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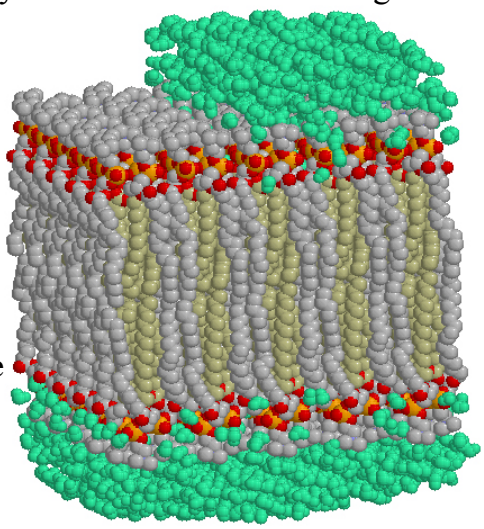
Phospho Lipids Cholesterols stabilized Bilayer Membrane

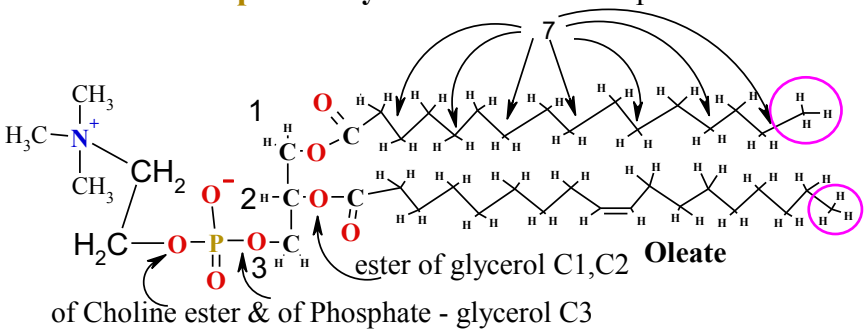
.....(-PO_4^--) **Phosphat**idyl **Choline** $^+$ MW=760.10 g/mol

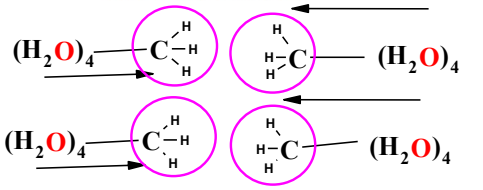
Phospho Lipids mass fraction of Membranes to make 33.3% mass fraction (**1/3**) of total 100%.



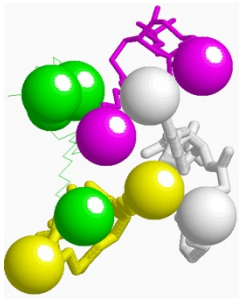
London Force (Wan der Walls) Bonding Energy in contact point between hydrogen atoms **H-H** is weak -2 kJ/mol. Investigations shows great number of contact points in **lipids bilayer membrane** molecules like as in picture to right **Phosphat**idyl **Cholines**. Green water molecules atoms isolate both side of bilayer membranes. Close packing bilayer membrane **Phosphat**idyl **Choline** MW=760.10 g/mol touch with two methyl groups six hydrogen atoms.

Choline Phosphate Glycerol 7 contact points **Palmitate**





7 contact points between palmitate and oleate methylene group >**CH2**:**2HC**< two hydrogen atoms London Force (Wan der Walls) Bonding Energy is **E**= -2•7•2=-28 kJ/mol.
Oleate **C18:1 unsaturated** double bond between **C** atoms is cis isomer ⌞C=C⌟ (trans is unhealthy)!



1) Each **chain** surrounded chains as **gray**, yellow and green three closest neighbors with energy $\mathbf{E_I}$= 3*-28 = -84 kJ/mol. **Phosphat**idyl **Choline** has two hydrocarbon **chains** with total London Forces energy $\mathbf{E_{II}}$=2*-84= -168 kJ/mol.

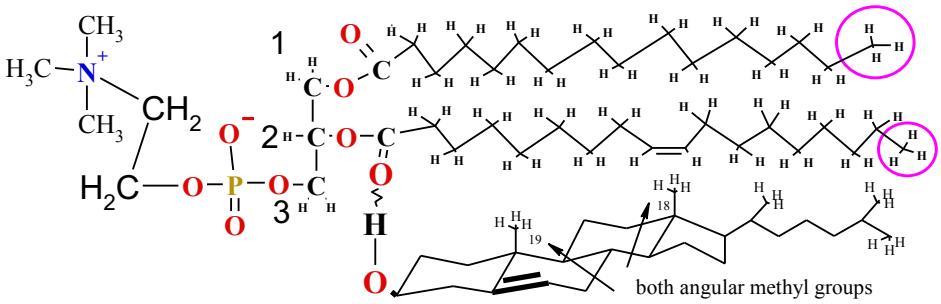
2) Non polar Fatty Acid tails with two methyl groups **–CH_3** in Interior are in close contacts on middle of Interior between two mono Layers of **Bilayer Membrane**. Three **hydrogen** atoms of methyl group touch to neighbor mono Layer methyl groups three **hydrogen** atoms make **Hydrophobic** intermolecular forces. Both side **Water** tetramer structures $(\mathbf{H_2O})_4$ of Exterior side **Membrane** press together mono Layers with **Hydrophobic** force -10 kJ/mol per each contact point. Six contact points **E**nergy $\mathbf{E_{hydr}}$=6*-10= -60kJ/mol of **Hydrophobic** force for each phospholipid $\mathbf{E_{hydrophobic}}$=- 60/2= -30 kJ/mol. Single phospholipid in membrane distributed London Forces -2 kJ/mol for 84 contact points -168 adds **hydrophobic** energy -30 kJ/mol total sum is $\mathbf{E_{total\_sum}}$= -168 +(-30)= -198 kJ/mol on **Phosphat**idyl **Choline** molecule. **Membrane** fragment total London Forces (Wan der Walls) energy is $\mathbf{E_{Walls}}$=200*-168= -33600 kJ/mol. **Hydrophobic** force energy -6000 kJ/mol between bilayer 100+100 **phospho lipid** methyl groups **–CH_3** of fatty acids.

Lipid Bilayer Membrane waterless Interior is impermeable so work as **isolating** cell wall for compartment components **water** molecules and **water solutes**: as salts and **water** soluble organic molecules. Cell wall thickness 56Å, 5,6 nm relates to **water** molecule size 1.4 Å covers **membrane** thickness 56/1.4=40 times as nano device forms living organism membranes. If house wall thickness 40 human tall size 1.7 m, then wall thickness would be 68 m so home doors would 68 m long tunnels in walls between rooms in our home.

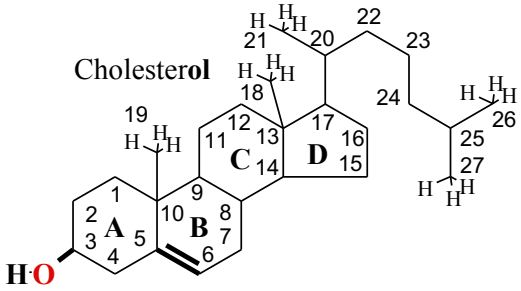
Lipids bilayer membrane waterless interior is impermeable **isolating** cell wall for **water** medium compartment molecular components and **solutions**: salts , soluble organic compounds. Similar as houses walls separate rooms. To entrance in rooms we uses doors, but **membranes** are equipped with **transport** and **signaling** enzymes (proteins): For entrance in the cell compartment **membranes** are penetrating channels **transport enzymes** (**proteins**): for $\mathbf{H_2O}$, $\mathbf{O_2}$, **CO**, **NO**, $\mathbf{C_3H_8O_3}$ Aquaporins, for charged ionic Particles: Proton channels for $\mathbf{H^+}$, Bicarbonate channels for $\mathbf{HCO_3^-}$. Sodium $\mathbf{Na^+}$, Potassium $\mathbf{K^+}$, Magnesium $\mathbf{Mg^{2+}}$, Calcium $\mathbf{Ca^{2+}}$, Chloride $\mathbf{Cl^-}$, sugars Glc , Gal , Man $\mathbf{C_6H_{12}O_6}$, 20 proteinogenic Amino Acids and so as for other solutes in biological **water solutions**. Organic regulated opened thermodynamic system through the cellular membranes organize life of organisms. That is the Medical Chemistry studies destiny of Biochemistry and Physiology.

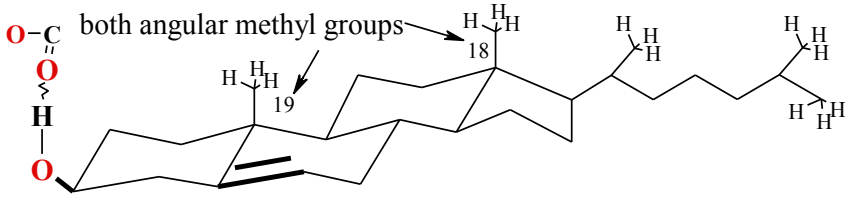
1. 1/3 mass fraction of **membranes** in cells as well organelles constitute **phospho lipids** as **Phosphat**idyl **Choline**. Intermolecular forces binding energy make $\mathbf{E_{bound}}$=-198 kJ/mol **phospho lipid membranes** liquid therefore can be mechanically broken, as liquid due to gravitation, pressure and movement. **Cholesterol** content make **membranes** stronger and flexible to prevent destruction with following cytosol leaking of **water** molecules as well solution of: salts , organic compound molecules.

2. Second third part of **Membrane**s mass constitutes hydrocarbon 27 carbon steric frame steroid.



Lipid - Cholesterol molecule. Four rings of the steroid are labeled **A**, **B**, **C** and **D**. Angular methyl **–CH₃** groups labeled 18 and 19 as well tail fork, rod, splinter are good clutch fixing close hydrocarbon chains in **membrane**. Double bond between carbon atoms >**C=C**< 5 and 6 to frame steroid molecule solid and inflexible. Alcohol **HO**- at carbon 3 but hydroxyl group **HO**-





forms hydrogen bond >**C=O**...**HO**- with carboxyl oxygen of fatty acid Oleate or one another fatty acid carboxyl oxygen >**C=O**.

Cholesterol as Steroid makes **membranes** unbroken, flexible and so prevent following leaking of **water** molecules and of **water** solution components: salts and **water** soluble organic molecules. The **Cholesterol/Phospho Lipid** C/PL mole ratio of human red blood cell membranes ranges from a normal value of 0.9–1.0 (since 1978 first publication Journal of Cellular Biochemistry 2004 V8, 4, p 413-430). If Cholesterol amount decreases up to 0,5= C/PL , then membranes leak cell content out, but if Cholesterol amount increases up to 1,5= C/PL , then membrane becomes solid, inflexible and squeeze channels, aquaporins, but receptors becomes inactive due to absence conformational flexibility.

