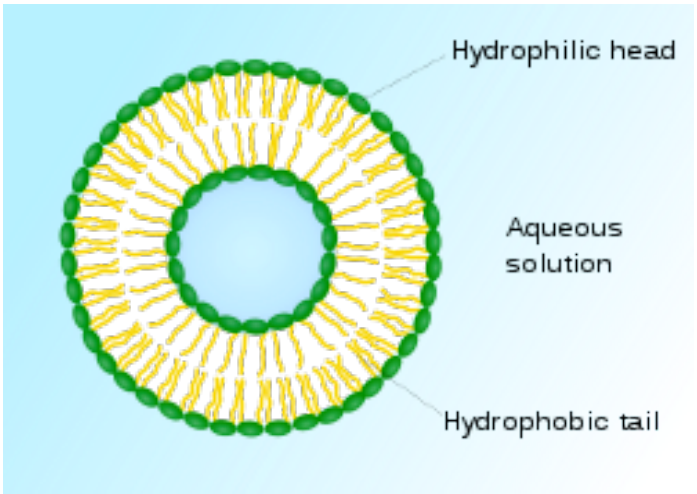
LIPOSOMES

The word liposome derives from two Greek words: lipo ("fat") and soma ("body"); it is so named because its composition is primarily of phospholipid.

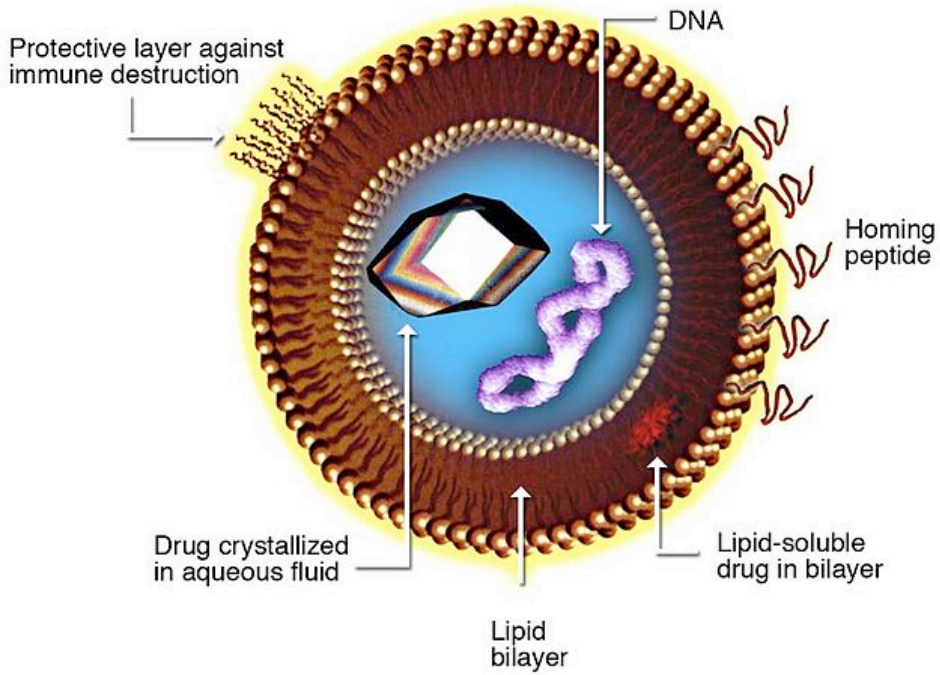
Liposomes were first described by British haematologist Alec D. Bangham in 1961 (published 1964), at the Babraham Institute, in Cambridge.

Liposomes can be easily distinguished from micelles and hexagonal lipid phases by negative staining transmission electron microscopy.

A **liposome** is an artificially-prepared spherical vesicle composed of a lamellar phase lipid bilayer.



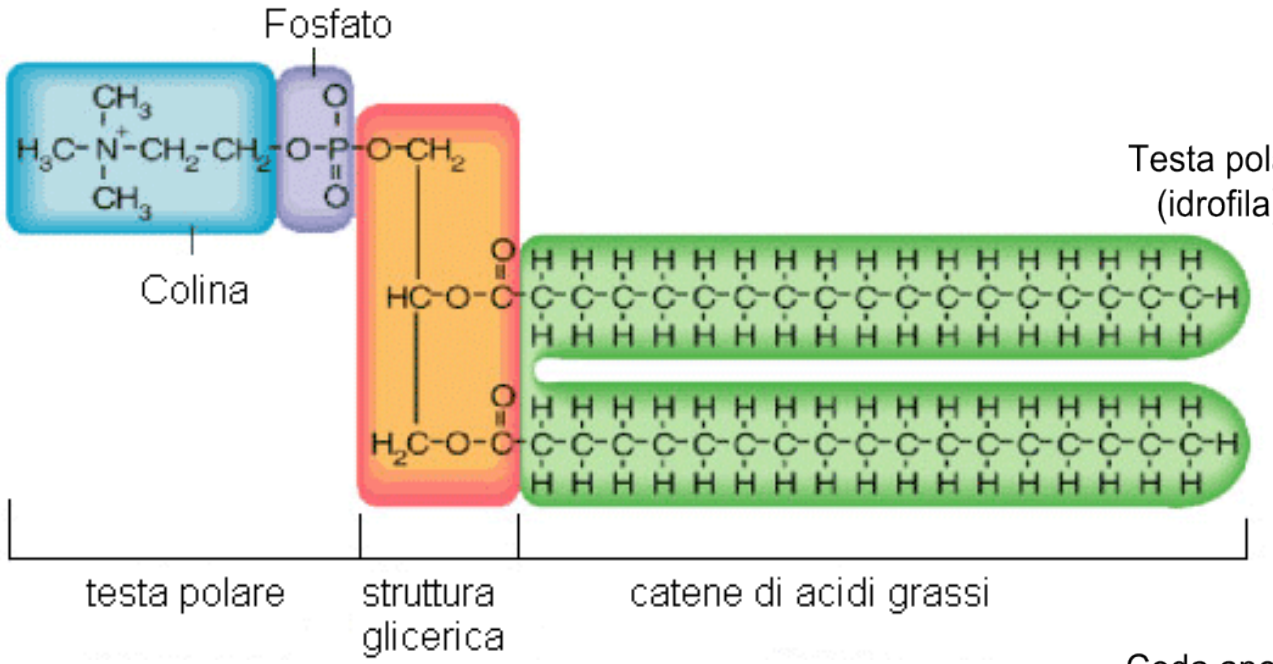
Liposome for Drug Delivery

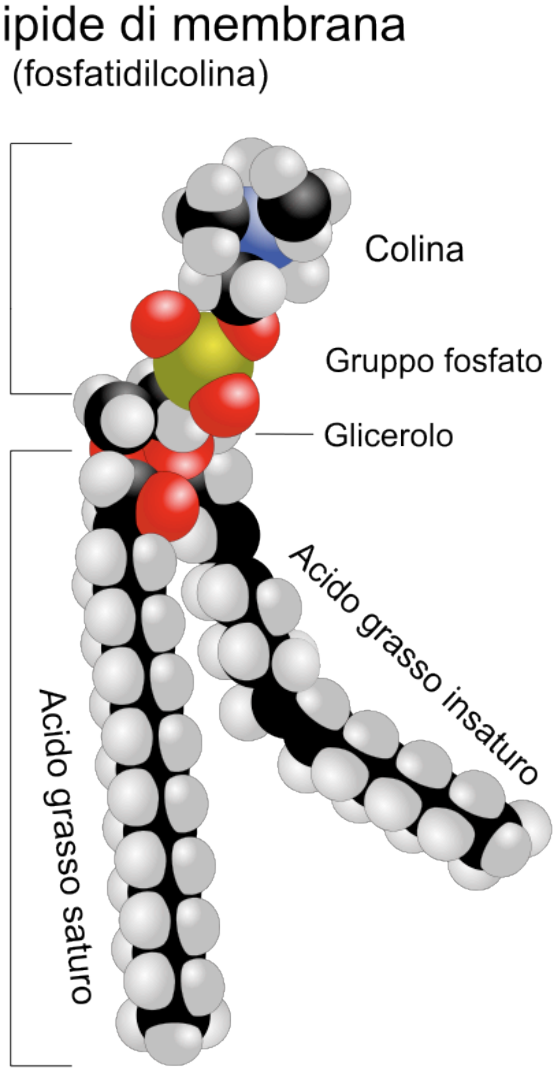


Liposomes are composite structures made of phospholipids and may contain small amounts of other molecules. Though liposomes can vary in size from low micrometer range to tens of micrometers, unilamellar liposomes, as pictured here, are typically in the lower size range with various targeting ligands attached to their surface allowing for their surface-attachment and accumulation in pathological areas for treatment of disease.

fosfolipidi – struttura

sono i principali costituenti strutturali delle membrane cellulari, doppio strato fosfolipidico

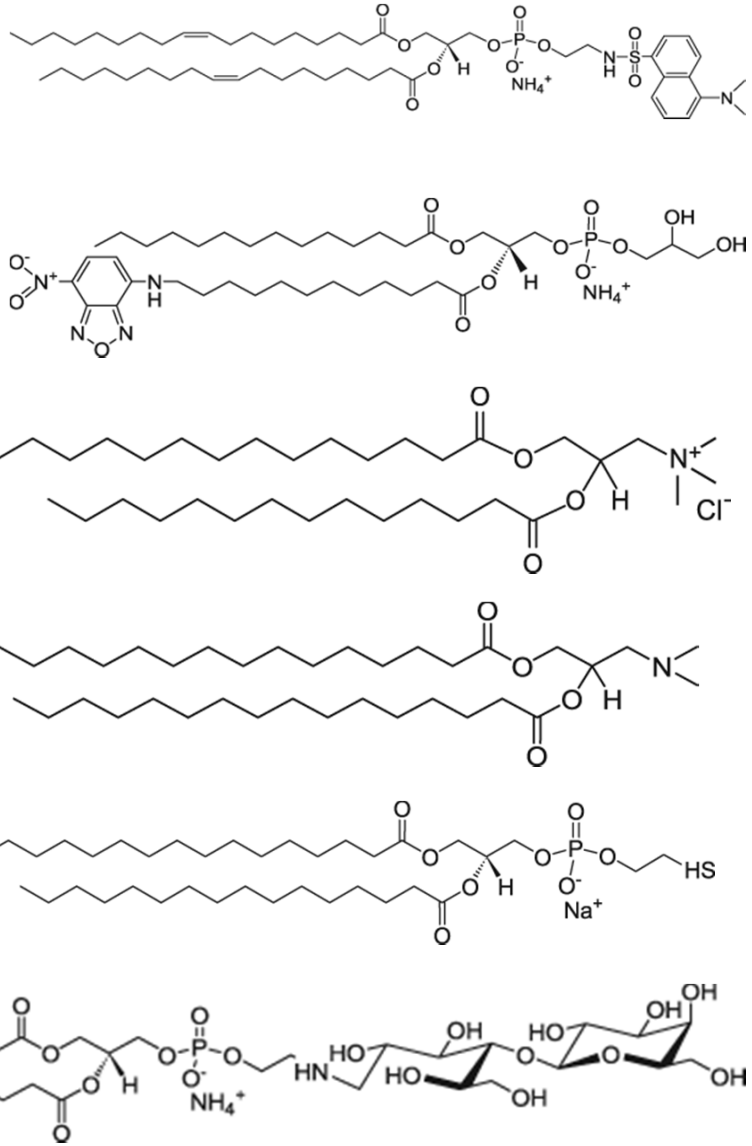




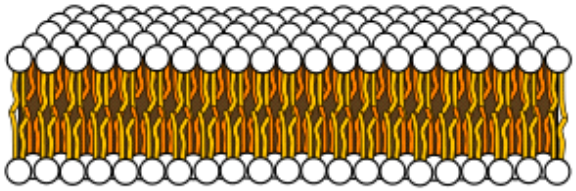
il glicerolo è esterificato in posizione 1 e 2 con acidi grassi e in posizione 3 con acido ortofosforico. L'acido ortofosforico, oltre alla esterificazione con il glicerolo, presenta una seconda esterificazione con un alcol (amminoalcol o un amminoacido con gruppo alcolico o uno zucchero). Di conseguenza i diacil-fosfolipidi vengono indicati con il prefisso fosfatidil-, cui segue il nome del composto esterificato con il gruppo fosfato (es. fosfatidil-colina, fosfatidil-etanolammina, fosfatidil-inositolo)

