may get less) (50)
zwitterion headgroup (10)
use of diacyl lipids (10)
longer acyl groups for high pressure (10)
unsaturated acyls for both high pressure and cold temperatures (10)
physico-chemical difference between pressure and cold explained clearly (10)

Question Two.

Two molecular species (a and b) have similar molecular weights. Species a has a permeability coefficient of 10^{-4} cm s^{-1} and no net charge. Species b has a permeability coefficient of 10^{-9} cm s^{-1} and a net charge of +ve 1. If the concentration outside of a 10 μm square cell is 10 mM and 1 mM inside, what is the flux of species a? At what electrical potential will the flux of species b equal that of species a?

Guidelines: Equations and constants are provided. Please be sure that you show units. This is an important internal check, both for you and for me.

Here are the constants and other values we require

$$> R := 1.987 \cdot 10^{-3} \frac{[[kcal]]}{[[mol]][[K]]} : T := 293[[K]] : F := 23.06 \frac{[[kcal]]}{[[mol]][[V]]} : \psi := -0.12\,[[V]] :$$

Now we need to consider the magnitude of the fluxes, using species concentrations in units of mols per cubic meter. First, for the uncharged species a

$$> P_a := 10^{-6} \frac{[[m]]}{[[s]]} : P_b := 10^{-11} \frac{[[m]]}{[[s]]} : C_o := 0.01 \cdot 10^3 \frac{[[mol]]}{[[m^3]]} : C_i := 0.001 \cdot 10^3 \frac{[[mol]]}{[[m^3]]} :$$

$$> solve(J = -P_a \cdot (C_o - C_i), J)$$

$$-\frac{9.00 \times 10^{-6} [[m]]\,[[mol]]}{[[s]]\,[[m^3]]} \qquad (1)$$

This is an inward flux of 9 umol m^{-2} s^{-1}, or 90 nmol cm^{-2} s^{-1}.

For species b, with one net positive charge, we can guess by using different values of the potential. It soon becomes clear that a very large potential must be used, given the low permeability coefficient of the charged species.

$$> solve\left(J = P_b \cdot \frac{F \cdot (-2275[[V]])}{R \cdot T} \cdot \frac{C_o - C_i \cdot \exp\left(\frac{F \cdot (-2275[[V]])}{R \cdot T}\right)}{1 - \exp\left(\frac{F \cdot (-2275[[V]])}{R \cdot T}\right)}, J\right)$$

$$-\frac{9.01 \times 10^{-6} [[m]]\,[[mol]]}{[[s]]\,[[m^3]]} \qquad (2)$$

About -2.4 kiloVolt. The is extremely negative (and would cause breakdown of the membrane)

scoring (/10): set-up (6/10); correct answer (4/10)

If we evaluate the flux at a more normal potential (-100 mV), the flux is very small compared to the neutral species.

$$> P_b \cdot \frac{F \cdot \psi}{R \cdot T} \cdot \frac{C_o - C_i \cdot \exp\left(\frac{F \cdot \psi}{R \cdot T}\right)}{1 - \exp\left(\frac{F \cdot \psi}{R \cdot T}\right)}$$

The flux is 10^4 lower than the uncharged molecule. So, a very large negative potential is necessary to 'pull' the cation into the cell. A large negative potential means that exp(FΨ/RT)~0. Setting the expenential terms to zero simplifies the equation, allowing an accurate estimate of the potential

$$-\frac{4.79 \times 10^{-10} [[m]]\,[[mol]]}{[[s]]\,[[m^3]]} \qquad (3)$$

Scoring (___/100):
neutral flux equation setup and value (50)
equation for cation flux (constant field equation) (20)
setup (20)
answer (10)

Equations relevant to Membrane Transport: Geometry, Diffusion and Flux

Sphere Area : $4 \bullet \pi \bullet r^2$ Sphere Volume : $\frac{4}{3} \bullet \pi \bullet r^3$

Cylinder Area : $4 \bullet \pi \bullet r \bullet h$ Cylinder Volume : $\pi \bullet r^2 \bullet h$

Cube Area : $6 \bullet \mathrm{h}^2$ Cube Volume : h^3

Fick's Diffusion : $J = D \bullet \frac{dc}{dx}$ Fick's Diffusion : $\frac{dc}{dt} = D \bullet \frac{d^2 c}{dx^2}$