Membrane total mass **100%=33.3%+33.3%+(20%)** Aquaporins **+13.3%** other proteins)

I) 1/3 part constitute **Phospholipids** which mass fraction of **Membranes** to make **33.3%** of total mass 100%;

II) second **1/3** part **Cholesterols** which mass fraction of **Membranes** to make **33.3%** of total mass 100%;

III) third **1/3** part **Membranes integral Proteins** which mass fraction to make 33.3% of total mass 100%

Bulk mass fraction **20%** goes to **Aquaporins** for other remains **13.3%** are constitute four type **Proteins**:

1. Glycoproteins with linked **O**- glycoside bonds **Immunological** marker L-fucose **Fuc** and **Immunological determinants** including blood groups **A**, **B**, **AB**, **0** located outside in extra cellular space for leucocytes-scanners host bodies recognition. Leucocytes are scavengers non-host bodies binding to remove from Host organism.

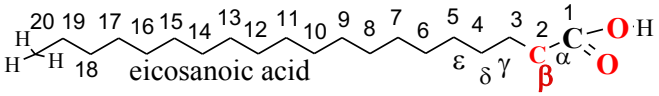
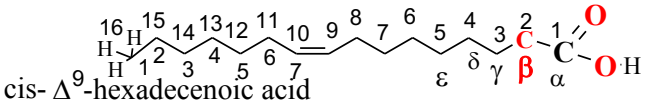
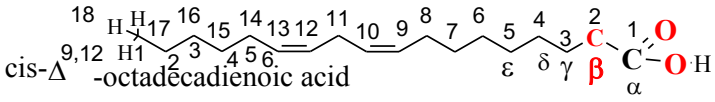
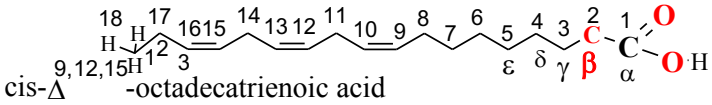
2. Cell Structural building blocks **cytoskeleton** and structural integral membrane **proteins**;

3.Transport enzymes (**channels**) integral membrane **proteins**, 20% **Aquaporins** for $\mathbf{H_2O}$,$\mathbf{O_2}$,**NO** transport;

4. Receptors enzymes (**Membranes integral Proteins**) of the Signal transduction Pathway components for biological communication inside the cells, between the cells and or tissues, as well between living organisms.

Table. Glycerol- LIPIDS Classification of Lipids

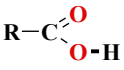
Lipid	Function
Fatty acids	Metabolic fuel; component of several other classes of lipids
Triglycerides	Main storage form of Fatty acids and chemical energy
Phospholipids	Component of membranes; source of Arachidonic acid for Eicosanoids production; inositol triphosphate and diglyceride membrane inner location for signal transduction
Sphingolipids	Component of membranes molecule linkage location site in extracellular space
Cholesterol	Component 1/3 fraction of membranes; precursor of bile salts and steroid hormones
Bile salts	Lipid digestion and absorption; main product of cholesterol metabolism
Steroid hormones	Intercellular signaling molecules, that regulate gene expression in target cells
Eicosanoids	Regulation of physiological functions:prostaglandins, tromboxans, leucotrienes, prostocyclin
Vitamins **KEDA**	Vision **A**; calcium metabolism **D**; lipids antioxidant molecules receiver **E**; blood coagulation K_1; Bone Phospho Apatites $Ca_3P_3O_{10}OH$ correct Structure calcium Ca^{2+} ions coenzyme K_2
Ketone bodies	Metabolic fuel

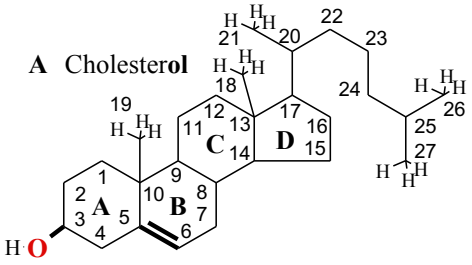
Table. **A** Some natural fatty acids.	**Carbon atoms**	**Structural Formulas.** *Fatty Acids break down in mitochondria through beta carbon oxidation reaction producing* CO_2, H_2O *and life energy*	**IUPAC Systematic name**	**Common Name** Notional name	**mp(°C)**
		Saturated fatty acids no double bonds between **C&C** atoms			
palm oil	16**C**	$CH_3(CH_2)_{14}\ CO_2H$	hexadecanoic acid	palmitic acid	63
Greek *stear* fat	18**C**	$CH_3(CH_2)_{16}\ CO_2H$	octadecanoic acid	stearic acid	70
Arachis Peanut	20**C**		eicosanoic acid	arachidic acid	77
		Unsaturated fatty acids			
Palm oil	$C_{16:1}$ ω-7		cis- Δ^9-hexadecenoic acid	palmitoleic acid ω-7	-1
Latin *oleum* oil	$C_{18:2}$ ω-6 linseed oil		cis-$\Delta^{9,12}$-octadecadienoic acid	linoleic acid **essential** ω-6	-5
Latin *linum* flax, and *oleum*	$C_{18:3}$ ω-3 } oil		cis-$\Delta^{9,12,15}$-octadecatrienoic acid	α-linolenic acid **essential** ω-3	-11

Omega unsaturated fatty acids count start from tail methyl H_3C– group. **Essential** are ω-6 and ω-3

TABLE. **Essential Fatty Acid Nomenclature**					
	Abbreviation System				
Descriptive Name	Numeric	Δ	n	C:=	ω
Palmitate	16:0				
Palmitoleate	9—16:1	16:01 Δ 9	16:1n-7	16:01	ω–7
Linoleate	9,12—18:2	18:02 Δ 9,12	18:2n-6	18:02	ω–6
α-Linolenate	9,12,15—18:3	18:03 Δ 9,12,15	18:3n-3	18:03	ω–3

Cholesterol transport with **StAR** to the cholesterol-poor outer mitochondrial membrane (OMM) appears to involve **cholesterol transport** proteins **StAR**. Then on the inner mitochondrial membrane (IMM) Cytochrome P450scc (CYP11A1) initiates steroid genesis by converting cholesterol to pregnenolone. Acute steroidogenic responses are regulated by **cholesterol delivery** from OMM to IMM, triggered by the steroidogenic acute regulatory **StAR** protein. Chronic steroidogenic capacity is determined by CYP11A1 gene transcription. **StAR** mutations cause congenital lipoid adrenal hyperplasia, with absent steroid genesis, potentially lethal salt loss, and 46,XY sex reversal. **StAR** mutations initially destroy most, but not all steroid genesis; low levels of **StAR**-independent steroid genesis are lost later due to cellular damage, explaining the clinical findings. Rare P450scc mutations cause a similar syndrome. This review addresses these early steps in steroid biosynthesis.

Cholesterol having hydroxyl group **HO**- constitute 1/3 mass fraction of membranes. Cholesteryl esters as cholesterol alcohol group attached to an acyl  are resulting in apolar **cholesteryl ester-cholesterin**.

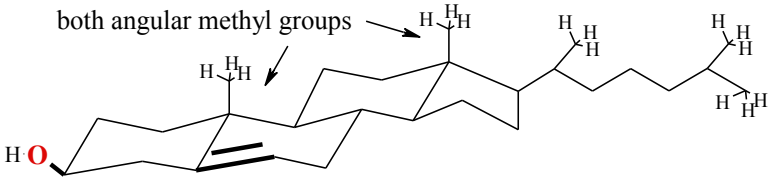


Cholesterin is deposed and stored in hepatic and extra hepatic (like leucocytes) cells as small droplets.

B. Acyl group is attached to the alcohol **HO**- via a low-energy ester bond -**O**-.

Steroid **alcohol** = cholester**ol** cholesteryl **ester – cholesterin**

Cholesterols make up 1/3 mass fraction of membrane mass, but cholesteryl esters – cholesterin do not.



Cholesterol four rings of the steroid are labeled **A**, **B**, **C** and **D**. Double bond between C5 and 6 atoms and alcohol **HO**- at C_3.**Cholesterol** is roughly planar with both angular methyl groups–CH_3 labeled 18 and 19above the plane of the molecule.

Steroid hormones are made from cholesterol, primarily derived from lipoproteins or lipocalins that enter cells via receptor-mediated endocytosis. In endo-lysosomes, cholesterol is released from cholesterol esters by lysosomal acid lipase (LAL; disordered in Wolman disease) and exported via Niemann-Pick type C (NPC) proteins (disordered in NPC disease).These diseases are characterized by accumulated cholesterol and cholesterol esters in most cell types. Mechanisms is known for trans-cytoplasmic cholesterol transport, membrane insertion, and retrieval from membranes with lipocalin proteins. Cholesterol esters and "free" cholesterol are enzymatically interconverted in lipid droplets.

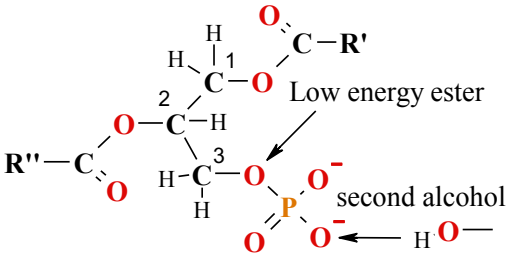
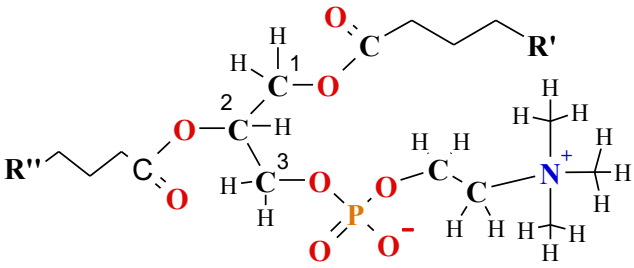
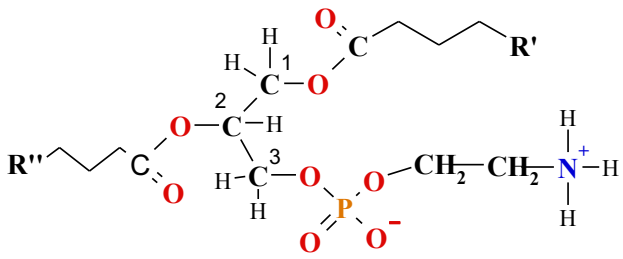


Figure. Phosphatidate.
Acyl groups are attached in ester linkage at C 1 and C 2 and phosphate is attached in ester linkage at C 3. Each of these bonds is of the low-energy variety.