Self-assembled system

Large library of building blocks

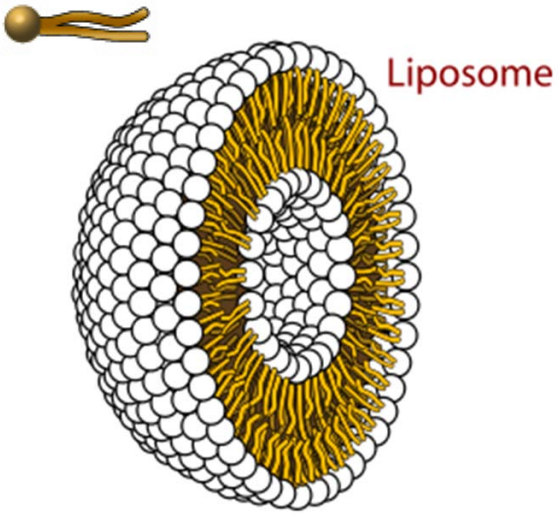


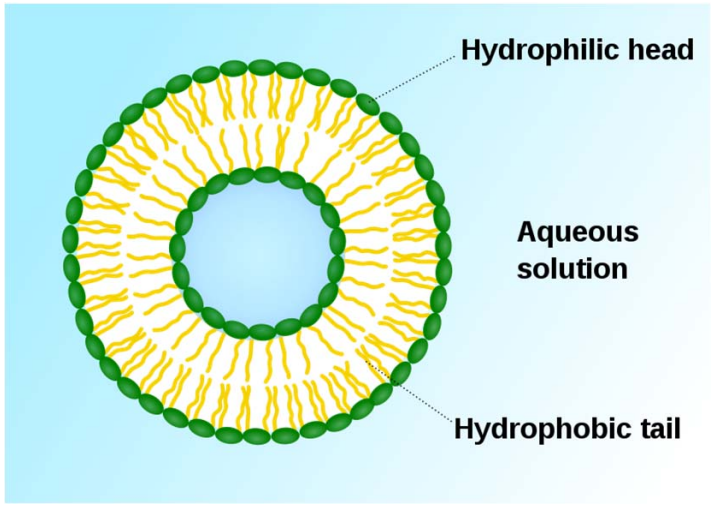
Double layers

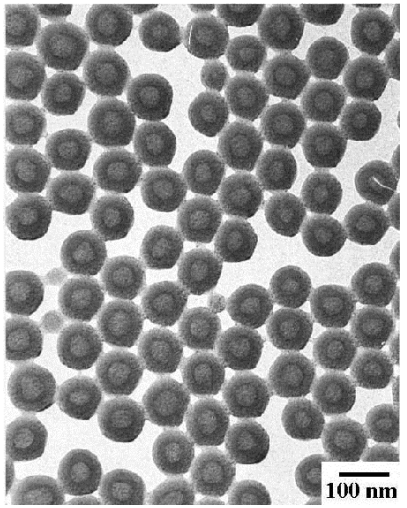


Doppio strato

Se un tensioattivo possiede due code idrofobiche, queste rendono la sua struttura cilindrica, favorendo un impaccamento a doppio strato

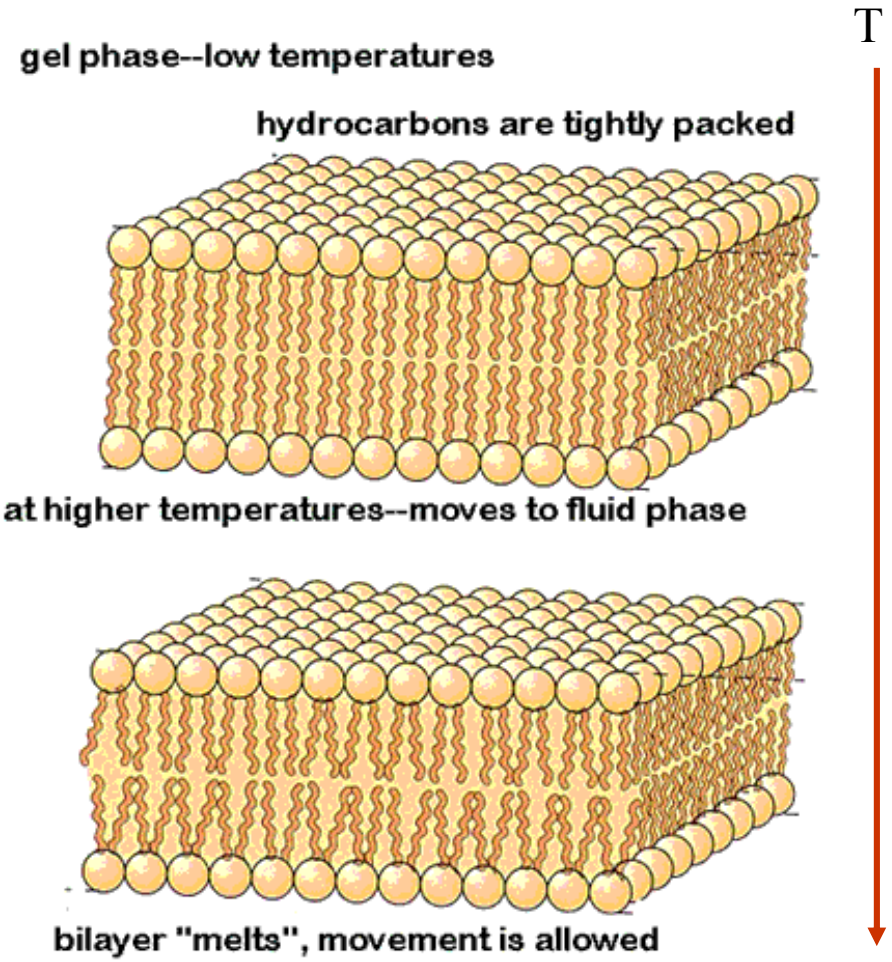


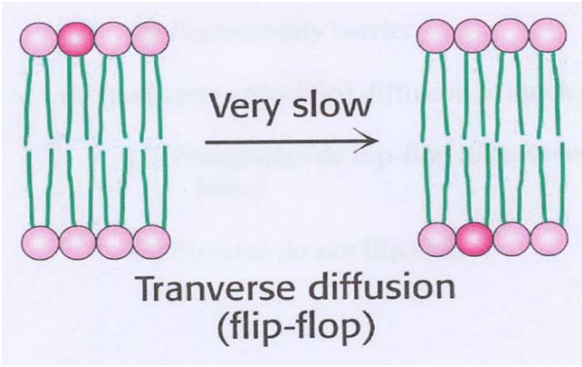




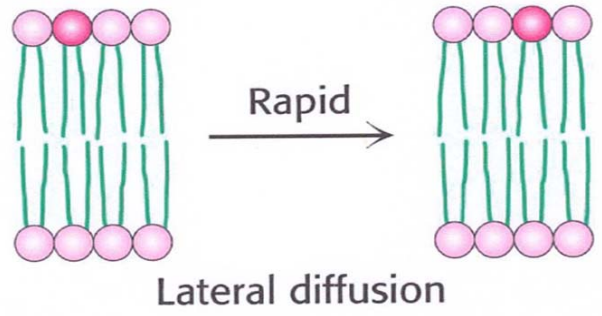
Vescicola/liposoma

Vesicles and liposomes





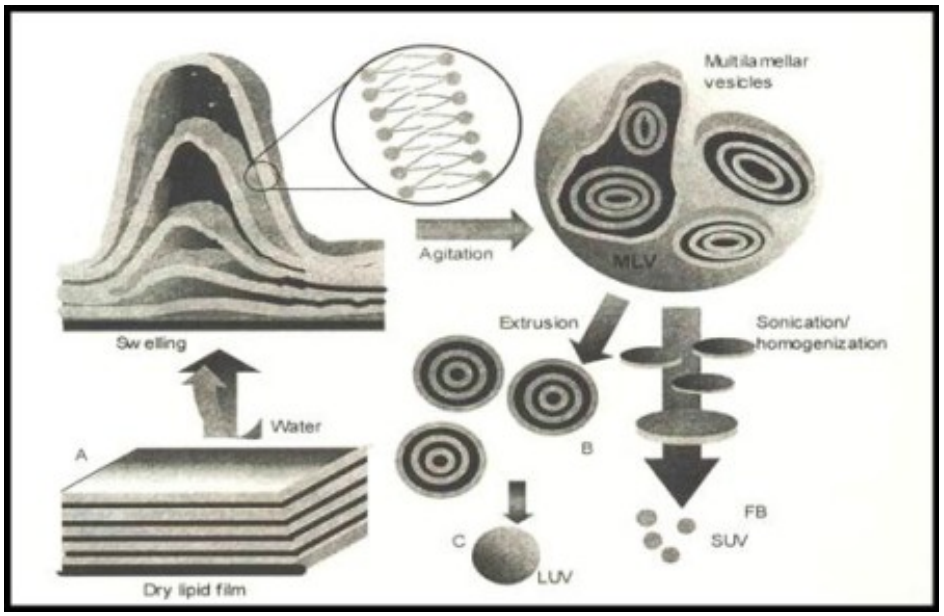
Il passaggio di un tensioattivo da una parte del doppio strato alla parte oppost è sempre molo lento. Il movimento dalla stessa parte dello strato è rapido.