Einstein's Random Walks : $D = \frac{1}{2} \bullet \frac{\Delta^2}{\tau}$, $\langle x^2 \rangle = 2 \bullet D \bullet t$, and $\langle r^2 \rangle = 6 \bullet D \bullet t$

Membrane Diffusion : $J = P \bullet (c_{outside} - c_{inside})$

Membrane Diffusion : $J = -(uRT) \bullet \frac{dc}{dx} - (zFuc) \bullet \frac{d\Psi}{dx}$

Membrane Diffusion : $J = -P \bullet \left(\frac{zF\Psi}{RT}\right) \bullet \left(\frac{c_o - c_i \bullet e^{zF\Psi/RT}}{1 - e^{zF\Psi/RT}}\right)$

Nernst Equation : $\Psi = \left(\frac{RT}{zF}\right) \bullet \ln\left(\frac{c_o}{c_i}\right)$

Ohm's Law : $V = I \bullet R$, $I = g \bullet V$, $R = \rho \bullet \left(l/A\right)$ and $J = I/(zF)$

Radial Diffusion : $C(r) = C_\infty \bullet \left(1 - \frac{a}{r}\right)$, and $J(r) = -D \bullet C_\infty \bullet \left(\frac{a}{r^2}\right)$

Radial Currents : $I_m = 4 \bullet \pi \bullet a^2 \bullet \beta$, and $I_d = 4 \bullet \pi \bullet a \bullet D \bullet C_\infty$

Dimensionless relations $P_e = \frac{2 \bullet a \bullet \nu}{D}$ and $R_e = \frac{\rho \bullet \nu \bullet l}{\eta}$

Goldman - Hodgkin - Katz (GHK) equation

$$\Psi = \frac{RT}{F} \ln\left(\frac{P_H c_H^o + P_{Na} c_{Na}^o + P_K c_K^o + P_{Cl} c_{Cl}^i}{P_H c_H^i + P_{Na} c_{Na}^i + P_K c_K^i + P_{Cl} c_{Cl}^o}\right)$$

Equations relevant to membrane capacitance

$Q = C \cdot \Delta E$ (coulombs) = (coulombs/volt) (volt)

Charge (Q) for a spherical cell of radius r:

$Q = \frac{4}{3} \cdot \pi \cdot r^3 \cdot c \cdot F$

c is the concentration of net charge.

Capacitance of a spherical cell of radius r:

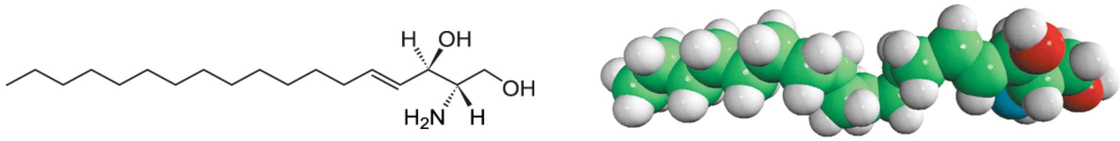
$C = 4 \cdot \pi \cdot r^2 \cdot C'$ C is the capacitance per unit area

(about 1 microFarad per square centimeter for cells).