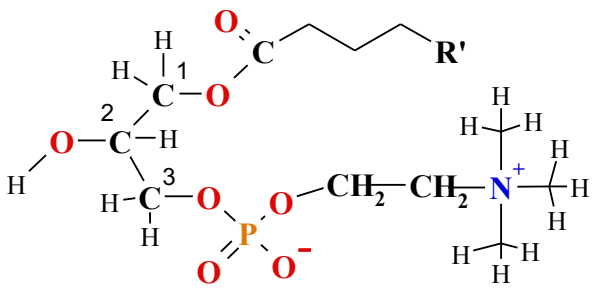
with second hydroxyl group **HO**- second ester bond -**O**- see below



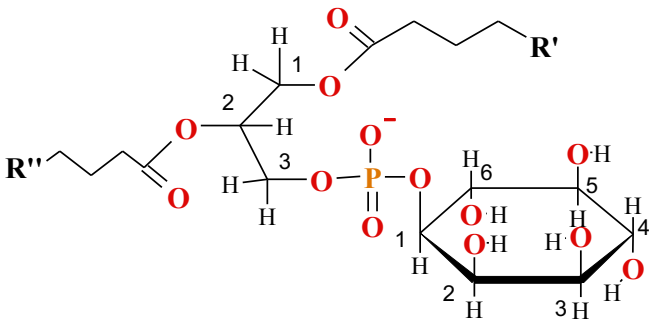
a lecithin (a Phosphatidyl **choline**$^+$)



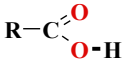
a cephalin (a Phosphatidyl **ethanolamine**$^+$ pH=7,36 proton N^+H_3))



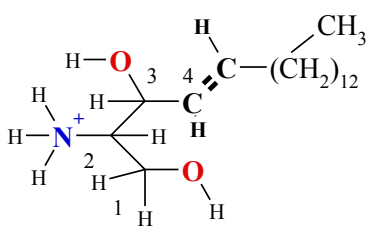
Lyso - phosphatidyl choline$^+$



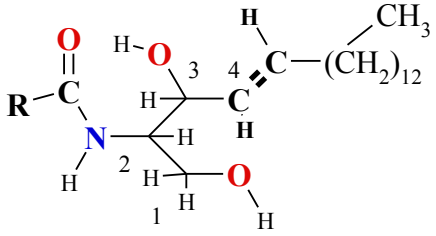
Phosphatidyl **inositol**

FIGURE. Representative glycero lipids. A nonsystematic name for phosphatidyl choline is lecithin. The one 1-*alkyl* phospholipids on platelet activating factor contain an alkyl group attached via an ether bond to the C1 carbon atom. The other compounds contain an acyl group  attached to alcohol **HO**- at C 1.

B. Sphingolipids are derivatives of **sphingosine**, an amino alcohol.

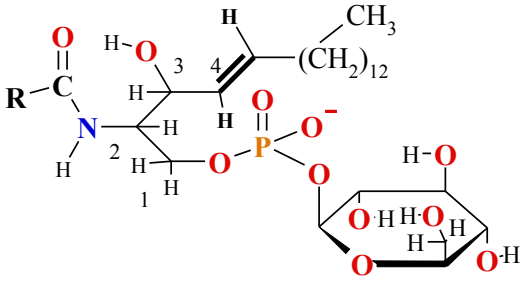


Sphingosine

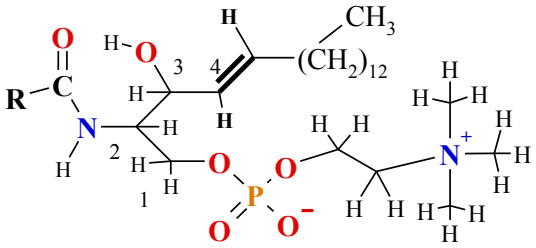


Ceramide

FIGURE. Sphingosine and representative sphingolipids. Sphingosine is a C18 compound with hydroxyl groups —**OH** on C^1 and C^3, an amino group —$\mathbf{NH_3^+}$ on C^2 and a trans double bond = at C^4



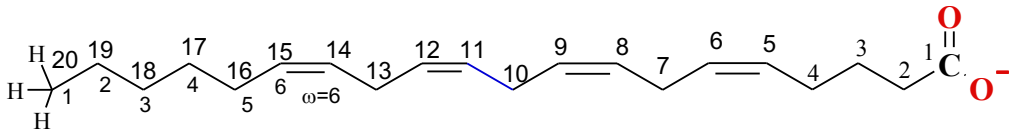
Glucosyl ceramide **(cerebroside) oligosaccharide** ceramide**(ganglioside)**



Sphingomyelin

Arachidonic acid salt arachidonate is **Phosphat**idyl **Choline** fatty acid ester component in membranes

Four **Eicosanoids** are produced in enzymatic lipid peroxidation using initial compound arachidonate.
Prostaglandins (**PGs**),
Thromboxanes (**TXs**) and
Prostacyclins (**PGIs**),
Leukotrienes (**LTs**).

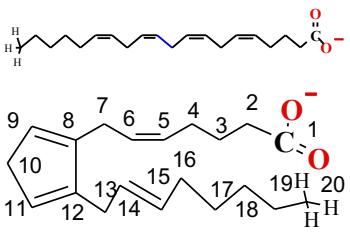


Essential ω=6 fatty acid 20-carbon compounds (Greek *eikosil* , "twenty") with four cis double bonds.

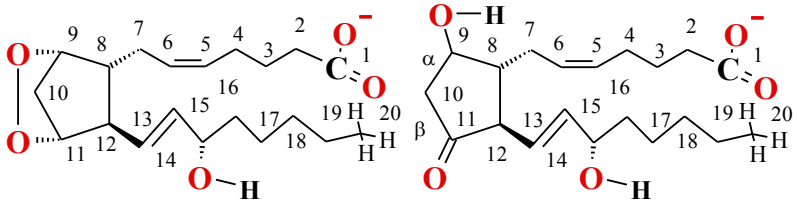
Almost all mammalian cells except erythrocytes produce one or more of **eicosanoids**,:
PGA_2, PGE_1, PGE_2, PGE_3, $PGF_{2\alpha}$, PGD_2, PGH_2, TXA_2, TXB_2 PGI_2, LTE_4 .

Enzymatic transformation of arachidonate in Cyclo Oxygenase COX begins with cross-link between C^8 —C^{12}.

This step is target of anti-inflammatory and anti-clotting human blood medicine: Aspirin, Ibuprofen, Tylenol, Paracetamol, Warfarin, which blocks cross-link between C^8—C^{12}.=>



If cross-link done COX hem **peroxidase** iron(III) Fe^{3+} by donor acceptor bond adsorbs radical oxygen **singlet** molecule •::O-:-O::• produce first **Eicosanoids**.
PGH_2 In COXI and PGD_2 in COXII Peroxidation of cross-linked



arachidonate between C^8—C^{12} start at C9 and C11 •::O-:-O::• with following peroxidation at C15 producing hydroxyl group –**OH**.
Arising **Prostaglandin** molecules produce swelled size tissue inflammation physiological reaction with strong pane.

Thromboxane is the initiating factor for blood clotting closing the damaged blood vessels.

If anti-inflammatory and anti-clotting human blood medicine: Aspirin, Ibuprofen, Tylenol, Paracetamol, Warfarin, which blocks cross-link between C^8 —C^{12} are used than:

No **Prostaglandin** and **Thromboxane** molecules arising and
No produce swelled size tissue inflammation physiological reaction with strong pane
No initiation for trombs formation in blood vessels.
Symptoms of produced swelled size tissue inflammation physiological reaction with strong pane removed,
Symptoms initiation for trombs formation in blood vessels are removed.

Lipid **peroxidation** is pollution formed radicals initiated chain reaction with creation of multiple parallel reactive and toxic specees in products. The possible process parallel **chain** reaction scheme is depicted as follows:

Important is to know that **water** plus **O=O** is source medium of peroxide formation agents: **metal$^{(n)+}$** ions, high energy ionization - radiation **~hν**, peroxisomes enzymes **Aldehyde OxidoReductases**.

Let us start from arachidonic acid salt 4 double bonds = ω6 fatty acid Eicosanoid in Membrane Bilipid Layer:

Oxygen **O=O** present oxidizing power as for agent is strong and is consequently working, which **Initiate** in life organisms bodies two different factors (**1.**, **2.**) of chain reactions and its activity depends on agents concentration: Inspiration of pure oxygen **O=O** oxidative peroxidation risk due to pollution increases five times**.**

(1) Production of radicals **R•**

1. Production of radicals **R•** from precursor **RH** by **metal$^{(n)+}$** ion as **Oxidant** (**Fe^{3+}**, **Mn^{4+}**, **Cu^{2+}**, etc).

R÷O÷O÷H + **metal$^{(n)+}$** (which transfer **H^+** and **e^-** to **Oxidant**) => **peroxide R÷O÷O•** + **metal$^{(n--1)+}$** + **H^+**

2. Production of radicals **R•** from precursor **RH** at presence of oxygen **O=O high energy radiation** (**~hν**)

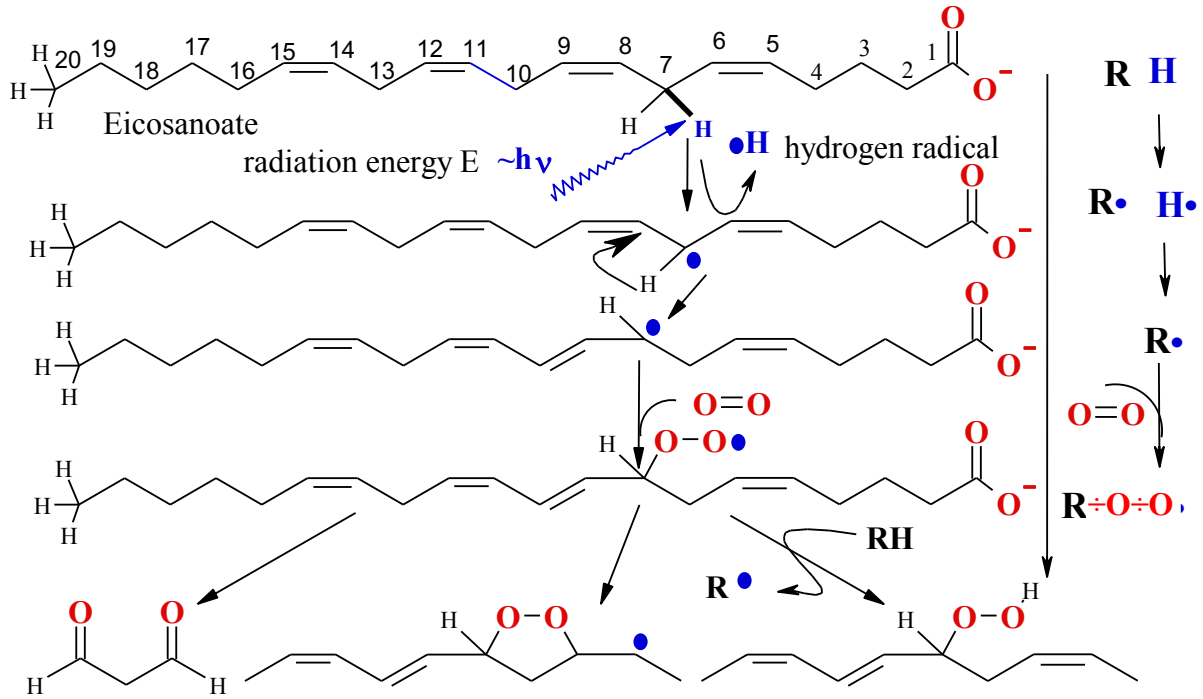
Homolytic separate **R÷H** about **H•**& **R•** as **Oxidant** separate electron pair in two free electrons • • at **H•** and **R•** **R÷H** + **~hν**=> **R•** +**H•** similar as **Oxidant metal** ions hydrogen ion accept free electron **H^+** + **e^-** =**H• is** radical.