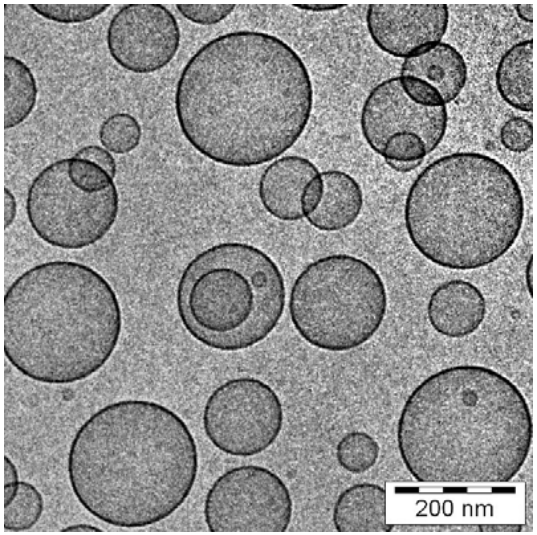


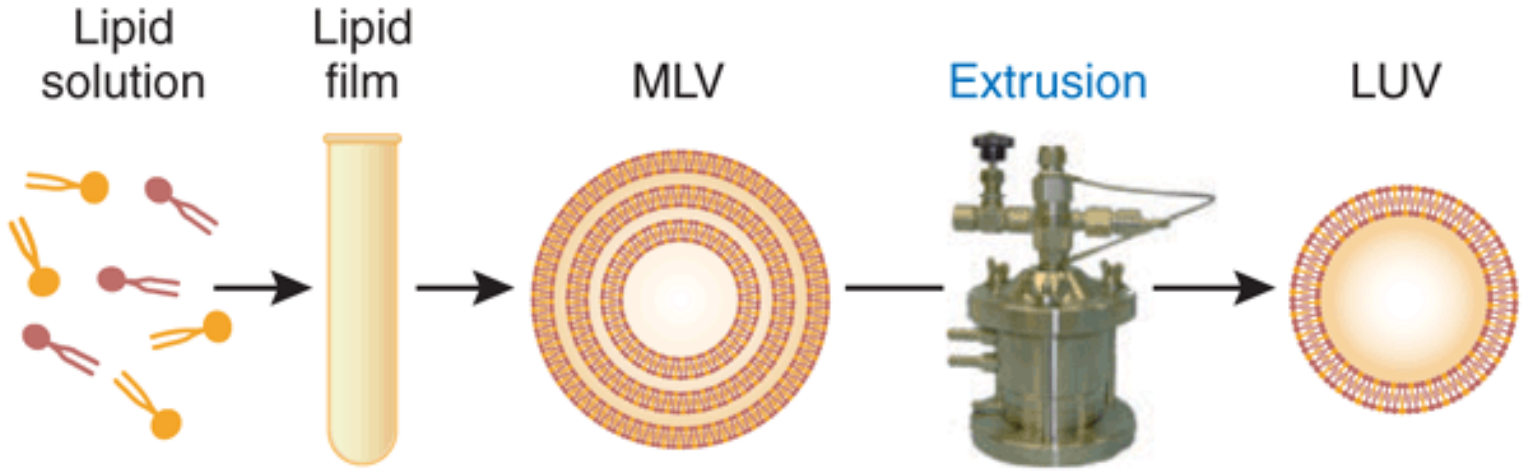
A bassa T le catene idrocarburiche sono completamente estese ed impaccate (**fase gel**), ad alta T le catene diventano più mobili (fase fluida). La transizione avviene ad una determinata T detta di transizione di fase.

Liposomes: synthesis

Classical methods: sonication and extrusion







Immergendo i fosfolipidi in acqua possiamo constatare che le teste di questi interaggiranno con l'acqua mentre le code (idrofobiche) si disporranno a forma di micelle in cerchio (per esempio come quando mettiamo del detersivo in acqua) non interagendo con l'acqua. Nonostante l'insolubilità in acqua, i fosfolipidi possono disperdersi in acqua, dando luogo ad emulsioni.

Inoltre, le loro caratteristiche strutturali (forma allungata; marcata asimmetria; carattere polare presente in una porzione ristretta della molecola, con netta suddivisione della porzione polare da quella apolare; possibilità di formare due ordini di legami, interazioni forti tra le teste polari e interazioni deboli tra le code alifatiche) consentono ai fosfolipidi di presentare fasi liquido-cristalline. La fase liquido-cristallina (o di cristallo liquido) è una fase intermedia (o mesofase) tra la fase solida e quella liquida, per cui manifesta alcune proprietà caratteristiche del primo stato (es. disposizione ordinata delle molecole, che si allineano secondo l'asse longitudinale, con tendenza all'allineamento anche dei centri di gravità delle molecole; in contrasto con la disposizione casuale caratteristica dello stato fluido) ed alcune del secondo (es. mobilità delle molecole all'interno del piano di allineamento). La proprietà dei fosfolipidi di formare fasi liquido-cristalline è alla base della struttura delle membrane cellulari.

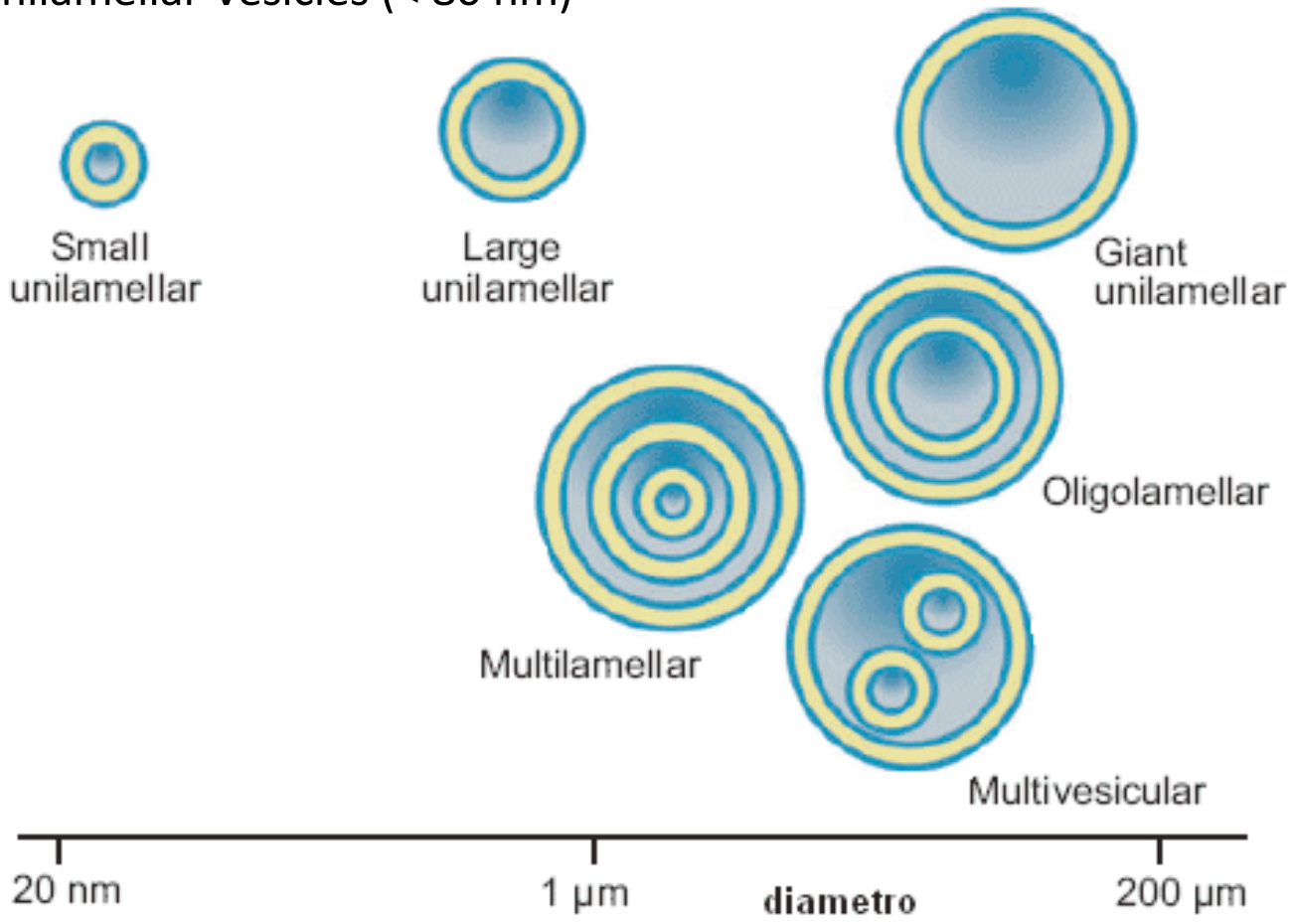
Le fasi liquido-cristalline più frequenti e meglio conosciute formate dai fosfolipidi comprendono: fase lamellare monomolecolare, fase micellare (particelle sferiche), fase esagonale (particelle cilindriche) HI, fase lamellare bimolecolare e fase esagonale invertita HII, a queste vanno aggiunte le meno frequenti fasi cubica e rombica.

Il tipo di fasi liquido-cristalline assunte dai singoli fosfolipidi dipende da diversi fattori. In primo luogo da **temperatura** e **concentrazione**, quindi dalla **forma dei fosfolipidi**, che è data dalla conformazione della testa e della coda e dal loro ingombro sterico. L'ingombro della testa dipende dalla sua carica, dal grado di idrofilia e dalle repulsioni o attrazioni elettrostatiche con le teste delle molecole adiacenti. Nel caso delle code, la lunghezza ed il grado di insaturazione delle catene alifatiche determinano il volume occupato dalla coda fosfolipidica. Infatti, la presenza di un doppio legame in conformazione cis in una delle catene alifatiche causa un inginocchiamento della catena, aumentando l'area occupata dalla molecola. La temperatura ha un'influenza rilevantissima, poiché essa determina il grado di agitazione termica delle catene alifatiche e, quindi, modifica fortemente il volume occupato dalle code.

E' possibile classificare i liposomi in base alle loro diverse e più rilevanti caratteristiche:
le dimensioni,
la lamellarità (numero di doppi strati lipidici di cui è composto il liposoma)
ed il metodo di preparazione adottato.
Questi parametri, oltre che definire le differenze
fra i liposomi, comportano significative caratteristiche, quali: stabilità, farmacocinetica,
biodisponibilità. Queste caratteristiche possono influenzare notevolmente le possibilità di
utilizzo dei liposomi.

diversi tipi di liposomi

GUV Giant Unilamellar Vesicles
LUV Large Unilamellar Vesicles (> 80 nm)
MLV Multilamellar Vesicles
MUV Medium size Unilamellar Vesicles
OLV OligoLamellar Vesicles
SUV Small Unilamellar Vesicles (< 80 nm)



Accanto a questa classificazione dei liposomi, se ne aggiunge una funzionale, che evidenzia più immediatamente le particolari caratteristiche:

liposomi sensibili al pH: la loro particolare struttura li rende sensibili ad eventuali abbassamenti di pH. A pH 6.5 i lipidi che li costituiscono si protonano e favoriscono la liberazione del farmaco. Questo comportamento è praticamente vantaggioso in quanto nelle zone dove sono presenti tumori, a causa del tessuto necrotico che va formandosi con la crescita del tumore, si verifica spesso un sensibile abbassamento del pH rispetto a quello fisiologico (Smallbone et al., 2005).

liposomi termosensibili: ad una temperatura critica (generalmente intorno a 38-39 °C) diventano permeabili al farmaco, rilasciandolo. Questo comportamento può essere utile se, dopo la somministrazione, si provvede a riscaldare (tramite ultrasuoni) la zona dove è presente la massa tumorale in modo da ottenere il maggior rilascio di farmaco solo nella zona da trattare. Questo porta a numerosi vantaggi: innanzitutto il farmaco è concentrato nella zona interessata (quindi necessitano dosaggi più bassi), secondariamente si riducono eventuali tossicità in altri distretti come reni e fegato (Mills et al., 2004).