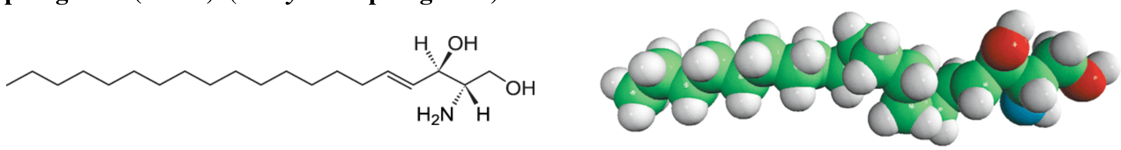
Symbol	Value	Units	Comments
GAS CONSTANT			
R	8.314	J mol^{-1} K^{-1}	R is the Boltzmann constant times Avogadro's Number ($6.023 \cdot 10^{23}$)
	1.987	cal mol^{-1} K^{-1}	
	8.314	m^3 Pa mol^{-1} K^{-1}	
RT	$2.437 \cdot 10^3$	J mol^{-1}	At 20 ºC (293 ºK)
	$5.822 \cdot 10^2$	cal mol^{-1}	At 20 ºC (293 ºK)
	2.437	liter MPa mol^{-1}	At 20 ºC (293 ºK)
RT/F	25.3	mV	At 20 ºC (293 ºK)
2.303 • RT	5.612	kJ mol^{-1}	At 20 ºC (293 ºK) used for $\log_{10}$
	1.342	kcal mol^{-1}	At 20 ºC (293 ºK) used for $\log_{10}$
FARADAY CONSTANT			
F	$9.649 \cdot 10^4$	coulombs mol^{-1}	F is the electric charge times Avogadro's Number
	$9.649 \cdot 10^4$	J mol^{-1} V^{-1}	
	23.06	kcal mol^{-1} V^{-1}	
CONVERSIONS			
kcal	4.187	J (joules)	Joules is an energy unit (equal to 1 Newton•meter)
Watt	1	J sec^{-1}	
Volt	1	J $coulomb^{-1}$	
Amperes	1	coulomb sec^{-1}	
Pascal (Pa)	1	Newton $meter^{-2}$	Pascal is a pressure unit (equal to 10^{-5} bars)
Siemens	1	Ohm^{-1}	Siemens (S) is conductance, the inverse of resistance (Ohm)
PHYSICAL PROPERTIES			
η_w	$1.004 \cdot 10^{-3}$	Pa sec	viscosity of water at 20 ºC
ν_w	$1.004 \cdot 10^{-6}$	m^2 sec^{-1}	kinematic viscosity of water at 20 ºC (viscosity/density)

Source: Nobel, Park S (1991) Physicochemical and Environmental Physiology

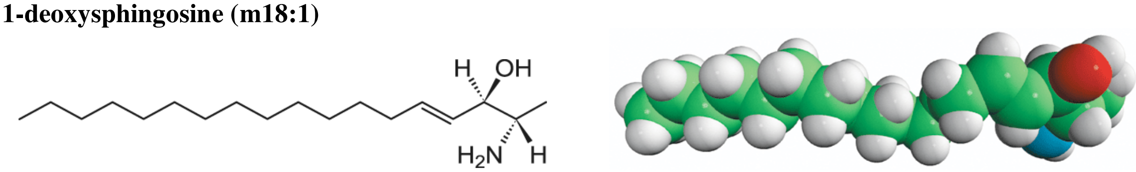
Sphingosine (d18:1) (D-*erythro*-Sphingosine



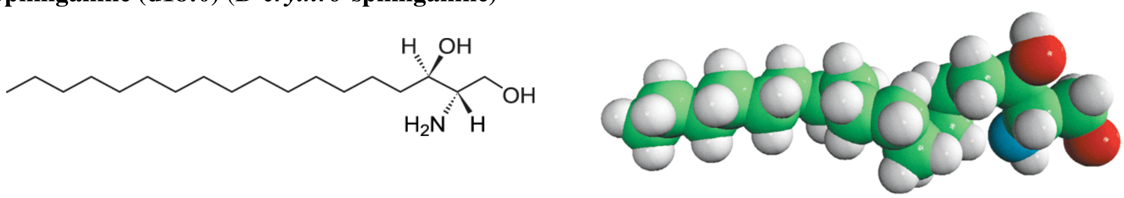
Sphingosine (d20:1) (D-*erythro*-Sphingosine)



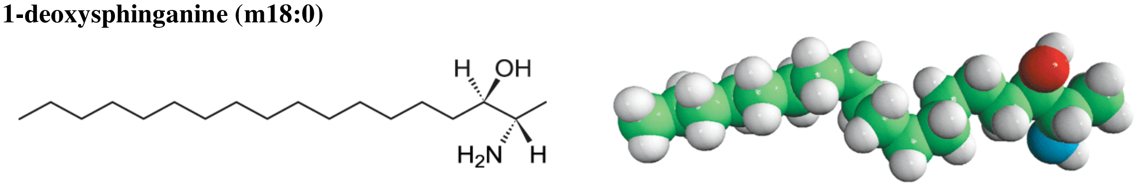
1-deoxysphingosine (m18:1)