(2) **Propagation** (new radical **R•** production):

peroxide R÷O÷O• + **R÷H => peroxide R÷O÷OH + R•**
R• + **O=O** => **peroxide R÷O÷O•** , etc.

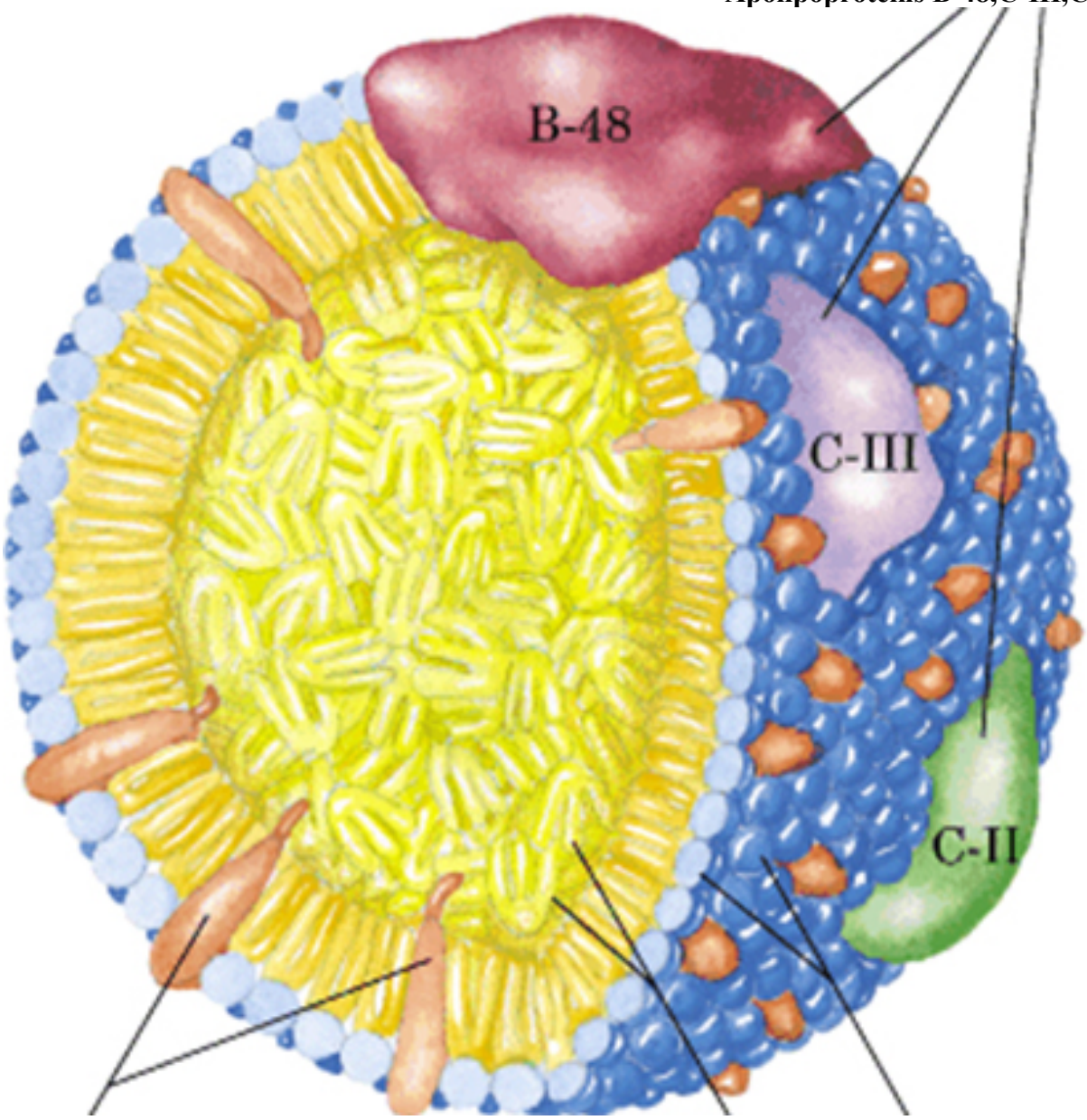
(3) **Termination** (recombination radical **R•** and **R÷O÷O•** attraction and joining):

peroxide R÷O÷O• + **peroxide R-O-O• => peroxide R÷O÷O÷R + O=O**
peroxide R÷O÷O• + **R•** => **peroxide R÷O÷O÷R**
R• + **R•=> R÷R**



Malonil aldehyde **Endoperoxide** **Hydro peroxide ROOH** does undergo **oxidation**.

FIGURE. NON-ENZYMATIC **Lipid peroxidation**. The reaction is initiated **R•** by high energy radiation (**~hν**), by heavy metal ions **Fe^{3+}**, **Cu^{2+}**,
Malonil aldehyde is only formed by fatty acids with **3** or more **>3** double bonds and is used as measure of lipid **peroxidation** together with ethane from the terminal 2-carbon of **ω3** fatty acids and pentane from the terminal 5-carbon of **ω6** fatty acids.



Apolipoproteins B-48,C-III,C-II **figure 17-2**

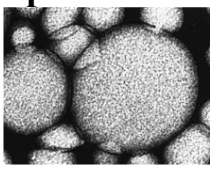
Molecular structure of a chylomicron. The surface is a layer of **phospholipids**-**cholesterol** complex with **head groups** facing the aqueous phase. **Triacylglycerides** sequestered in the **interior** (**yellow**) make up more than **80%** of the mass. Several **apolipoproteins** that protrude from the surface (**B-48**, **C-lll**, **C-ll**) act as signals in the uptake and metabolism of **Lipoprotein vesicle** content. The diameter of **Chylomicrons** ranges from about **100 nm** to about **500 nm** comprise up to 10^6 million molecules of **Fats**, **Cholesterin**, **Phosphatidyl Choline**

Cholesterol **Triacylglycerides** **Phospholipids** like **and Choleseryl esters**

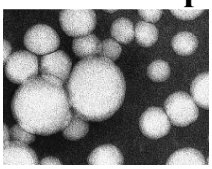
The **remnants** of **chylomicrons**, depleted of most of their **triacylglycerides** but still containing **cholesterol** and **apolipoproteins**, travel in the blood to the liver, where they are taken up by **endocytosis**, mediated by **receptors** for their **apolipoproteins**. **Triacylglycerides** that enter the liver by this route may be **oxidized** to provide energy and also to provide precursors for the synthesis of **ketone bodies**, as described in Biochemistry studies. When the diet contains more **fatty** acids in excess than are needed immediately for fuel or as **ketone bodies**, the liver converts them to **triacylglycerides**, which are packaged with specific **apolipo - proteins** into **VLDLs, LDL**. The **VLDLs, LDL** are transported in the blood to adipose tissues, where the **triacylglycerides** are removed and stored in **lipid droplets** within adipocytes. **Choleseryl esters** and **Cholesterol** metabolizing within **HDL** vesicles have been up taken in liver and extra hepatic cells.

Six blood plasma transport forms of Lipids in Lipoprotein vesicles and Lipocalins

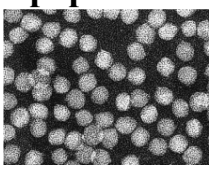
Albumin 7 Fatty acid and **Water** insoluble drug transport