Preparation of Liposomes

The correct choice of liposome preparation method depends on the following parameters;

- the physicochemical characteristics of the material to be entrapped and those of the liposomal ingredients;
- the nature of the medium in which the lipid vesicles are dispersed
- the effective concentration of the entrapped substance and its potential toxicity;
- additional processes involved during application/delivery of the vesicles;
- optimum size, polydispersity and shelf-life of the vesicles for the intended application; and,
- batch-to-batch reproducibility and possibility of large-scale production of safe and efficient liposomal products

Formation of liposomes and nanoliposomes is not a spontaneous process.

Lipid vesicles are formed when phospholipids such as lecithin are placed in water and consequently form one bilayer or a series of bilayers, each separated by water molecules, once enough energy is supplied.

Liposomes can be created by sonicating phosphatidylcholine rich phospholipids in water.

- Low shear rates create multilamellar liposomes, which have many layers like an onion.
- Continued high-shear sonication tends to form smaller unilamellar liposomes. In this technique, the liposome contents are the same as the contents of the aqueous phase. Sonication is generally considered a "gross" method of preparation as it can damage the structure

of the drug to be encapsulated. Newer methods such as extrusion and Mozafari method are employed to produce materials for human use.

- **Liposomes are used as models for artificial membranes.**

Liposomes as drug carriers

Liposomes can also be designed to deliver drugs in other ways. Liposomes that contain low (or high) pH can be constructed such that dissolved aqueous drugs will be charged in solution (i.e., the pH is outside the drug's pI range). As the pH naturally neutralizes within the liposome (protons can pass through some membranes), the drug will also be neutralized, allowing it to freely pass through a membrane. These liposomes work to deliver drug by diffusion rather than by direct cell fusion.

Another strategy for liposome drug delivery is to target endocytosis events. Liposomes can be made in a particular size range that makes them viable targets for natural macrophage phagocytosis. These liposomes may be digested while in the macrophage's phagosome, thus releasing its drug. Liposomes can also be decorated with opsonins and ligands to activate endocytosis in other cell types.

The use of liposomes for transformation or transfection of DNA into a host cell is known as lipofection.

Evolution of liposomes for drug delivery

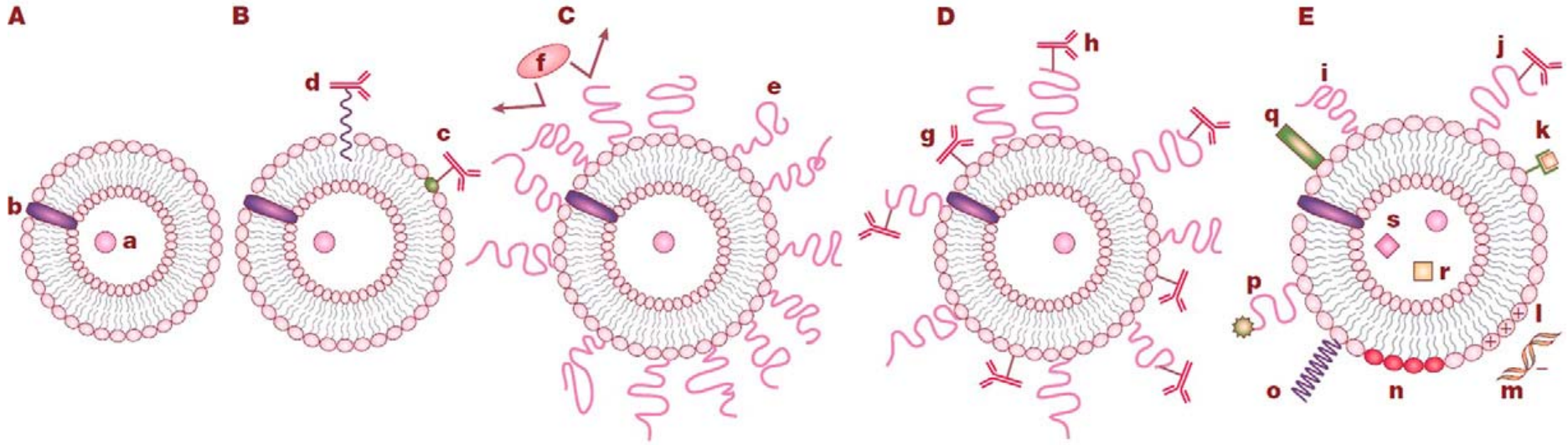
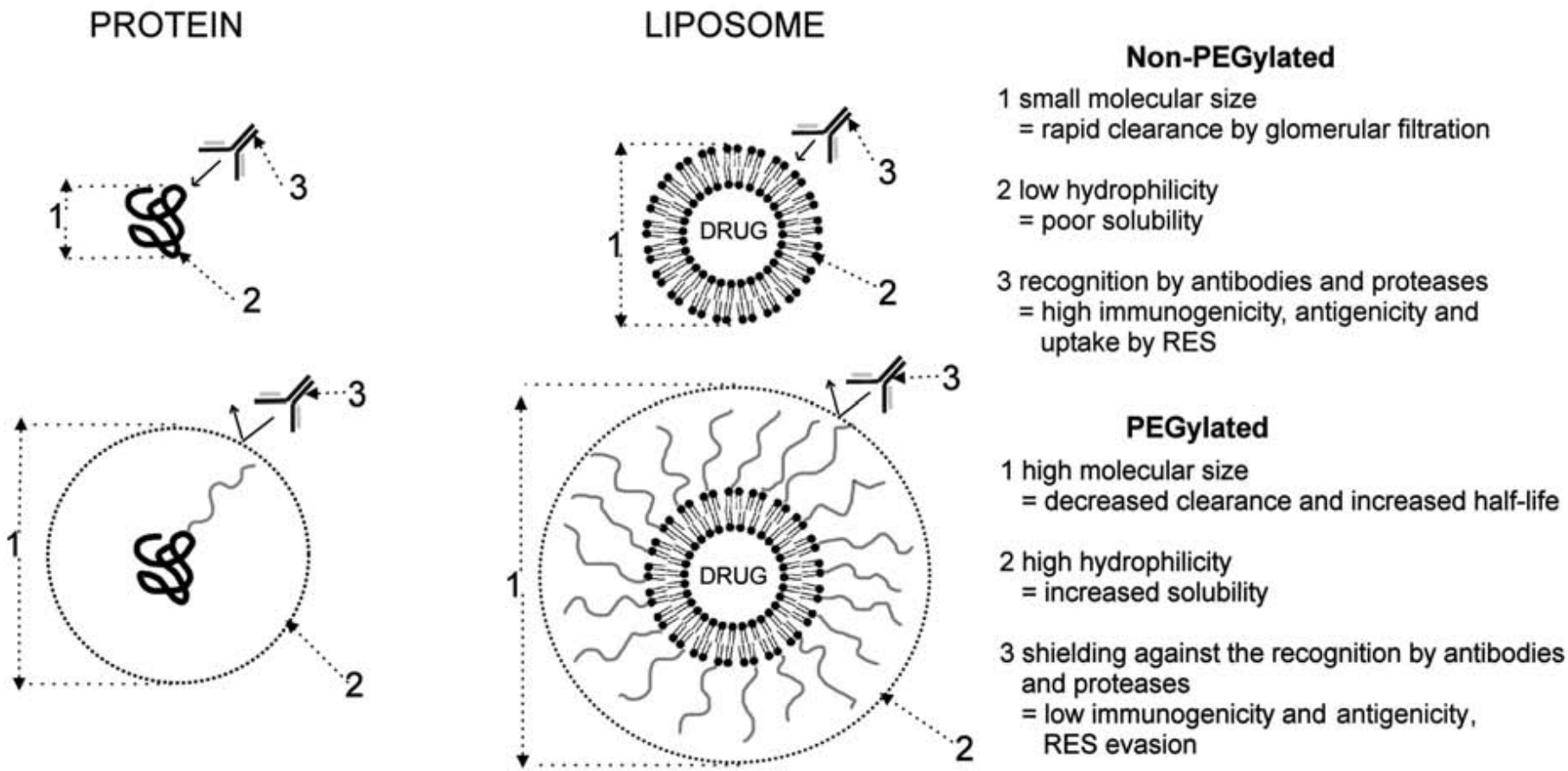


Fig. 2. Evolution of liposomes. (A) Early traditional liposomes with water soluble drug (a) entrapped into the aqueous liposome interior, and lipophilic drug (b) incorporated into the liposomal membrane. (B) Antibody-targeted immunoliposome with antibody covalently coupled (c) to the reactive phospholipids in the membrane, or hydrophobically anchored (d) into the liposomal membrane after preliminary modification with a hydrophobic moiety. (C) Long-circulating liposome grafted with a protective polymer (e) such as PEG, which shields the liposome surface from the interaction with opsonizing proteins (f). (D) Long-circulating immunoliposome simultaneously bearing both protective polymer and antibody, which can be attached to the liposome surface (g) or, preferably, to the distal end of the grafted polymeric chain (h). (E) New-generation liposome, the surface of which can be modified (separately or simultaneously) by different ways. Among these modifications are: the attachment of protective polymer (i) or protective polymer and targeting ligand, such as antibody (j); the attachment/incorporation of a diagnostic label (k); the incorporation of positively charged lipids (l) allowing for the complexation with DNA yielding lipoplex structures (m); the incorporation of stimuli-sensitive lipids (n); the attachment of a stimuli-sensitive polymer (o); the attachment of a cell-penetrating peptide (p); the incorporation of viral components (q). In addition to a drug, liposomes can be loaded with magnetic particles (r) for magnetic targeting and/or with colloidal gold, silver particles or fluorescent molecules (s) for microscopic analysis. Reproduced from 20: Torchilin VP. Nat Rev Drug Discov. 2005;4(2):145–160.