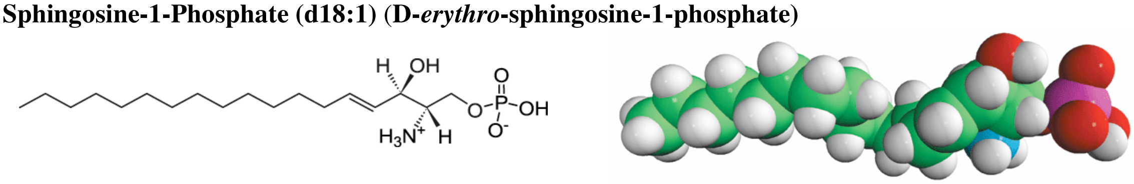
Sphinganine (d18:0) (D-*erythro*-sphinganine)



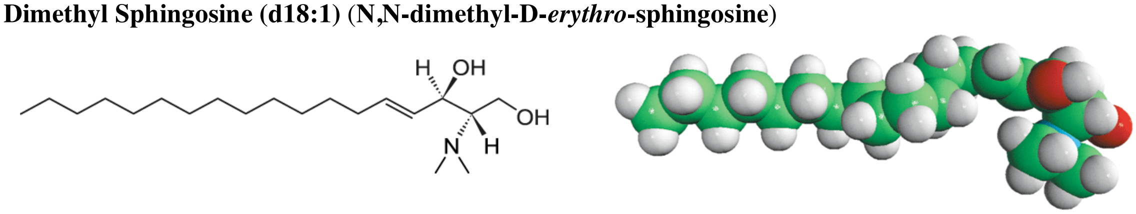
1-deoxysphinganine (m18:0)



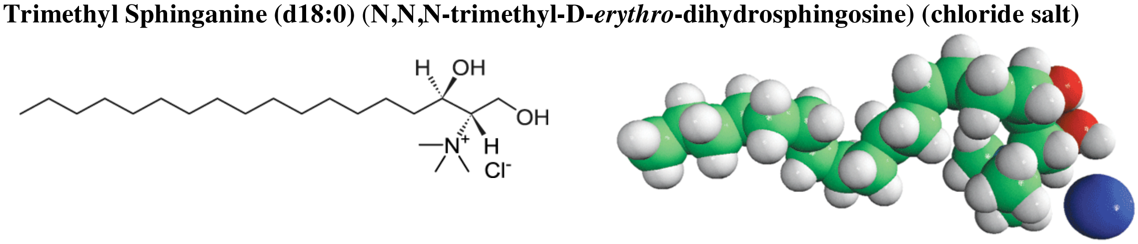
Sphingosine-1-Phosphate (d18:1) (D-*erythro*-sphingosine-1-phosphate)



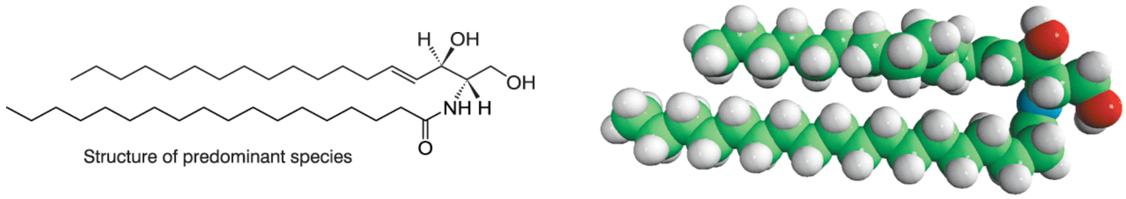
Dimethyl Sphingosine (d18:1) (N,N-dimethyl-D-*erythro*-sphingosine)



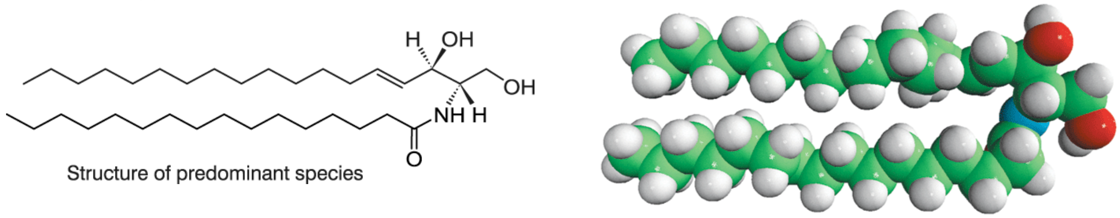
Trimethyl Sphinganine (d18:0) (N,N,N-trimethyl-D-*erythro*-dihydrosphingosine) (chloride salt)