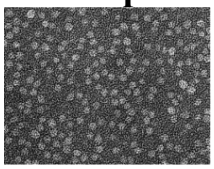
80…200 nm **Chylomicrons**



28…70 nm very low density **lipoproteins** **VLDL**



20…25 nm low density **lipoproteins** **LDL**



8…12 nm high density **lipoproteins** **HDL**

Greek Hylē - means Substance

Chylomicron - Substance of **micron size**

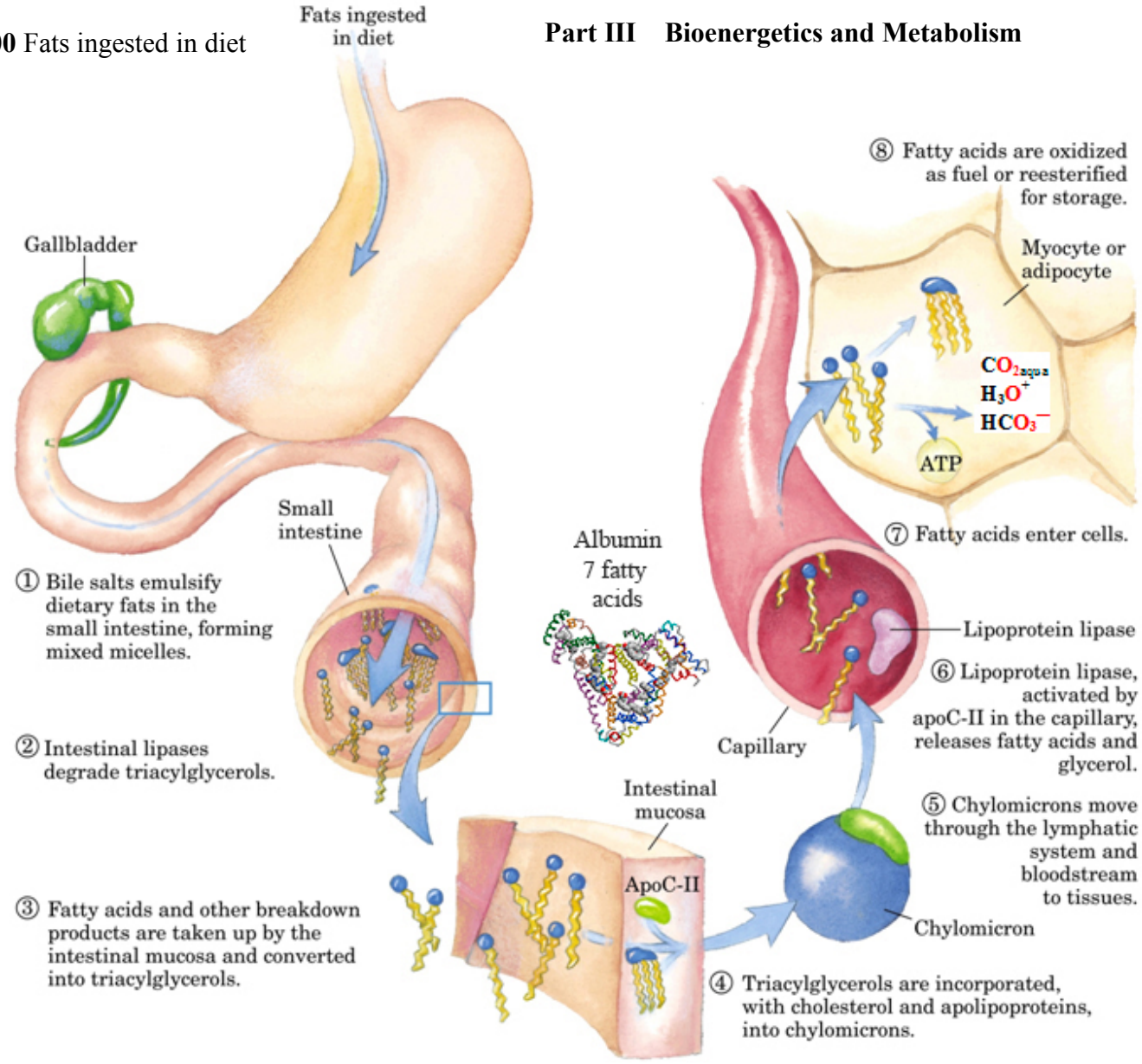


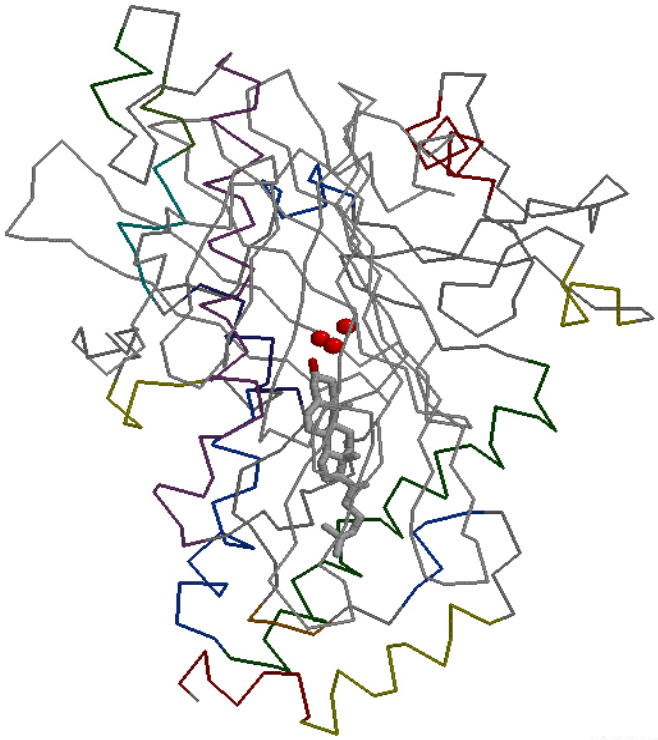
Figure. Processing of dietary lipids in vertebrates. Digestion and absorption of dietary lipids occur in the small intestine, and the fatty acids released from triacyl glycerides are packaged and delivered to muscle and adipose tissues. The eight steps are discussed in the text.

These products of **lipase** action diffuse into the epithelial cells lining the intestinal surface (the intestinal mucosa) (step (3)), where they are reconverted to **triacyl glycerides** and packaged with dietary **cholesterol** and specific proteins into lipoprotein aggregates called **chylomicrons** (Fig. 17-2; see also Fig. step (4)).

Apo lipoproteins are **lipid-binding proteins** in the blood, responsible for the transport of **triacyl glycerides**, **phospholipids**, **cholesterol**, and **cholesteryl esters** between organs. **Apo lipoproteins** ("**apo**" designates the **protein** in its **lipid**-free form) combine with lipids to form several classes of lipoprotein particles, spherical aggregates with hydrophobic lipids at the core and hydrophilic protein side chains and lipid head groups at the surface. Various combinations of lipid and protein produce particles of different densities, ranging from **chylomicrons** and **very low-density lipoproteins** (**VLDL**) to **high-density lipoproteins** (**HDL**). HDL join esterify outstanding cam Cholesterol molecule which protrude on membrane surface as insoluble. So avoid atherosclerosis and keep healthy cardiovascular state.

The protein moieties of **lipoproteins** are recognized by **receptors** on cell surfaces. In lipid uptake from the intestine, **chylomicrons**, which contain **apolipoprotein C-II** (**apoC-II**), move from the intestinal mucosa into the lymphatic system, from which they enter the blood and are carried to **PS*** (phospho lipase) and adipose tissue (step (5)). In the capillaries of these tissues, the **extracellular** enzyme **lipoprotein lipase,** activated by **apoC-II**, hydrolyzes **triacyl glycerides** to **fatty acids** and **glycerol** (step (6)), which are taken up by cells in the target tissues (step (7)). In muscle, the **fatty acids** are **oxidized** producing CO_{2aqua} ; H_3O^+ ; HCO_3^- and ATP molecules rich with **energy** for life, but in adipose tissue, they are reesterified for storage as **triacyl glycerides** (step (8)) and storing in **fat droplets**.

Lipocalins water transport of Cholesterol, Steroid hormones, vitamins K, E, D, A



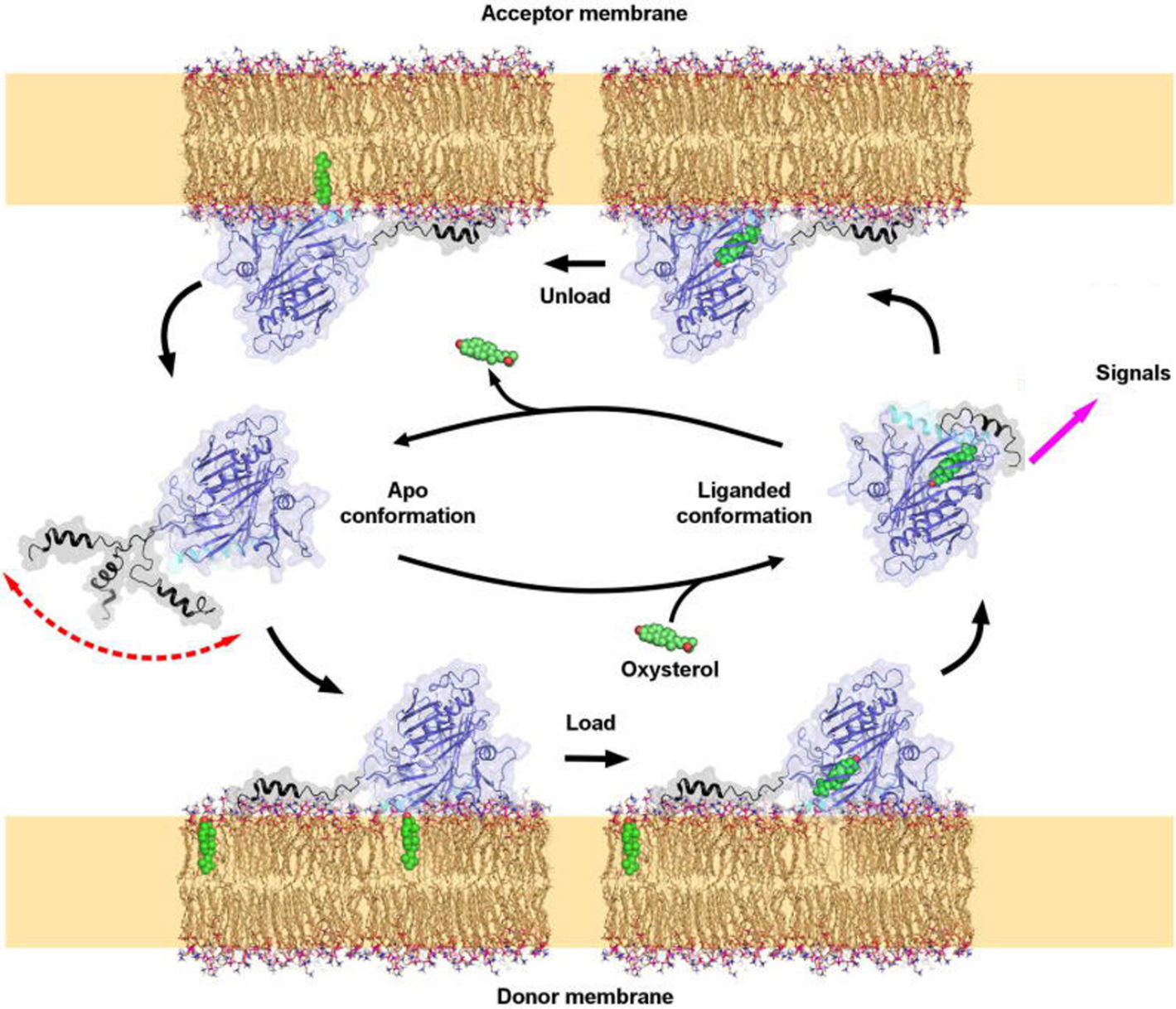
OSBP (oxy-sterol binding protein) oxy-sterol transport protein involved in cholesterol metabolic transport across membranes surface load from and unload to membranes, that keep homeostasis 33.3% mass fraction 1/3 of 100% membrane mass. **Lipocalins** like as **OSBP** mechanism is retinol **ORPs** and other **Lipocalins** for A,K,E,D vitamin transport proteins. Human has12 **OSBP** isoforms. Human isoform **OSBP4** loaded by cholesterol shown here:

Protein polypeptide chain backbone trace make 434 amino acids alpha carbon atoms from N-terminus Met1 up to C-terminus Leu434 .

OSB4 lipocalin molecule exterior surface around the lid of the tunnel three tentacle helixes contains ten highly conserved basic positive charged residues Lys15, Lys173, Lys334, Arg344, Arg347, Lys348, Lys353, Lys407, Arg410, Lys411. $-NH_3^+$ attract to negative charged $>PO_4^-$ phosphate on surface with three tentacle alpha helixes..