Liposome-based cancer therapy

Carrier design: stealth properties



Non-PEGylated

1 small molecular size
= rapid clearance by glomerular filtration

2 low hydrophilicity
= poor solubility

3 recognition by antibodies and proteases
= high immunogenicity, antigenicity and uptake by RES

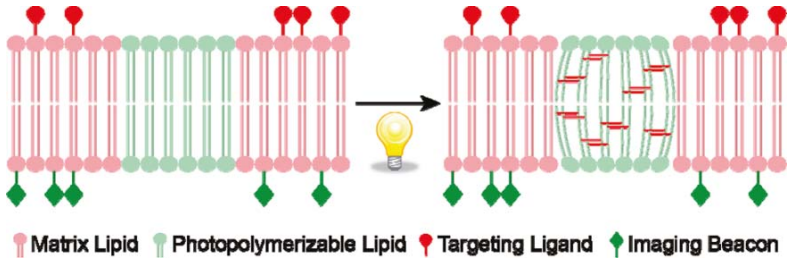
PEGylated

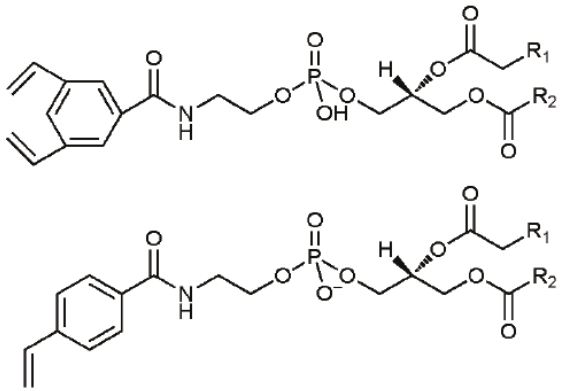
1 high molecular size
= decreased clearance and increased half-life

2 high hydrophilicity
= increased solubility

3 shielding against the recognition by antibodies and proteases
= low immunogenicity and antigenicity, RES evasion

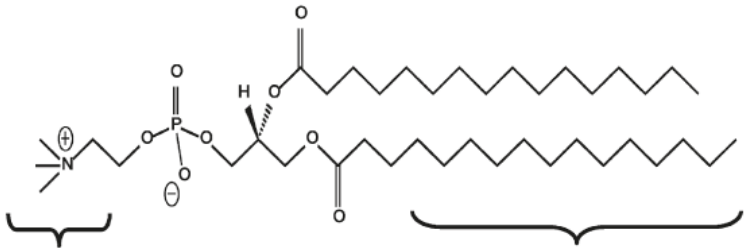
Evolutions: polymerizable liposomes





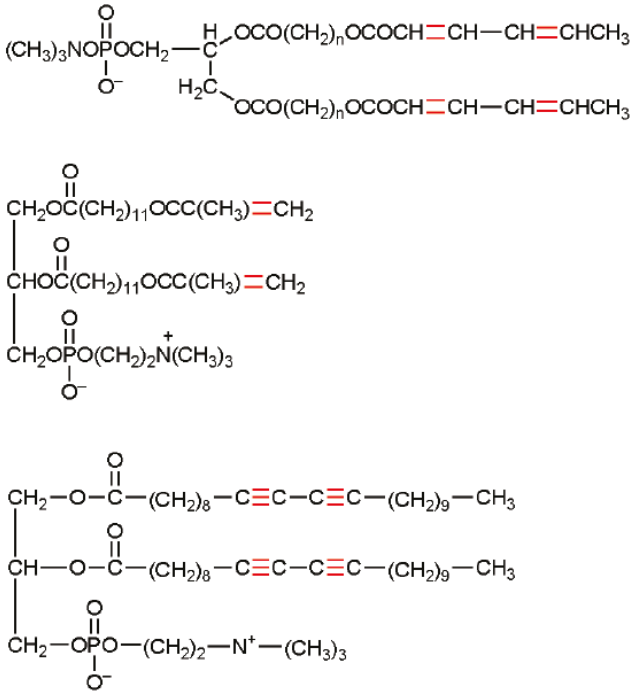
Free Radical Initiated Polymerization

Sites for Chemical Modifications in Phospholipids (photoreactive lipids)



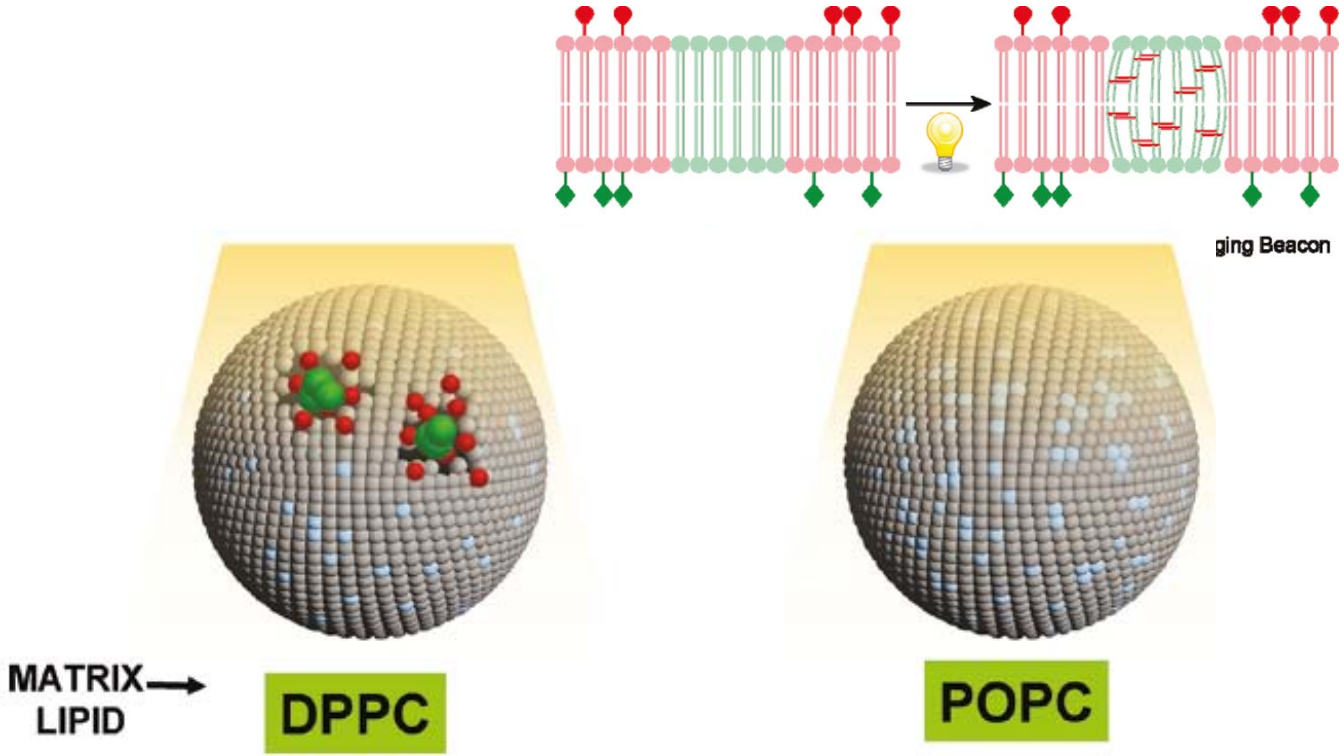
Head group Polymerization *(refs 33-37,70)*

Photo-polymerization *(Refs 12, 14, 17, 18, 30, 32, 39, 40,42,44,46)*



Fatty Acyl Chain Modified Polymerization

Evolutions: polymerizable liposomes

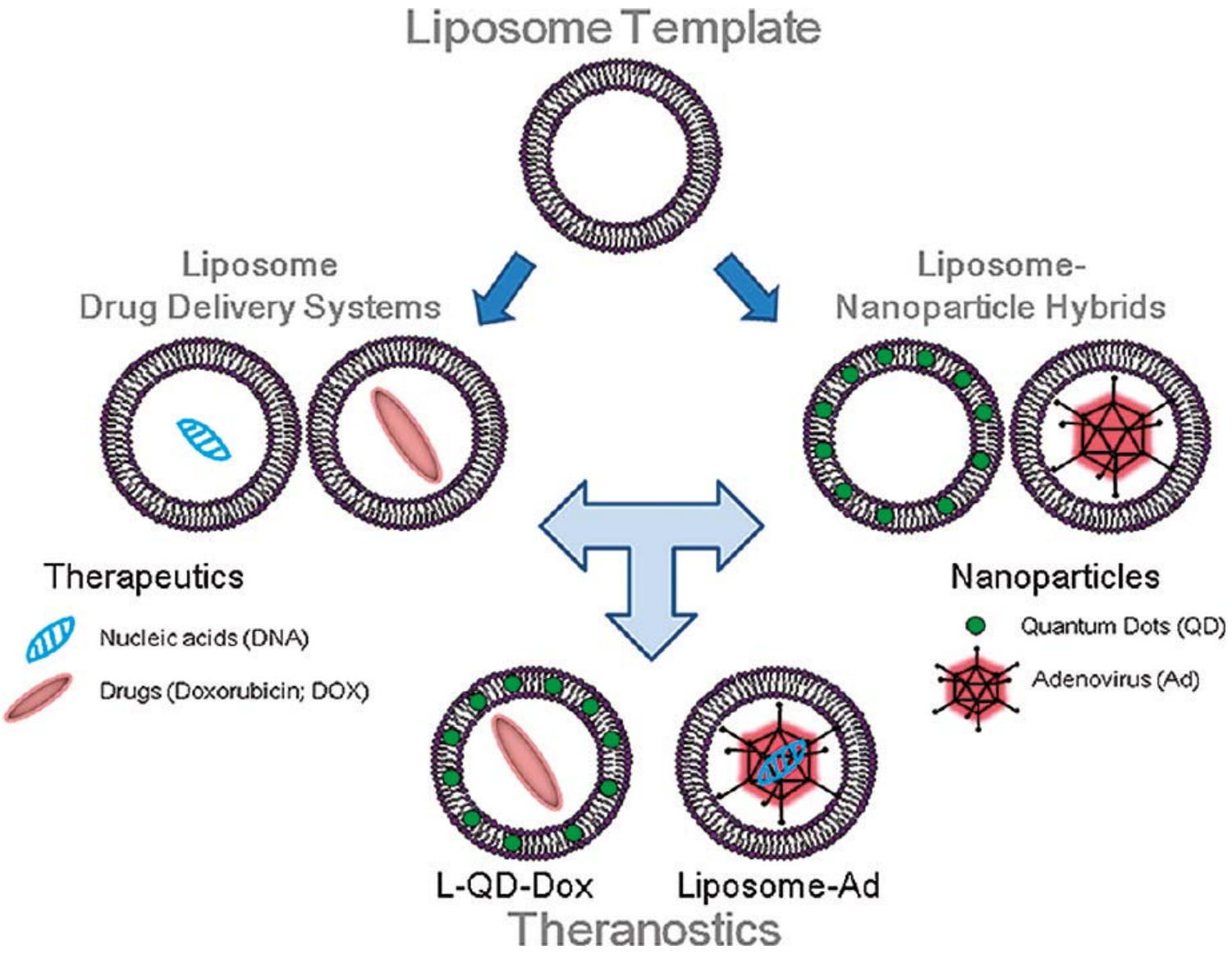


dipalmitoylphosphatidylcholine (DPPC)