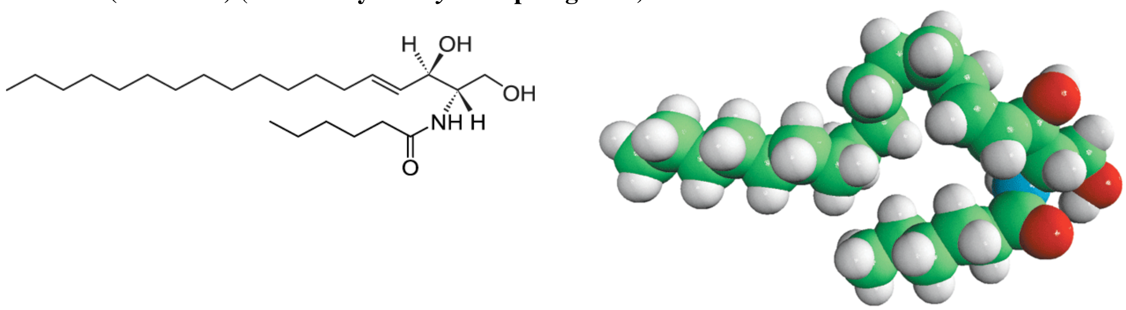
Ceramide (Brain, Porcine)



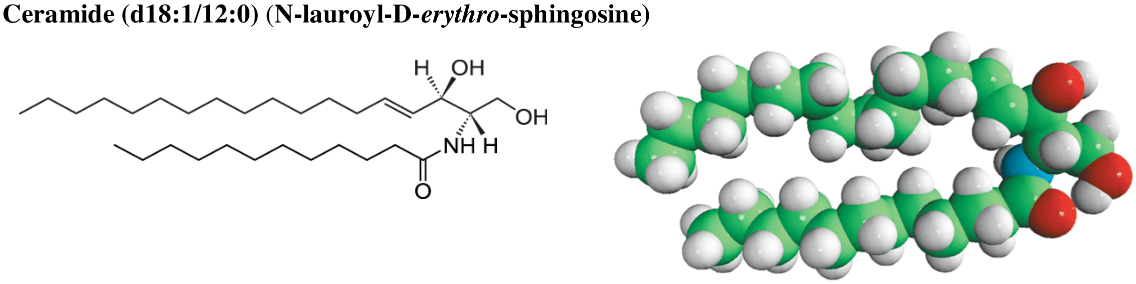
Ceramide (Egg, Chicken)



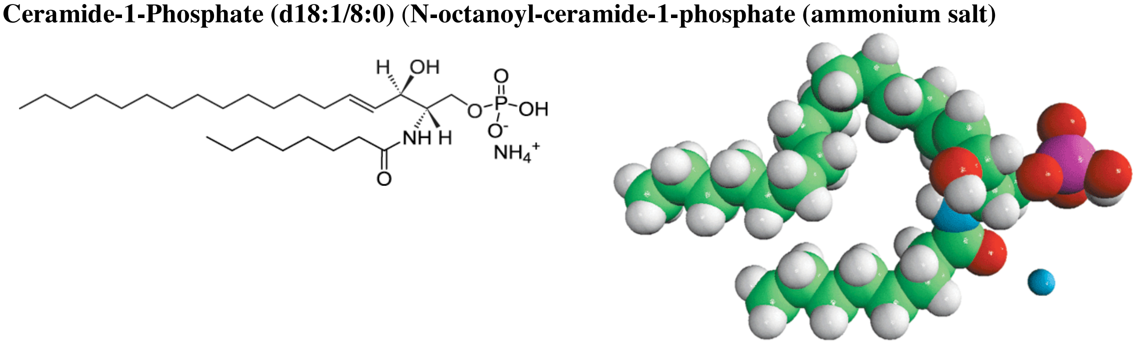
Ceramide (d18:1/6:0) (N-hexanoyl-D-*erythro*-sphingosine)



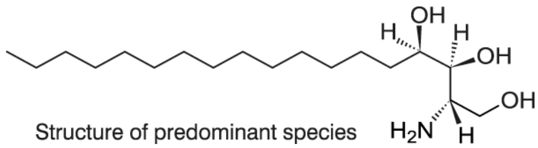
Ceramide (d18:1/12:0) (N-lauroyl-D-*erythro*-sphingosine)