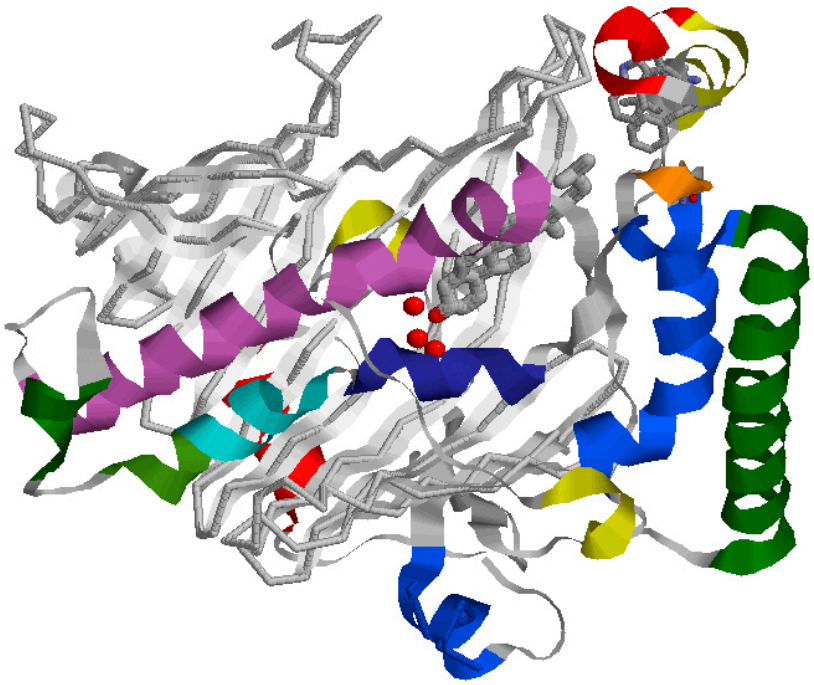
Published in Nature. 2005 September 1; 437(7055): 154–158

Steroids and vitamins K, E, D, A involved into transfer by **lipocalins** as water insoluble molecules.



In the bound conformation of Osh4, **sterol** ligands are inaccessible from the outside water molecules.

1ZHYMarz =>Steroid interaction complex with protein cholesterol head group dawn to beta sheet barrel floor. 115-293 beta sheet white 12 anti parallel strands
17 alpha α-helixes **H1**,H2,H3,H4,H5,H6,H7,H8,H9,H10,H11,H12,H13,H14,**H15**,H16,H17 CLR ligand .



Water molecules HOH2003,2004,2017,2064 near O-3. 20 that is hydrogen-bonded. Receptor activation domain protein has the ligand binding **LB** hydrophobic tunnel.

O-3 hydroxyl buried at the bottom of the tunnel and cholesterol tail chain touches the inner surface of the lid with amino acids Trp10,Phe13,Leu14,Ile17,Leu27,Ala29

The O-3-hydroxyl of cholesterol, ergosterol, and oxysterols binds to two water molecules and to the side-chain of Gln 96. This Gln96 is part of a hydrated cluster of polar side-chains at the bottom of the tunnel.
Gln96,Trp46,Tyr97,Asn165, and Gln181 comprise the remainder of the polar cluster at the tunnel bottom, and form water-mediated interactions with the O-3-hydroxyl.

30-117 consists of a two-stranded β-sheet and three α-helixes H4,H5,H6,H7,H8,H9 that form a 50 Å long anti parallel bundle,which runs the entire length of the barrel. The portion of the bundle distal to the lid fills the center of the barrel and thereby plugs the far end of the tunnel. The proximal portion of the bundle forms one wall of the tunnel, replacing the missing strands of the barrel.

There is a large C-terminal region residues 308-434 following the barrel red color. The exterior surface around the lid of the tunnel contains ten highly conserved basic residues
Lys15,Lys173,Lys334,Arg344,Arg347,Lys348,Lys353,Lys407,Arg410,Lys411 off.

Osh4 has a novel fold, the burial of its ligands in a central hydrophobic tunnel is reminiscent of the structures of other lipid binding and transport proteins.
Pro1,Ala5,Leu24,Leu27,Ala29,Pro31,Ile33,Leu39,Phe42,Leu93,Gly105,Pro110,
Leu111,Pro145,Pro146,Val147,Ala149,Ile167,Ala169,Phe171,Leu175,Leu177,
Val179,Phe182,Pro198,Pro199,Pro200,Ile203,Ile206,Leu207,Val208,Ala209,
Pro211,Phe212,Val213,Leu215,Leu290,Pro304,Leu305,Ala321

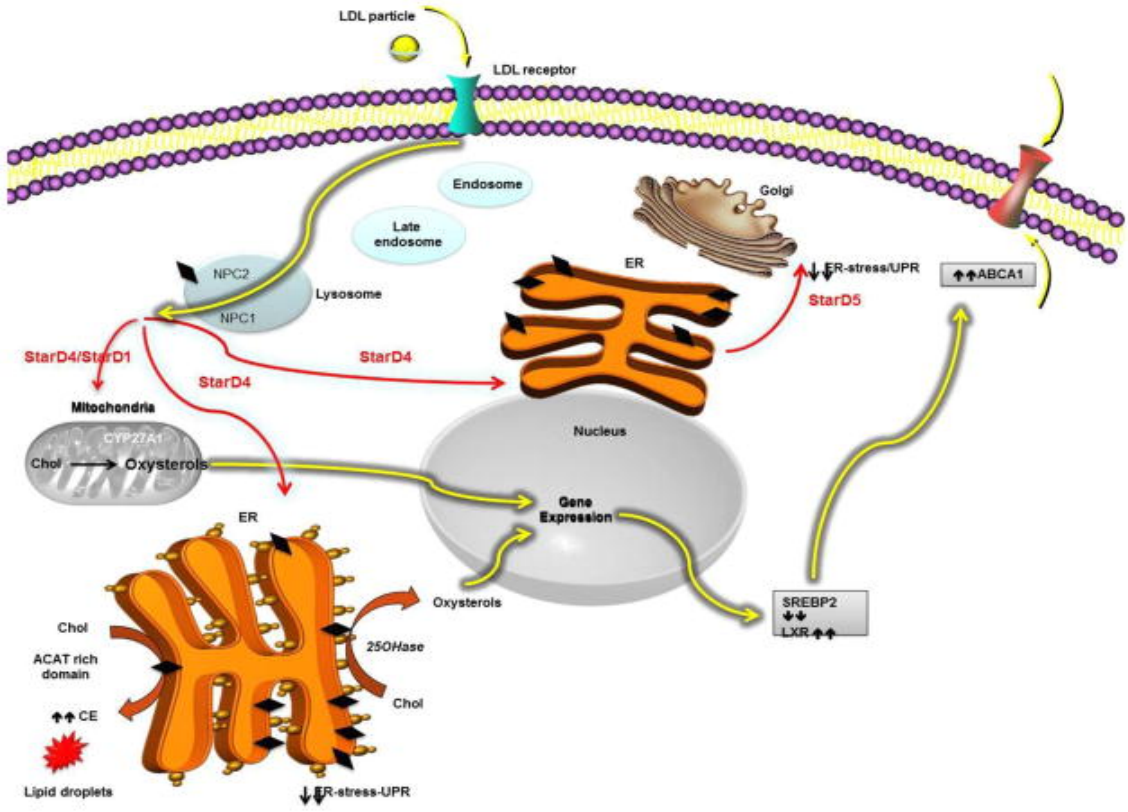


Figure 2 Schematic representation of intracellular cholesterol transport. A model for StarD4 and StarD5. Highlighted in red are potential functions of StarD4 and StarD5 in the distribution of free cholesterol (◆).

Steroidogenic acute regulatory protein (StAR) transport (START) intracellular
of steroids, bile acids. phospholipids, sphingosines, K, E, D, A vitamins transport in water

The steroidogenic acute regulatory protein-related lipid transfer (START) domain family are defined by the presence of a conserved 195÷240 amino acid sequence that folds into an α/β helix-grip structure forming a hydrophobic pocket for ligand binding. The mammalian START proteins bind diverse ligands, such as cholesterol, oxysterols, phospholipids, sphingolipids, possibly fatty acids and K,E,D,A vitamins, and have putative roles in non-vesicular lipid transport, thioesterase enzymatic activity, and tumor suppression. J Endocrinol March 1, 2012; **212**257-275

Fifteen mammalian proteins, STARD1-STARD15, possess a START domain for six subfamilies: cholesterol, 25-hydroxycholesterol, phosphatidylcholine, phosphatidylethanolamine and ceramides are ligands for STARD1/STARD3/STARD5, STARD5, STARD2/STARD10, STARD10 and STARD11, respectively. The lipids or sterols bound by the remaining 9 START proteins are unknown. Recent studies show that the C-terminal end of the domain plays a fundamental role, forming a lid over a deep lipid-binding pocket that shields the ligand from the external environment. The START domain can be regarded as a lipid-exchange and/or a lipid-sensing domain. Mammalian START proteins have diverse expression patterns and can be found free in the cytoplasm, attached to membranes or in the nucleus. They appear to function in a variety of distinct physiological processes, such as lipid transfer between intracellular compartments, lipid metabolism and modulation of signaling events. Mutation or misexpression of START proteins is linked to pathological processes, including genetic disorders, autoimmune disease and cancer.

A member of the STARD4 subfamily, was monitored. . Cholesterol stabilized STARD3-START against trypsin-catalyzed degradation, whereas cholesterol had no protective effect on STARD1-START.

Phosphorylated sphingolipids ceramide-1-phosphate (C1P) and sphingosine-1-phosphate (S1P) have emerged as key regulators of cell growth, survival, migration and inflammation. C1P produced by ceramide kinase is an activator of group IVA cytosolic phospholipase A2α (cPLA2α), the rate-limiting releaser of arachidonic acid used for pro-inflammatory eicosanoid production, which contributes to disease pathogenesis in asthma or airway hyper-responsiveness, cancer, atherosclerosis and thrombosis. To modulate eicosanoid action and avoid the damaging effects of chronic inflammation, cells require efficient targeting, trafficking and presentation of C1P to specific cellular sites.

Physiological function remain in the trafficking of cholesterol and in its cellular homeostasis [14, 15].

Possible medical and industrial applications The concepts reviewed represent novel approaches to further define intracellular cholesterol transport. We suggest that the newly described proteins of the StarD4 subfamily, with their distinctive regulations and localizations, alone or in association with other proteins, play unique and relevant roles in intracellular cholesterol movement and metabolism in a variety of tissues of relevance to human health. Furthermore, future studies on the role of these proteins will give a better understanding of cholesterol metabolism needed to lay the groundwork for the development of better therapies for cholesterol related disorders (i e. atherosclerosis or Niemann-Pick disease) and UPR related diseases (i.e. Huntington's disease and Alzheimer's disease).