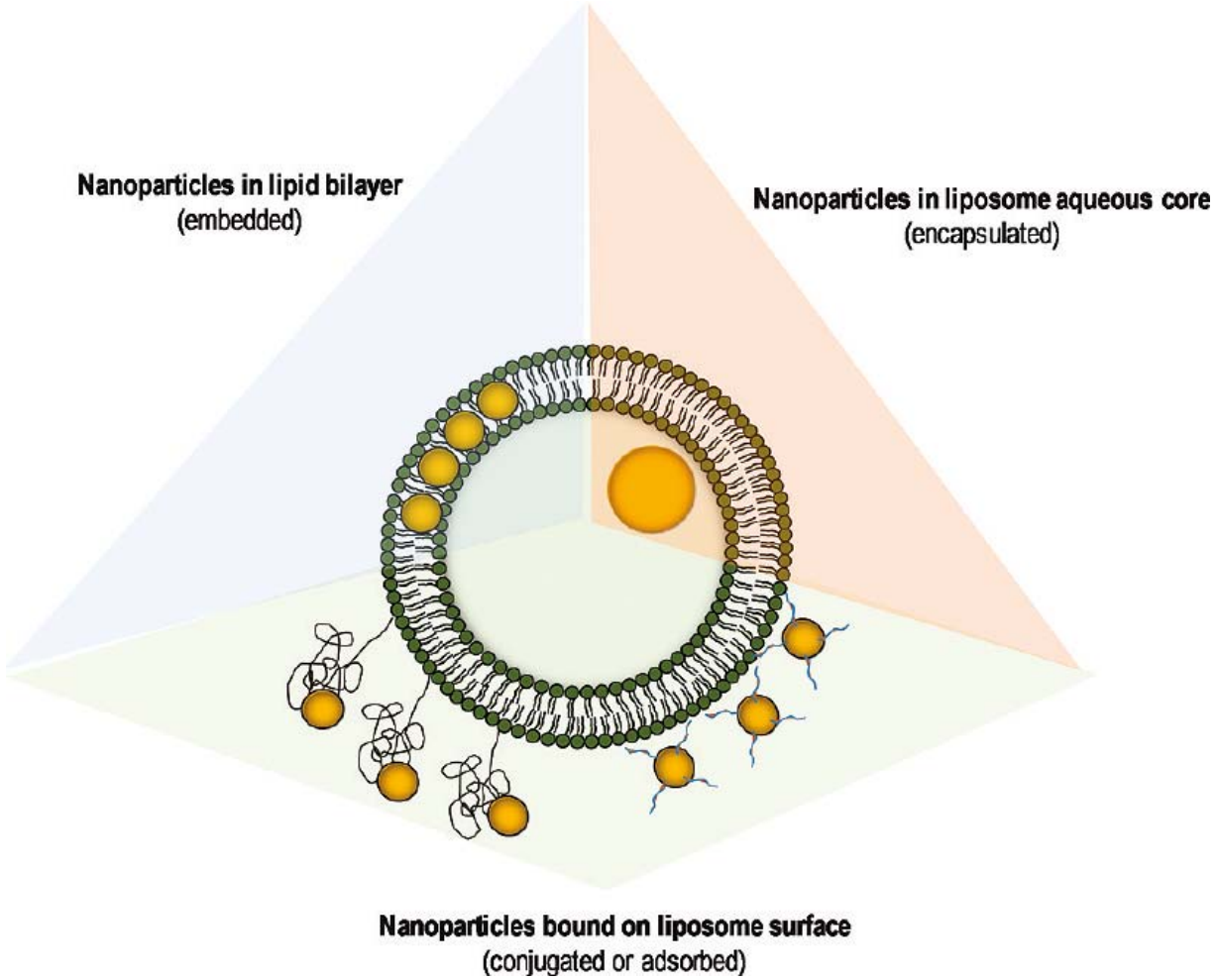
1-palmitoyl-2-oleoyl-*sn*-phosphatidyl choline (POPC)

FIGURE 3. Cartoon depicting effect of bulk (matrix) lipids on self-assembly of a polymerizable lipid, $DC_{8,9}PC$ in the lipid bilayers. Gray, matrix lipid (left panel, DPPC (T_m, 41 °C); right panel, POPC (T_m, −2 °C)). Blue, light-activated $DC_{8,9}PC$ (T_m, 44 °C). $DC_{8,9}PC$ clustering in DPPC results in light-induced activation of molecules (shown in blue) that leads to $DC_{8,9}PC$ polymerization. This results in release of drugs (green) or imaging molecules (red). Right panel, $DC_{8,9}PC$ is not clustered in POPC molecules; light treatment results in activation of $DC_{8,9}PC$, but no polymerization and hence no release of contents. Adapted from refs 12 and 20.

Evolutions: nanoparticles liposome hybrids

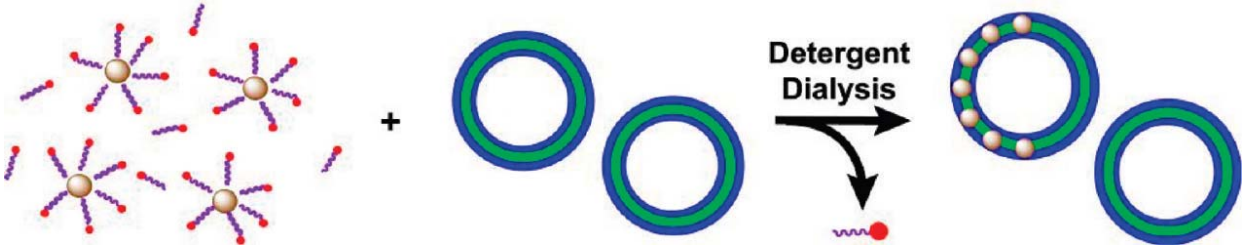


Evolutions: nanoparticles-liposome hybrids

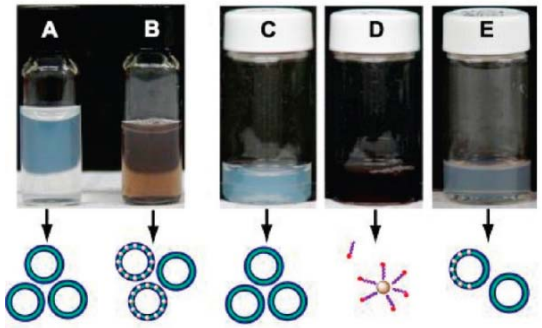


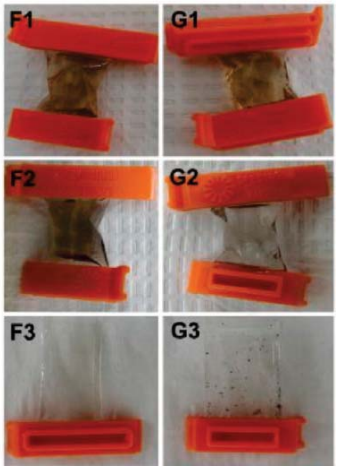
Embedding nanoparticles in the double layer