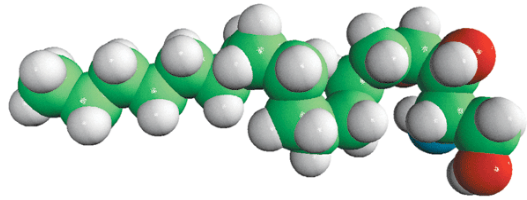


Ceramide-1-Phosphate (d18:1/8:0) (N-octanoyl-ceramide-1-phosphate (ammonium salt)

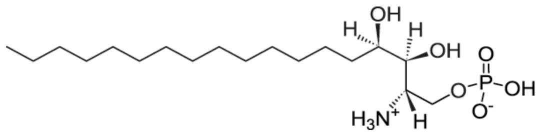
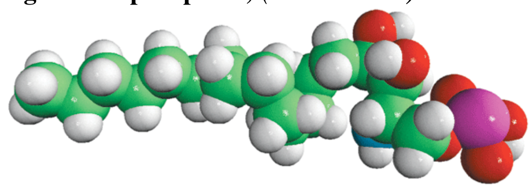


D-ribo-Phytosphingosine (**4-hydroxysphinganine**) ***(Saccharomyces cerevisiae)***

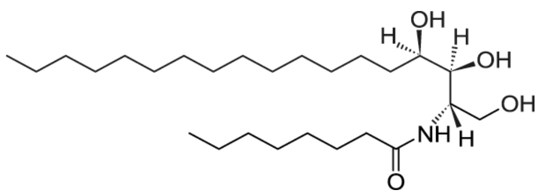
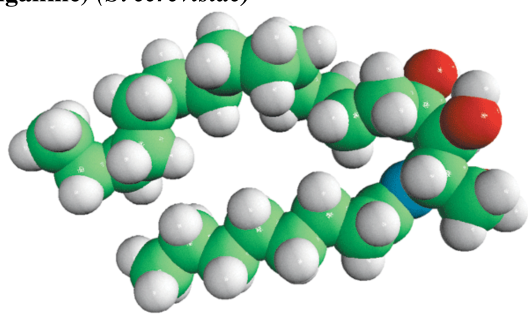




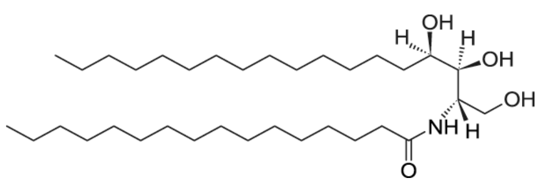
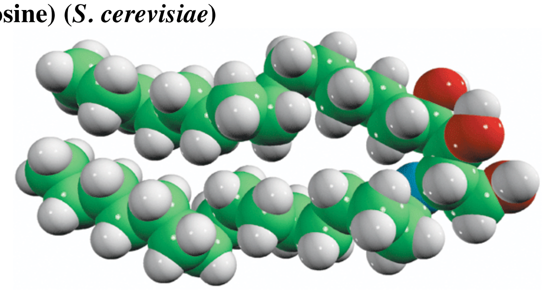
D-ribo-Phytosphingosine-1-Phosphate (**4-hydroxysphinganine-1-phosphate**) ***(S. cerevisiae)***

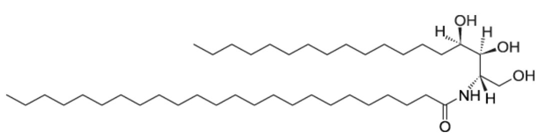
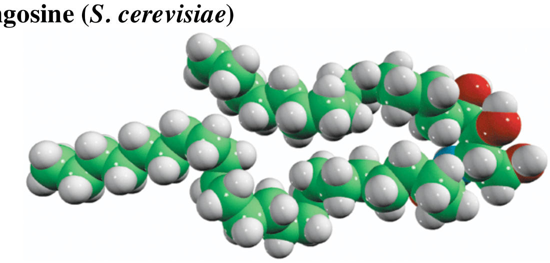
N-08:0 Phytosphingosine (**N-octanoyl 4-hydroxysphinganine**) ***(S. cerevisiae)***

N-16:0 Phytosphingosine (**N-palmitoyl-phytosphingosine**) (***S. cerevisiae***)

N-24:0 Phytosphingosine)**N-lignoceroyl-phytosphingosine** (***S. cerevisiae***)

Phytosphingosine Phosphocholine (**4-hydroxysphinganine-1-phosphocholine**) (***S. cerevisiae***)