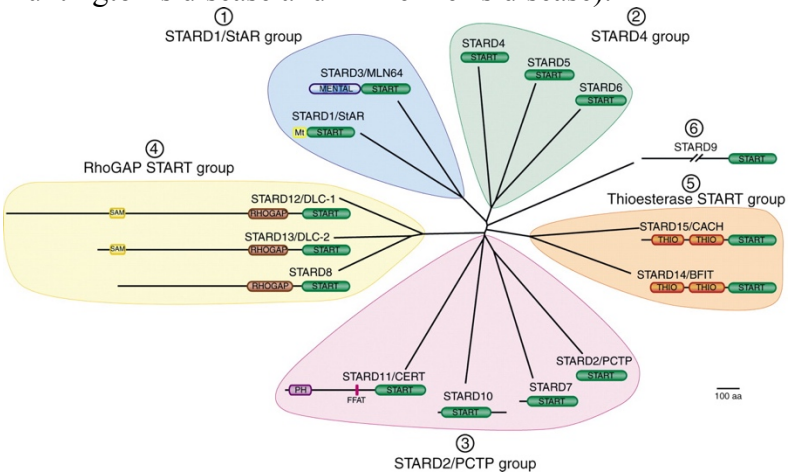


Fig. 1. Phylogenetic tree and the 15 START-domain proteins in humans. START domain sequences were aligned by the Eclustalw program (Genetics Computer Group, Madison, WI). The phylogenetic tree was drawn with the drawtree software [J. Felsenstein, 1993, PHYLIP (Phylogeny Inference Package) v.3.5c, Department of Genome Sciences, University of Washington, Seattle, WA]. Abbreviations: Journal of Cell Science 2005 118: 2791-2801. Mt, mitochondrial targeting motif; MENTAL, MLN64 N-terminal domain; PH, pleckstrin homology domain; FFAT, two phenylalanines in an acidic tract motif responsible for ER targeting; RHOGAP, Rho-GTPase-activating-protein domain; SAM, sterile alpha motif; THIO, acyl-CoA thioesterase domain. Journal of Cell Science 2005 118: 2791-2801 1EM2, 1LN1

Table 1 Characteristics of the mammalian START 4 domain protein subfamily members

Physical map positions (chromosome, position in megabases, Mb) in the mouse and human genomes are based on the Ensembl database (www.ensembl.org). Cellular location abbreviations used are: endoplasmic reticulum (ER). Lipid binding abbreviations used are: 7-α-hydroxycholesterol (7-α-OHchol), 25-hydroxycholesterol (25OH), cholic acid (CA) and chenodeoxycholic acid (CDCA).

StarD4 subfamily members			
Other name (s)	**StarD4** CRSP	**StarD5**	**StarD6**
Chromosomme/Position **Mouse**	18/33.4 Mb	7/73.3 Mb	18/70.8 Mb
Human	5/110.5 Mb	15/77.6 Mb	18/52 Mb
Subcellular location	Cytosolic[a], ER[a],[b], mitochondria[b][A,B]	Cytosolic, ER, Golgi[a], Nucleus[C–F]	Cytosolic[c], mitochondria[b] [G,H]
Tissue distribution *	Liver, macrophages, keratinocytes, kidney*[A,B]	Kupffer cells, peripheral macrophages, kidney proximal tubules*[D,E]	Nervous system and testis germ cell[G, I–K]
Lipid binding	Cholesterol, 7-α-OHchol, 7-hydroperoxycholesterol [#],[e][C, L–N]	Cholesterol, 25OH, CA and CDCA [d],[e][C,O,P]	Cholesterol[d],[e] [N]
Regulation	Regulated in response to sterol levels by SREBP pathway[B, Q]. Early phase of ER stress[R]	Induced in response to ER stress [D,F,Q]	Potential regulation under neurotoxic conditions[G,I, J]

* Tissue distribution: Restricted expression, note that STARD4 and STARD5

J Endocrinol March 1, 2012; **212**257-275 1EM2, 1LN1

Table 1. Human START proteins, their ligands, and the available crystal structures.

Group	Protein	Ligand	PDB entry
1 - StAR	STARD1	cholesterol [5]	3P0L; ligand-free (this study) 212 AA
	STARD3/MLN64	cholesterol [5]PLoS One. 2011;6(6)2I93+CLR,	1EM2; ligand-free [5] 213 AA
2 - START	STARD4	cholesterol [19]	1JSS (mouse); ligand-free [7] 222 AA
only	STARD5	cholesterol, 25-hydroxycholesterol [19]	2R55; ligand-free (this study) 213 AA
	STARD6	cholesterol [40]	2MOU to be published 2014 222 AA
3 - PCTP	STARD2/PCTP	phosphatidyl choline [41]1LN3,1LN1	, 1LN2; DLP complex[1] [6] 210 AA
	STARD7	phosphatidyl choline [42]	-
	STARD10	phosphatidylcholine/ethanolamine [43]	-234 AA 234 AA
	STARD11/CERT	ceramides [8] **4K80**, **4KF6**, **4K85**,	**2E3R**; C18-ceramide complex [8]; 2Z9Z;
	2E3M,**2E3N**,**2E3O**, **2E3P**,**2E3Q**,2E3S, **2Z9Y**,**2Z9Z**	**4K84**, **4K8N**, 4KBS, 4KBR 214 AA Nature. 2013 Aug 22;500(7463):463-7	C10-DAG complex[2] [8]; 3H3S; H15 complex[3] [9] and 10 more entries
4 - RhoGAP	STARD8	charged lipid?	-
	STARD12	charged lipid?	-
	STARD13	charged lipid?	2PSO; ligand-free (this study) 195 AA
5-Thioesterase	STARD14	fatty acid?	3FO5; PEG complex[4] (this study) 240 AA
	STARD15	fatty acid? PNAS. 2008	**2E3R**,2E3S,2Z9Y,2Z9Z,2E3Q, 2E3P, 2E3M,2E3N,2E3O,
6 -STARD9	STARD9	**?**Journal.PLoS One. 2011;6(6):e19521	3P0L,**1LN1**,1EM2,**1JSS**,**2R55**,2E3O,2PSO,3FO5

Central amphiphatic tunnel is reminiscent of the structures of mentioned lipid binding .

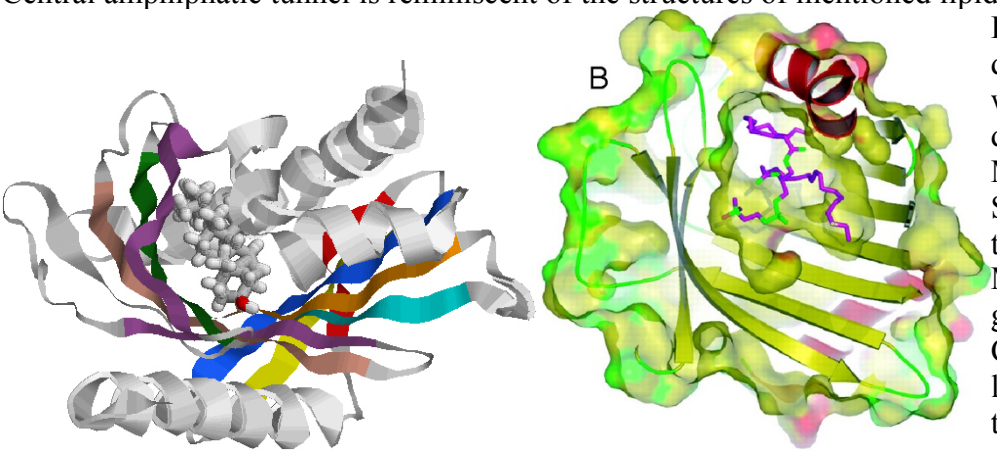
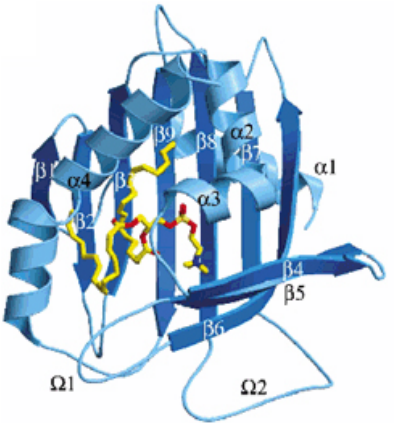


Fig. 2. Structure of the START domains of MLN64 (A) and PCTP with its ligand (B). (A) Ribbon diagram of the START domain of MLN64 (ID code: 2I93.PDB). Secondary structural elements and the C- and N-termini are labeled. MLN64 has a central β-sheet gripped by N-terminal (α1) and C-terminal (α4) α-helices (red), the latter being closely packed above the curved sheet.

(B). Cut-away view of the molecular surface of the START domain of PCTP complexed with(DLPC,dilinoleoyl-*sn*-glycerol-3-phosphorylcholine) a phosphatidylcholine molecule (ID code:

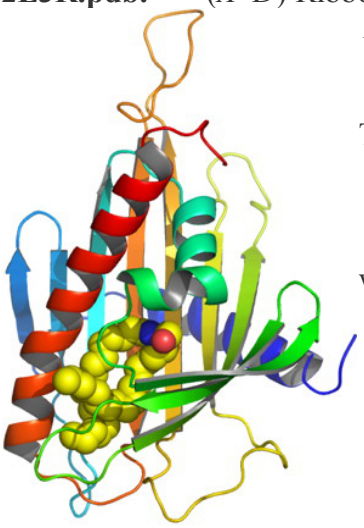
1LN1.PDB). The DLPC molecule (shown in stick representation) is located in the hydrophobic. Journal of Cell Science 2005 118: 2791-2801 1EM2.pdb, 2I93.pdb, 1LN1.pdb



The closest analogy is to the chole**sterol**-binding steroidogenic acute regulatory protein (StAR) transport (START) domain proteins MLN64 and StarD4, the phosphatidyl choline (PC) binding START domain protein PC-TP, and the mammalian phosphatidyl inositol transfer proteins. In these structures the ligands are completely sequestered from solution. For the ground prominence is water-mediated interactions.
Central amphiphatic tunnel is reminiscent of the structures of mentioned lipid binding.