- 50 nm POPC liposomes
- 1.8 nm gold nanoparticles coated with 1-dodecanethiol



Co-extrusion

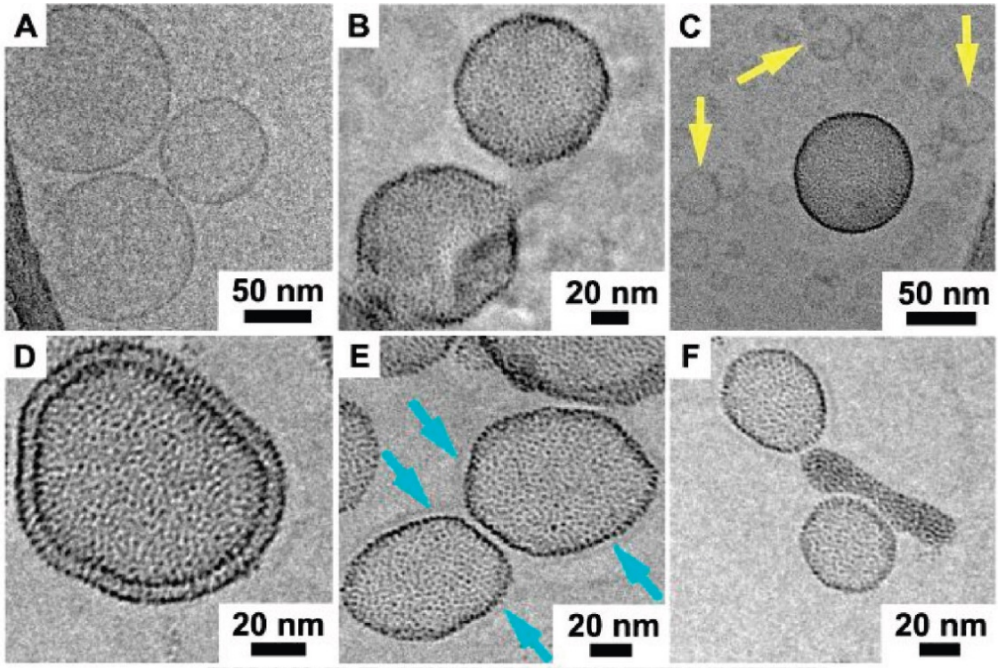




Dyalisis

Embedding nanoparticles in the double layer

Co-extrusion

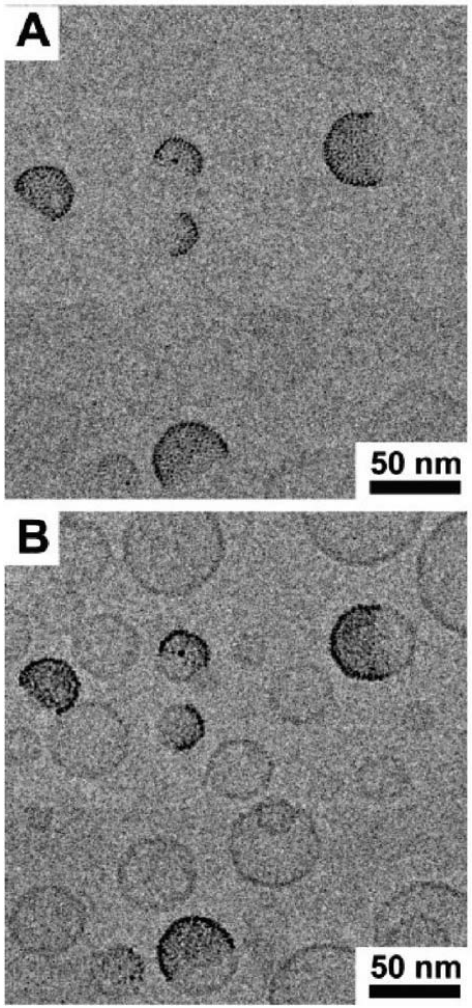


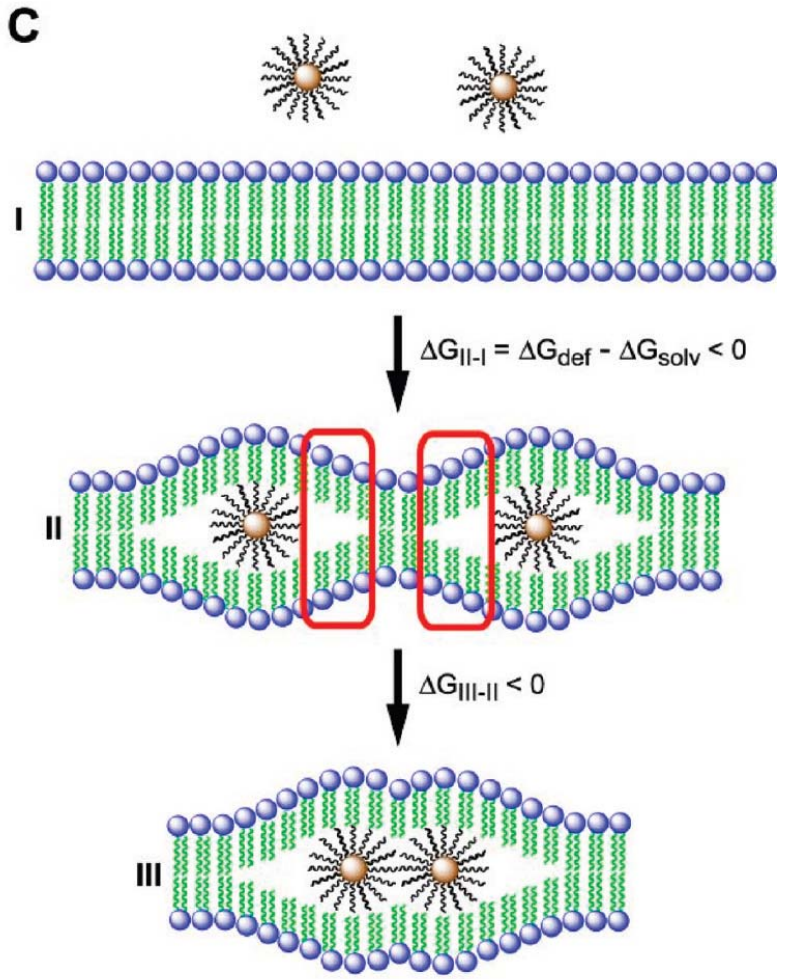
Lipid:np 1500:1

Lipid:np 100:1

Embedding nanoparticles in the double layer

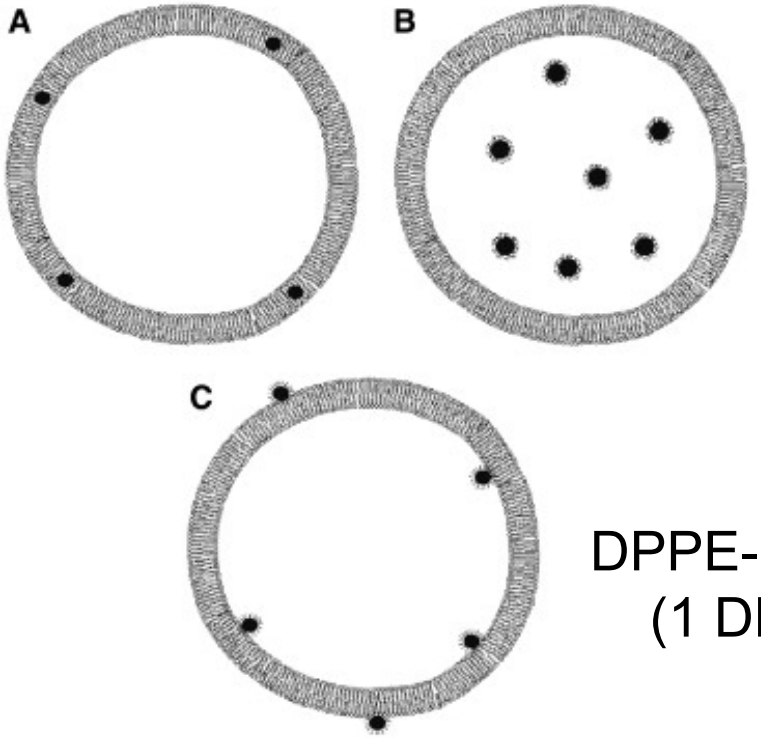
Dialysis





Phototriggered drug release

1-hexanethiol

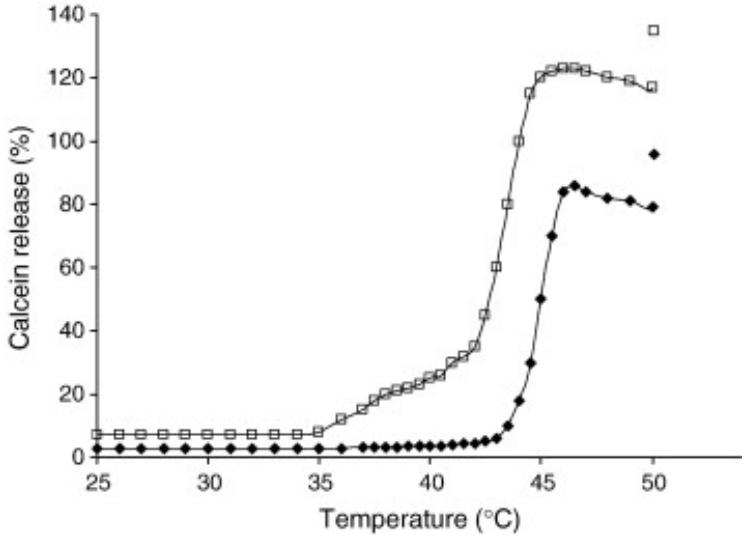


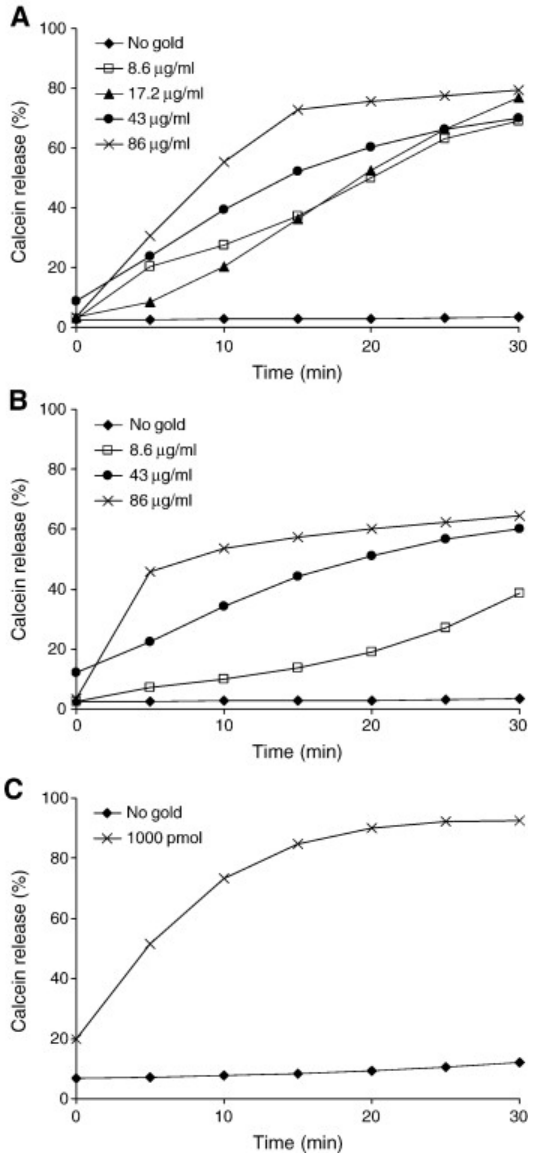
Mercaptosuccinate

DPPE-Nanogold®-particles
(1 DPPE per particle)

Prepared by co-extrusion

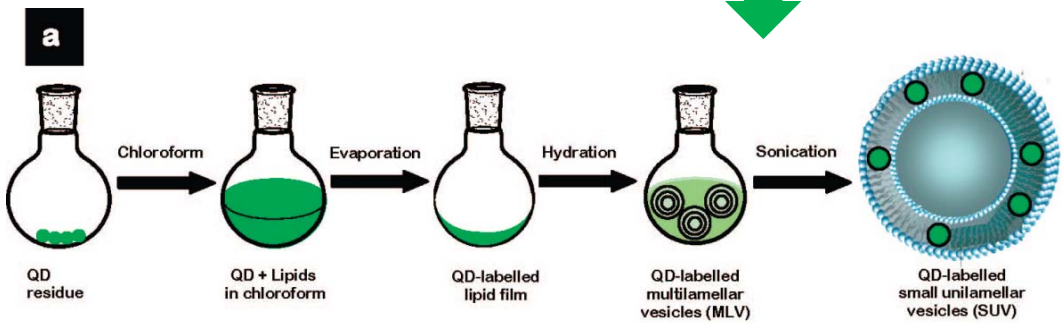
Phototriggered drug release

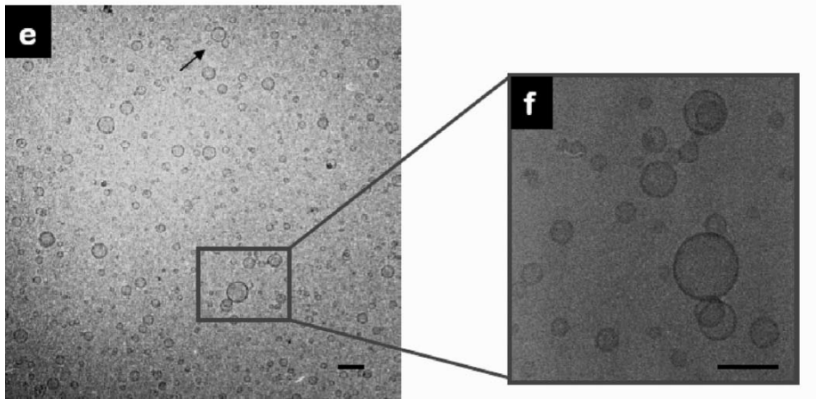




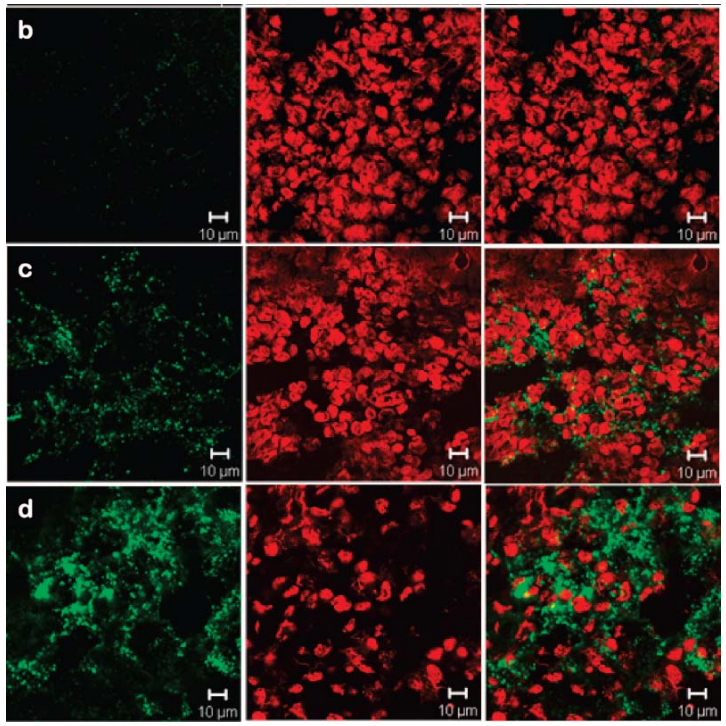
Nanoparticles-liposome hybrids

		Nanoparticle type	Lipid composition	Hybrid average diameter	Hybrid Functionality	Theranostic activity	Ref
Bilayer Embedded NP	Gold	Dodecanethiol coated Au NP (2nm)	PC	50-60nm	Cell membrane probe	No	19
	Gold	Hexanethiol capped Au NP (2.5nm)	DSPC:DPPC	200-500nm	UV light-induced drug release	No	20
	Gold	Stearylamine coated Au NP (3-4nm)	DPPC	20-200nm	Stabilize liposome membrane	No	21
	Iron	Oleic acid coated SPIO (5nm)	DPPC	150-200nm	Radiofrequency-induced drug release	No	22
	QD	TOPO-capped CdSe QD (2-4nm)	DMPC:DOTAP:DPPE-PEG_{2000}	20-100nm	QD solubilization Cell labeling *in vitro*	No	23
	QD	TOPO-capped CdSe/ZnS QD (2-4nm)	DOTAP:DOPE:Chol DSPC:Chol:DSPE:PEG_{2000}	80-100nm	Cell labeling *in vitro* and *in vivo* Cell imaging and drug delivery	Yes (Doxorubicin)	24,25





Xenografted tumor slices (gree: QD, red: nuclei)