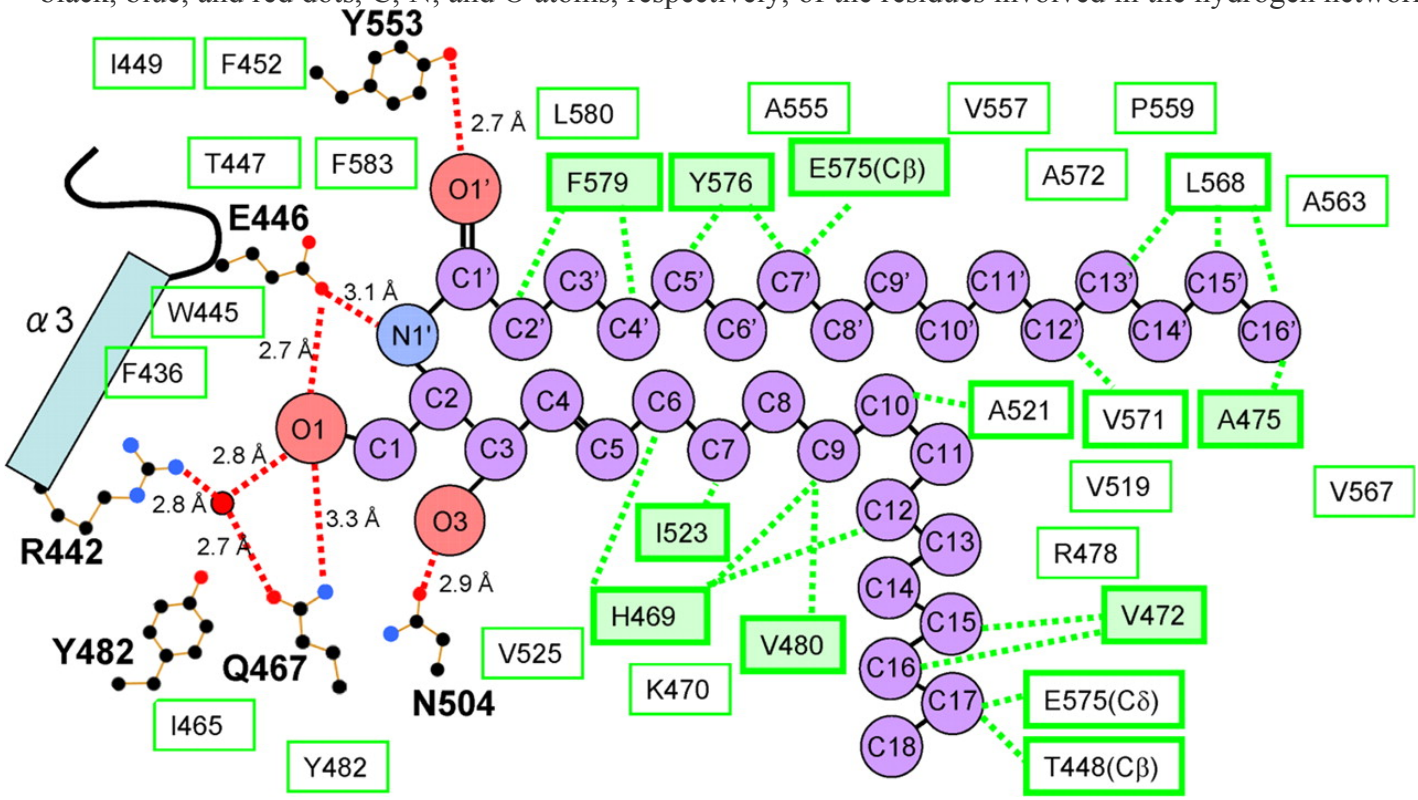
Nature Structural Biology **9**, 507 - 511 (2002) **1LN1.pdb** 1LN2 and 1LN3

Fig. 6. Temperature factors and a putative entrance to the amphiphilic cavity 2E3R.pdb. (*A–D*) Ribbon diagrams of the CERT START domain colored according to the crystallographic *B*-factors for the apo-CERT START domain and in complex with C_6-, C_{16}-, and C_{18}-ceramide, respectively. The ceramide molecules are drawn as filled spheres. (*E*) Ribbon representation of the CERT START domain in complex with C_{18}-ceramide. The structure is rotated by 45° around the *y* axis with respect to those shown in *D*. α3 and Ω1 loop are colored cyan and magenta, respectively. C_{18}-ceramide is drawn as space-filling spheres, in which yellow, blue, and red spheres represent C, N, and O atoms, respectively. (*F*) Molecular surface of the CERT START domain in complex with C_{18}-ceramide, drawn in the same orientation as in *E*. The hydrophobic surface is painted green, and the residues in α3 and Ω1 loop are highlighted as dotted spheres in cyan and magenta, respectively. C_{18}-ceramide is drawn as in *E*.



PNAS. 2008 2E3M, 2E3N, 2E3O, 2E3P, 2E3Q, **2E3R**, 2E3S, 2Z9Y, and 2Z9Z

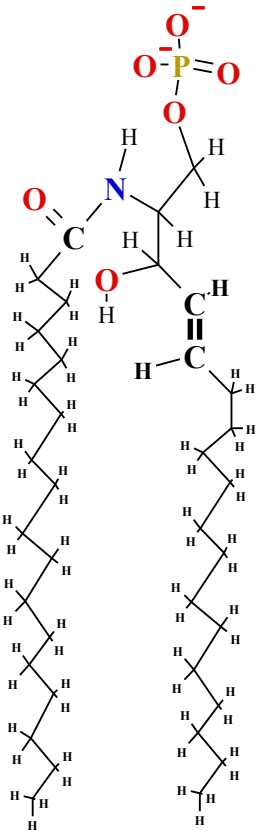
Fig. 2. Schematic representation of C_{16}-ceramide recognition by the CERT START domain. Residues lining the amphiphilic cavity are shown. Red dashed lines, hydrogen bonds; red circles, water molecules; black, blue, and red dots, C, N, and O atoms, respectively, of the residues involved in the hydrogen network.

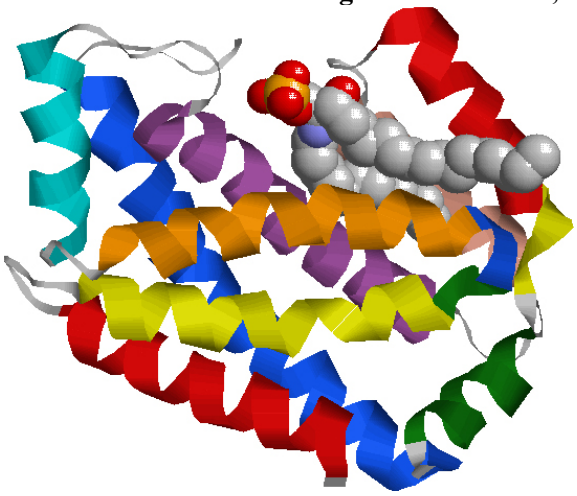


Green boxes indicate residues contributing to the hydrophobicity of the cavity in general, whereas green boxes with thick borders indicate those with direct hydrophobic interactions, which are represented as green dashed lines. Among these, eight amino acid residues, which are common to all of the C_6-, C_{16}-, C_{18}-ceramide complex structures, are indicated by thick-bordered green boxes filled with light green.

eukaryotes Nature. 2013 Aug 22;500(7463):463-7. 4K80, 4KF6, 4K85, 4K84, 4K8N, 4KBS, 4KBR

Figure 2 **CPTP conformation and functional recognition of C1P. a,** CPTP lipid headgroup recognition center residue interaction with phosphate and amide groups of bound 16:0-C1P (spacefill). Hydrogen-bonds = dashed lines. CPTP Cα backbone is light gray; side chains; and oxygen and nitrogen, red and blue, respectively. Water molecules are pink spheres. **b**, C1P transfer by CPTP point lines of phosphate headgroup recognition cavity. wtCPTP (gray). **c,** Non-polar residues forming hydrophobic pocket that accommodates 18:0-C1P sphingosine and acyl chains. **d**, C1P transfer by CPTP point mutants (violet) of the hydrophobic pocket. wtCPTP (gray); Side-chains shown in panel c. Data in b and d represent the mean ± s.d. of three independent experiments. **e**, Conformational changes in hydrophobic pocket upon 18:0-C1P binding. Side-chains (lavender; stick) of apo-CPTP and human CPTP with bound 18:0-C1P (yellow; ball-and-stick).





f, g, Surface electrostatics of hydrophobic pocket opening at lipid headgroup recognition sites in apo-CPTP (f) and CPTP/18:0-C1P complex (g). **h,** 18:0-C1P chemical structural formula.

i, j Crystal structures of CPTP (ribbon) with bound 18:0-C1P in sphingosine-in i (CPK); sphingosine conformations. **k,** Superposition of bound 18:0-C1P in sphingosine- conformation.

The hydrophobic pocket is lined by ~25 nonpolar residues, mainly Phe, Leu, Val, and Ile that prevent water entry while ensheathing the ceramide aliphatic chains. Mutation of L43, L118, or L146 to positively-charged R or V57 or V158 to high polarity N compromises hydrophobic pocket functionality and strongly diminishes C1P transfer. More conservative mutation (e.g. W117A) only moderately reduces C1P transfer, while F42A near the pocket bottom stimulates C1P transfer. Mutation near the entry portal (I53N) or in the flexible α1–2 loop (F50R) is well tolerated (75–80% active) (Fig. 2d). Ceramide entry is oriented by hydrogen bonding of the lipid amide oxygen and nitrogen with H150 and D56, respectively. Hydrogen bond disruption between lipid amide nitrogen and D56 (D56V) moderately slows C1P transfer, but H150 mutation (H150L) abolishes activity. Super positioning of apo- and 16:0-C1P/CPTP structures (rmsd 1.4 Å) shows K60, R106 and R110 nearly identically positioned in the positively-charged surface cavities. Yet, large conformational differences exist for VI53, W36, W119 and F52 (Fig. 2e) due to closer packing of certain α-helices in apo-CPTP (Fig. S2d,e). Many Leu and Phe are repositioned, reducing the solvent accessible (SA) volume (40 $Å^3$) (Table S4) and effectively collapsing the hydrophobic pocket (Fig. 2f,g) compared to 18:0-C1P/CPTP complex (364 $Å^3$).

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Cellular retinaldehyde-binding protein (CRALBP) is essential for mammalian vision by routing 11-cis-retinoids for the conversion of photo bleached opsin molecules into photosensitive visual pigments. The arginine-to-tryptophan missense mutation in position 234 (R234W) in the human gene RLBP1 encoding CRALBP compromises visual pigment regeneration and is associated with Bothnia dystrophy. Our structural model of wild-type CRALBP locates R234 to a positively charged cleft at a distance of 15 A from the hydrophobic core sequestering 11-cis-retinal.

Fig. 1. Monomeric structure of CRALBP. (*A*) Ribbon diagram of wild-type CRALBP bound to 11-*cis*-retinal. The helices of the N-terminal domain **H1**,**H2**,**H3**,**H4**,**H5** C-terminal helices **H14**,**H15**,**H16**; the helical gate **H11**,**H12**,**H13** β-strands are indicated in yellow. The position of R234 is indicated as CPK space fill, the 11-*cis*-retinal ligand is shown as CPK space fill in the cavity. The cavity surface was calculated with VOIDOO (33). (*B*) View after rotation of *A* by 90° on the vertical axis. Images were generated with Chemscape-Raswin. Proc Natl Acad Sci U S A. 2009 Nov 3;106(44):18545-50. 3HY5