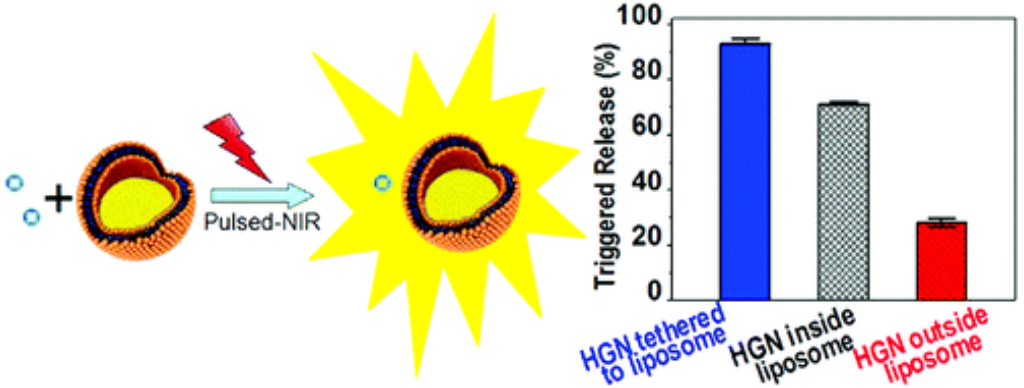
DSPC
5 m

DOTAP
5 m

DOTAP
24 h

Nanoparticles-liposome hybrids

Encapsulated NP	Gold	Gold nanoshell (100nm)	PC:Chol:DPPE-PEG_{2000}	N/A	Phototherapy-induced hyperthermia	No	26
		Hollow gold nanoshell (30-40nm)	DPPC	400-500nm	Laser-induced drug release	No	27
	Ceramic	$Y2O3:Er^{+3}$ (150nm)	DPPC:Chol:DPPG	500nm	NIR imaging	No	28
	QD	COOH-PEG-QD (25nm)	DOPC:DC-Chol DSPC:Chol:DSPE-PEG_{2000}	80-100nm	Cell labeling and imaging Tumor targeting	No	29,30
	Iron oxide	Magnetite (Fe_3O_4)	TMAG:DLPC: DOPE	N/A	Cell sorting and gene delivery	No	31
		Dextran Magnetite (Fe_3O_4) (5-10nm)	SPC:Chol:PS	N/A	Targeted drug delivery	No	32
		Citrate stabilized Maghemite (γFe_2O_3) (7.7nm)	EPC: DSPE-PEG_{2000}	200nm	MRI imaging	No	33
	Lipid	DSPC:Chol liposomes (50nm,200nm)	DPPC, DSPC	0.3-2μm	Drug delivery	No	34
	Polystyrene	Sulphate and amidine polystyrene NP (100-300nm)	DODAB, DODAC, DHP, PC	100-200nm	Nanoparticle stabilization Biosensor constructs	No	10,35



Nanoparticles-liposome hybrids

		Nanoparticle type	Lipid composition	Hybrid average diameter	Hybrid Functionality	Theranostic activity	Ref
Surface conjugated NP 	QD	Streptavidin-QD	DOTAP:DOPE:DSPE$_{2000}$-biotin	100nm	Multicolor cell imaging	No	36
		Carboxylated CdSe/ZnS Qd chemically linked to amine functionalized PEG$_{2000}$-DSPE (4nm)	DSPC:Chol:DSPE-PEG$_{2000}$	200nm	Imaging and therapeutic modalities	Yes (Doxorubicin)	37
	Gold	Citrate coated Au NP (13nm)	EYPC:DDAB EYPC:DSPE-PEG$_{2000}$	200nm	Increase liposome colloidal stability	No	38
		DPPE-Nanogold (1.4nm)	DPPC:Chol	90 nm	Drug delivery and imaging system	No	39
		DPPE-Nanogold (1.4nm)	DSPC:DPPC	200-500nm	Light-induced drug release	No	20
		Hollow gold nanoshell (30-40nm)	DPPC	400-500nm	Laser-induced drug release	No	27
		PEG-maleimide-functionalized Au NP (64nm)	SOPC:DOPE	120-620nm	Cell membrane probe	No	40
Surface adsorbed/complexed NP 	QD	DNA-QD conjugate	Lipofectamine2000	N/A	Cell labeling and gene delivery	Yes (pDNA)	41
		PEG-QD	Lipofectamine2000	N/A	Co-delivery of siRNA and QD	Yes (siRNA)	42
	Gold	COOH-Au (4nm)	EPC:DOTAP	92nm	Stimuli-responsive (acid) NP-stabilized liposomes	No	43
		Hydrophilic Au NP (300nm aggregates)	DPPC:DOTAP:Chol	5µm	NIR-induced drug release	No	44
		DDAB coated Au NP (9nm)	DOTAP, Lipotap	N/A	Gene delivery	Yes (pDNA)	45
	Polystyrene	COOH- polystyrene NP (20nm)	DLPC	N/A	Liposome stabilization	No	46

immunoliposomi: vengono creati e studiati in maniera tale che disaggreghino e liberino il farmaco quando entrano in contatto con una cellula che presenti un antigene specifico. Questo comportamento è realizzato integrando nella membrana fosfolipidica del liposoma anticorpi monoclonali specifici per antigeni tumorali. In questo modo, il legame tra antigene e anticorpo avvicinerà in maniera determinante il liposoma alla cellula bersaglio liberando il farmaco preferenzialmente su quest'ultima (Park, 2002).

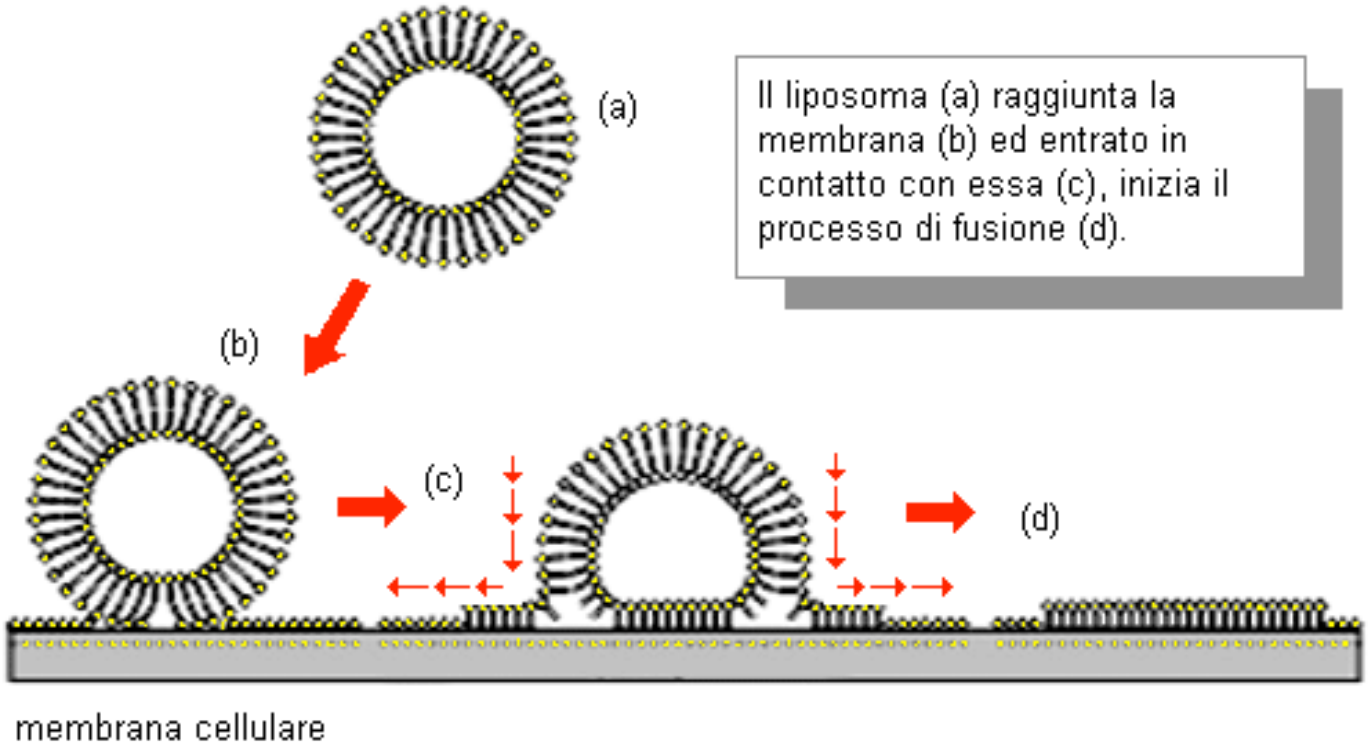


Table 1. Liposome-based drugs on market, Table 1

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3260950/table/t1-ijn-7-049/

Table 2. Liposome-based drugs in clinical trials, Table 2

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3260950/table/t2-ijn-7-049/

Table 1
Marketed liposomal and lipid-based products, plus a selection of products in clinical development.

Product	Drug	Indications	Year approved	Reference
Approved products				
AmBisome (Gilead)	Amphotericin B	Fungal infections Leishmaniasis,	1990 (Europe), 1997 (USA), 2000	[255,256]
Doxil/Caelyx (Johnson & Johnson)	Doxorubicin	Kaposi's sarcoma Ovarian cancer Breast Cancer Multiple myeloma + Velcade	1995 1999 2003 (Europe, Canada) 2007	[93,257–259]
DaunoXome (Galen)	Daunorubicin	Kaposi's sarcoma	1996 (Europe), 1996 (USA)	[260]
Myocet (Cephalon)	Doxorubicin	Breast cancer + cyclophosphamide	2000 (Europe)	[261]
Amphotec (Intermune)	Amphotericin B	Invasive aspergillosis	1996	[262]
Abelcet (Enzon)	Amphotericin B	Aspergillosis	1995	[263]
Visudyne (QLT)	Verteporphin	Wet macular degeneration	2000 (USA), 2003 (Japan)	[250]
DepoDur (Pacira)	Morphine sulfate	Pain following surgery	2004	[264]
DepoCyt (Pacira)	Cytosine Arabinoside	Lymphomatous meningitis Neoplastic meningitis	1999	[265,266]
Diprivan (AstraZeneca)	Propofol	Anesthesia	1986	[267]
Estrasorb (King)	Estrogen	Menopausal therapy	2003	[268]
Lipo-Dox (Taiwan Liposome)	Doxorubicin	Kaposi's sarcoma, breast and ovarian cancer	2001 (Taiwan)	[269]
Marqibo (Talon)	Vincristine	Acute lymphoblastic leukemia	2012 (USA)	[270,271]

No PEG

sphingomyelin/cholesterol
Targets MPS

- ✓ Liposomes are one of the most well-established nanoscale drug delivery systems, with several promising formulations now in clinical use. Doxil is a liposomal product delivering doxorubicin for ovarian cancer, AIDS-related Kaposi's sarcoma, and multiple myeloma.

- ✓ Comprised of amphiphilic phospholipids that self-assemble to form bilayers to enclose an aqueous phase, liposomes are an ideal candidate for dual drug delivery since they have the unique capability of entrapping both lipophilic and hydrophilic drugs.

- ✓ In cancer-based applications, they make use of the EPR effect to passively target tumors, and are easily modifed with targeting ligands, allowing them to actively target the tumor.